Papers on Anthropology

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PAPERS ON ANTHROPOLOGY
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CENTRE FOR PHYSICAL ANTHROPOLOGY

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PREFACE

This collection is dedicated to the 6th Baltic Conference of Morphology in Tartu.

The editorial board is particularly happy to extend 75th birthday congratulations to Leiu Heapost PhD, a student of Juhan Aul, long-term Chair of the Anthropology Section of the Estonian Naturalists' Society, Senior Researcher at the Department of Archaeobiology at the Institute of History.

Next year the venerable Old Anatomical Theatre will be renovated. The History Museum and the Faculty of Medicine jointly strive to continue historically and scientifically dignified activities here after the renovation. For this, the Faculty of Medicine will apply its current structural unit – the Medical Collections – which are meant to be not only a tourist attraction but also the site for constant cooperation between the anatomists and anthropologists for their joint research and communication with foreign scientists. During the last five years, three doctoral theses have been defended in Riga as a result of cooperation initiated here.

The contribution of the Centre for Physical Anthropology to cooperation, however, is its internationally recognized collection of research articles – *Papers on Anthropology*. Each year Baltic morphologists and their European colleagues publish their research articles here. The collection is annually indexed in seven European and one American database.

In the future, new centres of theoretical and applied research could be accommodated in the Old Anatomicum. The Institute of Anatomy is ready, if necessary, to give our collection a broader approach so that it could represent the Old Anatomicum as a whole under the new title *Papers of the Old Anatomical Theatre*.

In any case, the editorial board recommends the organizers to follow a very valuable recent trend of European museums – a skilful combination of tourism and research.

Prof. Helje Kaarma

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MEDICAL COLLECTIONS IN TARTU OLD ANATOMICAL THEATRE – FROM AUGUST RAUBER'S ANATOMY MUSEUM TO A MULTIFUNCTIONAL RESEARCH AND EDUTAINMENT CENTRE

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In 1890 Professor of Anatomy August Antonius Rauber founded a museum of anatomy at the Old Anatomical Theatre in Tartu (Dorpat) in order to illustrate his lectures and to improve the students' knowledge. As Rauber lectured in German whereas most of the students were Russians, illustrating lectures with models and original specimens was of great significance. Rauber himself was very skilful at anatomical preparation and he also trained the preparator Aleksander Reinvald who was of Estonian ethnicity. He writes, "Seitdem führt dieser Studiensaal ein fröhliches, nie unterbrochenes Dasein, hat sich in der Studentenschaft fest eingebürgert und wird voraussichtlich in Zukunft nicht wieder von der Bildfläche verschwinden" (Rauber, 1895) (Since then, the lecture hall is uninterruptedly teeming with happy life; it has found its rightful place among the students and, in all likelihood, will not disappear from the scene in the future). Rauber considered the museum most essential for the training of medical students and wrote about it in 1895: "Even the first impression of the hall is of significant educational value for the young medical student. It can be very well compared with the influence of the art museum on a young susceptible soul, which exceeds a certain limit where the scientific influence of the exhibits prevails. The first impression is festive and majestic, much more powerful than what a first-year student expects to see at the Anatomical Theatre. Thus, even the unexpectedness of the sight exerts a favourable influence. Many of them are so overwhelmed by the richness of the material that a certain amount of time is needed until an understanding is reached. Thus, the first impression is of great value for the entrant and

makes an excellent beginning. During the semesters of clinical studies the student will experience the infinite practical value of anatomy. …. The painfully acquired anatomical knowledge will fade too quickly or disappear completely if one does not constantly work on its consolidation. Here, the museum of anatomy offers an excellent opportunity for avoiding such a danger" (Rauber, 1895). In the afternoons, after lectures, the exhibition was open for students of other faculties and townspeople. On Fridays, the exhibition was visited by housewives.

In his 1895 publication *Über die Einrichtung von Studiensälen in anatomischen Instituten*, Professor Rauber emphasized that people from other fields should visit the collections. "The exhibition hall which entirely lacks the frightening aspect is appropriate for students of other faculties for acquiring knowledge about their bodies through visualized teaching. If such an exhibition hall is able to exert its positive influence outside the Faculty of Medicine and encourage non-medics to study anatomy, it is, in my opinion, a sufficiently great achievement to justify their foundation at the institutes of anatomy in the future" (Rauber, 1895).

Unfortunately, a great number of exhibits of Rauber's museum, although not all of them, have been lost in the turmoil of wars. In September 2005, a medical exhibition was opened in the same Old Anatomical Theatre again, now under the name of the Medical Collections of the Faculty of Medicine of the University of Tartu. The exhibits include those that have survived from the time of Prof. August Rauber's museum. Some of them have been described in his famous textbook *Lehrbuch der Anatomie des Menschen*. The major part of the collection consists of exhibits on pathological anatomy: dissection and operation materials classified according to organ systems, wallcharts and wax moulages, numerous pathological bone specimens and skulls. The embryology collection shows specimens of normal development of human and animal foetuses but also malformations and wax models. The collection of the Estonian neurosurgeon Ludvig Puusepp (1899–1942) who worked in Russia before 1920 demonstrates several specimens of brains in fluid. The pharmacological display introduces scientists from Rudolf Buchheim (1820–1879), who in 1846 founded the first experimental laboratory of pharmacology in the world, to Lembit Allikmets (1936–) who was a long-time dean of the Medical Faculty, is an honorary citizen of Tartu and has done a lot for the

development of medicine in Tartu. The collection of forensic medical specimens demonstrates different specimens related to intoxication, accidents, murder and suicide. The radiology collection demonstrates X-ray equipment from different periods and also has a radiograph of hand from year 1896. Even Professor August Antonius Rauber himself is present again – his full-size wax figure is sitting behind his own writing-desk.

Usually different collections as memory institutions collect items that have lost their original function. The case is different, however, with the medical collections of the University of Tartu. With every information poster and specimen, the display reminds every interested visitor of the great contribution that the scientists who have worked at the Old Anatomical Theatre and collected and produced these specimens have made to Estonian as well as world science. A medical professional from every corner of the world is familiar with eponyms such as Burdach bundle (*fasciculus cuneatus – cuneate fasciculus*), Boettcher cells (*epitheliocytus glandularis externus basalis*), Kupffer cells (*macrophagocytus stellatus*), Rathke's pouch (*saccus hypophysialis*), Rathke's bundles (*trabeculae carneae*), Reichert's cartilage, Reichert's recess (*recessus cochlearis*), Volkmann's canal (*canalis transversus*, *canalis perforans*), Thoma ampulls, Hueck's ligament (*ligamentum pectinatum anguli iridocornealis*), Rauber's hepatic cord (*a. hepatica propria*), Rauber's layer, Rauber's ligament (*ligamentum atlantoaxiale accessories*), Reissner's membrane (*membrana vestibularis*), Reissner's duct (*ductus cochlearis*), Pirogov's triangle (*trigonum linguale*), Waldeyer's-Pirogov tonsillar ring, Bidder's organ, Bidder's knot, etc. They come and feel thankful towards these men. They are also grateful that such a collection has been preserved and that old specimens have not been destroyed or thrown away in shifting political and academic winds.

Medical exhibits, however, have retained their original function – they contribute to the enrichment of knowledge of medical students. As a new function, the specimens of medical collections have become objects for international comparative biomedical studies. For example, when researching for her doctoral thesis *Identifikation von Mutationen im Tumorsuppressorgen p53 und des Bakteriums Helicobacter pylori in Magenkarzinomen histopathologischer Präparate aus verschiedenen medizinhistorischen Sammlungen* in 2011, Dr. Katharina Licht from

Georg-August-University of Göttingen also used samples of gastric cancer from the Medical Collections of the University of Tartu, and as a result of her study she concluded that both, the changes of the mutation spectrum as well as of the localization of the mutations, indicate that the influences on the development of gastric carcinomas have changed during the last century. In addition to the large number of old specimens of different pathologies the Medical Collection in the Old Anatomical Theatre in Tartu also have unique exhibits of diseases which are rare nowadays or exhibits of terminal conditions of diseases which are not allowed to progress to that state in modern societies.

For practising doctors, the museum is a place for refreshing their memory and comparing symptoms. The museum has also acquired a number of new functions. The same exhibition is used to playfully teach very young children (of the 4th school year) to know their anatomy and physiology. The exhibition has become a part of the education system through which school students and adults learn about the hazards of risk behaviour (smoking, overconsumption of alcohol, drug addiction, unsafe sex, wrong nutrition, etc.). Attempts are made to get schoolchildren interested in biology and medicine, which has caused great competition among those who would like to take up studies of medicine at the university. The medical exhibition has also become a place for self-reflection where adherents of a healthy way of life from the whole world get assurance that their behaviour is correct. As the feedback questionnaires and interviews with visitors show, in many cases the exhibition makes those who practise risk behaviour seriously reconsider their lifestyle and can provide impetus for changing their lifestyle and prolonging their lifespan. The exhibition has also been well received by disabled people as it gives them strength to cope with their lives. Specimens, models and posters tell the story of study and discovery of the human organism that has been increasingly dependent on the development of technology. The exhibits have been of great interest for children and adult artists, and their pictures inspired by the exhibition have been of great interest for visitors.

In conclusion, we can say that in the 21st century the study hall that initially was meant to illustrate lectures has become a multifunctional place of edutainment. It has become a place where families and children spend their free time; school and university students study; doctors recall the time of their studies and revise what they have learned.

Researchers study specimens of diseases. The collections have become a place where the exhibits influence people's emotions, giving them confidence or inspiring them to make changes in their lives.

The authors are of the opinion that the collections should retain all of their present functions because each specimen is special and different. Dividing the collections up into separate parts and displaying them at different places would destroy the wholeness of the exhibition and jeopardize the preservation of many rare specimens for the visitors.

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A SHORT OVERVIEW OF THE WORK OF ANTHROPOLOGISTS OF THE OLD ANATOMICAL THEATRE

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The anthropologists of the University of Tartu have continued Juhan Aul's work in all its directions. As Juhan Aul considered it important to assess the physical development of great numbers of schoolchildren, to pay greater attention to anthropometric studies of women and regularly publish research papers on anthropology [1–5], his advice has been followed.

In order to coordinate anthropological research, Juhan Aul founded the Anthropology Section of the Estonian Naturalists' Society. Its 70 years of activities have been described by J. Kasmel and T. Kasmel [9, 10].

When the Republic of Estonia had regained its independence, it became possible, in 1993, to establish the Centre for Physical Anthropology at the Institute of Anatomy of the University of Tartu. The fifteen years of activities of the Centre have been described by H. Kaarma [8].

In 1995, financed by the Ministry of Social Affairs of the Republic of Estonia, the Estonian Anthropometric Register was founded. At present, the register is located at the Centre for Physical Anthropology. It contains more than 100,000 units of anthropometric data on Estonians' body build.

Large-scale detailed anthropometric studies of schoolchildren, adult men and women and thorough statistical analysis of data under the supervision of Emeritus Professor Ene-Margit Tiit have enabled us to solve the problem of schoolchildren's, conscripts' and women's body structure, of inclusion of classical constitutional types into the general structure. It became clear that there exists a statistically significantly correlated system of anthropometric variables where the leading variables are height and weight. Changes in relations between height

and weight lead to systematic changes in length, breadth and depth measurements, circumferences, proportions and body composition characteristics. Classical types like pycnics and leptosomes also belong to this system, and changes in their body build are also related to changes in relations between their height and weight. Based on the above-mentioned, the anthropologists of Tartu created a five-class classification of body build. The latter has been successfully applied to classify the anthropometric variables of young women, conscripts, and schoolchildren aged 7–18 years [6,7,11,12].

C. Raschka presented this classification in his book *Sportanthropologie* [16] as a new Estonian system in constitutional typology.

The body build structure system created in Estonia can be proposed to physicians and health promoters who can use body build data for analysing the data of their speciality.

The Centre for Physical Anthropology has also taken care of compiling height and weight norms of adult Estonian men and women. The corresponding norms have been published [15] and, based on them, the limits of the five-class height-weight classification for each annual age group of men and women have been presented [13]. We recommend this classification to family physicians and specialists on different diseases for comparative classification of their patients' body build data.

The anthropologists of the Old Anatomical Theatre have also compiled the most recent height-weight norms of Estonian schoolchildren which date from 2006–2009 [14]. They have been discussed in comparison with the previous norms of 1998. These will also be used to create somatotypic height-weight classifications for 7–18-year-old boys and girls. These will be given to the Ministry of Social Affairs, family physicians, and school doctors and nurses for use. Summing up what has been said above, we can confirm that the anthropologists of Tartu are ready to continue their current trends of research and cooperate with Latvian and Lithuanian colleagues.

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LEIU HEAPOST – 75

Mart Viikmaa

Leiu Heapost is the third great Estonian anthropologist next to and after Juhan Aul (1897–1994) and Karin Mark (1922–1999). She, however, is the most versatile of our anthropologists. She has published studies on somatology, age-related anthropology, paleoanthropology and population genetics of Estonians and several other peoples. On the occasion of Leiu Heapost's recent 75th birthday, it is natural to look back – where has she come from and how has she become like she is?

Leiu was born on Muhu island into the family of a farmer and fisherman on Mihkli farm in Rootsivere village on 13 March 1936. The family was wealthier and more educated than the average in Muhu. Leiu speaks with excitement about the drawings in his father's diaries. Her father was also a masterful smith and cabinetmaker. Her mother came from Tüü farm in Pallasma village. This was also the home place of her mother's two uncles who were schoolteachers. The family also had an "official artist" (like Leiu says), i.e. a woman who had graduated from art school.

Leiu began her education at Piiri school, also on Muhu Island, but completed secondary school in Orissaare in Saaremaa (1956). After leaving secondary school, Leiu had a few gap years to think about her future prospects. She worked as head of Tamse village library then. Her father recommended her to study medicine. Leiu, however, did not find this profession close to her heart. She decided in favour of biology.

It could also have happened otherwise. Leiu had the skills and vision necessary for an artist. She definitely had hereditary talent for that; her

two sisters became artists indeed. Luckily for Estonian anthropology, this did not happen – Leiu was saved for science.

In 1958 Leiu became a student of biology at Tartu State University. Being delighted about the lectures of Prof. J. Aul, Head of the Department of Zoology, Leiu approached him with the wish that he would become her supervisor. Juhan Aul had recently started studies on anthropology of Estonian children's physical development. As a second-year student, Leiu began to accompany Prof. Aul on his expeditions to conduct anthropological measuring of schoolchildren. Sometimes she also joined K. Mark on her expeditions to the settlement areas of Finno-Ugric peoples and their neighbours. Thus, Leiu acquired perfectly the technique and methodology of physical anthropology and collected material for her term and graduation papers.

Nonetheless, Leiu did not forsake art either. In parallel with her studies of biology, she practised at the art studio of the university and listened to Prof. Voldemar Vaga's (1899–1999) lectures on art history. She still speaks with great enthusiasm about those lectures and the accompanying demonstrations.

Leiu graduated from the university in 1963. Her graduation thesis discussed anthropology of school students' physical development. Thus the small number of Estonian anthropologists was replenished by a new capable researcher. Unfortunately, in Soviet Estonia (just like now), the administrators of science did not consider anthropology an essential branch of science. There were no teaching or research positions for anthropologists either at the university or in the institutes of the Academy of Sciences.

As Prof. Aul did not want to lose the trained colleague who would help him in his research, he applied for an additional position of a senior laboratory assistant for his department. Leiu filled this position until 1970. Along with participation in J. Aul's research expeditions, she was engaged in statistical analysis of the data. Because of her abilities as an artist, she was used as the illustrator of textbooks written by the lecturers of the department (although her name is not mentioned there).

Along with all that, Leiu found time for studying school students of Tallinn.

In 1970 the Estonian Academy of Sciences opened postgraduate studies in anthropology. Leiu stood as a candidate and got a student place. She continued her study on anthropology of Tallinn school-

children at different ages she had begun during her Tartu period and defended her candidate's dissertation *Physical Development of Talllinn Schoolchildren* in 1976. (The materials of the dissertation were later published as a monograph). At that time, from 1974, Leiu was already a staff member at the Institute of History. She has worked in this institution up to the present, filling the positions from junior research fellow to leading research fellow. Even now, she is still working at the institute as a senior research fellow. As an active member of the academic community, Leiu has participated in several research societies (Estonian Naturalists' Society, Society of Geneticists and Selectionists of the Soviet Union, Estonian Society of Human Genetics, European Anthropological Association). She is a member of the international editorial board of the collection *Papers on Anthropology*.

Leiu has perhaps been the most inquisitive and versatile among the Estonian anthropologists. She is the initiator of systemic population-genetic research of Estonians and one of the first Estonian paleo-anthropologists in both craniology and osteology. As early as in 1966, she began, among the school students of Tallinn, in addition to somatometric studies, collection of genetic data and determination of frequency of antigen systems of several blood group systems and a few physiological characteristics (taste sensitivity to phenylthiocarbamide and red-green colour blindness). In the 1970s–1980s she broadened these studies to many samples (40 in total) all over Estonia. She initiated close cooperation with the geneticists of the Institute of General and Molecular Pathology at the University of Tartu to use their material equipment and potential for analysis.

Along with studies of Estonians, Leiu Heapost and Karin Mark, in cooperation with Soviet and Finnish anthropologists, participated in research expeditions to the Volga area, Vologda region, Western Siberia and Transcarpathia. Leiu has also collected comparative population-genetic data from Vepsians and Latvians.

Leiu has presented her research results at several international conferences of anthropologists; her papers have been published, in addition to the Soviet Union and Estonia, in a number of foreign countries (Finland, Sweden, Poland, Germany, Hungary, Belgium). The list of her printed works includes more than 100 publications. Along with the above-mentioned monograph, her largest work is the chapter on population genetics written in cooperation with Karin Mark and Galina

Sarap in a book on Estonians' ethnogenesis (1994). At present she is supplementing and editing the manuscript of her deceased colleague K. Mark *Physical Anthropology of Finno-Ugric Peoples*. Some of these materials have been published as separate articles.

Leiu's studies have given a remarkable contribution to the fact that anthropologically Estonians are one of the most thoroughly researched peoples in the world.

Leiu's population genetic analysis has confirmed and broadened the conclusions of physical anthropology on Estonians' great biological variability. She has found that in within the whole of Estonia the main scale of differences runs in the East-West direction. Based on the greatest differences, four main groups of Estonians can be differentiated: inhabitants of the Western Estonian islands, the West Estonian continental area, North-East Estonia and South-East Estonia. Other regions form transitional zones between them. She has noted that the Setu dialect area is very close to other South-Estonian samples in its genetic structure. On the contrary, some samples from the western part of Võru County clearly differ from their neighbours, and the inhabitants of Muhu Island essentially differ from those of Saaremaa (the former being closer to Northeastern Estonians).

Leiu's research results have made her critically reappraise the Mongoloidness index created by K. Mark as a Mongoloid addition to the genetic structure of Finno-Ugric peoples. Leiu considers those "additions" traits of the peculiarities of the Finno-Ugric original population where Mongoloid and Europoid features might have appeared in an original combination.

These viewpoints contain appeals for further studies and theoretical analysis. The scientist whose works raise such problems has been successful in her research.

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HUMAN BONES IN SALME I BOAT-GRAVE, THE ISLAND OF SAAREMAA; ESTONIA

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ABSTRACT

In the autumn of 2008 human and animal bones came to light during the cabling works in the village of Salme, on the island of Saaremaa, Estonia. Some days later a contour of an ancient boat was discovered. The ancient boat, as well as human and animal bones inside it, dates to the second half of the 7th century or the beginning of the 8th century. The osteological analyses of the human bone material featured the specific quality of this burial-skeletal remains of seven men, which are unknown in the boat- and ship-burials around the Baltic Sea and in the broader context of Northern Europe. The absence of bones of dogs and horses, which are very common in Scandinavian boat- and ship-burials, is also exceptional.

Key words: *boat-grave, human bones, osteology, bioarchaeology.*

INTRODUCTION

The burials in boats or ships were widely spread in Northern Europe (including the British Isles) at the end of the first millennium, during the Vendel Era and especially the Viking Age [25]. Human burials in boats and ships may be inhumations as well cremations. The burial customs, related to cremation and boat/ship vary a lot. For example, one form of cremation burials known from the Viking Age Birka was scattering the cremation deposits with pyre remains to the burial place. This type of cremation burials sometimes contained boat rivets, indicating that probably the boat was burned together with human remains [28]. The same kind of archaeological finds are known from Estonia. From the

graves of Lagedi XIII, Aseri I, and Rae I cremains with numerous boat-rivets have been found [16, 19] as for the Lagedi grave there is also a possibility that a boat with human remains was cremated at the burial place [19]. Boat graves with inhumations are so far not known from Estonia, as well as from other Baltic states. The Salme boat is the first find of the kind in Estonia. Radiocarbon dating of osteological materials and wood from the boat confirmed that the burial was one unit and dated from the second half of the 7th century or the beginning of the 8th century [17]. In the summer of 2010, 30 metres from the Salme I boat-grave another boat burial with inhumations was exposed [27]. The archaeological investigation of the Salme second boat-grave will continue in the summer of 2011, therefore it is not comprehensively discussed in the present paper.

MATERIAL AND METHODS

In the autumn of 2008 human skeletons came to light during the cabling works in the Salme village, Saaremaa, Estonia (Figure 1). The construction work was suspended and archaeological rescue excavations began. Unfortunately, most of the human bones were already taken out by that time. After a week of archaeological excavations the contour of ancient boat remains was discovered and it became evident that an important discovery for Estonian archaeology had been made – an ancient boat with inhumations. The human osteological material from the Salme boat was mainly commingled when it arrived at the institute to be analysed. Fortunately a lot of photographs were taken during the excavation.

The bones were analysed as commingled ones, using the method of recurrent bone fragments. The sex of skeletons was determined according to the widely used osteologic standards [24, 33, 7, 8, 5, 22].

The age of the buried people was determined on the basis of tooth wear, the method was chosen as the best preserved diagnostic parts of skeletons were maxilla and mandible. Three different methods [24, 33, 7] were used for age determination and then most recurrent age range was counted as most probable. For estimating the severity of deposits of dental calculus and alveolar resorption the scale proposed by Brothwell [7] was used. For stature reconstruction two methods were used: Trotter & Gleser [30] and Gerhards [9]. The placement and plausible positions

of bodies inside the boat contour were concluded on the basis of photos taken during the archaeological works and the information derived from the bone assemblages packed as one unit was taken into account. Comparing the photos of skeletons and bones, collected as one unit, some conclusions concerning the placement and positions of the bodies in boat were possible.

Figure 1. Location of the Salme village.

RESULTS AND DISCUSSION

Analyses of commingled human bones: the minimum number of individuals, their age and sex.

After the construction work was stopped, the preliminary analysis of the recovered human bones was conducted on site. It indicated the presence of the remains of at least five individuals. The analyses of the whole osteologial material increased the number to seven.

The excavation area was divided into three parts: the cable trench area – the disturbed area, from where most of the commingled human bones were gathered; the edge of the cable trench where presumably intact upper bodies and craniums were found, and the area around the single limestone withinin the boat contour, from where also commingled bones and some articulated bones were found. At first the mandibles and

maxillas were paired. The loose teeth were put in right positions and the pair of the upper and the lower jaw was determined according to the congruity of occlusal surfaces of upper and lower teeth. It became evident that there were well-preserved jaws of seven individuals (except one cranium, the mandible of which is lost), 4 of these were for certain related to craniums and 3 not, as the latter was discovered among the commingled bones of the disturbed area of the boat (Table 1). All the craniums were more or less broken, especially those in the higher layers of the soil. Second, the innominate bones were paired. It was possible to pair fragments of 6 innominate bones, and to determine 7 left ones (Table 1). Third, foot bones and the distal parts of tibiae were analysed – the minimum number of seven people was affirmed (Table 1). Other fragments of post-cranial skeleton were less recurrent than the above presented ones, because of the overall poor preservation of the bone material.

Table 1. Analyses of commingled bones – the minimum number of individuals

Diagnostic parts of skeletons	Number of individuals		Cable trench area		Around the limestone		On the edge of the cable ditch	
Cranial part of skeleton								
Maxilla and mandible (pair) related to craniums	4		–		1 (the mandible is absent)		3	
Maxilla and mandible (pair) without related cranium	3		3		–		–	
Post-cranial skeleton								
Side	Left	Right	Left	Right	Left	Right	Left	Right
Innominate (fragments)	7	6	6	5	1	1	–	–
Calcaneus	7	5	5	4	2	1	–	–
Talus	4	7	2	6	2	1	–	–
Distal end of tibial bone	7	6	6	5	1	1		

The sex determination was carried out taking into account all the possible descriptive diagnostic traits on craniums and post-cranial skeletons. It led to the conclusion that all 7 skeletons were of male sex. The age of men was determined on the basis of tooth wear. Three different methods were used and the most recurrent age range was concluded as most plausible (Table 2).

Table 2. The results of age and sex determination

Skeletons	**Sex**	**Dental age in years**			**Most recurrent age range**
		Brothwell 1981	Gerassimov 1964	Miles 1963	
S1	male	25–35	30–35	25–35	**30–35**
S2	male	17–25	18–25	18–24	**18–25**
S3	male	20–30	25–35	25–30	**25–30**
S4 *	male	20–30	20–30	18–24	**20–25**
S5	male	25–30	25–30	18–24	**25–30**
S6	male	35–45	35–40	36–38	**35–40**
S7	male	40–45	40–50	42–46	**40–45**

* – mandible absent

Pecularity of dental and alveolar pathologies

Dental and alveolar pathologies were also recorded and are presented in Table 3. It is noteworthy that only one case of dental caries was found, but regardless of the age of men the periapical abcess, alveolar resorption (periodontal disease) and new bone formation in alveolar sockets were quite common amongst them. These pathologies refer to the periodontal disease, which could have been caused by scurvy. The men buried in the Salme boat were probably seafarers [17]; some health indicators on their skeletons suggest the possibility of scurvy. Scurvy was quite a common disease in prehistoric times, but quite difficult to diagnose in its early stages on the basis of skeletal materials. Skeletally, the evidence for scurvy consists of new bone formation, potentially anywhere on the skeleton [29]. The periodontal disease with new bone formation in maxillar orbits, jaws and tibial bones may refer to scurvy. Vitamine C deficiency predisposes to bleeding into skin and beneath the periosteum; most commonly the gums swell and bleed, and this leads

Table 3. Dental and alveolar pathologies (stages after Brothwell 1981)

Skeleton Age	Dental calculus	Dental caries	Periapical abcess /periodontal abcess	Alveolar resorption
S1 30–35	1	–	Left M^1 /M^2 Left M_1/M_2 I^1/I^2	1
S2 18–25	1	Right M_1	Left M_2	2
S3 25–30	1	–	–	2
S4* 20–25	1	–	–	1
S5 25–30	0	–	–	1
S6 35–40	1	–	Left M^2 Right M^2	2
S7 40–45	1	–	Right M^1 /M^2	2

* – mandible absent, I – incisor, M – molar

to the development of peridontal disease [29]. The symptoms of scurvy and its severity on skeletons vary a lot as described by different authors. Scurvy is differently expressed on adults' and subadults' skeletons, it has been also described that different parts of skeletons may have been involved with different severity [23, 21, 31]. In British (Abingdon, Poundbury) and in Peruvian skulls the porosity of the bone tissue was most prominent on the palate, the alveolar sockets of maxilla were the second most affected area in British samples, the orbital roof in the Peruvian one [23]. Scurvy was quite well expressed on the post-cranial skeletons of South-African mineworkers and surprisingly only in half of the cases the periodontal disease was evolved [31]. The scurvy was probaly present as subclinical condition in numerous inhabitants of Northern Europe in the past. The sailors had subclinical condition of scurvy before they ever got on the ship. The rapid development of the symptoms of scurvy in sailors is caused by specific environmental conditions [15]. Maat [21] for example has demonstrated that in Low Countries scurvy was of endemic nature, appearing at the end of winter and early spring. The study of skeletal remains of the 17th century Dutch whalers confirmed the presence of scurvy amongst them, the evidence of bleeding into joints and gums was found [20]. The frequency of scurvy may arise also during wars, but is far more widespread and linked to much greater range of activities than the recent focus on voyages and wars would suggest [6]. The sailors, seafarers and soldiers

spent long periods at sea or far from fresh food supplies. The deficiency of vitamine C causes the inflammation of gums and periodontal disease. Cold weather could have triggered the spreading of bacteria and inflammation into deeper layers of alveolar tissues. Finally it reached the tissues around the tooth root and chronic periapical abcess developed. This kind of abcess of the alveolar bone without the involvment of the pulp cavity suggests periodontal abcess [26]. In its early stages scurvy may affect gums – swelling, bleeding and being infectious [21]. The skeletons in the Salme boat indicated new bone formation in alveolar sockets, periodontal disease, periodontal abscesses and some pitting on cranial bones and on the palate.

Some remarks on the body height of men

There is not much information about the stature of the Middle and the Late Iron Age men in Estonia, there are reconstructions of body height based on two male skeletons from the Maidla second stone-grave of the 5th–7th cc in Läänemaa; both men were quite tall according to Trotter & Gleser formula [30]: first between 172–176 cm and the second between 175–178 cm [1]. Five humeral, one tibial and two femoral bones were measurable in the Salme osteological material. Using the stature reconstruction models, compiled by Gerhards [9] and Trotter & Gleser [30], it became evident that the deceased men had been quite tall (Table 4), especially S1 and S3.

The men buried in the Salme boat were quite tall in the context of Saaremaa men through centuries of the second millenium, the mean stature over 175 cm for males has been common for Saaremaa since the second half of the 20th century, but not in earlier times [11, 12, 13]. Unfortunately there is no data of the body height of men from the second half of the first millenium in Saaremaa. The men in the Salme boat were quite tall – 175–180 cm, likewise few men from some 5th–7th centuries Läänemaa in Estonia. There is not much information available concerning the osteology of skeletons recovered from Scandinavian boat-graves, the reason being the poor preservation of bones. Unfortunately in many Swedish Vendel Era boat-graves with inhumations the human bones are badly preserved, sometimes totally absent, thus the number of actual human burials in a boat is often not known [4]. So far only the stature of the man buried into the boat in Scar (Orkney) has been estimated, he was also quite tall, 181 cm [10].

Table 4. The reconstructed body height of men in Salme boat (cm)

Method Bone	Gerhards (2000)	Trotter & Gleser (1952)
Humerus S1	179.7	182.9
Humerus S2	175.5	176.7
Humerus S3	178.5	181.3
Tibia commingled bones	174.6	177.1
Femur (pair)	178.3	180.6
commingled bones	178.3	180.6

The placement and the position of human bones in the boat.

The placement of human bones in the boat and their body positions were not documented on site, the reconstruction of body positions is based on photos. The human remains were located mostly in the stern area of the boat. Unfortunately this part of the boat has been disturbed repeatedly in the course of earlier construction and cabling works. The photos, taken during the excavation, exposed three craniums with skeletons of upper bodies in initial positions on the edge of the cable trench.

Skeleton S1 – a male at the age of 30–35 years is more facing the stern, the body has slumped to the left – like from a sitting postion, his vertebra is quite horizontal in the cervical and at the beginning of the thoracic area. The body is leaning backwards, the head has fallen towards the chest, shoulders are up – a relaxed position. On the right side of S1 frontal part of the cranium there is a burning mark. Possibly it is caused by the activities which have taken place later, maybe during various construction works, for example, due to the welding work on the site. The skeletons were not deep and the heat of a welding set may have caused charring of the bone surface. There are no other signs of burning on other bones (Figure 2). **Skeleton S2** – male at the age of 18–25 years is facing the bow. The body is lying on his right side and leaning on S1 right shoulder and chest – like fallen asleep, exhausted (Figure 2). **Skeleton S3** – a male at the age of 25–30 years is facing the bow again

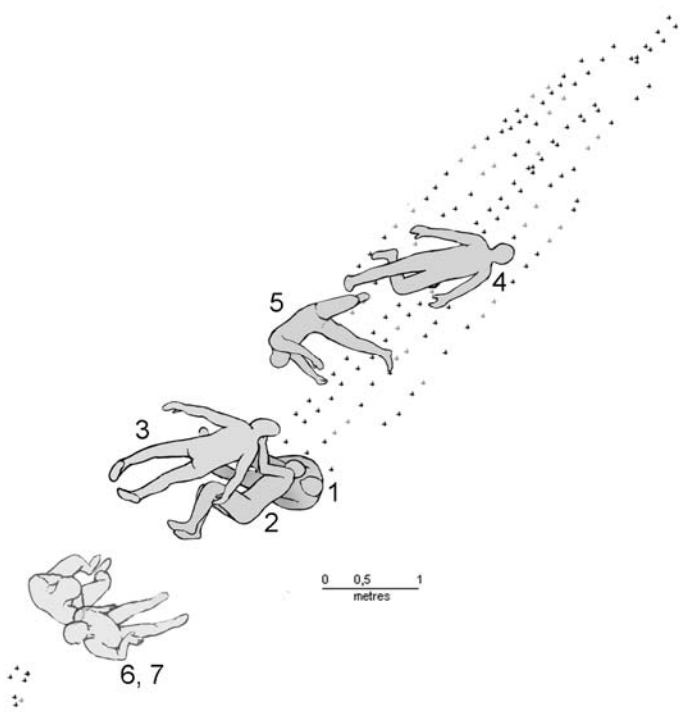

Figure 2. The placement and possible positions of bodies in the Salme boat. Drawing: Jaana Ratas. The boat contour [17], adapted.

and is lying on his face, the head leaning on the right cheek, or more obviously on the mandible; arms stretched out quite far from the body and shoulder-blades moved towards each other. The problem concerning postures of skeletons S1, S2 and S3 is that the lower parts of skeletons were destroyed and the directions of vertebras and the positions of lower extremities were not observable. The above-mentioned information could have been crucial to understand the initial positions of the bodies (Figure 2). **Skeleton S4** – the position of a man at the age of 18–25 years is more dubious. Next to the limestone (in the middle of the boat) was a cranium (mandible was not found) and partly on the limestone the left innominate bone articulated with the proximal part of the femoral bone was found. Some fragments of fibula and tibia also seem to be in the initial places, these could also be related to S4 (Figure 2). It is

noteworhy that beside the innominate bone one of the gaming pieces was found, which could have been in the pocket or in the hand of S4. The S4 body was lying more on the left side, the position also may refer to the sitting position as initial. Unfortunately, there is no firm evidence to argue that the sitting position of corpses was a part of burial ritual. On the other hand, the skeletons of Tuna –Alsike boat-graves VI, XII [2] are often found in a similar position – on one body side, with flexed knees and for these skeletons also the initial sitting position has been suggested [2]. In the Nabberör boat-grave the initial sitting position has been also supposed for two skeletons [18]. In the Valsgärde boat-grave the deceased have been placed to see the fairway [3], in the Salme boat such delibrate positioning of bodies towards one direction cannot be observed.

The position of **skeleton S5** is totally fictional. The only intact part of **skeleton S5** was the left foot at the edge of the cable trench and it was parallel to the ground – like a foot of a sitting, crouching or standing man. The left tibia and fibula should have been vertical to explain such a position of the foot. The leg could have been in this position, for example, due to the inner construction of the boat or things stored in the boat. When a body slumps from a sitting position, or when a corpse is recumbent, a leg cannot remain in such a position unless it is jammed between the construction elements or things (Figure 2). The paired mandible and maxilla of a male at the age of 25–30 years was found in the stern area (from the cable trench area) amongst commingled bones, the intact foot is probably related to the younger adult found mostly from the cable trench area.

The skeletons **S6** – a male at the age of 35–40, and **S7** – a male at the age of 40–45 were also found from the stern or the cable trench area amongst commingled bones. These men were the oldest of the seven. It is known that the older and more experienced men were responsible for steering. The placement and hypothetical body positions of men in the Salme boat are presented in Figure 2.

The Salme boat-grave is unique regarding the remarkable number of human burials inside the boat and their sex – 7 burials and all male skeletons. Two boat burials with inhumations are known in Northern Europe, where the number of individuals in one boat-grave is equal or exceeds three – Nabberör in Öland and Scar in Orkney [10, 18], but in both cases individuals of different sex have been determined and in the

Scar boat-grave amongst the others the remains of a child have been found. The Naberrör boat-grave from the Vendel Era contains four burials: the one in the centre of the boat with the remains of several artefacts has been suggested as the central or main burial, the second in bow area and other two in the stern area were less equipped [18] and have been even suggested to be the sacrifice of slaves, Anderbjörk in Hemmendorf [14]. The Viking Age boat-burial from Scar contained three burials – all buried at the same time with grave goods typical of such burials. The woman had died probably in her seventies, the man in thirties and the child around 10 years of age [10]. In the summer of 2010 the second boat-grave was discovered in the Salme village. Twenty-eight human skeletons have been found in the second boat-grave of Salme so far. We hope that further archaeological investigation on the site will provide us additional data to enlighten these mysterious objects and the events of the 7th–8th centuries in the Salme village, the Island of Saaremaa.

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CHANGES OF THE CHICKEN CHORIOALLANTOIC MEMBRANE AND THE BEHAVIOUR OF TRANSPLANTED GLIOBLASTOMA

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ABSTRACT

Glioblastoma is the most common brain malignancy and is marked by an extremely poor prognosis, despite advances in surgical and clinical neuro-oncology. Glioblastomas are very heterogenic in their biological and morphological features and they are widely investigated.

Existing in vivo glioblastoma models are based on the inoculation of glioma cells or cell lines into the rodent brain, the dog brain or the use of transgenic mice causing spontaneous tumors. These models suffer from the variable growth rate and poor penetration, and are limited by the difficulty of obtaining morphological data. In our research we suggested the model in which the native human glioblastoma was transplanted into the chicken embryo chorioallantoic membrane. The glioblastoma was transplanted into the embryo's chorioallantoic membrane on the seventh – ninth day, when it was fully developed and could ensure the nutrition of the tumor. Transplantation was successful if the glioblastoma survived at least for 24 hours together with the embryo. The chorioallantoic membrane after transplantation showed thickenning. Between 48 and 120 hours after transplantation the thickness of the membrane changed from 2x to 5x. Starting from 144 hours after transplantation the thickness of the membrane diminished. The tumor transplanted into the chorioallantoic membrane ingrows in it in the zones where the epithelium of the membrane was mechanically removed. The tumor keeps its proliferative activity until 48 hours of transplantation,

afterwards the proliferative activity is noticed in the chorioallantoic membrane until 120 hours of transplantation. This shows that the main processes take place in the zone where the tumor adheres to the chorioallantoic membrane.

The human glioblastoma transplanted on chicken chorioallantoic membrane repeated all the essential stages of tumor growth, which are also typical of other mammal models. This model reflects the morphological and biological features of the glioblastoma, allows to evaluate the invasivity, the progress of the tumor, and to investigate new medicines.

Key words: *chorioallantoic membrane, glioblastoma, chicken embryo.*

INTRODUCTION

Glioblastoma is the most common brain malignancy and is marked by an extremely poor prognosis, despite advances in surgical and clinical neuro-oncology [1, 12, 19, 20]. Glioblastomas are very heterogenic in their biological and morphological features. Despite the major advances in science, a lot of questions regarding glioblastomas are still without an answer – what are the main etiological and risk factors of glioblastoma, what molecular mechanisms are involved, what factors are responsible for poor prognosis and resistance to therapy [7]. The factors mentioned above constitute factors the reason why these malignancies are still so much investigated.

Glioblastoma models existing in vivo are based on the inoculation of glioma cells or cell lines into the rodent brain, the dog brain or the use of transgenic mice causing spontaneous tumors. However, these models suffer from the variable growth rate and poor penetration, and are limited by the difficulty of obtaining morphological data. These models do not reflect properly the interaction between the tumor and the host that occurs in the human, the accurate invasion processes, the vasculature, the gene expression profiling, and stroma interactions [2, 4, 6, 9].

The chicken embryo chorioallantoic membrane assay is a well established method for studying cancer, cell biology, immunology and genetics. Heterologous tissue transplantation into the chorioallantoic membrane of the fertilized chicken egg was first reported by Murphy in 1912. In 1934 Burnett and Ferry showed that many viruses can be grown on the chicken chorioallantoic membrane [15]. This extra-

embryonic well vascularised membrane is created by the fusion of chorion and allantois. The model of the chorioallantoic membrane is used for the investigation of growing tumors (lung, prostate, skin, brain) and viruses, the endometriosis research, the research of angiogenesis, for new medicines and therapeutical targets [3, 5, 8, 11, 13, 14, 17, 18, 21].

Nowadays the experimental models for tumors, especially for the glioblastoma, are under research. These new models should reflect the morphological and biological features of the glioblastoma, should allow to evaluate the invasivity, the progress of the tumor and to investigate new medicines. All these models should not be expensive. The model of the chicken chorioallantoic membrane has all the mentioned features. It is important that chicken embryos are immunodeficitic and there are no reactions of transplant rejection. This model for the glioblastoma research is used rarely [9, 16].

The objectives of this work: To evaluate changes of the chicken embryo chorioallantoic membrane, which appear after the transplantation of the glioblastoma and the changes of the membrane ensuring survival of the transplanted glioblastoma and to determine the invasivity of the glioblastoma into chicken chorioallantoic membrane.

MATERIALS AND METHODS

The glioblastoma tissue was taken from the patients (n=15) operated in the Department of Neurosurgery of *Lithuanian University of Health Sciences Kaunas Clinics.* All the patients had clinical and radiological diagnosis of the glioblastoma and gave the permission to participate.

The model of the chicken chorioallantoic membrane

Overall 300 of fertilized eggs (Hisex Brown, the Vievis Poultry Farm) were used for these particular experiments, 20 eggs were used for one tumor transplant. Overall fifteen tumors were transplanted into the chorioallantoic membrane; ten of them were glioblastomas (the tumor stands at the 4^{th} stage of malignancy according to the World Health Organization (WHO) classification), 200 eggs were used for the transplantation of glioblastomas into the chorioallantoic membrane. The diagnosis of the glioblastoma was confirmed by the pathologist.

Eggs were inserted into the incubator (SIEPMANN AB) for three days. The temperature in the incubator was constantly kept at 37.8 °C

and the humidity was 60%. The incubator was ventilated with the room air, while the eggs were turned back and forward every hour. The first incubation day was called the zero day. The eggs were taken out on the third-fourth incubation day and the rectangular 1×1.5 cm hole was screwed in their shell. The third-fourth day after incubation is the best time to make a hole, as the embryo has already formed, the chorioallantoic membrane begins to form, the protein is transparent and liquid, which makes it easy to take out with the disposable syringe. The hole was covered with a sterile plastic wrap in order to maintain humidity and so that the development and the vitality of the embryo could be observed. About 2 milliliters of protein was taken out from the blunt end of the egg with the sterile needle, so that the yolk did not reach the shell of the egg and the embryo was not hurt . The eggs were put back to the incubator until the seventh-ninth development day, when the chorioallantoic membrane forms, but they were not being turned anymore.

The glioblastoma tissue within 30 min was transported to the laboratory, cut into small pieces of 0.2×0.2 cm and transplanted into the chicken chorioallantoic membrane on the seventh – ninth day of the incubation. The chorioallantoic membrane in that period was fully developed and could ensure the nutrition of the tumor. The epithelium layer of the chorioallantoic membrane was destroyed with the sterile stick to improve the penetration of tumor cells [16]. After the transplantation, the hole in the shell was covered with the wrap again and the eggs with transplants were put back into the incubator. The tumors with the transplanted glioblastoma were observed every day and the vitality of the embryos and transplants was tested. The dead embryos with the transplanted glioblastoma were removed from the incubator immediately.

Histology and immunohistochemistry

Transplanted on chorioallantoic membrane tumors were cut together with the membrane during certain time intervals – every 24 hours. Tissue samples were fixed in 4% neutral buffered formaldehyde. The tissue was dehydrated, embedded into paraffin and 3–5 μm thick sections were performed. After that sections were stained with hematoxylin and eosin, and the imunohistochemistry for glial fibrillar protein was performed. Immunochemical staining allows to differentiate

the human tumor tissue from the chorioallantoic membrane tissue. Histological slides were observed with the optic microscope "Zeiss – standart 25" using the MC DX photocamera.

RESULTS

The macroscopic view of transplanted glioblastomas

Transplantation was successful if the glioblastoma survived at least for 24 hours together with the embryo. 158 (79%) transplants survived for 24 hours during the experiment, 130 (65%) of transplants survived for 48 hours, 105 (52.5%) transplants survived for 72 hours, 74 (37%) transplants survived for 96 hours, 54 (27%) transplants survived for 120 hours, 26 (13%) transplants survived for 144 hours, 18 (9%) transplants survived for 168 hours, 9 (4.5%) transplants survived for 188 hours (Table 1). It also should be noted that a part of embryos together with the transplant did not survive also because they were taken away for histological and immunohistochemical tests.

Changes of the chorioallantoic membrane

The chorioallantoic membrane is a very vascular fetal membrane composed of the fused chorion and the adjacent wall of the allantois. Different sizes of blood vessels are abundant in the mesenchyme and the chorioallantoic membrane is a good nutritional environment for the glioblastoma. (Figure1)

There are changes in the normal chorioallantoic membrane that take place during transplantation, but on the whole the membrane does not change obviously. Comparing the normal membrane after 48 hours and 120 hours after transplantation, it is seen that the width of the membrane remains almost the same. Small vessels remain under the epithelial layer, while large ones are concentrated in the mesenchymal layer. This position of vessels helps for tumor nutrition. Though the drying of the membrane starts after 120 hours of transplantation, microscopically only the mesenchymal layer seems denser. After 144 hours from the transplantation the membrane is thinner because of drying, while the

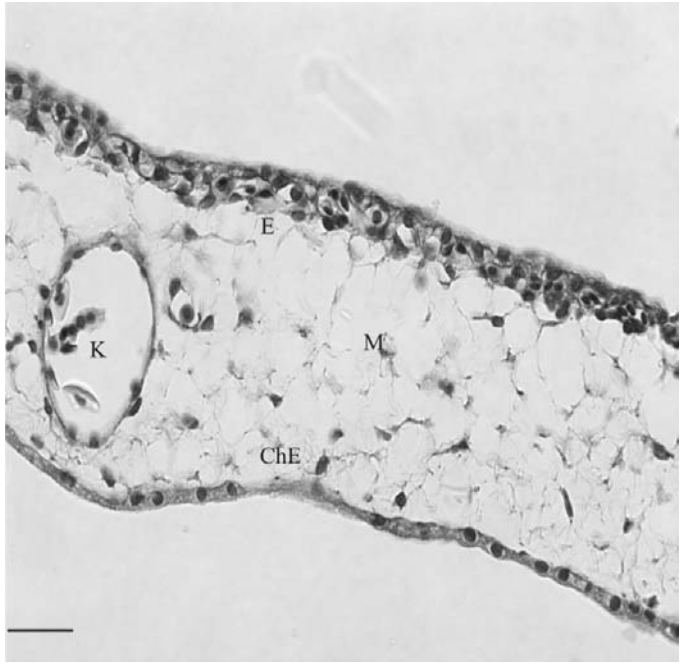

Figure 1. Normal chorioallantoic membrane. V – blood vessel, E – epithelium layer, M – mesenchymal layer, ChE – epithelium of the chorion. Magnification 40x. Scale bar – 20 μ.

epithelium has proliferated from both sides. After 188 hours of transplantation the membrane is thin, the epithelium layer has dried.
During incubation the chorioallantoic membrane becomes thicker not only in the glioblastoma fixation region, but also in the adjacent region of about 1 cm diameter. After 48 hours of transplantation the membrane becomes thicker 2x, after 72 hours the chorioallantoic membrane becomes thicker 4x, and after 120 hours – 5x (Figure 2). The membrane becomes more vascularised and the proliferation of the epithelium is noticed. The changes of the thickness of chorioallantoic membrane are shown in the Figure 3.

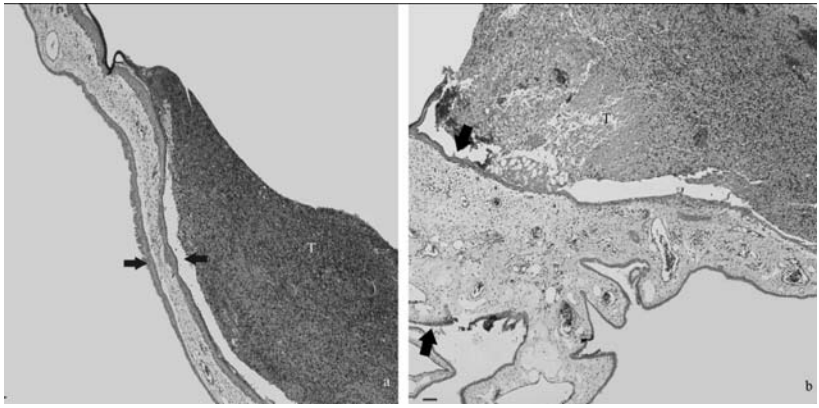

Figure 2. Thickness (arrows) of the chorioallantoic membrane after 48 hours (a) and 120 hours (b) from transplantation. T – tumor. Magnification 4x. Scale bar – 20 μ.

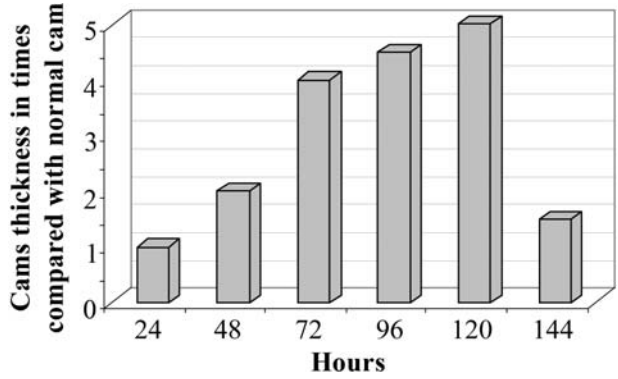

Figure 3. Thickness changes of the chorioallantoic membrane during different transplantation periods.

In the regions, where the epithelium of the membrane was removed, the reaction of the membrane is much more obvious – the small vessels are targeted to the epithelium and the transplanted tumor survives better, because the nutrition is better and the tumor cells pass the membrane easier. The small membrane vessels are entering the tumor, thus keeping it alive. We can distinguish the vessels of chicken from the nucleated erythrocytes.

In the regions, where the epithelium was not removed, the reaction of membrane is not so active, the vascularisation is smaller, epithelium proliferation is not so active.

Behaviour of the transplanted tumor

The tumor transplanted into the chorioallantoic membrane ingrows in it in the zones where epithelium was mechanically removed. The tumors keeps its proliferative activity until 48 hours of transplantation, afterwards the proliferative activity is noticed in the membrane until 120 hours of transplantation. This shows that the main processes take place in the zone where the tumor adheres to the chorioallantoic membrane.

After 48 hours of transplantation the week invasion of glioblastoma cells could be noticed, but the more noticeable invasion starts at 72 hours after transplantation (Figure 4). The borders of the tumor and the

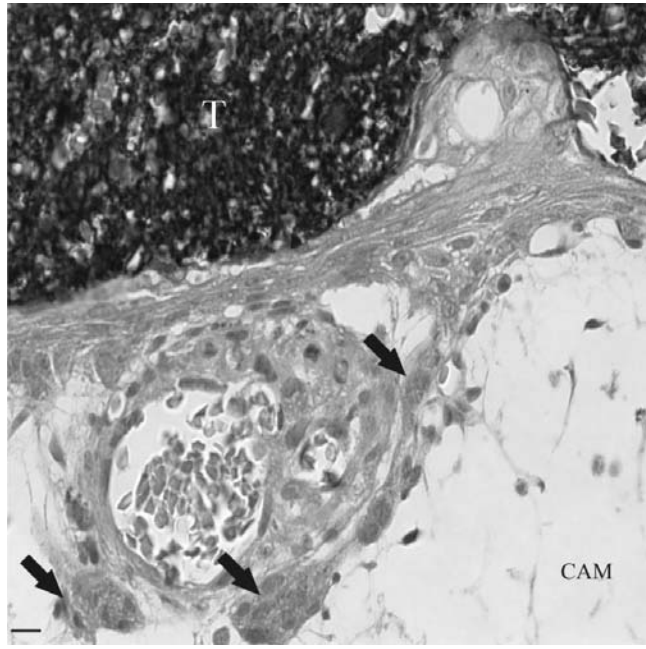

Figure 4. Invasion (arrows) of the transplanted glioblastoma (T) into the chorioallantoic membrane (CAM) after 72 hours of transplantation. Magnification 40x. Scale bar – 20 μ.

chorioallantoic membrane are still noticeable. The proliferation of epithelium is seen in the invasion zone. The fact that the cells invading the chorioallantoic membrane are of glial origin shows the positive immunohistochemical reaction to the glial fibriliar acid protein.

The most active invasivity of glioblastoma cells is seen at 120 hours after transplantation. Invading glioblastoma destroys the epithelium layer of the membrane and no more the boarders of the tumor and the membrane are distinguished (Figure 5).

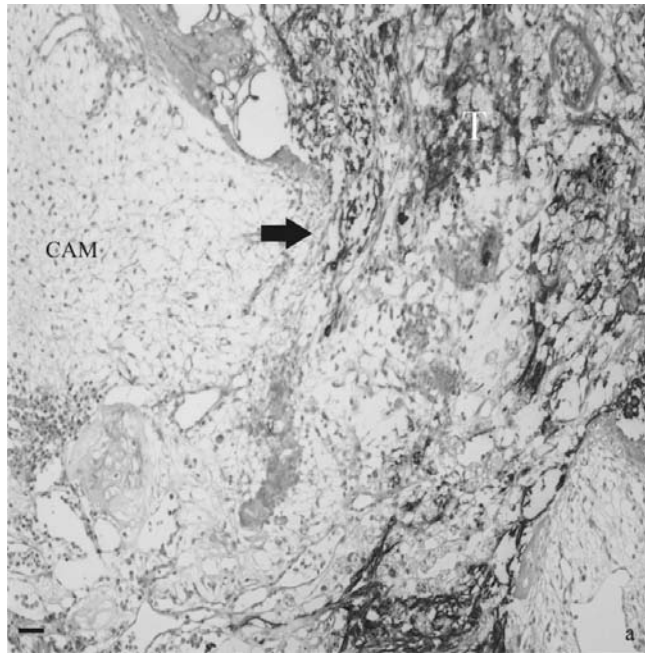

Figure 5. Invasion (arrows) of the transplanted glioblastoma (T) into the chorioallantoic membrane (CAM) after 120 hours of transplantation. Magnification 40x. Scale bar – 20 μ.

The glioblastoma invades the chorioallantoic membrane along its vessels. The glioblastoma vessels are also participating in the process by helping glioblastoma invasion. The formation of new glioblastoma vessels starts at 72 hours after transplantation, while being the most active at 120 hours after transplantation.

DISCUSSION

Referring to the data of our experiments, the tumors on the chorioallantoic membrane survived six days. Further survival of transplanted glioblastomas is limited because of the nourishing membrane becoming dry. This process takes place because of the natural process of embryo development – during the development of the embryo the outer layer of the chorioallantoic membrane is becoming dry and is not able to ensure sufficient nourishment of the transplanted tumor. The data obtained does not contradict other authors' data. Shoin, Yamashita et al. [16], who transplanted gliomas, also emphasize that the average survival of the tumor on the chorioallantoic membrane is seven days. The authors indicate that, wishing to prolong the time of tumor survival, it is possible to transfer it on the other chorioallantoic membrane. Other authors [9] do not indicate the duration of the tumor survival at all. Assessing the chorioallantoic membrane as an experimental model for the research of various anticancer medications, Vargas, Zeisser-Labouebe et al.[18] indicate that the experimental model has the same characteristics as the other models of mammals and tumors retain their growing characteristics; however, due to the development of the chicken embryo, the model is suitable only for the research of short duration. Therefore, most of the authors emphasize that the chorioallantoic membrane is a suitable model for short-term experiments, which coincides with our results obtained.

The growth of the tumor on the chorioallantoic membrane is accompanied by membrane thickening: the chorioallantoic membrane around the tumor changes significantly. The chorioallantoic membrane is significantly thicker in the area of tumor growth as well. The membrane starts thickening after 48 hours of transplantation, being the thickest at 120 hours from transplantation. The membrane became thicker 5 times compared to the normal chorioallantoic membrane. This coincides with other authors [9] who confirm that in the places where the tumor adheres to the chorioallantoic membrane, it gets thicker six times in comparison with the control group. These authors name this thickening as the edema of the membrane. In our case, this thickening was more proliferation than edema, because the number of cells and vessels in the membrane increased. The chorioallantoic membrane reacted to the transplanted tumor also with vascularisation and epithelium proliferation.

Glioblastoma cells invade into the chorioallantoic membrane, the process of invasion starts at 48 hours of transplantation and is most active at 120 hours of transplantation. Glioblastoma cells invaded the chorioallantoic membrane along membrane vessels. The membrane vessels grew into the tumor keeping it alive. According to Hagedorn, Javerzat et al. [9], tumor cells penetrated actively into the chorioallantoic membrane along the new blood vessels of chorioallantoic membrane which grew in the tumor. The transplants of melanoma also metastasized and melanoma cells penetrated into the chicken chorioallantoic membrane [10].

Glioblastomas transplanted on the chicken chorioallantoic membrane repeated all the essential stages of tumor growth, which are also typical of other glioblastoma mammal models.

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GFAP AND NF EXPRESSION IN BRAIN TISSUE IN CHILDREN AND ADULTS AFTER FATAL TRAUMATIC BRAIN INJURY

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ABSTRACT

Background and objectives. Still, there is almost no information about the role of biomarkers in the pathological processes of the brain in those patients, which die immediately after the injury, and those, which die several hours after the trauma. **Design and Settings**. A retrospective study. The human brain tissue material from the archive of the Institute of Anatomy and Anthropology in Riga Stradins University (RSU). **Methods**. We used the brain tissue material from the trauma and counterstroke spots of 28 patients. Brain tissue specimens were routinely fixed, embedded into paraffin, cut in 5 μm thick slides. For immuno-histochemistry we used monoclonal antibodies against NF proteins to detect axonal injury and monoclonal antibodies against GFAP to detect astrocytes. **Results**. Statistical correlation was seen between the lethal cases and survived in the brain tissue in the areas of counterstroke between lethal cases and survived for NF and GFAP presence (p=0.017) The data was compared, by dividing patients into groups of children and adults. Each of these groups was divided into 2 sub-groups. Statistically significant differences were noted between the lethal and the survived cases in the group of children for GFAP (Mann-Whitney U Test, p = 0.015) and in the group of adults for NF in the area of the counterstroke (Mann-Whitney U Test, p = 0.019). **Conclusions**. Higher quantities of intermediated filaments such as GFAP and NF are characteristic in the patients who survived after a head trauma in comparison to those, who died on the spot of the accident. Children under 2 years of age with severe head trauma have more dynamic glial cell reaction than other patients.

***Key words*:** *Severe traumatic brain injury; secondary brain injury; structural brain damage; reactive astrocytes; glial fibrillary acidic protein; neurofilament; astrogliosis.*

INTRODUCTION

The role of biomarkers in prognosticating therapy and the result of severe traumatic brain injury (TBI) have increased in the past decades [1]. The traumatic brain injury is a common cause of death and disability in children and adults of all ages. After the primary injury, the secondary injury to the brain follows. This process causes the multiple morphological damage of the brain tissue releasing a lot of substances, which play an important role in the recovery processes and in the outcome in general. Severe head trauma studies of the past couple of years are dedicated to exploring biomarkers and their role in the pathological processes that take place right after the moment of trauma; special attention is focused on those biomarkers that characterize the structural brain injury, thus determining neuronal, axonal and glial damage, and the regeneration potential. There are two main courses in these studies: 1. The biomarkers that characterize brain tissue damage, determining neuronal, axonal and glial damage, and the regeneration potential. 2. The mediators of the secondary brain damage are studied [11]. When the structural integrity of the nervous system is compromised, glial cells are activated to restore homeostasis [17]. Astrocytes are the multifunctional cells that play an essential role in homeostasis, and contribute to the information processing in physiological processes; they are also capable to generate a response to any kind of insult to the central nervous system (CNS). Within a few hours after virtually any type of brain injury, the surviving astrocytes in the affected region exhibit hypertrophy, and they proliferate in a process that is called reactive astrogliosis [5; 19]. Reactive astrocytes increase the expression of their structural proteins, glial fibrillary acidic protein (GFAP) [5] and neurofilament (NF) from intermediate filaments (IF) group. Immediately after the injury reactive astrocytes interweave their processes to form a glial scar that can impede axon regeneration. Most research is focused on the direct protection of neuronal cells; however, non-neuronal cells, like astrocytes can exert an active role in the pathogenesis of TBI [12]. Now the evidence has increased, and the modalities and dynamics of the

astrocytal response to damage are crucial to the outcome of brain pathology and the degree of neurological damage. Taking this into account, astrogliosis appears to be an appealing therapeutic target for the implementation of endogenous repair in the CNS [22].

There is almost no information about the role of biomarkers in the pathological processes of the brain in those patients, who die immediately after the injury, and those, who die several hours after the trauma. The aim of our work was to detect differences of brain tissue reactions in two different spots of the human brain, after traumatic brain injury in pediatric patients and in adults who died immediately after the trauma and those, who were treated for several hours after the accident.

MATERIALS AND METHODS

We used the material from the archive of the Institute of Anatomy and Anthropology in Riga Stradins University (RSU) brain tissue from the trauma and counterstroke spots of 28 patients (permission of the RSU Committee of Ethics Nr. E-9(2) – 17.12.2009.). Patients were divided into 2 groups. All patients had a severe head injury (SHI). The patients with multiple traumas were excluded. In the beginning all 28 patients were divided into 2 groups: 20 patients, who died in the place of the accident (7 children and 13 adults), and 8 patients, who survived and received therapy for different amounts of time (5 children and 3 adults). Then all 28 patients were divided into the following 2 groups: group 1 included children up to 18 years old: 7 children died on the spot of the accident (1, 4, 10, 11 years old, 2 children were 15 and 2 were 17 years old), and 5 children, who were hospitalized and received treatment for more than 24 hours (a 2-year-old, hospitalized for 48 hours, a 16-year-old, hospitalized for 78 hours, a 17-year-old, hospitalized for 36 hours and another 17-year-old, hospitalized for 168 hours).

Group 2 consisted of 16 adults, 18 to 61 years old, 13 of them died on the spot of the accident, but 3 were hospitalized after SHI and received therapy for more than 24 hours (a 36 year old, hospitalized for 15 days (360 hours), a 37-year-old, hospitalized for 11 days (264 hours), and a 40-year-old, hospitalized for 7 days (168 hours). In all the groups we analyzed GFAP and NF in the areas of impact and counterstroke.

Brain tissue specimens were fixed in 10% formaldehyde. Then tissue specimens were embedded into paraffin, cut in 5 μm thick slides. For

immunohistochemistry we used monoclonal antibodies against NF proteins to detect axonal injury (Monoclonal Mouse Anti – Human Neurofilament Protein Clone 2F11, working dilution 1:100, Dako, Denmark) and monoclonal antibodies against GFAP to detect astrocytes injury (Monoclonal Anti – Human Glial Fibrillary Acidic Protein Clone 6F2, working dilution 1:100, DakoCytomation, Denmark).

Sections were de-parafinised in xylene, and kept in absolute ethanol, then rinsed with PBS pH 7,4 (10 min), put into 4% citrate buffer solution, and placed in the microwave for 20 min (750W). After cooling and rinsing with PBS the tissue samples were covered in 150 µl 3% hydrogen peroxide (10 min.). After rinsing with PBS the primary antibody (30 µl) was applied for 2 hours, then LSAB + LINK (linked streptavidin antibody) was applied for 30 min, LSAB + KIT (streptavidin connected with enzyme peroxidase) was applied for 25 min, and DAB color reaction for 10 min. Finally, routine hematoxyline and eosine staining was performed in each sample (05B1003 Eosin Y Alcoholic Solution and 05M06002 Mayers Hematoxylin, Bio optica, Italy).

The semi – quantitative method was used for the quantification of NF- and GFAP-containing cells in the white substance of brain – on the spot of the trauma and on the spot of the counterstroke. The designations were as follows: 0 – negative reaction; 0/+ – occasional positive structures in the field of view; + – few positive structures in the field of view; ++ – moderate number of positive structures in the field of view; +++ – numerous positive structures in the field of view; ++++ – abundance of positive structures in the field of view [20].

Further data processing was done with SPSS (Statistical package for social sciences for Windows 17.0 ASV) software, using the non-parametrical statistical method, group and correlation methods.

RESULTS

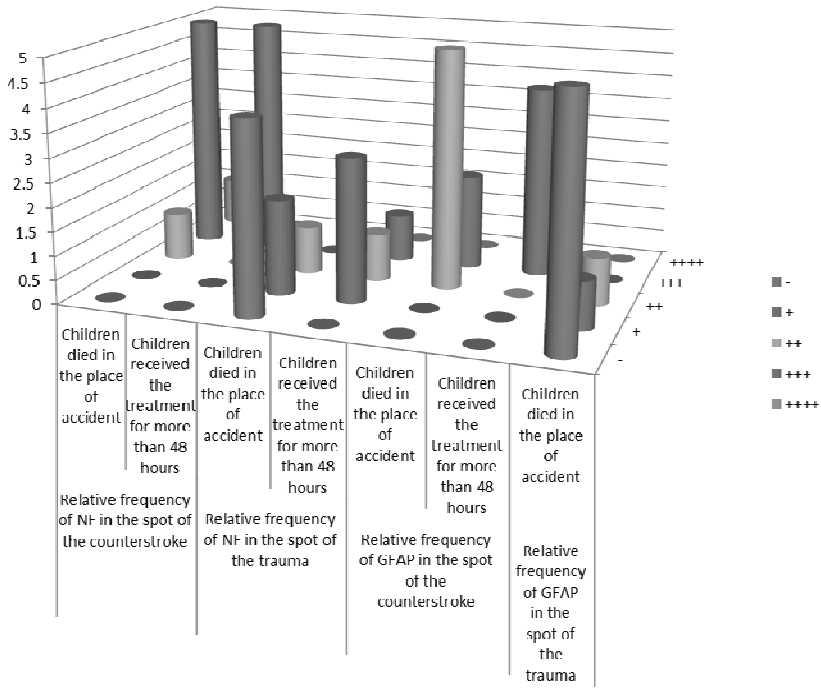
In the overview slides the tissue in the area of the impact was relatively less damaged than in the area of the counterstroke. Plethoric blood vessels and glial proliferation was noted in the surviving patients. In all the groups glial edema was seen.

From 20 patients who died in the place of the accident NF in the area of the direct impact was detected only in 3 children and no adults. From

8 patients who survived the trauma, in 7 we noticed NF in the area of the impact. NF in the area of the counterstroke was observed to be significantly higher in the survived patients compared with the patients who died in the place of the accident. When calculating the Spearmen correlation, a statistical correlation was seen in the NF presence in the brain tissue in the areas of the counterstroke between lethal cases and the survived ($p=0.017$). There was no statistically significant correlation in the NF presence on the spot of trauma between the survived and the lethal cases. The correlation coefficient "r" is low because out of 28 patients only 10 had a positive NF reaction in the area of trauma.

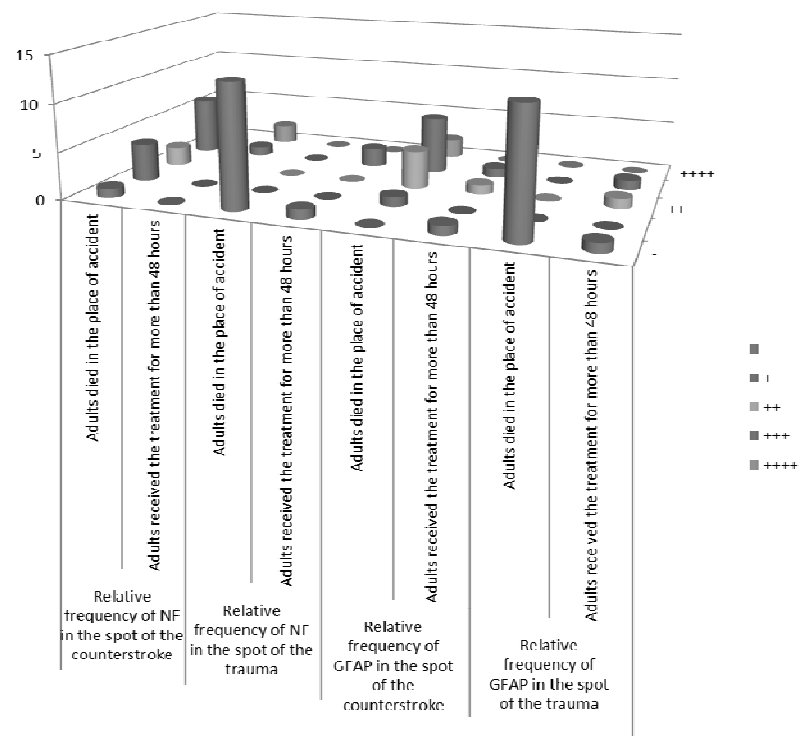
The data about GFAP is similar. We detected the presence of GFAP on the spot of trauma only in 2 children, who died in the place of accident, and in 7 survived patients (5 children and 2 adults). The quantity of GFAP in the area of the counterstroke was noted higher in all 28 patients, both lethal cases and survived groups. When calculating the Spearmen correlation, a statistically significant correlation was seen in GFAP presence in the brain tissue in the areas of the counterstroke between survived and lethal cases ($p=0.017$). There was no statistically significant correlation in GFAP presence on the spot of the trauma between the survived and lethal cases; from 28 patients only in 9 positive GFAP reaction was detected.

The data were compared, by dividing patients into groups of children (see Figure 1) and adults (see Figure 2), and each of these groups were divided into 2 sub-groups. The Mann-Whitney U Test and the Wilcoxon Signed Ranks Test were performed. Statistically significant differences were noted in the group of children, between lethal and survived cases in GFAP in the area of the counterstroke (Mann-Whitney U Test, $p = 0.015$ and Wilcoxon Signed Ranks Test, $p=0.030$). Statistically significant differences were noted in the group of adults, between lethal and survived cases in NF in the area of the counterstroke (Mann-Whitney U Test, $p = 0.019$ and Wilcoxon Signed Ranks Test, $p = 0.025$).



NF – neurofilament, GFAP – glial fibrillary acidic protein
Relative number abrevations:
–lack of cellss containing NF and GFAP structures
+ few positive structures
++ moderate number of positive structures
+++ numerous positive structures
++++ abundance of positive structures

Figure 1. Relative frequency of intermediate filaments GFAP and NF in the children who died in the place of the accident and who received the treatment for more than 48 hours.



NF – neurofilament, GFAP – glial fibrillary acidic protein
Relative number abrevations:
++ moderate number of positive structures
– lack of cellss containing NF and GFAP structures
+++ numerous positive structures
+ few positive structures ++++ abundance of positive structures

Figure 2. Relative frequency of intermediate filaments GFAP and NF in the adults who died in the place of accident and who received the treatment for more than 48 hours.

DISCUSSION

The primary brain damage occurs at the same time as the traumatic impact; within a few minutes, hours and days the secondary damage occurs, which is characterized macroscopically as posttraumatic

bleeding, and forming of a secondary contusion focus and the hypoxic zone of penumbre [7]. Microscopically it is characterized as a cascade of biochemical reactions, in which the damaged blood brain barrier (BBB) is actively enrolled, and it is acting as the neuro-inflammatory mediator producer [6]. Release of pro-inflammatory molecules can potentially induce further stress to compromised penumbral regions of the injured brain [23]. Aside of these processes the brain injury induces the activation of the intermediated filament (IF) structures. Glial cells participate in the inflammatory response. In particular, microglial cells are strongly activated following the injury and play an important role in the phagocytosis of the injured brain tissue [24]. Astrocytes are also activated in glial fibrillary acid protein (GFAP) expression. Astrocytes participate in the immune response of brain and form a permanent glial scar that physically and biochemically inhibits axonal regeneration and re-myelination. Reactive gliosis has been thought to be the major impediment to axonal re-growth after an injury, the formation of the glial barrier around a lesion site is also an advantage, because it protects the intact CNS tissue from secondary lesions [2]. In those first minutes of acute traumatic brain injury prominent reactive astrocytosis is seen. Astrocyte responses to injury are aimed at both protecting the nervous system, and sealing off damaged area, leaving the heavily injured zone to its natural degeneration, while preserving the less affected tissue. They may lead to reparative or destructive outcomes depending on the context in which they occur, for example, the extent and the type of injury, and the time point after damage [4].

In this study we examined the reaction of the white substance in the spot of the impact and the counterstroke by detecting the presence of two intermediated filaments in the structures of the brain tissue. IF in the human nervous system can be found both, in astrocytes and neurons, thus in the case of the fatal brain injury the information about the reaction of astrocytes was given by GFAP, and the presence of NF showed the damage of neurons [14].

Our result shows that in both, patients died on the spot of the injury and those who survived the injury moment NF and GFAP in the white substance of the brain was found less in the spot of the impact than in the spot of the counterstroke. In most patients, who died on the spot of the injury no NF and GFAP were found. Most of the studies concluded so far were made with animals, mostly rats, determining how quickly

after the injury astrocytal and microglial reaction appears, and measuring the presence of neurinflammatory cytokines. In rats the immune-reactive activation of microglia in peri-lesional regions can be seen only after 24 hours after the injury [25]. In our study in children younger than 2 years, who died on the spot of the injury we found the presence of NF and GFAP on the spot of the counterstroke.

Analyzing the data among groups, children versus adults, in children more pronounced amounts of NF and GFAP were seen in both groups, who died in the place of accident, and in those who were treated for more than 48 hours after the injury. We did not note the presence of NF and GFAP in any of the adults, who died in the place of the inury, but in some children, who died in the place of the accident, especially in those younger than 2 years, a moderate number of positive NF and GFAP structure on the spot of the trauma was noted in comparison with the spot on the counterstroke where higher number of positive NF and GFAP structures was seen. That could be explained by a specific brain tissue development features in children. The data about the brain development stages has shown that in children up to 2 years of age the central nervous system (CNS) is actively developing and maturing [18]. If during this period of life CNS has some impact of mechanical force, necrotic and apoptotic death of CNS cells is more dynamic than in the children older than 2 years and adults [13]. This way with a high probability we can speculate that the dynamic IF forming mechanism in the white substance of the brain in children right after the trauma shows the plasticity and self-protection of the brain by forming a glial scar, and restricting the primary injury and limiting the spread of the secondary injury, including the degrading impact of inflammatory mediators on the penumbral tissue and the healthy brain tissue. Also, several manifestations of activated glia must be taken into account. While the glial response can provide trophic end metabolic support to the damaged neurons, deactivating toxins and eliminating cell debris, there is a growing evidence that the response may also be detrimental to neurons, as activated microglia and astrocytes can produce a variety of potentially neurotoxic molecules that are implicated in neuronal degeneration [8;16]. It is possible that the survived patients, especially those who received therapy for a longer period of time parallel to dynamic IF production also inflammatory mediators and other neurotoxic substances were dynamically produced; they did not promote homeostasis in the

brain tissue, and accelerated brain tissue degeneration. The neuro-inflammation following acute brain trauma plays a prominent role in both the pathological and reconstructive response of the brain to injury [15].

The pathology of the traumatic brain injury is still extremely complex. For example, the glial activation in the injured brain certainly represents a disturbance of normal brain physiology or the predictor of pathological condition. It is still controversial as to whether reactive gliosis is harmful or beneficial to the acutely injured brain [9; 10]. For future ideal biomarkers will provide information on the pathobiology of traumatic brain injury and facilitate better monitoring of the progression of the secondary damage, the response to treatment and the prediction of outcome. But the initial characterization of biomarkers will be mainly based on the methods for measurement of such biomarkers in the blood serum. The development of reliable and accessible biomarkers is likely to change the way the clinical studies of head injury are conducted, resulting in more mechanism driven, optimally timed therapies [21].

CONCLUSIONS

1. Higher quantities of intermediated filaments such as glial fibrillary acidic protein (GFAP) and neurofilament (NF) are characteristic in patients who survived after the head trauma in comparison to those, who died on the spot of the accident.
2. Children under 2 years of age with severe head trauma have more dynamic glial cell reaction than other patients.

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COMPLEXITY OF THE FROG INTRACARDIAC NEURONS. INTRACELLULAR INJECTION STUDY

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ABSTRACT

The goal of this study was to determine the structure of intracardiac neurons in the frog *Rana temporaria*. Fifty-six intracardiac neurons from 8 animals were labelled ionophoretically by the intracellular markers AlexaFluor 586 and Lucifer Yellow CH. Among the labelled neurons, we found the cells of unipolar, bipolar, multipolar and pseudounipolar types. Multiple neuronal processes originated from the soma, the axon hillock and the initial segment of axon. With respect to the soma, the neuron contained (Mean ± SE) 3.5 ± 0.3 long and 5.5 ± 0.6 short processes. Most neurons had the spine, the bubble or the flake like extensions on their soma surface and were classified as Golgi I type neurons. Few Golgi II type neurons, the presumptive interneurons, were also found. Our findings contradict to a general view that the frog intracardiac ganglia contain only the adendritic neurons of the unipolar type. Our findings demonstrate that the frog intracardiac neurons are structurally complex and diverse. This diversity may account for the complicated integrative functions of the frog intrinsic cardiac ganglia.

***Key words*:** *heart, ganglion, cytology, nerve, dendrites, axon.*

INTRODUCTION

Neuronal morphology and function are closely related, since the shape of the neuron determines its connections with other neurons and target tissues. The complexity of dendrites and the soma size correlates to the

integrative capacity of the neuron [1]. It is considered that the neuron with complex dendrites receives more inputs than the adendritic neuron [1]. Likewise, the course of the axon may be indicative for the target specialization of the neuron. The neuron with a long axon extending to a distinct location is typically considered as a projecting neuron [2]. In contrast, the neuron with a short axon terminating proximal to the soma may be regarded as the interneuron or the local circuit neuron [2].

It has been shown recently that mammalian intracardiac ganglia contain functionally the diverse neurons including the parasympathetic, sympathetic, sensory and local circuit neurons [3–5]. Mammalian intracardiac neurons have variable morphologies, a different number of processes and multiple presynaptic inputs [3–6]. It is generally considered that the intracardiac neurons of amphibians exhibit surprising structural simplicity. The frog intracardiac ganglia contain only the adendritic neurons that receive a few presynaptic inputs and serve parasympathetic function [1]. It has been proposed that the frog intracardiac neurons could serve as a model to study the functional aspects of the intracardiac ganglia [1, 7]. Therefore, there is a current need to review the knowledge on the structure of the frog intracardiac neurons.

In the present study we use the intracellular injection technique to determine the structure of the frog intracardiac neurons. We report that the frog intracardiac neurons exhibit remarkable morphological diversity with respect to the soma and neuronal processes.

MATERIAL AND METHODS

The study was performed on 8 adult (20–35 g in weight) common frogs *(Rana temporaria)*. This investigation conforms both to the "Principles of laboratory animal care" (NIH publication No 86–23, revised 1985) and to the local guidelines for the use of experimental animals. Frogs were euthanized by the ether overdose.

Intracellular injection of neurons

The atria with interatrial septum, venal sinus and atrio-ventricular region were dissected and pinned flat in the Petri dish with the physiologic solution (pH 7,2; composition in mM: NaCl, 114; KCl, 4,8; $CaCl_2$, 1,8; $MgCl_2$, 1,44). The neuron somata were observed by a phase contrast

illumination on the microscope Axiovert 40 CFL (Zeiss). Intracellular fluorescent dyes Lucifer Yellow CH (Aldrich) and AlexaFluor 586 (Molecular Probes) were injected ionophoretically into the somata of neurons. The microelectrodes were made from borosilicate glass capillaries (0.86 mm inner diameter, Intrafil) with puller PC-10 (Narishige). The microelectrode was inserted into the neuron soma by the aid of micromanipulator ROE-200 (Sutter Instrument Company). The dyes were injected by applying 10 nA constant negative current for 10–15 min with Microionophoresis Dual Current Generator 260 (World Precision Instruments).

Microscopy of the injected neurons

Following the injection, the tissue was fixed in 4% paraformaldehyde in PBS overnight. Then the tissue was washed in PBS for 30 min and mounted on glass slides using the Vectashield mounting medium (Vectorlabs). The neurons were observed at 400X magnification by the fluorescent microscope Imager Z1 (Zeiss) equipped with an Apotome (Zeiss) and digital camera AxioCam MRm (Zeiss). We used the fluorescein isothiocyanate (FITC) and cyanine (Cy3) fluorescence filters to observe Lucifer Yellow CH and AlexaFluor586 labelled neurons, respectively. Measurements and reconstructions of neurons were made with AxioVision 4.7 image analysing software (Zeiss).

RESULTS

Neuron soma

Round, oval and ovoid shaped neurons predominated in the frog heart (Figure 1). In addition, we found spindle, crescent and triangular shaped neuron somata. The long axis of the labelled soma was 40 ± 1 μm, while the short axis 29 ± 1 μm (Mean ± SE). The soma occupied 1004 ± 61 μm^2 area, and the cytoplasm/nucleus ratio was 6.0 ± 0.3. The somata of most neurons were smooth, but some neurons contained numerous elevations and depressions of the soma surface (Figure 1).

Hillock and axon

Most of the neurons had the conical shaped axon hillock, the limits of which were hardly discernible (Figure 1). The axonal hillock of 9%

neurons, however, was well discernible and covered 135 ± 52 μm^2. The axon was a 2–6 μm thick process originating from the hillock. We could trace the axon for up to 1.6 mm. In 23% of neurons, the axon was bifurcated and had collaterals (Figure 1C). Most of the neurons were classified as Golgi I type neurons because they had a long prominent axon (Figure 1A-C). A few neurons (5%) were classified as Golgi II type neurons, as their processes terminated in close proximity to the somata (Figure 1D).

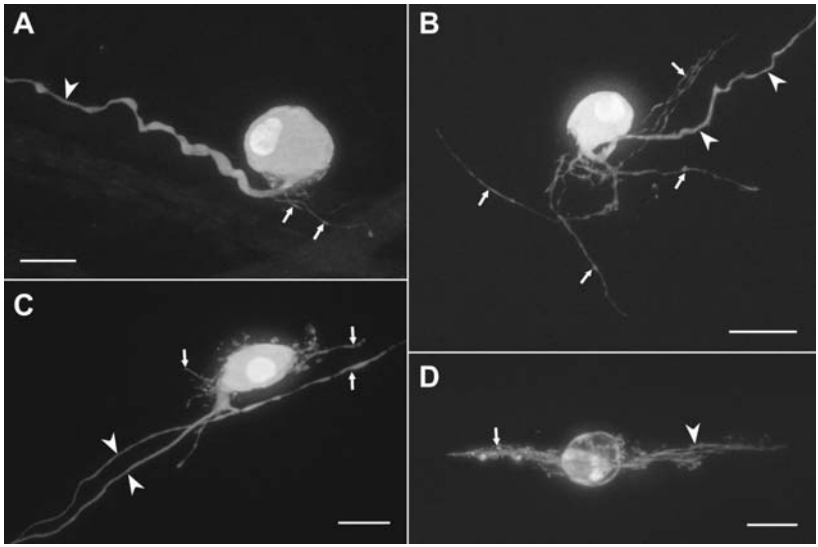

Figure 1. Frog intracardiac neurons labelled by the injection of Lucifer Yellow CH (A) and AlexaFluor 586 (B-D). Note the structural variability of the axon (arrowheads) and other processes (arrows). The processes of varying length originate from the axonal hillock, soma and proximal axon (A-C). Bubble shaped extensions of the soma surface are seen in C. Neurons in panels A-B are classified as the unipolar while in C-D as bipolar. The neuron in panel D is classified as of the Golgi II type, because all its processes terminate in close proximity to the soma. Bar = 25 μm.

Neuronal processes

Multiple neuronal processes originated from the soma, hillock and the proximal axon (Figures 1, 2). With respect to the long axis of the soma, the neuron contained 3.5 ± 0.3 long and 5.5 ± 0.6 short processes. The initial segment of the axon had 4.3 ± 0.6 processes. The hillock and soma had 1.7 ± 0.5 and 4.1 ± 0.6 processes, respectively. The average length of long processes was 86 ± 5 µm with the longest processes reaching up to 500 µm. The somata of 48% of the neurons had spine, bubble or flake like extensions (Figure 1C). Based on the position of the processes on the soma, 61% of neurons were classified as unipolar, 25% multipolar, 11% bipolar and 4% pseudounipolar (Figure 1).

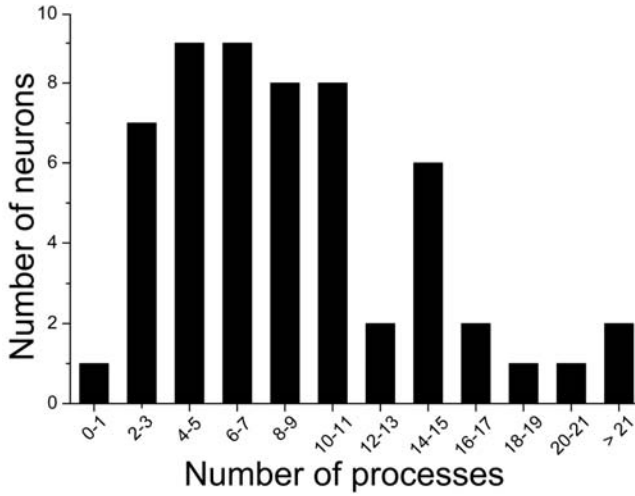

Figure 2. Number of processes among the labelled frog intracardiac neurons (n = 56).

DISCUSSION

Our study demonstrates that the frog intracardiac neurons contain multiple processes which originate from the soma, hillock and the proximal axon. A small proportion of the frog intracardiac neurons is devoid of long processes. The frog intracardiac neurons contain fine extensions of the soma, and differ in morphology of the soma, the

number and the length of processes. Both the Golgi I and II type neurons are present in the frog heart. Hence, we postulate that the frog intracardiac neurons are structurally more complex and diverse than it has been earlier considered.

Our findings contradict to a view that the frog intracardiac ganglia contain only adendritic neurons of the unipolar type [1, 8]. The diverse morphology of intracardiac neurons has been reported in mammals including rat, guinea pig and dog [3–6]. In addition, the diverse morphology of sympathetic neurons has been shown in frog [9–10]. It was proposed that the different morphology of the intracardiac neurons reflects their distinct functional specializations [4].

In respect to the functional model of the frog intracardiac ganglion, all the intrinsic neurons of this ganglion serve the parasympathetic function [1, 8]. The intracardiac neuron is innervated by 1–2 presynaptic axons [1]. The terminals of presynaptic axons contact and innervate the initial segment of the postsynaptic axon [1]. The short processes of the soma identified in the present study probably represent the dendrites and may be considered as the sites of synapses with the presynaptic axon terminals. The processes that originate from the hillock and the proximal axon could serve as the local axon collaterals. Since these local axon collaterals contact the adjacent neuronal somata, they may exert the modulation effect on the neighbouring intracardiac neurons [9]. These findings we obtained and the discovery of the Golgi II type neurons suggest the morphological basis for communication between the frog intracardiac neurons. It is likely that the frog intracardiac ganglia are not a simple relay for preganglionic fibers, but could serve as a more complex integration centers.

The intracellular injection technique has been used in our study. This technique allows us to trace the axons and dendrites of the individual nerve cells. Although more work should be done in combining the intracellular injection with both the electron microscopy and immunocytochemistry, the presented data would contribute towards an understanding of the structure of the frog intracardiac ganglion.

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GENDER DIFFERENCES IN THE HUMAN CERVICOTHORACIC GANGLIA

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ABSTRACT

Researchers still further have a morphological interest in sympathetic ganglia. Functionally, paravertebral ganglia are involved in many physiological and pathological aspects of neuropathic, vascular, visceral pain syndromes, the Raynaud's syndrome and hyperhydrosis. Several interventions on ganglia as surgical and chemical sympathectomy, the ganglion block, and the chemical or thermal sympatholysis are applied for treatment of the pathological conditions. Accurate knowledge about the structure and the location of ganglia is required for a successful after effect of these procedures. In scientific literature there are many facts about the structural variations of the sympathetic ganglia related to development, age, pathology, lateral asymmetry and gender. The last one is the indeterminate factor, and the data about it are few and controversial. Still we were missing the information about the gender role on morphometry of human sympathetic ganglia and particularly of the cervicothoracic ganglion. The goal of our present study was to evaluate gender differences in the human ganglia. In our study we found that male cervicothoracic ganglia were longer than female ganglia, 21.33±4.74 mm vs. 14.87±1.84 mm, wider 9.51±1.48 mm vs. 8.76±1.14 mm, and thicker than female ganglia 5.19±0.77 vs. 4.29±0.36 mm. The dissected ganglia exhibited the three main distinguishable shapes: spindle, dumbbell and inverted "L". We defined gender differences: the female ganglia were mainly of spindle shape (78%), whereas the male ones equally expressed all the three types of the shape (35%, 30%, and

35%). In summary, we determined the gender differences in human cervicothoracic ganglia. We note that these differences are important for interventions on ganglia.

***Key words*:** *Cervicothoracic ganglion, human, gender, anatomy.*

INTRODUCTION

Researchers still further have a morphological interest in sympathetic ganglia. Functionally, paravertebral ganglia are involved in many physiological and pathological aspects of neuropathic, vascular, visceral pain syndromes, the Raynaud's syndrome and hyperhydrosis. Several interventions on ganglia- surgical and chemical sympathectomy, the ganglion block, and chemical or thermal sympatholysis are applied as a possible treatment for pathological conditions [2]. The stellate ganglion block decreases the cerebral vascular tone for relieving cerebral vasospasm [4]. Accurate knowledge about the structure and the location of ganglia is required for a successful after effect of these procedures. In scientific literature there are many facts about structural variations of the sympathetic ganglia related to development, age, pathology, lateral asymmetry and gender. The last one is the indeterminate factor and the data about it are few and controversial. There were gender differences on the frequency of neuroaxonal dystrophy in diabetic human autonomic ganglia. Frequency was increased threefold in men in comparison to women [9]. Electrophysiological investigations had revealed the gender related differences in autonomic cardiac control and in clinical expressions of ganglia functions, such as human females are more prevalent to a variety of chronic pain than males [8]. The cadaveric studies determined pathology- dependent histological changes on the left stellate ganglion [3]. Still we were missing the information about the gender role on the morphometry of human sympathetic ganglia and particularly of the cervicothoracic ganglion.

The goal of our present study goal was to evaluate gender differences in the human cervicothoracic ganglia.

MATERIALS AND METHODS

We examined 41 cervicothoracic ganglia of 26 autopsied human subjects within 18–24 h delay. This study was approved by the Kaunas Regional Bioethics Committee. There were 15 males and 10 females, aged between 60 and 80 years (the mean age at death was 75 years). The cadavers displaying the pathology of the nervous system, traumatic lesions and surgical procedures in the neck were excluded. In supine the cadaver's position in the middle line of the neck skin incision was done. The sternocleidomastoid muscle was dissected and pushed laterally for the exposition of the carotid vagina. Soft tissue and muscles of the neck were removed, and subclavian and vertebral arteries were dissected. As skeletal landmarks for the dissection, the transverse process of the seventh cervical vertebra and the neck of the first rib were used [5]. Removed ganglia were grouped by gender and the side (right, left) (Table 1).

Table 1. Groups of human cervicothoracic ganglia.

Ganglia/Gender	Female	Male	Total
Right cervicothoracic ganglia	10	13	23
Left cervicothoracic ganglia	8	10	18
Total	18	23	41

The ganglia measurements: length, width, and thickness were made by using outside callipers (sensitivity 0.01 mm) and recorded in a specially created pro forma. The types of shapes were defined: spindle, dumbbell and inverted "L" by the appearance of the spindle, the constriction or the waist and the hook like form [7]. During dissection on ganglia the photographs were taken by the camera Fujifilm Finepix JV150.

Prepared cervicothoracic ganglia were fixed in paraformaldehyde 4% buffer solution and embedded in paraffin. For light microscopy 7 μm thick serial sections were cut and stained with cresylviolet for the histological confirmation of ganglia. Statistic evaluation was performed by using SSPS 13.0 software. Data differences were considered significant if the level of significance was at $p<0.05$.

RESULTS

Macroscopically, human cervicothoracic ganglia were greyish, enclosed in the connective tissue capsule, surrounded by fascia, adipose tissues and lymphatic nodes. Topographically, the ganglion was located close to the lateral border of the longus colli muscle. Above and laterally of it, we found the inferior trunk of brachial plexus and the anterior scalene muscle. The ganglion was located behind the vertebral artery. In all 26 dissections the cervicothoracic ganglion was formed by the fusion of two ganglia: the inferior cervical and the first thoracic. We did not dissect one right-sided and 4 left-sided ganglia because of the pathologies in the neck and the upper part of the thorax. The obtained dimensional results were tabulated in Tables 2, 3, 4, 5. We found that the mean length of the right and the left cervicothoracic ganglia were 19.05±3.62 mm and 17.41±2.68 mm, respectively. The mean total length for all the ganglia was 18.30 ±2.17 mm (Table 2).

Table 2. Length, width and thickness of cervicothoracic ganglia.

Variable	Ganglia (n)	Length (mm)	Width (mm)	Thickness (mm)
Left	18	17.41±2.68	8.65±1.47	4.56±0.51
Right	23	19.05±3.62	9.15±0.93	4.93±0.52
Left, right	17	17.55±2.18	9.21±0.80	4.78±0.42
Total	41	18.30±2.17	8.92±0.79	4.76±0.35
Male				
Left	10	19.29±6.02	9.29±2.31	5.14±1.14
Right	13	23.36±5.65	9.73±1.68	5.23±0.88
Total	23	21.33±4.74	9.51±1.48	5.19±0.77
Female				
Left	8	15.10±2.38	9.20±1.68	4.15±0.43
Right	10	14.63±2.32	8.31±0.78	4.38±0.50
Total	18	14.87±1.84	8.76±1.14	4.29±0.36

Table 3. Incidence of different shapes of cervicothoracic ganglia.

Shape	**Left**	n	**Right**	n	**Total**	n
Spindle	**55%**	11	**57%**	12	**56%**	23
Dumbbell	**25%**	5	**24%**	5	**24%**	10
Inverted "L"	**20%**	4	**19%**	4	**20%**	8

Table 4. Incidence of different shapes of male cervicothoracic ganglia.

Shape	**Left**	n	**Right**	n	**Total**	n
Spindle	**30%**	3	**38%**	5	**35%**	8
Dumbbell	**30%**	3	**31%**	4	**30%**	7
Inverted "L"	**40%**	4	**31%**	4	**35%**	8

Table 5. Incidence of different shapes of female cervicothoracic ganglia.

Shape	**Left**	n	**Right**	n	**Total**	n
Spindle	**88%**	7	**70%**	7	**78%**	14
Dumbbell	**12%**	1	**20%**	2	**17%**	3
Inverted "L"	**0%**	0	**10%**	1	**5%**	1

Male cervicothoracic ganglia were longer than female ganglia, 21.33±4.74 mm vs. 14.87±1.84 mm, wider 9.51±1.48 vs. 8.76± 1.14 mm, and thicker than female ganglia 5.19±0.77 vs. 4.29±0.36 mm. The dissected ganglia exhibited the three main distinguishable shapes: spindle, dumbbell and inverted "L" (Table 3), (Figure 1). The largest incidence was for the spindle shape, the smallest incidence was for inverted "L" shape ganglia. These results were obtained on both right- and left-sided ganglia. We defined the gender differences of ganglia. The female ganglia were mainly of spindle shape (78%; Table 4), whereas the male ones equally expressed all the tree types of shape (35%, 30%, and 35%; Table 5).

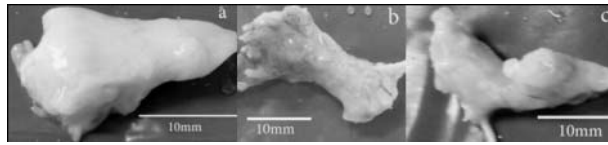

Figure 1. Shapes of human cervicothoracic ganglia: a – spindle; b – dumbbell; c – inverted "L" shape.

DISCUSSION

Saylam C.Y. et al. [10] examined 40 inferior cervical/cervicothoracic ganglia: 70% were located behind the vertebral artery, 25% postero-laterally and only 5% posteromedially to the vertebral artery. Sceletotopically, 40% of inferior cervical/cervicothoracic ganglia were

located at the level of the seventh cervical vertebra. Fifty-five per cent of inferior cervical/cervicothoracic ganglia were located more caudally: at the level of the intervertebral disc between the seventh cervical/first thoracic vertebra (25%) and at the level of the first thoracic vertebra (35%). Ateş Y. [1], using cadaver material and MRI scans, revealed that the longus colli muscle is an important landmark structure for the cervicothoracic ganglion block, and males have a thicker muscle at each vertebral level. We observed the similar position of ganglia. The means of the length and the width of ganglia were 11.3 ±4.6 mm (5.1–23 mm) and 8.2± 3.0 mm (3.5–15.6 mm) of 20 male cadavers, respectively [10]. Our measurements were correspondingly 18.30±2.17 mm (7–44 mm); 8.92±0.79 mm (1–15). We suggest that the cause for the disagreement in results is that we made the measurements of ganglia from individuals of both genders of precisely known age, whereas Saylam C.Y. could not determine the exact age and did not publish the data about gender differences. Zhang B. et al. [11] in their protocol defined the length 19.3±2.3 mm (14.7– 25.2), and the width 6.5±1.7 mm (4.1–8.5 mm) of human ganglia. For this study the material of 18 males and 7 females was used, but the authors did not give scientific data about it and the exact age. Conditionally we can consider that our obtained findings are similar. One more factor that is important for the rate of results is that Saylam C.Y. and Zhang B. et al. used cadavers already embalmed and fixed with the formaldehyde solution. Kalsey G. et al. [6] reported the average dimensions of human cervicothoracic ganglia: 2.0×1.0×0.3 cm. This group of researchers identified that ganglions in all the cases were the fusion of inferior cervical and the first thoracic ganglions. They made a set of ganglia shapes: oval, stellate, hourglass, elongated, comma, C or the club shaped. In the study of Pather N. et al. [7] we found a detailed morphological description of human ganglia. In comparison with our findings, Pather N. et al. [7] detected more inverted shape ganglia (45.3% vs. 20%) and less spindle shape ganglia (28% vs. 56%). The incidence of dumbbell shape is similar between Pather N. et al. [7] and our study (26.7% vs. 24%). Pather's study material was 48 human subjects: 31 fetuses (the mean age 34 weeks) and 17 adult subjects (the mean age 48 years). There is a wide range of age in the study of Pather N. et al. [7], and many factors may influence the structure of human ganglia during the developmental changes.

In summary, we determined the significant morphometric differences in the cervicothoracic ganglion between the male and the female. These gender differences may be important for surgical and physiological interventions on the human sympathetic ganglia.

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ANTHROPOMETRICAL AND SPORT CONSTITUTIONAL COMPARISON OF GERMAN MALE SOCCER PLAYERS AND MALE STUDENTS OF SPORTS SCIENCES

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ABSTRACT

The aim of the present study was to figure out differences between the body compositions of German soccer players and the students of sports sciences from Hessen (Germany). For this purpose 30 soccer players and 40 sports students were anthropometrically examined. The collective of the students majoring in physical education had the function as a reference group.

Both groups were divided into "experienced" and "less experienced" athletes. The soccer players were recruited from two different Hessian leagues, the players from the higher league were classified as "experienced" athletes and the players of the lower league were defined as "less experienced" football players. The reference group of sports students was also separated into "experienced" and "less experienced" groups. The participants who did workout for more than 10 hours a week, were categorized as "experienced" and the athletes who trained less than 10 hours a week were recorded as "less experienced". In general the students of sports sciences were involved in different sports like ball sports, martial arts, athletics and strengths events.

The measurements were taken under standardised conditions by the authors of this work and the results were analysed statistically (ANOVA). To analyse differences the skin fold measures, the Body-Mass-Index (BMI) and the AKS- Index (Lean-Body-Mass) were used. The comparison of the groups is based on the methods of German and Anglo-American schools of constitutional biology. The exact defined landmarks after Conrad, Knussmann, Parnell and Heath & Carter were

used to calculate the body constitution of the different groups and athletes.

The soccer players of the present study had an average body height of 178.8 cm +/– 6.0 cm and an average body weight of 71.9 kg. In comparison to the soccer players, the athletes of the reference group are taller and heavier. The average height of the students of sports sciences was 183 cm +/– 5.1 cm and they had an average weight of 80.2 kg. Nearly all the height and longitudinal dimensions of the reference group were significantly higher than in the group of soccer players. Depending on the performance level of both groups, there were no significant differences found.

***Key words**: soccer players, sportstudents, anthropometric measurements, constitutional body types.*

INTRODUCTION

The first soccer like games were played 2,500 years ago. There were different types of games in which the participants tried to take a ball to the opponent´s goal. These games were played without exact defined rules and did not have much in common with the modern way of soccer that is known from today.

The modern style of football was established in England in 1863 and developed rapidly in Europe. Over 40 years later the World Football Federation FIFA was founded. The primary responsibility of the FIFA was to develop international rules and regulations and to organize international matches.

Today soccer or football is the most common and famous of sports in the world. Maybe the simple rules and the easy way of playing this game is one of the main reasons why many people from different cultures and countries like to watch and to play soccer. Above all soccer developed into a big industrial sector and has a high value for the economic system of sports. Based on that, the importance of success is more and more in focus, only teams and players who are successful, are able to become famous and earn money. From that point of view, the classification of talent is really important and can help to get success.

The body constitution of soccer players can be a fundamental part of talent. Anthropometric studies can have a key role in talent searching and scouting for soccer and other sports. On closer examination, one of

the main questions of anthropometric researches is if there are special body constitutions or anthropometric sizes and variables for professional and successful soccer players. It is not only the research for a potential "optimum" body composition of soccer players. The focus of interest is as well on constitutional differences of soccer players from different performance levels.

From Knussmann's [6] point of view, the constitution of the human being can have an effect to general and specific sports skills. Furthermore his idea is that sports have an influence on the shape of the human body.

The present study tries to clarify the mentioned questions by focusing on soccer players and the students of sports sciences as a reference group. Therefore variable anthropometric measurements like height-, girth-, breadth – and body fat-measurements were collected as well as the German constitution typologies according to Conrad & Knussmann and the Anglo-American Somatotypes appropriate to Parnell and Heath & Carter were evaluated. Apart from that, the Body-Mass-Index and the AKS-Index (Lean-body-mass) were examined.

PARTICIPANTS AND METHODS

In this study 30 soccer players and 40 students of sports sciences as a reference group were examined. Each proband participated voluntarily and the data were used anonymously. Both groups were divided into "experienced" and "less experienced" athletes; 13 soccer players were recruited from the 5th German Soccer Division and were classified as an "experienced" group. The other 17 soccer players were playing in the 6th German Soccer Division and were rated as "less experienced". The ages of the soccer athletes ranged from 16 for the youngest participant to 23 years for the oldest soccer player.

The reference group is composed of 40 sports students. They were recruited from the Frankfurt Goethe University. Students of physical education have to pass different physical tests during their study and they have to be physically strong and well trained. In general the students of sports sciences were involved in different sports like ball sports, martial arts, athletics and strengths events. Collectively 25 students were classified as "experienced" who did workouts for more than 10 hours a week. In addition to that, 15 students of sports sciences

were defined as "less experienced", because of their lower training volume of under 10 hours a week. The training volume of the students was determined with interviews. The youngest participant of the reference group was 21 years old and the oldest student had an age of 37 years.

The anthropometrical measurements took place under standardized conditions, to exclude sources of error as much as possible. The heights and lengths were measured with an anthropometer, the breadths and widths with a pelvimeter, the circumferences with a measuring tape. The skin folds, which are important for the calculation of the body fat and also for some constitution typologies, were measured with a caliper. The body weight was measured with a digital scale of the brand Korona. The measurements were evaluated by the author of this study with always the same instruments and conditions. The statistical data were checked and analyzed using ANOVA. Furthermore the age of the participants, the training volume were interviewed and recorded.

The exact defined landmarks after Conrad, Knussmann, Parnell and Heath & Carter were conducted as the foundation of the examined and calculated measures. In addition to the mentioned constitutional typologies, the BMI (Body-Mass-Index) and the AKS-Index (Lean-Body-Mass-Index) were ascertained and calculated.

RESULTS

The results of the anthropometric data of all examined groups are summarized in Table 1. The students of sports sciences are taller and heavier than the soccer players. They seem to be more muscular, which is proofed by the higher AKS-Index results, but at the same time the body fat percentage is also higher. There were also significant differences of the circumferences. The reference group has the bigger girth results at the upper body and upper extremities. The additional parameters of the widths and the results of the single determinations of the constitution typologies also support the fact that the sports students are on average taller, bigger and heavier than the soccer players of this study.

With the main focus on the experience and performance level of the participants, the variations are less clear. There is a meaningful difference at the sitting height of the participants. In both groups the experienced athletes had significant higher sitting heights. Other

significant differences with the main focus on the experience are: min. forearm, girth of foot and width of humerus. In all the mentioned body parts, the experienced athletes showed higher values.

All the groups have on average a normal weight, which is demonstrated by the Body-Mass-Index (BMI) results. A normal weight is defined between a BMI of 18.5–24.9 kg/m^2. The group of the soccer players had a mean BMI of 22.4 kg/m^2 and the group of sports students demonstrated a BMI of 24.5 kg/m^2, which is significantly higher. The AKS-Index-Diagram is illustrated in Figure 1 and shows the relationship between the active body substance (musculature) and the body height. The sports students demonstrated higher AKS-Index findings in comparison to the soccer players, but the difference was not significant. These results underline the fact that the students of sports sciences seem to be heavier and more muscular than the soccer players.

According to the German constitution typology after Conrad, the soccer players are on average leptomorph-hyperplastic and there were no meaningful differences found between "experienced" and "less experienced" soccer players. The group of sports students has a metromorph-hyperplastic body type. Based on the experience level, there were no significant differences found. The classification of pyknomorphy and makrosomia after Knussmann orders both groups to leptomorph- makrosom type. The sports students showed significantly higher Makrosomia- results compared to the soccer players.

Regarding to the somatocharts after Parnell and Heath & Carter, the soccer players are almost all in the mesomorphic area. With this result on the main focus the soccer players of the present study showed an athletic and sporty body constitution. With respect to the experience level of the soccer players, there were no important differences observed.

The group of sports students showed a bigger spreading at the somatocharts after Parnell and Heath & Carter. Most of the participants of the reference group are mesomorphic, but some are located in the endomorphic area. There were no meaningful differences between experienced and less experienced sports students found. Figures 4 and 5 show the averages in the somatocharts after Parnell and Heath & Carter.

Table 1. Results of the body composition data of all the tested groups

	soccer players (experienced)	**soccer players (less experienced)**	**sports students (experienced)**	**Sports Students (less experienced)**
n	13	17	25	15
age (yrs)	18.2 +/– 2.3	17.1 +/– 0.6	23.3 +/– 3.6	25,4 +/– 3,5
height (cm)	179.4 +/– 6.3	178.3 +/– 6.0	184.1 +/– 4.6	181,2 +/– 5,5
sitting height (cm)	94.2 +/– 2.6	92 +/– 3.6	95.2 +/– 2.8	92,8 +/– 4,6
mass (kg)	74 +/– 3.1	70.2 +/– 2.9	79.7 +/– 3.2	80,9 +/– 3,4
AKS (AKS/ h^3)	1.09 +/– 0.1	1.08 +/– 0.1	1.1 +/– 0.0	1,11 +/– 0,1
BMI (kg/m^2)	23 +/– 1.6	22 +/– 2.6	24.4 +/– 2.1	24,6 +/– 2,3

Table 2. Results of skinfold measurements of all groups

group	experience level	triceps (mm)	forearm (mm)	suprailiacal (mm)	scapula (mm)	thigh (mm)	calf (mm)
soccer players	experienced	9.5	5.1	16.3	11.7	14.8	11.4
	less experienced	8.8	4.2	15.2	9.2	10.7	9.1
	complete group	9.1	4.6	15.7	10.3	12.5	10.1
sports students	experienced	10.8	5.6	20.3	14.7	13.9	11.2
	less experienced	12.2	4.8	23.2	14.9	14.5	11.9
	complete group	11.3	5.3	21.4	14.8	14.1	11.5

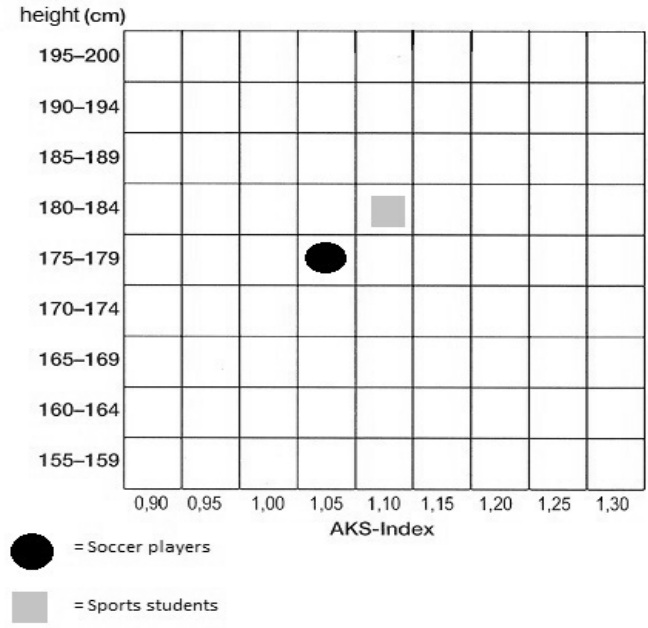

Figure 1. AKS-Index-Diagram with the averages of both groups (No significant differences between the experience level found).

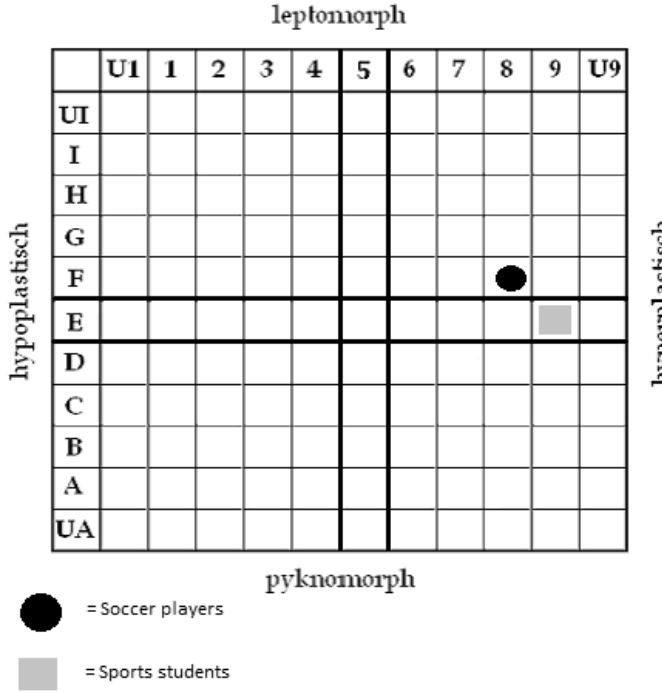

Figure 2. Chessboard pattern graphic after Conrad with the averages of both groups (No significant differences between the experience level found).

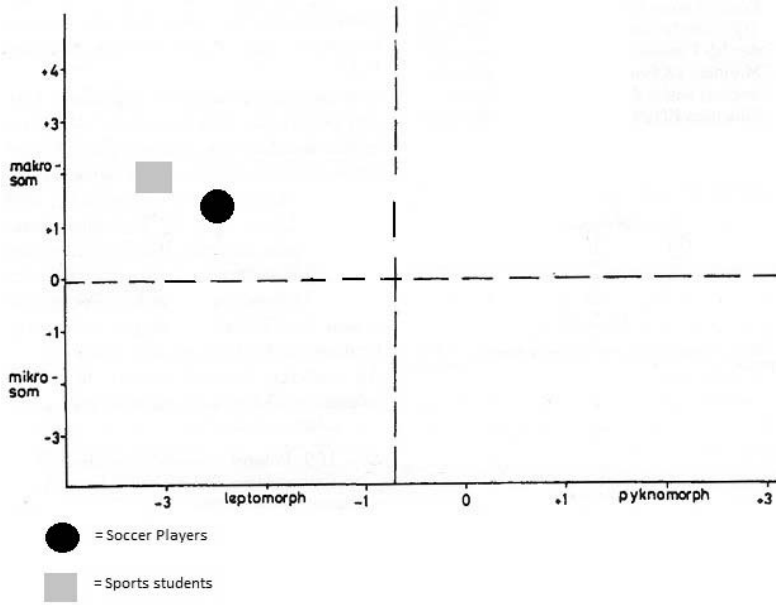

Figure 3. Graphic of constitution types after Knussmann with the averages of both groups. (No significant differences between the experience level found).

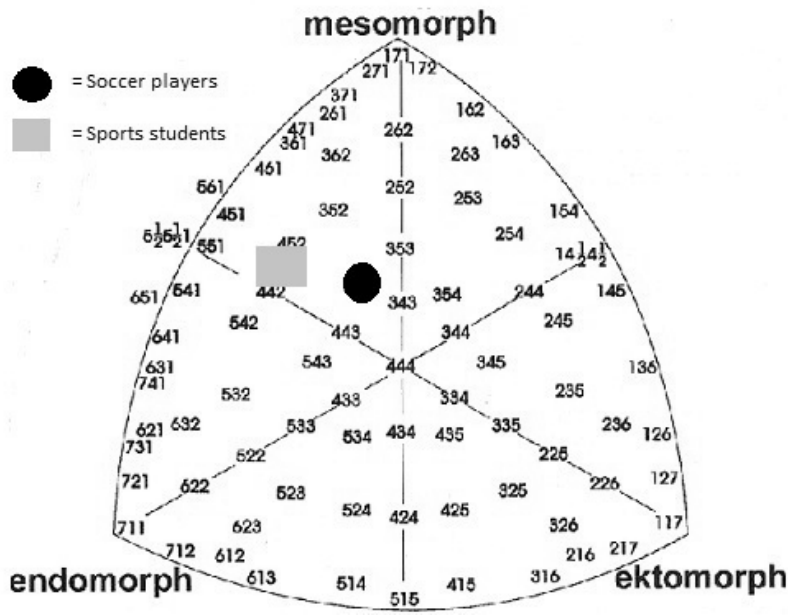

Figure 4. Somatochart after Parnell with the averages of both participant groups (No significant differences between the experience level found).

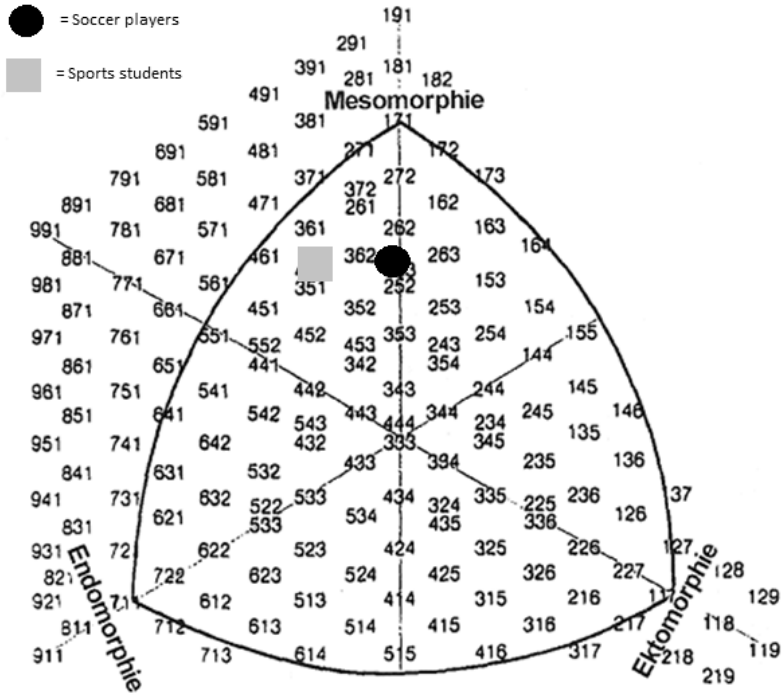

Figure 5. Somatochart after Heath & Carter with the averages of both participant groups (No significant differences between the experience level found).

DISCUSSION

The present study supports the conclusion of a clear difference between the anthropometrically examined soccer players and the students of sports sciences, according to the collected data.

The group of the soccer players demonstrates an average height of 178.8 cm comparing to the group of sports students, who have an average height of 183.0 cm. The participants of the reference group are clearly taller than the soccer players of this study. According to the experience level, the experienced athletes are taller than the less experienced sportsmen, but the difference is not significant. Corresponding to the Federal Office of Statistics the average height for 18 to 40

year old men in Germany is about 178 cm. In comparison to the findings of the present study, the soccer players are almost at the national average and the sports students are 5 cm over the above the nationwide average. In addition, another interesting fact is that the experienced athletes had significant higher sitting heights than the less experienced participants.

The reference group of sports students illustrate an average body weight of 80.2 kg. They are heavier than the group of soccer players who present an average weight of 71.9 kg. They have also higher AKS-Index results, which is a proof of a more muscular mass. After Matkovic et al. [9] and Luiz do Prado et al. [7], who examined professional soccer players, an AKS-Index around 1.13 AKS/h^3 is meaningful for professional soccer players [7; 9]. The soccer players from the present study, who play in lower divisions than the probands of Matkovic et al. and Luiz do Prado et al. show lower AKS-Index results. The mentioned findings illustrate a higher muscle mass of professional soccer players in comparison to the soccer players of this study. The professional players seem to be more athletically trained.

Other interesting findings are the lower body-fat values of the soccer players. All the skin fold results of the soccer players are lower than the findings of the reference groups. A soccer player has to be quick and fast to be a successful player. With this fact in mind, a high body-fat percentage could have a negative influence on the conditional aspects and the overall performance of a soccer player. In conclusion a low body-fat percentage could be important for an optimum performance of a soccer player. According to the performance level of the soccer players in the present study, there are no meaningful differences found.

The reference group of sports students has significantly bigger circumferences at the upper body and the upper extremities. A possible explanation for this result could be the training of the sports students, who are involved in different sports like athletics and strengths events. This contains more strength training than the training of soccer players who are more focused on technical and tactical training and on other conditional aspects. Another interesting result is the girth of the lower extremities as thigh and calf, because there is no significant difference between the soccer players and the sports students. This result shows the strong and muscular legs of the soccer players, although the students majoring in physical education have the higher weight and higher body-

fat. The soccer players have on average a significant smaller waist girth than the reference group, which is correlating with the lower skin fold results at this area. After Raschka a small waist in combination with a big shoulder breath is described as an "athletic shape" [10].

Corresponding to the results of the breaths, the sports students have on average the higher values. Nearly all breaths are significantly higher in comparison to the group of soccer players. The mentioned results highlight the stronger bone structure of the sports students. There are no meaningful differences found according to the experience level of the participants.

Regarding to the constitution theory of Conrad, the soccer players are on average leptomorph-hyperplastic. The group of sports students has a metromorph-hyperplastic body constitution. Based on the experience level, in both groups there were no significant differences found. Knussmann has the view that the leptomorph body type is suitable for a jumper and a runner, because of a low relative body weight, which is an advantage on locomotion [5]. Both mentioned aspects (jumping and running) are important aspects of soccer. The classification of pyknomorphy and makrosomia after Knussmann orders both groups to the leptomorph- makrosom type. The students of sports sciences have significantly higher Makrosomia- results.

Referring to Parnell and Heath & Carter both groups are on average in the mesomorphic area. The students of sports sciences have on average higher endomorphic values. This result means that they are more massive than the soccer players, which is proofed by the higher skin fold- results and the higher AKS-Index findings. The separation of the participants in experienced and less experienced sportsmen does not show any significant differences.

Summarizing both groups of this study has a sporty physique which is shown by the high mesomorphic values after Parnell and Heath & Carter. The sports students are taller and heavier than the soccer players, but the data of the soccer players contain lower skin fold measurement results, which illustrates a lower body- fat.

The hypothesis that there is a significant difference between the body constitution of soccer players and sports students, could be proofed in the present study. There were not any meaningful differences between the experience-level of the participants, however.

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AGE RELATED STRUCTURAL CHANGES IN HUMAN BASILAR ARTERY

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ABSTRACT

The basilar artery is the most important artery in the posterior cerebral circulation. In the cases of stroke due to acute basilar artery occlusion the mortality rate is significantly higher if compared to all the stroke cases. Arterial wall stiffening is characteristic of ageing arteries and in many investigations arterial wall stiffening is related to the loss of the elastic component in the arterial wall during ageing. In arterial stiffening the changes in the collagen content and the number of smooth muscle cells (SMC) may also play a role.

Basilar arteries were obtained within 24 hours postmortem from 89 human cadavers (44 male and 45 female). From the middle part of the basilar arteries histological slides were performed and area of collagen fibers, the number of smooth muscle cells and the thickness of the media were measured.

The morphometric analysis revealed the increase of collagen network area with the age. Analysing age-related changes in the number of SMC in male and female basilar artery media, we determined that in both genders the number of SMC in the media decreased. Changes in SMC number in both genders had a strong negative correlation with the age ($r = -0.93$ in the male group and $r = -0.95$ in the female group, respectively). In the analysis of the media thickness in different gender and age groups, its thickening was determined. The correlation with the age was of medium strength both in the male ($r=0.36$) and female ($r=0.4$) group. In all the cases this correlation was statistically significant ($p<0.05$).

Our morphometric findings that revealed the increased collagen area together with the decrease of the SMC number might be responsible for

the stiffening of the basilar artery in aging and contribute to the development of atherosclerosis and arterial hypertension.

Key words**:** *basilar artery, collagen fibers, media thickness, smooth muscle cells.*

INTRODUCTION

Arterial tree aging is different in large elastic arteries and in distal muscular arteries [11]. It is commonly recognized that arterial aging is related to the arterial wall stiffening which is a physiological phenomenon assessed by the increased pulse wave velocity or augmentation index [12, 13]. Experimental studies demonstrated that the effects of aging vary considerably along the cerebrovascular tree and the data obtained studying large arteries cannot be extrapolated to the smaller arteries of the circle of Willis [3].

The basilar artery is the most important artery in the posterior cerebral circulation. In the cases of stroke due to acute basilar artery occlusion the mortality rate is significantly higher if compared to all the stroke cases and the patients are about a decade younger [6]. Some authors consider that isolated basilar artery dissection is an under-recognized disease [14]. One of the methods to evaluate the arterial wall remodelling related to age is the morphometric investigation of the media. Scientific literature lacks information about the changes in the human basilar artery wall during aging. Fonck E. et al. [2] investigated another intracranial artery – the posterior cerebral artery and found considerable increase in the collagen content in the media with aging together with the decrease of smooth muscle cells (SMC) number.

Our aim was to investigate age-related changes in the basilar artery media by assessing the area of collagen fibers, the number of smooth muscle cells and media thickness.

MATERIAL AND METHODS

Basilar arteries were obtained within 24 hours postmortem from 89 human cadavers (44 male and 45 female, age from 20 to 84 years) at the Kaunas Department of M. Romeris University Institute of Forensic Medicine (the study was approved by the Kaunas Regional Ethics

Committee for Biomedical Research, Protocol Nr BE-2–8). The investigation included the cases of violent death without obstructive atherosclerotic lesions and the history of cerebral blood vessels disease. All the investigated cases were distributed into 3 age groups: < 40 years (young age group); 40–59 years (middle age group) and ≥ 60 years (old age group).

Tissue samples were obtained from the middle portion of the basilar arteries and fixed in the 4% neutral buffered formaldehyde. The tissue was dehydrated, embedded into paraffin and 5 μm thick sections were performed. After that sections were stained with hematoxylin and eosin, lisamin fast red, picro-sirius red and acid orsein methods. Histological slides were observed with the optic microscope "Zeiss – standart 25" using the MC DX photocamera, 10 pictures from every case were investigated morphometrically using "Image–Pro Plus" v. 5.1 semi-automated image analysis system. The following quantitative parameters: the area (%) of collagen fibers, the number of SMC and the thickness of the media were measured. The area of collagen fiber bundles was measured in percentage by calculating the total area of all the fibres and by dividing it by the area that we measured, magnifying x 100. The morphometric analysis of collagen fibers was performed using the picro-sirius red method and counting of smooth muscle cells – the lisamin fast red method (Figure 1).

SMC were calculated quantitatively in the constant media areas of the same size. Also, in ten samples of each case ten calculations of the media thickness (in μm) were made (100 calculations for each case). The slides stained with hematoxyline and eosine and acid orseine were used for the investigation of the histological structure of basilar arteries.
For the statistical analysis SPSS 15.0 for Windows (SPSS Inc., Chicago, IL) was used ("Statistika 6.0" program). The initial morphometrical data distribution of each case testing the zero hypothesis refered to χ^2 and Kolmogorov – Smirnov criterion was analysed. To compare histomorphometric parameters between two and more groups, we used the dispersive analysis using one and two factors method (ANOVA). Correlation between the analysed parameters was estimated by the correlation and regression analysis, $p \leq 0{,}05$ was considered as statistically significant.

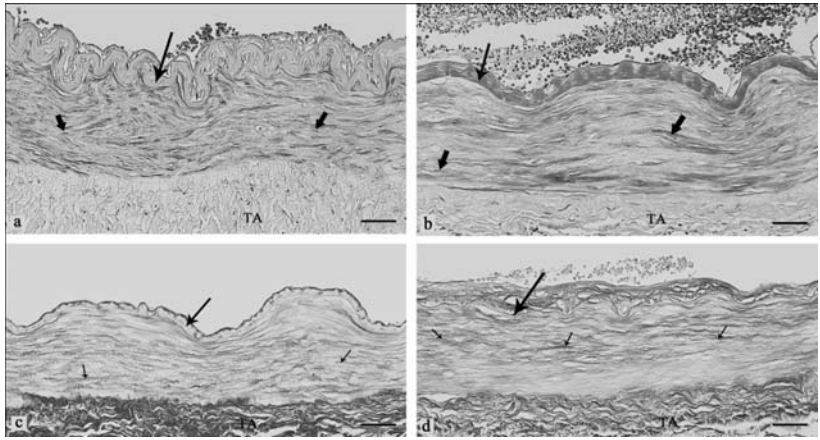

Figure 1. Histology of the basilar artery. a – male, age – 27 years; b – female, age 84 years, the diminished number of smooth muscle cells; c – male, age – 20 years; d – male, age – 65 years, the increased area of collagen fibers. TA – *tunica adventitia*, long arrows – the elastic membrane, short thick arrows (a, b) – the nuclei of smooth muscle cells, short thin arrows (lisamin fast red), (c, d) – the collagen fibers are stained in red (picro-sirius red); scale bar (a-d) – 50 μm, original magnification: 10x.

RESULTS

Collagen network changes in the basilar artery in ageing

The morphometric analysis revealed the increase of the collagen network area with the age. The area of collagen bundles was 3.4% higher in the male middle age group than in the young group ($p<0.05$). the difference between the old and middle age male groups the collagen area in media was 8.5% ($p<0.00001$). There was 11.9% higher area of collagen network in the old age group than in the young age male ($p<0.001$) (Figure 2). The analysis of the female groups showed that there was significant difference of increase in the collagen network area in the basilar artery media between all the age groups ($p<0.001$). The area of collagen was 7.8% higher in the middle age group than in the young female and 5.6% lower than in the old age group. We found 13.4% higher collagen area percentage in the old female group than in the youngest one.

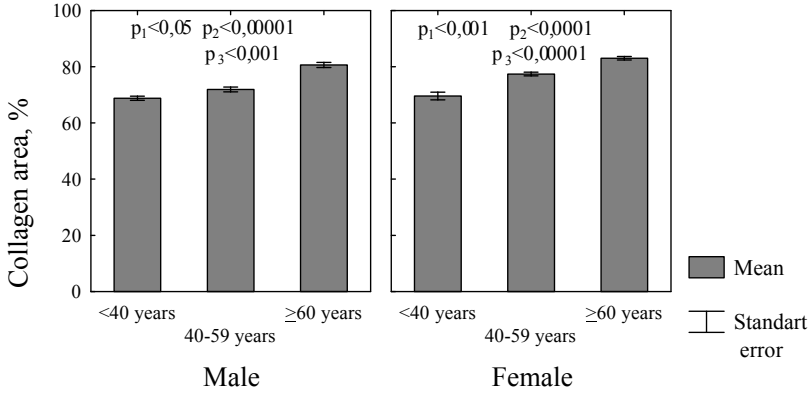

Figure 2. Male and female collagen network area in the basilar artery media in all the age groups. p_1 (<40 years and 40–59 years); p_2 (40–59 years and ≥60 years); p_3 (<40m and ≥60 years).

Analyzing collagen network area of the same age male and female groups, we noticed that the collagen network area was higher in the female groups. This difference was statistically significant ($p < 0.05$) in the 40–59 age group.

Age-related changes of the smooth muscle cells number

Analysing age-related changes in the number of SMC in male and female basilar artery media, we determined that in both genders the number of SMC in the media decreased. Changes in SMC number in both genders had a strong negative correlation with the age (r=–0.93 in the male group and r=–0.95 in the female group).

We observed a statistically significant decrease in the SMC number in the old age group, where SMC decreased twice in comparison to the youngest group ($p<0.001$) (53% of male, and 51% of female). The morphometric analysis of the data has shown that the number of SMC in the middle-aged group of male was lower by 26% than in the youngest group, but their number was higher by 36% than in the group of male older than 60 years ($p<0.0001$). The SMC number in the middle-aged female group was lower by 25% than in the group of female younger than 40 years and by 34.5% than the old age group of female ($p<0.001$) (Figure 3). Such identical tendencies in the decrease of SMC in male

and female of the same age demonstrate that the decrease in SMC in the basilar artery is similar in both male and female.

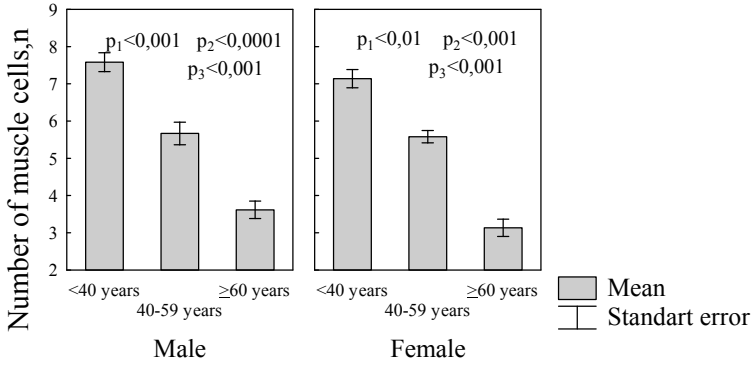

Figure 3. Number of muscle cells in the basilar artery media. p_1 (<40 years and 40–59 years); p_2 (40-59 years and ≥60 years); p_3 (<40 years and ≥60 years).

The SMC number in the male basilar artery media was slightly bigger; this difference decreased with age. The number of SMC in the media of the male youngest group was higher by 10% than in the female group of the same age. It was higher by 8.3% in the middle-aged group of male and just by 5% in the old age group. This difference is statistically significant only between the youngest groups ($p<0.05$).

With the age the collagen content increases in the tunica media, while the SMC number decreases (Figure 4). In both gender groups the correlation between the collagen area and the SMC number in the arterial wall was statistically significant ($p<0.05$). In the male group a medium correlation was observed ($r = –0.5$), whereas the correlation between the changes of the two parameters in the female group was strong ($r= –0.7$). Such results suggest that in the majority of cases the decreased SMC number is gradually replaced by collagen fibers, which causes thickening of the media.

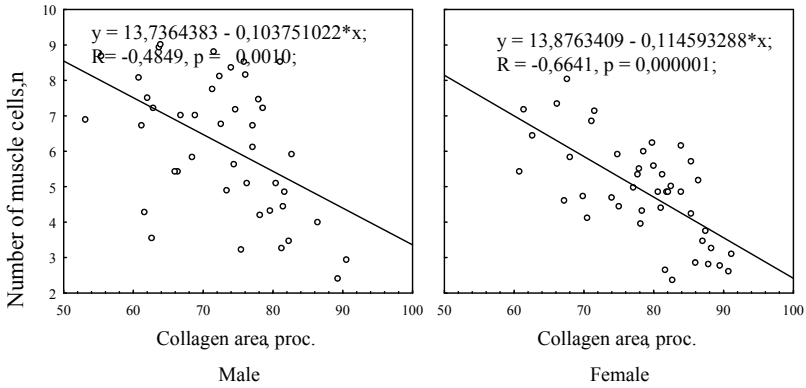

Figure 4. The collagen area and the smooth muscle cells number correlation.

Age-related changes of the media thickness

In the analysis of the media thickness in different gender and age groups, its thickening with the age was determined. The correlation with the age was of medium strength both in the male (r=0.36) and female (r=0.4) group. In all the cases this correlation was statistically significant ($p<0.05$).

The media thickness in the male group younger than 40 years differed by 14.4% from the middle-aged group ($p<0.001$) and by 22.5% from the oldest group ($p<0.0001$). In the male group over 60, the media thickness was thicker by 7% than in the middle-aged group, and difference was statistically significant ($p<0.0001$).

The media thickness in female was smaller by 46.7% in the youngest group as compared to the middle-aged group, and smaller by 59% as compared to the oldest group. The media thickness was bigger by 8% in female over 60 than in the middle-aged group. In the female groups the differences in the media thickness were statistically significant ($p<0.001$). (Fig 5).

Similar percentages reflecting the changes of the media thickening in the oldest groups of different genders (in contrast to the younger groups) show that the changes in the media thickness differ between male and female more extensively in the younger age. Media thickening was sharper in the first two age groups of female than in those of male. After

the age of 60 the rate of the changes almost equalled. The difference in the media thickness between the male younger than 40 and the older ones was just 14%. The correlation between the changes in the media thickness and the collagen area was of medium strength in the male (r=0.38) and female (r=0.48) groups (Figure 6).

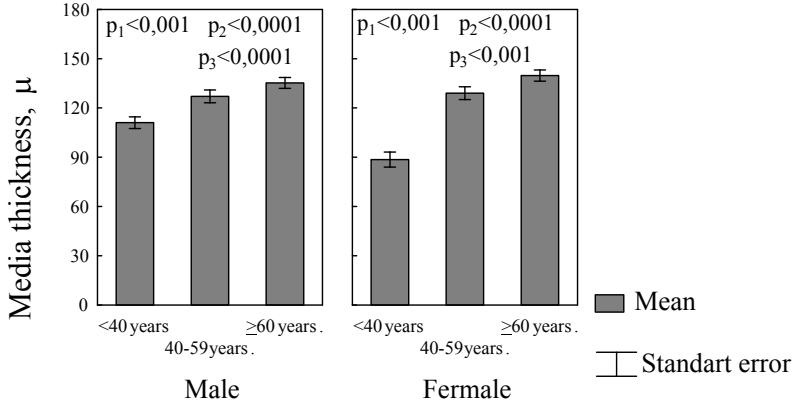

Figure 5. Media thickness in male and female in all the age groups. p_1 (<40 years and 40–59 years); p_2 (40–59 years and ≥60 years); p_3 (<40 years and ≥60 years).

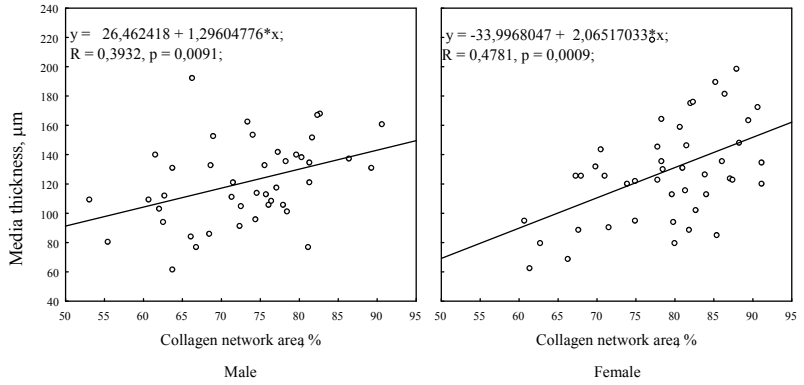

Figure 6. Collagen fibers area and media thickness correlation in the basilar artery media.

The correlation was statistically significant in both gender groups ($p<0.01$). Such results show that the changes in the media thickness can be related to the increase of the collagen content in the basilar artery.

DISCUSSION

We determined that the human basilar artery collagen network area was increasing with the age. In our previous report we found that the perimeter of collagen bundles and their number were decreasing with age in both gender groups [4]. Changes and the regeneration of collagen network are going on constantly. Even in old age collagen fibers are fragmentating and forming again [5]. The area of the network increases, but the perimeter and the number of bundles decrease with the age. Similar age-related changes were noticed in the studies analyzing collagen network structure changes in other blood vessels and the heart [1, 7]. While ageing collagen looses its toughness and the fibers start to stiffen and thicken [5]. With an increase of the amount of collagen, the elasticity of blood vessels decreases. The age-related structural evolution of arteries, with the predominance of collagen over elastin and reticulin, could be the reason of their fragility [7].

Our data also show the significant decrease of smooth muscle cells number in the media of the basilar artery during ageing. Statistical data have revealed that the decrease in the SMC number had a strong statistically significant negative correlation with the age ($p<0.05$). Fonck E. et al. [2] investigated the age–related changes of the posterior cerebral artery and also found simultaneous diminishing of smooth cells number and the increase of the collagen content in the media of this artery.

When the thickness of the media of the basilar artery increases, the SMC number decreases. Similar data were obtained in some previous studies that analysed other cerebral arteries in relation to ageing [10]. We assume that the decrease in the SMC number does not have a direct influence on the media thickness. It is most likely that when the SMC number decreases, collagen fibers become thicker, which changes the properties and structure of the vascular wall.

In similar investigations of other arteries, researchers also relate the media thickening with the age, but concerning different age groups, they do not regularly obtain statistically significant data [8]. The correlation

between the changes in the media thickness and the collagen area was of medium strength in the male and the female. This correlation was statistically significant in both gender groups ($p<0.01$). Such results show that the changes in the media thickness can be related to the increase of the collagen content in the basilar artery. Our results support other researchers' conclusions [9].

Our morphometric findings that revealed the increased collagen area together with the decrease of SMC number might be responsible for the stiffening of the basilar artery in ageing and contribute to the development of atherosclerosis and arterial hypertension.

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TASTE SENSITIVITY TO PTC AND COLOUR BLINDNESS IN ESTONIANS

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ABSTRACT

The article discusses the genetic characterisation of Estonians on the basis of the traits of PTC and colour blindness in Estonian population samples from various parts of Estonia.

The taste sensitivity to PTC was studied in 2559 Estonian school-children from 24 localities and of colour blindness in 4300 males from 33 localities. Results: The frequency of nontasters was 25.4% on average; *t* gene frequency (50.4% for Estonians) varied between 36% and 65%. PTC nontasters frequency is higher on West-Estonian islands and in Western Estonia compared to other parts of Estonia. The frequency of colour blindness *cb* gene (5.3% for Estonians) is higher in North, North-East and East Estonia (6–8%); its mean frequency on the rest of the territory is lower. The genetic diversity in various traits seem to be a trace of the historical development of the Estonian nation.

***Key words*:** *PTC, colour blindness, population genetics, Estonians*.

INTRODUCTION

Taste sensitivity to phenylthiocarbamide (PTC) and colour blindness belong to those physiological traits that directly prove genetic diversity of people concerning the perception of the world; they are highly informative genetic markers in the studies of human diversity.

Determing taste sensitivity to PTC begun as early as in 1931, after the chemical substance was synthesized by chemist A. L. Fox. On describing the taste of PTC it appeared that the people tested were divided into two groups; most people tasted it bitter, but to others it was

without taste. So it was possible to discover polymorphism in the taste sensitivity to PTC, and to divide the human population into "tasters" and "nontasters" [4].

Later the ability to taste the PTC was found to be inherited as a simple Mendelian dominant trait, and nontasting is a simple recessive characteristic, but there are references that the threshold of taste sensitivity is higher in heterozygotes than homozygotes [8]. For studying taste sensitivity, a standard method using serial dilutions of PTC was developed. It appeared that the "tasters" varied greatly according to their sensitivity to taste PTC; everybody has their own threshold (the lowest concentration of PTC where the bitter taste appears), and taste sensitivity in populations has a bimodal distribution, one mode – "tasters", the other – "nontasters" [7].

Recent genetic PTC studies have revealed that the ability to taste PTC or not is conveyed by a single gene that codes for a taste receptor on the tongue. The PTC gene, TAS2R38, was discovered in 2003 [15]. Phenotypic variance in PTC sensitivity is accounted for the presence of just two common alleles: a tasting allele and a non-tasting allele, and the frequencies of these alleles in human populations correspond well to frequencies estimated from phenotype data [21].

Inability to clearly identify different colours of the spectrum is widely known as colour blindness. An individual with normal colour vision is capable to distinguish all the primary colours and blend them into different tones of colours. In the case of partial colour blindness, an individual does not distinguish clearly, in most cases, between red and green colour. These colour vision deficiencies are named protanomaly and deuteranomaly, but the inability to perceive red and green colours – protanop and deuteranop. Red-green colour blindness is a sex-linked trait; the corresponding genes are situated in the X chromosome, but normal colour vision is dominant in relation to colour blindness. Therefore, colour blindness is expressed mainly in men. Red and green colour pigments are present at the tip of the long arm of the X chromosome Xq28 [17].

In the distribution of colour blindness and PTC taste sensitivity, regional and racial differences occur. Colour blindness is more frequent among the Caucasoid peoples than among the Mongoloids [9]. PTC nontasers appear more frequently among Caucasoid populations. In Mongoloid populations, as Japanese and Chinese, nontasters are con-

siderably rare and, vice versa, tasters are found there much more frequently [18].

These data have also been referred to in studies of Estonians, and erroneous statements about the occurrence of PTC nontasters in Estonia have been made; the corresponding studies still have to be started [19]. Population genetic studies of 10 polymorphic systems (blood groups, PTC, colour blindness) of the Estonian population have been studied by the author of the present paper, and genetic analyses have been given earlier [11, 12, 5:570, 6:622]. The aim of the current study is to give an overview of the diversity of taste sensitivity to PTC and colour blindness among Estonians.

MATERIAL AND METHODS

The material used in the present study was gathered by the author mainly during the anthropological expeditions of the Institute of History of the Academy of Sciences in the 1970s and 1980s. The subjects were of Estonian descent; their parents and grandparents came from the same district. Tests were carried out on both schoolchildren and grown-up men. In the case of taste sensitivity to PTC, only the schoolchildren's material (2559 individuals from 24 regional locations) was used to avoid possible errors in the case of older people, and so that the data would be wholly comparable all over Estonia. The ability to detect the bitter taste of PTC was tested using 15 concentrations of PTC solutions, following the technique of Harris and Kalmus [7]. The concentrations of the solutions, in boiled tap water, were obtained by means of the formula $2.6\times2\text{-}n$ g/l (where n is the number of the solution), whereby in the case of the strongest concentration n=0, in the weakest – n=15. Tasting was started from the solution with the lowest concentration (no.15). Between every different solution, pure water was given. The threshold for each subject was the lowest concentration at which he was able to distinguish the PTC solution from pure water. Approximately 2% of schoolchildren did not even feel the bitter taste of the strongest solution (n=0). These people are marked with a negative sign in Fig.1. The distribution of the thresholds shows a typical bimodal shape (Fig.1). The threshold for schoolchildren was solution no. 5. The subjects who felt the taste of PTC solutions no. 15–6 were regarded as "tasters", of no. 5–0 as "nontasters".

Colour blindness was studied among 4300 males from 33 districts. The colour vision test for red-green colour deficiency among men and schoolboys was carried out by using polychromatic tables by J. Rabkin [24]. The test was conducted in daylight inside a room, avoiding direct sunlight.

RESULTS AND DISCUSSION

As regards to PTC tasting ability, the data on Estonians exibit a bimodal distribution of the threshold (Fig. 1). Among the 2559 individuals studied, 74.6% were tasters, 25.4% did not feel the bitter taste of PTC. The percentage and gene frequency of nontasters is given in Table 1. The frequency of nontasters of PTC, *t* gene, varies in Estonian different local samples between 36% and 65%, with the mean frequency 50.4%) (Table 1, Fig. 2). In the West Estonian islands the *t* gene frequency is higher (60%) in comparison to the other parts of Estonia. It is also comparatively high in some other westernmost parts of West and South-West Estonia (56–57%). In the East Estonian region (in Alatskivi), in the area between Lake Võrtsjärv and Lakes Peipsi-Pihkva (Pskov) and in some locations of inner Estonia, the frequency is lower (40–53%) (Fig.2).

Frequency of nontasters of PTC in Estonians is lower in comparison with the Finns; however, on the West Estonian islands and on the West Estonian coast, the percentage of nontasters of PTC is higher, being similar to that of other Finno-Ugric peoples, such as Komis, Maris, Hungarians but also non-Finno-Ugric peoples like Lithuanians, Russians, Swedes, et al. (Table 1, Table 2). Frequency of *t* gene in Latvians from Varakļāni is more similar to that of West-Estonian islands, from Alūksne to that of the South-East Estonian population. The frequency of nontasters is somewhat lower in Lapps from Inari and Kola Peninsula. The frequency of *t* gene is lower in the Mongoloid peoples – in Evenks 24%, Chinese 21%, etc. At that, taste sensibility to PTC is much higher in Mongoloid peoples who distinguish even a very weak solution (no.28) from pure water [25].

Table 1. Frequency of PTC nontasters (% and *t*-gene)

Population group	N	Phenotype		Genotype	
		n	%	*t*	σ
1. Kuressaare	116	38	32,76	.5724	.0381
2. Orissaare	113	47	41.59	.6449	.0359
3. Muhu	103	35	33.98	.5829	.0400
4. Haapsalu	110	36	32.73	.5721	.0391
5. Lihula	107	21	19.63	.4431	.0433
6. Tõstamaa	75	21	28.00	.5292	.0489
7. Audru	80	20	25.00	.5000	.0484
8. Pärnu-Jaagupi	99	31	31.31	.5596	.0416
9. Märjamaa	100	15	15.00	.3875	.0461
10. Kehra	100	22	22.00	.4690	.0441
11. Kunda	109	28	25.69	.5069	.0413
12. Iisaku	154	34	22.08	.4699	.0356
13. Järva-Jaani	83	13	15.66	.3957	.0504
14. Suure-Jaani	101	43	42.57	.6525	.0377
15. Alatskivi	92	17	18.48	.4299	.0471
16. Elva	96	21	21.88	.4677	.0451
17. Võnnu	67	15	22.39	.4732	.0538
18. Põlva	224	59	26.34	.5132	.0286
19. Värska	106	17	16.04	.4005	.0445
20. Meremäe	123	34	27.64	.5257	.0383
21. Valga	98	13	13.27	.3642	.0470
22. Abja	97	20	20.62	.4541	.0437
23. Viljandi	104	18	17.31	.4161	.0446
24. Kilingi-Nõmme	102	32	31.37	.5601	.0410
1–24 in total	2559	650	25.40	.5040	.0085

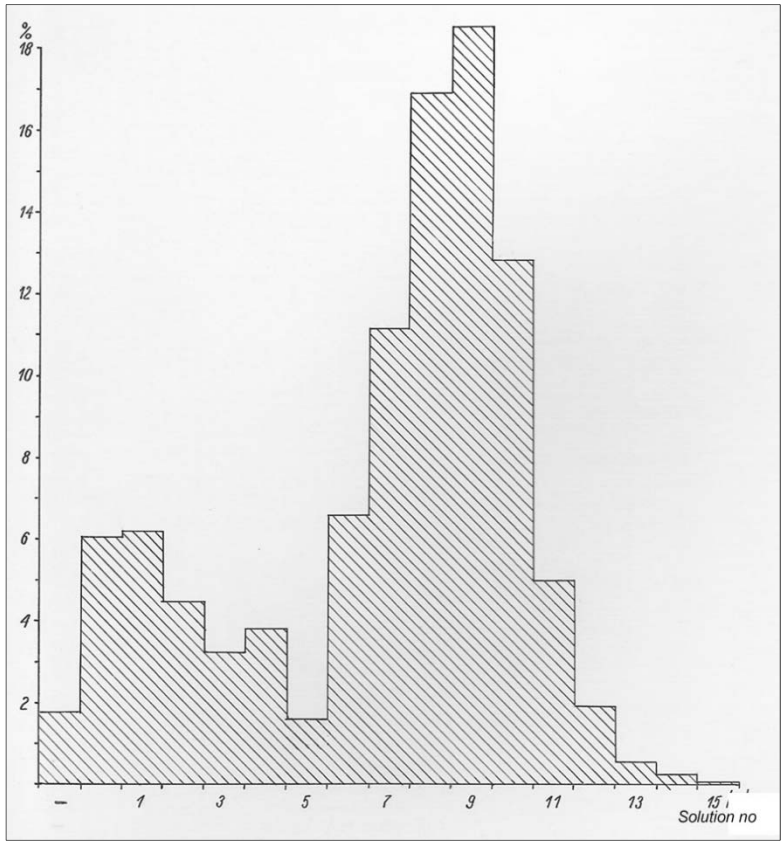

Fig. 1. Taste sensitivity to PTC.

Table 2. Frequency of PTC nontasters in various populations (% and *t*-gene)

Population	N	%	*t*-gene	Sources
Estonians	2559	25.4	.504	[11]
Finns	811	35.0	.592	[14]
Finns (Helsinki)	202	29.2	.541	[1]
Finns (Oulu)	761	22.1	.470	[2]
Lapps (Inari)	184	15.8	.396	[23]
Lapps (Kolta)	149	36.2	.602	[23]
Lapps (Kola Peninsula)	124	12.5	.353	[28]
Vepsians	176	28.4	.533	[11]
Komi	302	36.8	.606	[23]
Mari	321	26.5	.514	[23]
Hungarians	401	32.2	.567	[3]
Hungarians (Transcarpathian)	203	31.5	.562	Author's data
Latvians (Alūksne)	109	24.8	.498	[11]
Latvians (Varakļāni)	109	43.1	.657	[11]
Lithuanians	163	31.9	.565	[26]
Belarusians	2694	35.6	.597	[26]
Russians	245	32.1	.567	[22]
Swedes (Åland)	124	33.1	.575	[13]
Swedes	509	33.8	.581	[20]
Kyrgyz	640	19.6	.443	[22]
Evenks	137	5.8	.241	[25]
Chinese	239	4.6	.214	[16]

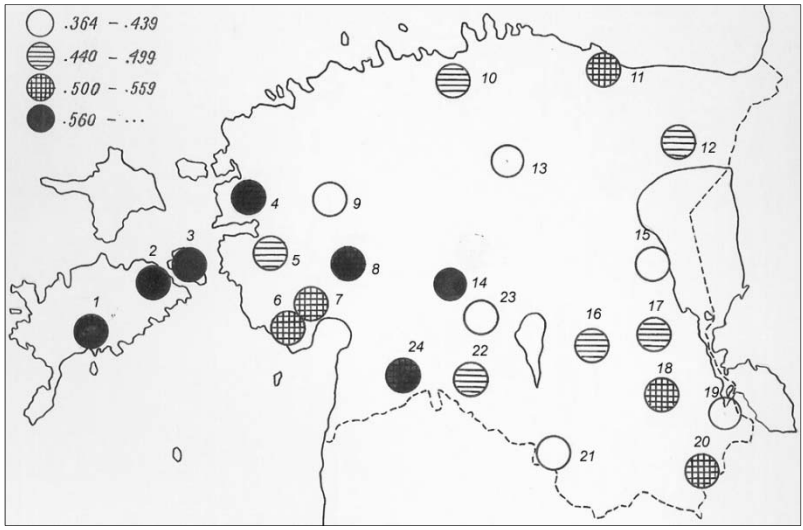

Fig. 2. Distribution of nontaster (*t*) gene.

Colour blindness. In Estonia, the colour blindness has been studied among male population (grown-up men and schoolboys). For testing colour blindness in Tallinn schools, 7.17% of schoolboys from 293 tested were found to be colour blind; among them – deuteranomaly 3.1%, protanamaly 1.35%, deuteranop 1.36% and protanop 1.36% [10]. These data correspond well with the earlier data by R. I. Serebrovskaya (in 235 males 3.0% of deuteranomaly and 1.3% of protanomaly) [27].

Table 3 shows the percentage of incidence of colour blindness. The frequency of colour blindness varies in different Estonian local samples between 2% and 9%, with the average 5.3%. The frequency is higher in East and North-East Estonia (6–8%). The frequency of *cb* gene in West Estonia is comparatively high as well (6,2%) and its frequency decreases towards South-East Estonia (Fig. 3). The whole South-East is characterised by low occurrence of colour blindness (on average 4.3%). It is low on the West Estonian islands and in Central Estonia as well.

Table 3. Frequency of colour blindness

Population	N	Phenotype		Genotype	
		n	%	*cb*	σ
1.Saaremaa, Muhu	382	14	3.66	.0366	.0096
2.Hiiumaa	102	5	4.90	.0490	.0214
3.Haapsalu	210	13	6.19	.0619	.0166
4.Lihula	158	9	5.70	.0570	.0184
5.Tõstamaa	43	4	9.30	.0930	.0443
6.Audru, Pootsi	178	11	6.18	.0618	.0180
7.Pärnu-Jaagupi	132	8	6.06	.0606	.0208
8.Rapla	182	8	4.40	.0440	.0152
9.Keila	137	8	5.84	.0584	.0200
10.Tallinn	293	21	7.17	.0717	.0151
11.Kehra	75	5	6.67	.0667	.0288
12.Rakvere	99	8	8.08	.0808	.0274
13.Kohtla-Järve	99	5	5.05	.0505	.0220
14.Iisaku	147	10	6.80	.0680	.0208
15.Väike-Maarja	100	8	8.00	.0800	.0271
16.Paide	94	2	2.13	.0213	.0149
17.Suure-Jaani	56	3	5.36	.0536	.0301
18.Põltsamaa	101	4	3.96	.0396	.0194
19.Jõgeva	65	5	7.69	.0769	.0330
20.Alatskivi	55	4	7.27	.0727	.0350
21.Elva	91	4	4.40	.0440	.0215
22.Otepää	99	3	3.03	.0303	.0172
23.Võnnu	37	2	5.40	.0540	.0372
24.Põlva	295	15	5.08	.0508	.0128
25.Antsla	102	5	4.90	.0490	.0214
26.Võru	103	5	4.85	.0485	.0212
27.Värska	49	3	6.12	.0612	.0342
28.Petseri	102	2	1.96	.0196	.0137
29.Meremäe	210	8	3.81	.0381	.0132
30.Valga	105	6	5.71	.0571	.0226
31.Abja, Karksi	196	14	7.14	.0714	.0184
32.Viljandi	100	3	3.00	.0300	.0171
33.Kilingi-Nõmme	103	5	4.85	.0485	.0212
1–33 in total	4300	230	5.35	.0535	.0034

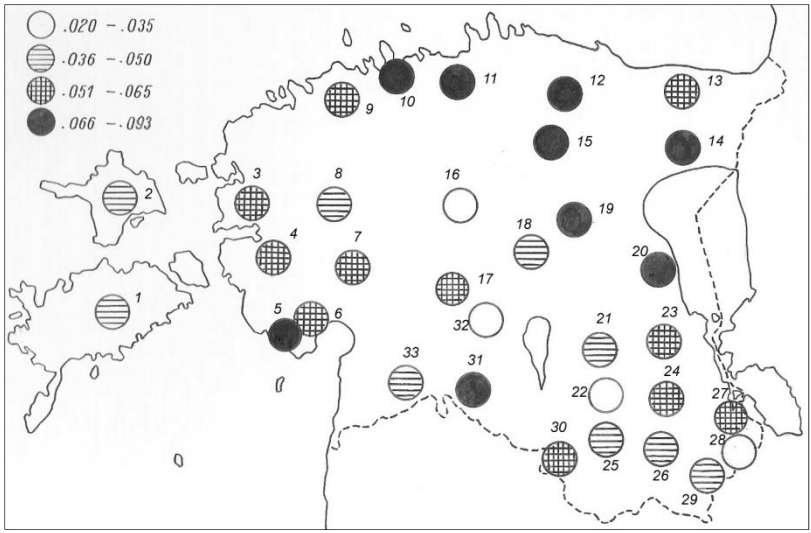

Fig. 3. Distribution of colour blindness (*cb*) gene.

In the distribution of colour blindness, regional and racial differences occur. In Europoid populations colour vision deficiency is higher than in Mongoloids [9]. In comparison with some other European populations, the mean percentage of Estonians' colour blindness is comparatively low (Table 4). Frequency of colour blindness in Latvians in Alūksne and Varakļāni is similar to that of Central and South-West Estonians. On the territory of Northern Eurasia the mean frequency is 5.5%, varying in the limits of 0.0%–24.0% [6:303]. Territory with the frequency below the mean (0.0%–4.3%) embraces the central and north-eastern regions of Siberia. Towards north and south and southwest the frequency increases. The highest frequency of colour blindness is found on the territory of Belarus with a nucleus in Grodnensk region – 24.1% [6:303].

Table 4. Frequency of colour blindness in various populations

Population	N	%	Sources
Estonians	4300	5.3	[11]
Estonians	235	6.2	[27]
Latvians (Alūksne)	90	4.4	[11]
Latvians (Varakļāni)	76	4.0	[11]
Vepsians	103	7.8	Author's data
Hungarians (Transcarpathia)	201	4.5	Author's data
Norwegians	9049	8.0	[9]
Scots	464	7.8	[9]
Germans	1000	7.5	[9]
American Indians	392	2.0	[9]
Congo Blacks	929	1.7	[9]

Thus, the survey showed genetic heterogeneity in the traits of taste sensitivity to PTC and colour blindness in Estonians; the greatest genetic differences were observed in the West-East direction (as in the other genetic characteristics of polymorphic systems [11,12]). On the West Estonian islands and on the West Estonian coast the percentage of nontasters of PTC is higher than anywhere else in Estonia. Frequency of colour blindness is higher in North, North-East and East Estonia; its mean frequency on the rest of the territory is lower. The genetic diversity in various traits seem to be a trace of the historical development of the Estonian nation.

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DEXAMETHASONE-INDUCED T-LYMPHOCYTE APOPTOSIS IN DIFFERENT LYMPHOID ORGANS

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ABSTRACT

The aim of our study was the comparison of the synthetic glucocorticoid dexamethasone (DEXA) influence on T-lymphocytes in central (thymus) and peripheral (spleen, lymphatic nodes) immune organs. For that reason therapeutic doses of DEXA were used followed by histological, histochemical (TUNEL) as well as computerized histomorphometrical investigations.

In the study 36 young adult Wistar rats performed. 1 – 7 days after 3 days injection of DEXA (total dose 1,2 mg/rat i.p.) the material was taken for futher investigations. First days after DEXA administration in therapeutic doses the number of apoptotic cells was considerably increased in the cortical part of thymus. No significant changes were in rest of thymus as well as in peripheral immune organs. 7 days after DEXA-induced injury the number of apoptotic cells had decreased almost to the normal level.

Our investigations conclude that the most sensitive for the dexamethasone-induced T-lymphocyte apoptosis is cortex of thymus while the changes in medullary area of thymus and peripheral immune organs – spleen and perithymic lymphatic nodes- are less significant. Week after drug cessation the apoptotic changes are almost at normal level in both types of lymphoid organs.

Key words: *apoptosis, dexamethasone, thymus, spleen, lymphatic nodes*

INTRODUCTION

Apoptosis is an active process of cellular self-destruction, called programmed cell death, that is implicated in both normal and pathologic tissue changes (1). Apoptosis is frequently present in tumours and tissues treated with a variety of toxic stimuli (2). As changes in normal apoptotic processes may cause diseases and disorders in homeostasis the investigations of apoptosis have increased recently (3–5).

Increase in apoptosis in immune organs is involved in enhancement of the immune response**.** It is widely known that central immune organ thymus is vulnerable to radiation and immunotoxic chemicals (6–8). Still there are no comparative studies on apoptosis of central and peripheral immune organs – comparison of apoptosis on T-lymphocytes in different lymphoid organs.

Thymocyte apoptosis plays a physiological role in the T-cell selection in the thymus (9) Apoptosis occurs in the cells of the thymus cortex where it is accelerated by glycocorticoid hormones (4). Probably therefore for the study of thymocyte apoptosis dexamethasone-model is one of the best characterised experimental models (10–11).

The aim of the present study was to compare the apoptotic changes in different immune organs using synthetic glucocorticoid dexamethasone in therapeutic dosages.

MATERIAL AND METHODS

36 male young adult Wistar rats with a weight of 200–220 g were investigated on 1 to 7 days after dexamethasone (DEXA) administration. The guidelines for the care and use of the animals were approved by the Ethical Committee of the University of Tartu. The rats were housed in an animal room, controlled at temperature, 22±2ºC; humidity, 55±15%; ventilation (all-fresh-air system); 12 h light/dark cycle and fed a certified diet "Dimela" (Finland R-70 and R-34) and water ad libidum. Animals were acclimated for 7 days before dosing.

The rats were injected with DEXA 1,2 mg/rat i.p. on the 1st, 4th and 7th days and sacrified 1, 3, 5 and 7 days after last injection. The animals were euthanized by decapitation under anesthesia with i.m. injection of ketamine 50 mg/kg b. w. and diazepam 5 mg/kg. The lymphoid organs – thymus, perithymic lymphatic nodes (mediastinal lymph nodes) and spleen, were carefully removed. One-half of each organ was frozen in

liguid nitrogen and stored at –80ºC until used for histochemistry (TUNEL). The remaining parts were used for routine histology as well as for lymphocyte detecting by histomorphometry: the lymphoid organs were fixed in 10% buffered formalin, and embedded in paraffin, thereafter 7 μm thick sections were stained with haematoxylin – eosin, Heidenhain's iron haematoxylin and Feulgen. For better understanding of apoptotic changes, mostly occurring in the cortical and medullary cells in the thymus, computerized morphometry was used: the pictures of slices stained for routine histology were photographed by a light-microscope Olympus BX-50, saved electronically and the further process was performed with the computer program Adobe Photoshop 5.0 under a simultaneous visual control of light-microscopy (11). The areas of thymolymphocytes were observed and analysed with the help of Adobe Photoshop selecting different colours (apoptotic/non-apoptotic): the painted areas were summarised in pixels and the proportions of different cells were calculated in percents.

Statistical analysis was performed using one sample t-test and the unpaired t-test (GraphPad Quick Calcs: Analyze continous data) at the level of significance p less than 0,05 ($p<0,05$) with the Newman-Keuls multiple comparison test.

Computerized histomorphometry is widely used in the quantitative analysis (12–15).

For histochemistry frozen tissue sections were stained for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) with a standard in situ Cell Death Detection Kit – POD (Roche Molecular Diagnostics, U.S.A.), according to the manufacture's guidelines (16–19). The apoptotic cells were detected by TUNEL; each experiment set up by TUNEL assay without terminal transferase served as negative control.

RESULTS

The apoptotic cells (Ao) were detected in rat thymus, spleen and perithymic lymphoid tissue(mediastinal lymph nodes). For better understanding of the apoptotic changes in thymus, the TUNEL-positive cells were detected separately in cortical and medullary part of thymus (Fig. 1).

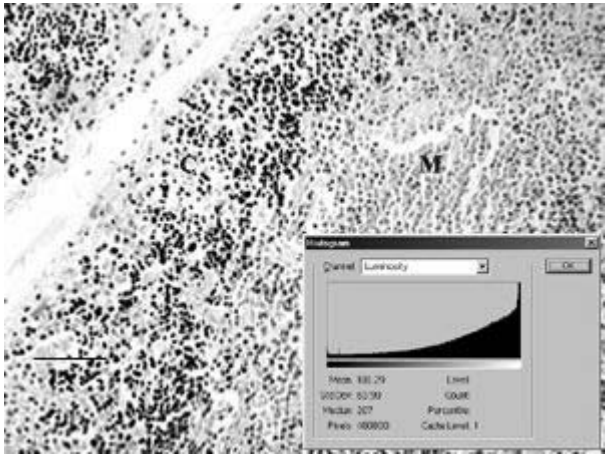

Figure 1. Group of apoptotic cells in outer cortex (C) of thymus lobe; medullary area (M) remains lightly stained. Iron haematoxylin. Bar: 100 μm.

During the first days after DEXA administration the number of Ao cells detected by Photoshop analysis and TUNEL-positive cells were the largest in the cortical part of thymus (Table 1). In compare to the rest of thymus and secondary lymphoid organs the remarkable increase of the percentage of Ao cells and amount of TUNEL-positive cells in deep cortex were noted (Fig. 2).

The high number of Ao cells and TUNEL-positive cells in cortical part of thymus continued up to 5^{th} days whereas the changes in medullary area of thymus as well as in spleen and perithymic lymphatic nodes remained almost at control levels. In spleen the highest increase of Ao cells was noted during first days after injury in germinal centres of splenic nodules and around central artery (Fig. 3). Among the different parts of lymphatic nodes the highest increase of Ao cells was in paracortex compared to cortical and medullar parts (Fig. 4).

7 days after cessation of DEXA administration the expression of apoptosis was at almost normal levels (Table 1).

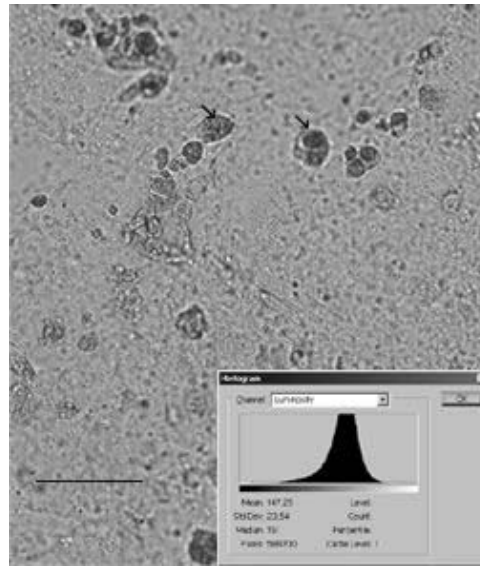

Figure 2. The large number of the apoptotic TUNEL-positive cells (indicated by arrows) in thymus cortex 1 day after the i.p. DEXA injection in rat. Histogram on the whole field (769600) pixels. Bar: 100 μm.

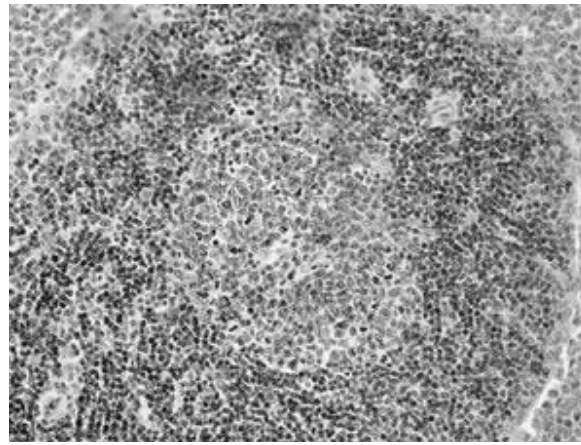

Figure 3. Spleen 1st day after injury: destruction of follicles, depletion of parenchyma and distribution of the apoptotic cells mainly in outer part of splenic nodule. Haematoxylin-eosin. Bar: 100 μm.

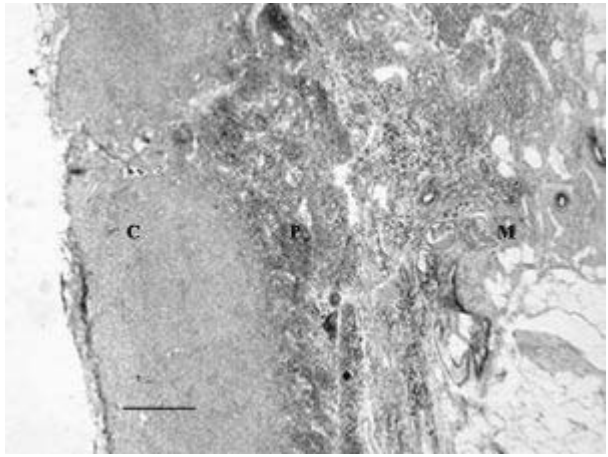

Figure 4. Distribution of apoptotic cells in cortical (C), paracortical (P) and medullary (M) area of lymph node 1 day after the i.p. DEXA injection in rat. Increase of apoptotic cells in paracortical area. Haematoxylin-eosin. Bar: 500 μm.

Table 1. Areas of apoptotic cells (APC) in rat thymus 1–7 days after the DEXA injection 1,2 mg/rat i.p. (percentage of total cell area ±SEM). Computer field in use: 16,7%; 118306 pixels (=100%)

Days after DEXA injection	**Cortical area (inner cortex)**	**Medulla**
1	48,0±1,9*	2,3±0,3
3	28,1±1,2*	1,9±0,2
5	13,4±0,5	2,4±0,3
7	9,2±0,7	1,6±0,1
Control 6,3±0,8	1,2±0,1	

*differences between the values of this group are significant ($p<0,05$)

Our findings suggest that for the dexamethasone induced apoptosis the most sensitive in compare to different lymphoid organs are T-lymphocytes locating in the cortical part of thymus, especially in deep cortex. Week after the drug cessation the apoptotic changes become almost at normal level in both types of immune organs.

DISCUSSION

Apoptosis is a genetically mediated programmed cell destruction where cells die, shrink, and disintegrate in the absence of any reactive inflammation. Autoreactive T- and B-lymphocytes are also eliminated by apoptosis, ensuring homeostasis for organism. Dearangements of the process of apoptosis can lead to deformities and tumors (20). Apoptosis is frequently present in tissues treated with a variety of toxic stimuli (2).

Endogenous glucocorticoids (GC) take part in homeostasis. Overflow of exogenous GC have caused appearance of thymic atrophy, inhibition of mitotic activity of cortical thymolymphocytes and fall the number of cells. Synthetic GC dexamethasone (DEXA) in therapeutic dose caused thymus atrophy enhancing the number of Ao cells, especially in the cortical area (2,21).

In our experiments the synthetic glycocorticoid dexamethasone in therapeutic doses was used to compare the apoptotic changes in primary and secondary lymphoid organs. The countings of Ao cells and histochemistry by TUNEL reaction after DEXA administration showed the clear difference of apoptotic changes in different immune organs, moreover the different localization of apoptosis of a particular organ. For example one day after DEXA injection the percentage of Ao cells of total cell area in rat thymus were in cortical area 48,0±1,9 and in medullary area 2,3±0,3 compared with control values 6,3±0,8 and 1,2±0,1 respectively.

Ao cell values are more high compared to TUNEL-positive cells, because the Ao cell group consists besides of darkly staining proper apoptotic cells also of some necrotic and mitotic cells, which are non-differentiated in photoshop-based image analysis. Still, the number of Ao cells, markers of lymphocyte proliferative rate are widely used (22–27).

TUNEL-positive cell specifity varies with tissue and degree of injury. After therapeutic doses of DEXA TUNEL-sensitivity of thymolymphocytes is 100% in correlation with histology. TUNEL specifity after predominantly necrotic injury do not exceed 70% of detected apoptotic cells (28). In mouse kidney the apoptotic signal detected with TUNEL assay may be false-positive (29).

The TUNEL-positive cells are apoptotic cells *in situ*, because the chromosomal nick ends are directly labelled (7,10). Therefore our indirect detection of Ao cells by computerized histomorphometry

simultanously with TUNEL assessment, is quite correct for the measurement of apoptotic thymolymphocytes.

Our experiments showed that after DEXA administration the most sensitive were T-lymphocytes in central immune organ thymus. Sensitivity of thymolymphocytes to DEXA-induced apoptosis varies with exo- and endogeneous factors. DEXA activated thymocyte apoptosis requires a sequence of events including interactions with the GC receptor, phosphatidylinositol-specific phospholipase C, acidic sphingomyelinase activation (30).

The initiation of apoptosis needs triggering of primed cells into apoptosis itself – stimuli that trigger primed cells will not initiate apoptosis in unprimed cells. The immature T-cells of the thymus cortex are primed for apoptosis and receptor occupancy triggers apoptosis in them. In contrast, the mature T-cell is not primed and responds to the same ligand by initiating cell replication instead of apoptosis in medullary area of thymus (1). Probably the same reason occurs also in peripheral immune organs. More investigations (genetic, electron-microscopic etc.) for solving the questions are required in future.

Our investigations conclude that the first days after DEXA-administration in therapeutic doses the most sensitive for the dexamethasone-induced T-lymphocyte apoptosis is the cortical area of thymus while the changes in medullary area of thymus as well as in peripheral immune organs are not remarkable. In spleen the most sensitive parts were the splenic nodules and in lymph nodes the paracortical area.

The apoptotic changes caused by synthetic glycocorticoid dexamethasone are reversible. 5–7 days after drug cessation the apoptotic changes become almost at normal(control) level in both types of lymphoid organs.

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HAND OSTEOARTHRITIS AND AGING: THE RESULTS OF A LARGE-SCALE CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Because osteoarthritis (OA) is associated with morbidity and mortality, we hypothesized that radiographic hand OA would generally be associated with chronic systemic morbidity and it will be less prevalent in longevity populations than in non-longevity populations.

Aims: 1) to evaluate the association between chronic systemic morbidity and radiographic hand OA; 2) to compare the prevalence and the mode of the development of radiographic hand OA in three longevity populations (Abkhazian, Azerbaijani and Georgian) vs. two non-longevity populations (Russian and Chuvashians).

Methods: Radiographic hand OA was evaluated using the left hand radiograms in 14 joints according to the Kellgren and Lawrence's (K-L) grading system. Each individual was characterized by the total number of affected (K-L≥2) joints (NAJ). The prevalence of hand OA was defined as the presence of at least one affected joint.

Morbidity data were attained from their medical records and divided into 9 categories by a research physician. The longevity index was calculated as a ratio of the number of individuals aged >90 years versus the number of people aged >60, expressed in per mills (‰). The population with the longevity index >40‰ was considered as the longevity population. Statistical analyses included the prevalence estimation and ANOVA.

Results: Radiographic hand OA was statistically significantly and positively associated with the ischemic heart disease. A significant

difference in the age standardized prevalence of hand OA was found between each pair of the studied samples, except between the Chuvashians, Russians and Georgians and between the Azerbaijanis and Abkhazians. The lowest prevalence was found in the Abkhazians followed by the Azerbaijanis and Georgians. The highest prevalence was found in the Chuvashians. ANOVA showed significant differences between the age-adjusted means of NAJs. The lowest age-adjusted NAJ was found in the Abkhazian population followed by the Azerbaijanis and Georgians. The highest NAJ was found in the Chuvashians.

Conclusions: The results of our study showed association between ischemic heart diseases and hand OA. Longevity populations showed lower hand OA prevalence and NAJ compared to a non-longevity population, that can be interpreted as that longevity populations age slower. Additional follow-up studies are needed to verify this hypothesis.

***Key words*:** *Ageing, hands, longevity, osteoarthritis, biological ageing.*

INTRODUCTION

Osteoarthritis (OA) is the most common form of joint disease and one of the most frequent morbidities in the elderly [17]. Radiographic hand OA is a particularly frequent condition in any population, and its prevalence and severity have been shown to be associated with age [15] and ageing [8]. Livshits et al. [18] performed a standard three-factor variance decomposition analysis on Chuvashian pedigrees and found that the main factor influencing the extent of OA development was age, contributing about 73% of the total variance. A study employing samples from the TwinsUK Adult Twin Registry [26] found significant differences in leucocyte telomere length between the subjects with and without hand OA. The authors suggested that there were potential shared mechanisms between OA and ageing, thus implicating oxidative stress and low-level chronic inflammation in both conditions. In addition, there are numerous studies that found the association of OA with chronic morbidity [7, 10, 11] and even with increased mortality [9]. Schellevis et al. [23] found that OA had the highest rate of comorbidity. As the population ages, individuals with arthritis may represent a subgroup with disproportionate increases in disability relative to the general population [5]. Based on aforementioned studies, we postulated that the individuals

suffering from some form of chronic systemic morbidity would show higher levels of hand OA.

If, as we showed before, hand OA is associated with age, chronic morbidity and mortality, the logical assumption can be that in longevity populations radiographic hand OA generally is less prevalent and would develop at a later age in longevity populations versus non-longevity populations. Longevity populations were comprehensively studied [1, 3] and many studies have reported that centenarians escape the major age-related diseases [2, 6]. However, we found no studies of the prevalence and the severity of OA in the longevity population.

The aims of our study were: 1) to evaluate the association between chronic systemic morbidity and radiographic hand OA; 2) to compare the prevalence and the mode of the development of radiographic hand OA in three longevity populations (Abkhazian, Azerbaijani and Georgian) vs. two non-longevity populations (Russian and Chuvashians);

METHODS

Sample

In the present study, we benefited from the unique data collection from the Institute and Museum of Anthropology, Moscow University. Several ethnic samples were collected from different rural areas of the USSR using the same selection and data collection procedures. There were no significant differences in the level and the accessibility of medical services between samples. Data were collected during several annual expeditions undertaken by the Institute and the Museum of Anthropology in the 1980s. The aim of these expeditions was to collect data for the USSR countrywide study of environmental adaptation in humans. The samples chosen were representative of the general population in each specific area. The focus was on the historical stability of a traditional farming community with little occupational diversity. For generations, most of the population lived under the same environmental conditions and were unexposed to an outside genetic flow [21].

To evaluate the association between OA morbidities, we used the data collected in native Chuvashians residing in small villages in the Chuvasha Autonomies of the Russian Federation.

To evaluate the association between hand OA and longevity, five population samples were chosen. The population samples with a

longevity index (LI) of >40‰ were considered longevity populations [21]. According to Kozlov et al. [16], who conducted extensive research on longevity in the same area, among the four samples chosen, three were longevity populations: Abkhazian, Azerbaijani, Georgian, and one non-longevity sample: Russian [16]. LI is calculated as a ratio of the number of individuals >90 years versus the number of people >60 years old, expressed in per mills (‰). This index was previously used in numerous publications including the recent [16, 19, 21].

Data on age, chronic morbidity and medical treatment were obtained from the participants' medical records and completed during the interview. Age was also verified by an identification document. Various anthropological measurements and left hand x-rays were taken. There were no individuals using hormone replacement therapy in the studied samples. Individuals with known posttraumatic, rheumatoid or psoriatic arthritis were not included in this study.

Morbidity definitions

An experienced research physician divided the morbidity data into 9 categories, similar to the previously used categories in several comorbidity investigations [6, 24–26), without noting the physiologic severity of each chronic condition (see Appendix 1).

Appendix 1: Diseases included in each studied group of morbidities

Ischemic heart diseases	Angina pectoris, Ischemic heart disease, Myocardial infarction
Organic heart deceases	Cardiosclerosis, Myocarditis, Rheumatoid myocarditis
Rheumatologic diseases	Rheumatism, Ankylosing spondylitis, Systemic lupus erythematosus
Diabetes	Diabetes
Renal diseases	Pyelonephritis, Nephrolithiasis
Hepatitis	Hepatitis
Hypertension	Hypertension
Pulmonary diseases	Chronic bronchitis, Emphysema
Gastrointestinal diseases	Chronic colitis, Chronic gastritis, Peptic ulcer, Gastroectomy

Radiographic assessment of OA: Single plain radiographs of the left hand, in the postero-anterior position with the x-ray source located

60 cm above, using a standard radiographic technique as described in detail by Pavlovsky [21], were obtained from each study participant The same equipment was used in all the expeditions. An experienced and specially trained researcher read each x-ray. The intra-observer reliability of the K-L scores (kappa statistics) was at least 0.84 ($p<0.01$), based on 20 repeated measurements.

OA development was evaluated according to Kellgren and Lawrence's (K-L) grading scheme, utilizing the photographs from the Atlas of Standard Radiographs [14]. The K-L score for each joint (ranging from 0 to 4) represented the accumulation of degenerative changes. OA development was separately evaluated on 14 joints of the left hand, i.e., 4 distal interphalangeal (DIP), 4 proximal interphalangeal (PIP), 5 metacarpophalangeal (MP), and 1st interphalngeal (IP-1). Joints scored as K-L$\geq$2 were considered affected. Each individual was characterized by the number of affected joints (NAJ). The prevalence of hand OA was defined as the presence of at least one affected joint.

Statistical Analysis

To evaluate the difference in the mean values of age-adjusted NAJ between the individuals affected vs. non-affected with the specific disease, we used one-way analysis of variance (ANOVA) with hand NAJ as a dependent variable and the individuals affected vs. non-affected with the specific disease as an independent (grouping) variable. This analysis was performed only in the Chuvashian sample, because most detailed and reliable information on morbidity was collected in this sample.

The prevalence of OA (at least one affected joint) and the mean NAJ were then calculated according to age groups ($\leq$35, 36–50, 51–65, >65 years). Using the χ^2 test, we compared the prevalence of hand OA between longevity and non-longevity samples, after the standardization for age, in each sample. The age distribution in the total sample was used as the standard. Applying the one-way ANOVA (Scheffe) procedure, we compared the NAJ, adjusted for age and sex, between the studied population samples.

RESULTS

The descriptive statistics of the studied samples is presented in Table 1. As mentioned above, three sampled populations fulfilled the criterion

for longevity populations (LI >40‰): Abkhazians (LI=60–70‰), Azerbaijanis (LI=50–60‰) and Georgians (LI=40–50‰); and two samples were of a non-longevity population: Russians and Chuvashians (LI=10–20‰). The samples in tables 1, 2 and 3 were arranged in accordance with their LI. Each sample comprised individuals with a wide range of ages. The mean age (±SE) for Abkhazians was 43.47±0.62; Azerbaijanis 48.29±0.98; Georgians 52.11±1.14, Russians 43.75±0.45 and the Chuvashians 48.93±0.58. The prevalence of at least one joint with OA and the mean NAJs in each studied sample is presented in Table 1.

Table 1. Description statistics of the studied samples

Studied group	Longe-vity index* 90+/60+ (‰)	Sample size	Sex (% of males)	Mean age±SE [years]	Age range [years]	Prevalence of hand OA (%)	# of affected joints mean ± SE
Chuva-shians	10–20	819	52.99	48.93±0.58	18–86	48.00±0.49	2.33±0.14
Russian	10–20	1071	43.60	43.75±0.45	18–90	49.86±0.76	1.74±0.08
Geor-gians	40–50	271	32.84	52.11±1.14	18–99	61.99±1.43	2.27±0.16
Azer-baijanis	50–60	290	44.48	48.29±0.98	19–90	29.15±1.21	1.28±0.12
Abkha-zian	60–70	590	52.37	43.47±0.62	18–94	43.10±1.01	0.72±0.06

* The intervals of longevity index as were estimated in an extensive screening study [16].

Table 2 presents the prevalence of radiographic hand OA (at least one affected joint) in the studied ethnic samples according to four age groups (≤35, 36–50, 51–65, >65). The prevalence of hand OA in each age group was lower in populations with high LI and vice versa. The results of the χ^2 test (data not presented) showed significant differences ($p<0.003$) in the age-standardized prevalence of hand OA between each pair of the studied samples, except between Chuvashians, Russians and

Georgians and between Azerbaijanis and Abkhazians ($p>0.05$). The lowest age-standardized prevalence was found in Abkhazians followed by Azerbaijanis and Georgians. The highest prevalence was found in the Chuvashian population.

Table 2. Prevalence (%) of radiographic hand OA in different ethnic samples according to age groups

Sample	≤35	36–50	51–65	>65
Chuvashians	5.30	21.60	72.40	94.60
Russians	21.70	45.52	81.90	99.06
Georgians	13.79	40.58	89.47	94.03
Azerbaijanis	8.22	26.74	66.29	88.09
Abkhazians	3.89	21.95	60.71	82.69

Table 3 shows the mean NAJ (±SE) in the studied samples according to age groups and age-adjusted means in each sample. In each age group, the samples with a higher LI showed lower mean values of NAJs. ANOVA showed significant differences ($p<0.01$) between the age-adjusted standardized means of NAJs. The post-hoc comparison (the Scheffe test) demonstrated a significant difference ($p<0.01$) between each pair of samples, except between Azerbaijanis and Abkhazians ($p>0.05$). The lowest age-adjusted mean NAJ was found in Abkhazians followed by Azerbaijanis and Georgians. The highest NAJ was found in Chuvashians.

Table 3. Mean number of affected joints (±SE) of radiographic hand OA in different ethnic samples according to age groups

Sample	≤35	36–50	51–65	>65
Chuvashians	0.06±0.02	0.36±0.09	3.42±0.21	6.55±0.45
Russians	0.32±0.04	0.89±0.06	3.24±0.18	6.72±0.29
Georgians	0.22±0.08	0.83±0.16	2.32±0.20	5.44±0.34
Azerbaijanis	0.08±0.03	0.39±0.08	1.82±0.20	4.04±0.45
Abkhazians	0.04±0.01	0.33±0.05	1.55±0.19	3.15±0.31

The most common morbidities in the Chuvashian population were gastrointestinal diseases (107 individuals or 13%), the ischemic heart disease and hypertension (79 individuals or 9.6%). The results of ANOVA are also shown in Table 4. Statistically significant differences

between the mean radiographic hand OA scores in affected vs. non-affected individuals were found only for the ischemic heart disease (p=0.022) and a group of gastrointestinal diseases (p=0.043). The mean values of hand OA scores were higher in the individuals affected by the ischemic heart disease and lower in the individuals affected by the gastrointestinal disease compared to non-affected persons.

Table 4. Analysis of variance of hand OA scores in the Chuvashian population. The mean values and standard deviations (SD) of age-adjusted hand OA score of affected vs. non-affected individuals

Disease	P-value	Category	Mean	SD	N
Ischemic heart disease	**0.022**	Affected	0.18	1.34	79
		Non-affected	–0.02	0.95	740
Organic heart diseases	0.202	Affected	–0.32	0.99	38
		Non-affected	0.02	0.99	781
Rheumatologic diseases	0.841	Affected	0.00	0.71	45
		Non-affected	0.00	1.00	774
Diabetes	0.509	Affected	0.29	1.62	6
		Non-affected	0.00	0.99	816
Renal diseases	0.667	Affected	0.10	0.77	21
		Non-affected	–0.00	1.00	798
Hepatitis	0.134	Affected	–0.18	0.91	67
		Non-affected	0.02	1.01	752
Hypertension	0.475	Affected	–0.08	1.22	79
		Non-affected	0.01	0.97	740
Pulmonary diseases	0.455	Affected	–0.02	1.31	61
		Non-affected	0.00	0.97	758
Gastrointestinal diseases	**0.043**	Affected	–0.16	0.85	107
		Non-affected	0.02	1.01	712

N – number of individuals in each category; Significance of variance (p-values) indicated between the means of hand OA scores in affected vs. non-affected individuals. Significant p (<0.05) appear in bold.

DISCUSSION

In every population and at any age, it is possible to find individuals with higher or lower than average hand OA. The main hypothesis underlying

this study was that the extent of age-associated skeletal changes (such as radiographic hand OA) might be, inter alia, under the influence of some adverse functional conditions and morbidities. Different measures of skeletal aging and among them OA, are strongly correlated with the status of the vital health systems and ultimately with survival rates. Indeed, in previous studies, the individuals with OA had a significantly higher risk of comorbid conditions such as the cardiovascular disease [7], hypertension, chronic pulmonary diseases [20], peptic ulcer and renal diseases [5], gastritis and phlebitis [10]. In the present study, we found that radiographic hand OA is positively associated with ischemic heart morbidity. To support this finding, Philbin et al [22] found that the individuals with OA had an adverse profile of metabolic risk factors for the coronary heart disease. Compared with controls, OA patients had a higher mean body mass index, systolic blood pressure and fasting blood glucose, and lower mean high-density-lipoprotein (HDL) cholesterol. Singh et al. [24] also reported that the patients with OA often have risk factors for the cardiovascular disease, including the respiratory disease, hypertension and a low HDL cholesterol level.

In a systematic review, Hochberg [9] found moderate evidence of increased mortality among the persons with OA compared with the general population. The association between the lifespan and OA development can also be seen in animals [25]. The animals with long life expectancy (monkeys) postpone OA development, while the animals with short life expectancy, (mice) show early OA. Primary OA development occurs after a reproductive active life expectancy, indicating that primary OA is not directly time-related. Evolutionary controlled age-related processes are involved in the development of bone and cartilage degeneration and, therefore in primary OA. OA apparently is not a simple wear and tear process but a process of biological ageing [25].

The rate of degenerative changes in the skeleton, such as hand OA, may reflect an individual's biological resistance, immunity, the functional or health status in reference to his or her chronological age. We also believe that chronic morbidities and different body compositions may influence skeletal aging through changes in the metabolism of bone and joints. Therefore, age-related skeletal changes can serve as an index of biological ageing. Unlike the non-skeletal

markers, proposed as biomarkers of aging (such as physiological and blood chemistry measurements), bone characteristics are relatively stable and not prone to circadian or seasonal rhythms [12, 13].

The present study suggests that longevity populations showed lower hand OA prevalence and NAJ compared to a non-longevity population, that can be interpreted as that longevity populations age slower. We suggest that the same phenomenon occurs on an individual level. Franceschi et al., in their comprehesive review on ageing and longevity [4], demonstrated that centenarians (and long-lived individuals, in general) were not the best example for the age cohort, but rather the individuals who better biologically and psycho-socially adapted to the environment. Unfortunately, the cross-sectional design of our study did not allow us to evaluate this hypothesis. Additional follow-up studies are needed to verify this hypothesis.

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ANNUAL REPORT OF THE ESTONIAN NATURALISTS' SOCIETY (2010)

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Hereby we give the short version of the annual report of the Estonian Naturalists' Society for the year 2010.

Our Society was founded in 1853 and it was associated with the Estonian Academy of Sciences on 23 January 1998.

The Society lists 749 active members, 14 honorary members and 635 trustees, thus being one of the biggest societies in Estonia.

The Society consists of the following subunits:

1. Assembly of Ecology
2. Estonian Teriological Society
3. Estonian Malacological Society
4. Jakob von Uexküll Centre.
5. Section of Botany
6. Section of Entomology
7. Section of Forestry
8. Section of Geology
9. Section of Anthropology
10. Estonian Mycological Society
11. Section of Theoretical Biology
12. Lake Commission
13. Section of Amateur Meteorologists
14. Commission of Natural Education
15. Commission of Terms in Ecology
16. Round Table of Nature Conservation
17. Commission of Botanical Rarities
18. Library Commission

19. Commission of Plant Names
20. Assembly of Honorary Members
21. Commission of the Observational Networks
22. Commission of the History of Natural Sciences

GENERAL ASSEMBLIES

In 2010 nine General Assemblies with scientific presentations and one special meeting were held:

- January 28 – Helle Mäemets: "About estimating the quality of lakes".
- February 25 – "The Baer day".

Presentations

1) Erkki Tammiksaar: "K.E. V Baer and Russian espionage – myth and reality".
2) Maarja Öpik: "The diversity of fungi forming arbuscular mycorrhiza".
   - March 25 – Riinu Rannap: "Threatened amphibians in Estonia and their protection management", the review meeting of 2009.
   - April 15 – a special meeting, the Board of ENS answers the members' questions.
   - April 29 – Silver Rattasepp: "About culture using evolutionary terms".
   - May 27 – Ivo Leito: "About acids and bases: from different viewpoints".

Kai Reemann: "About the library and the electronic database of the library ENS".

- September 30 – Peeter Olesk: "Strategy of writing textbooks and professor Julius Tehver".
- October 28 – Ain-Elmar Kaasik: "Multidimensional medicine".
- November 25 – 100 anniversary of Neeme Mikelsaar in the Centre of Limnology.

Presentations

1) Tarmo Timm: "Life with Neeme Mikelsaar".
2) Ain Järvalt, Ervin Pihu, Teet Krause: "The surveys of fishery of inland waters yesterday and today".

3) Ain Järvalt: “The overview of the activities of the Centre of Limnology”.
   - December 16 – Olav Renno: “Revival of the nature conservation in Estonia after the World War 2 (1944–1966)”.

SECTIONS’ MEETINGS

Section of Botany

February 3 – Inga Hiiesalu: “The hidden world of plants – belowground diversity”.

December 8 – Malle Leht: “About XXIII Conference-Expedition of the Baltic botanists in Haapsalu”.

Estonian Teriological Society

May 21 – Lauri Klein: “Roads and crossing points for the animals”.

Section of Forestry

February 25 – Tiit Maaten: “Review of plantations of experiments created by the instructions of professor E. Pihelgas”.

April 22 – Marek Metslaid and Kajar Köster: “Studies about storm damages and forest fires”.

June 15 – Heino Kasesalu: “Review of Valdek Ritslaid’s life and activities”.

November 3 – Meelis Seedre: “Dynamics of carbon in boreal mixed forest”.

December 15 – Mats Varik: “About the growth dynamics in *Alnus* and *Betula* stands”.

Section of Geology

January 21 – Vincent Perrier: “Geology of France”.

March 4 – Igor Tuuling: “On the hiking trails of Patagonia”.

April 20 – Randal Keynes: “Recollections on Charles Darwin’s inquisitive mind”.

April 22 – Ann Kraut: “The presentation with photos of some stones: how I visited Tenerife and what I saw”.

May 20 – Martin Liira: “А вы что, геологи? – possibilities of ski hiking in Russia”.

November 18 – Oive Tinn, Leho Ainsaar and Vincent Perrier: "Geological expedition to Canada, the island of Anticosti".
December 9 – Vincent Perrier: "Dinosaurs and other vertebrate bearing deposits from France".

IMPORTANT EVENTS AND CONFERENCES

1. The 11th Estonian conference on ecology "Diversity and ecosystems", Tartu, 8–9 April. (In the framework of the UN International year of biodiversity). http://www.lote.ut.ee/geo/okoloogiakonverents. (together with the University of Tartu Centre of Excellence FIBIR, Institute of Ecology and Geosciences, Natural History Museum and the Estonian University of Life Sciences)
2. Science day dedicated to Karin Mark "Gender and gender roles". April 9, Tallinn (together with the Tallinn University Institute of History and the NGO Centre of Archeology).
3. Mushroom practice, the island of Vormsi, May 13–16.
4. The Spring School of Theoretical Biology "Theory of movement". May 21–23, on the Kopra farm, Viljandi County (together with the University of Tartu Institute of Ecology and Geosciences, and the Natural History Museum).
5. Gathering of the friends of mosses, May 22–23, on Kesselaid.
6. Tha Naturalist's Day XXXIII, June 19, in Tartus and in landscape reserve of Vapramäe-Vellavere-Vitipalu.
7. The Summer School of Ecosemiotics with the Institute of Philosophy and Semiotics of the University of Tartu. July 10–11, Nüpli.
8. The 23rd Conference-Expedition of the Baltic Botanists "Semi-natural habitats". July19–22, in Haapsalu.
9. Gathering of amateur meteorologists and thunder observers. July 24, in Tartu.
10. Mushroom practice, on Vormsi, September 16–22.
11. The Autumn School of Teriology, in Ähijärve, September 17–19.
12. The Autumn School of Geology VI "Global changes". October 4–10, in Roosta, Läänemaa.
13. The Conference commemorating Juhan Aul (1897–1994). October 14.

14. The Conference commemorating Neeme-Õnneleid Mikelsaar (1910–1980, November 25, the Centre of Limnology.
15. The Meeting of Mycological Society "Actiones". December 11, Tallinn, the Estonian Museum of Natural History.

PROJECTS

The Society participated in fulfilling the projects funded by the Environmental Investment Centre, Tallinn Botanic Garden and the Ministry of Environment.

LIBRARY AND PUBLICATIONS

In December 2010 there were 161,891 printed items in ENS library. Within a year the library reviewed 154 new books and items of 187 periodicals. The publications were exchanged in the reporting year with 54 institutions and organisations from 18 countries.

Publications

1. Öpik, M. and Puura, I. (eds.) 2010. Scola Biotheoretica 35 "Theory of Origin". 128 pp. (together with University of Tartu Institute of Ecology and Geosciences and the Natural History Museum).
2. Preeden, U. and Laumets, L. (eds.) 2010. Schola Geologica 6 "Global changes" 152 pp. (together with University of Tartu Institute of Ecology and Geosciences, Tallinn Technical University Institute of Geology and the Department of Mining).
3. Folia Cryptogamica Estonica 47. 107 pp. (with the University of Tartu)
4. The 23rd Conference-Expedition of the Baltic Botanists. Abstracts and Excursion Guides. Haapsalu, Estonia, July 19–22, 2010. 92 pp. Tartu Ülikool, Eesti LUS, EMÜ, PKÜ, EL

Internet journal

Ingerpuu, N., Vellak, K. (eds.) 2010. Friend of mosses 13. 24 pp. (http://www.botany.ut.ee/bruoloogia/Samblasober13.pdf)

OTHER ACTIVITIES

- ENS and The Estonian Environment Information Centre are developing the Nature Observation Database. The overview of 2010: http://eelis.ic.envir.ee/lva/LVA.aspx?type=Artikkel&content=607836056
- The commission of plant names continued to complement the database of plant names. During 2010 altogether 226 records were entered.
- ENS with the Estonian Fund for Nature and Estonian Green Movement made an accusation against Finnish governmental institutions in the case of the Nord Stream pipeline.
- Seven seminars from the series "From natural scientists to teachers of natural sciences" were held.

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FINGER AND PALMAR DERMATOGLYPHICS IN MUZEINA BEDOUINS FROM SOUTH SINAI: A QUALITATIVE TRAITS

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ABSTRACT

Qualitative finger and palmar dermatoglyphics traits of 218 individuals (170 males and 48 females), belonging to the Muzeina Bedouins- the nomadic tribe, a small isolate with a high degree of consanguinity from the South Sinai Peninsula were studied. The highest frequencies of pattern whorl (W) on the 4th finger followed by an ulnar loop (UL) on the 3rd finger for both hands in both sexes were observed. Similarly, the highest occurrence of pattern combinations W-W (67.7%) was followed by the UL-UL (65.2%) in both sexes. Finger and palmar patterns show homogeneity in nature except the 3rd finger and the 4th palmar area, which have a significant sex difference. The present results are not exactly similar with our previous studies on other populations – Chuvashian (Karmakar et al 2007), Indians (Karmakar et al 2002), Turkmenians (Karmakar et al 2010), perhaps due to a major ethnic difference and a high inbreeding level.

Key words: *Dermatoglyphics, pattern types, Bedouins*.

INTRODUCTION

The digital pattern types (polygenic nature) are frequently used to characterize human populations. In anthropological research (Cummins & Midlo 1961, Igbigbi & Msamati 1999, 2005, Nagy & Pap 2005, Gasiorowski 2005), because of the prenatal origin of the dermatoglyphic patterns (Babler 1978) that remain unchanged during postnatal life. Qualitative palmar dermatoglyphic traits are considered to be largely under genetic control (Pons 1954, Glanville 1965, Karev 1991). The inter-population variability of palmar dermatoglyphics has also been ascertained (Pons 1952, Plato et al 1975, Plato & Wertelecki 1972, Malhotra 1979, Vrydagh-Laoureux 1979, Fox et al 1987, Francis 1991, Gualdi-Russo et al 1994). Recently, qualitative traits on palmar dermatoglyphics alone have been utilized to perform the cluster analysis (Kamali et al 1992), the correspondence analysis (Martin 1991, Arreta et al 1992), or the correlation analysis to establish inter-population relationships (Sanna & Floris 1995, Sanna et al 1998). Another well-known important aspect is that dermatoglyphic sexual dimorphism differs in diverse populations. Cummins & Midlo (1961) pointed out that "the usual sexual distinction may be leveled or even inverted in some populations". Females almost universally differ from males as revealed from several studies on dermatoglyphic characters in various racial samples. Compared to males, females exhibit narrower ridges, lower frequencies of whorls and radial loops, and higher frequencies of arches and ulnar loops on the fingertips (Cummins & Midlo 1961, Schauman & Alter 1976). Regarding palmar configurational areas, generally females have patterns more frequently on the hypothenar and the inter-digital areas compared to males (Cummins & Midlo 1961).

The qualitative dermatoglyphic traits study is very important in Muzeina Bedouins, a highly inbreeding group. The main objective of the present study is therefore to provide information of qualitative finger and palmar patterns in Muzeina Bedouins and to compare the present result with our previous studies on Indian populations (Karmakar et al 2002a, b), the Chuvashian population of Russia (Karmakar et al 2007, 2008) and Turkmenian populations (Karmakar and Kobyliansky 2010).

MATERIALS AND METHODS

The sample and analyses of prints

The Muzeina tribe inhabited for centuries in the Sinai desert, which was especially occupied by the Bedouins and they originated mainly from the Saudi Arabian Peninsula (Kobyliansky and Hershkovitz 1997). The Muzeina tribe is characterized by strong biological isolation, they rarely intermix and show preference for the first-cousin marriages and the inbreeding coefficient is 0.09. The sample contains the data of 218 individuals (170 men and 48 women).

Finger and palmar prints were collected using the ink and roller method of Cummins & Midlo (1961). Similarly, the dermatoglyphic qualitative characteristics were analyzed according to the criteria and methods of Cummins & Midlo (1961). Dermatoglyphic traits include four types of finger patterns (UL, RL, A, W) and the palmar pattern was present and absent in 5 palmar areas namely: Hypothenar, Thenar/I, II, III, IV interdigital areas. The analyzed variables are digital pattern types which were classified into three major categories named 'Whorls', 'Loops' and 'Arches' according to Galton (1892). All the types of true whorls like concentric, single spiral, double spiral, accidental, etc. and also all the types of composite whorls like twin loops, central pocket loops, lateral pocket loops, crested and knot-crested loops are grouped under the broad category of 'whorls'. On the other hand radial and ulnar loops are categorized into 'Loops'; both simple and tented arches are grouped into the category 'Arches'. Thus, these three groups of finger patterns according to Galton (1892) are represented in an overall picture of the pattern distribution in fingers. However, in the present report, loops were classified into ulnar loops, radial loops separately, based on Cummins and Midlo (1961), and thus four digital patterns were considered in the present analysis. The symmetry of pattern types on homologous fingers and the diversity of finger pattern types present on the ten fingers were analyzed. Ten possible combinations between four types of finger patterns were considered in this analysis.

RESULTS

Finger patterns

The *frequencies of digital pattern types are presented in Table 1*. The most frequently observed pattern type is the whorl (W) 49.4% in males and 50.3% in females for both hands followed by an ulner loop (UL), 46.3% in males and 45.8% in females whereas the pattern arch (A) and radial loop (RL) are less frequent than the UL and W. Arches are 1.7% in males and 1.6% in females, while radial loops 2.9% in males and 2.4% in females. Thus the order of pattern types is W>UL> RL>A, both in males and females within this population.

Table 1. Frequency in the percentage (%) of finger pattern types, by sex and hand

Pat-tern	Left fingers					Left	Right fingers					Right	Both
Type	I	II	III	IV	V	hand	I	II	III	IV	V	hand	hands
						Males							
A	0.0	8.1	1.4	0.9	0.0	2.1	0.0	5.3	0.5	1.0	0.0	1.3	1.7
RL	0.0	9.0	0.9	1.9	0.0	2.4	0.0	12.5	0.9	2.4	0.9	3.3	2.9
UL	42.2	48.1	65.4	28.9	49.5	46.8	45.0	43.3	70.6	24.9	45.0	45.8	46.3
W	57.8	34.8	32.2	68.2	50.5	48.7	55.0	38.9	28.0	71.8	54.0	49.5	49.1
						Femles							
A	0.0	6.1	0.0	2.5	0.0	1.7	0.0	6.1	1.2	0.0	0.0	1.5	1.6
RL	0.0	11.0	3.7	2.5	1.3	3.7	1.2	1.2	0.0	4.9	1.3	1.7	2.4
UL	36.6	47.6	64.2	17.5	42.3	41.7	48.8	53.7	79.3	19.5	37.2	47.8	45.8
W	63.4	35.4	32.1	77.5	56.4	52.9	50.0	39.0	19.5	75.6	61.5	49.0	50.3

In males, for the left hand, the highest occurrence of W (48.7%) followed by UL (46.8%) in males and W (52.9%) followed by UL (41.7%) in females. Similarly, for the right hand the highest frequency of W (49.5%) and UL (45.8%) are found in males; while in females W (49.0%) and UL (47.8%) respectively. In the case of A and RL for the left and the right hand, RL is greater than A in both sexes. Separately for the left and the right hand the order of pattern types is also W>UL> RL>A, for both in males and females are clear.

The order of pattern frequency for the individual fingers. The order of pattern types decreases from finger to finger in the following order for W: IV (68.2%)>I (57.8%)> V (50.5%)> II (34.8%)> III (32.2%) in

males; and IV (77.5%)>I (63.4%)> V (56.4%)> II (35.4%)> III (32.1%) in females for the left hand. Similarly for the right hand in males W is IV (71.8%)>I (55.0%)> V (50.0%)> II (38.9%)> III (28.0%); and in females IV (75.0%)> V (61.5%)> I (50.0%)> II (39.0%)> III (19.5%) respectively. Pattern UL in males for the left hand the preponderance order on different fingers is: III (65.4%)>V (49.5%)>II (48.1%)>IV (28.9%)>I (42.2%); for the right hand is III (70.6%)>V (45.0%)> I (45.0%)> II (43.3%)>IV (28.9%). For females in the left hand is III (64.2%)> II (47.6%)>V (42.3%)> I (36.6%)> IV (17.5%); for the right hand is III (79.3%)> II (53.7%)> I (48.8%)> V (37.2%)> IV (19.5%) respectively.

Thus the order of pattern frequency of W decreases from finger to finger for the left and the right hands in both males and females in the following order IV > I > V > II > III; for UL is III>V>II>I>IV with a slight difference in both hands and sexes.

Compared to W and UL, A, and RL are less frequent in both sexes and for the left and the right hands; but the trend of the maximum frequency of these two patterns is found in II, III, III, and IV interdigital areas both in males and females.

Pattern combinations on the digital pairs presented in Table 2. The frequency of symmetrical patterns regarding finger pairs is similar between the right and the left hands in both sexes. Among 10 possible combinations the highest occurrence of W-W (67.7%) was followed by the UL-UL (65.2%) combination for the five categories of finger combinations. Among the five pairs of fingers, the maximum frequency of W-W, 67.7% for the IV-IV pair, was followed by 52.7% for the I-I pair, 46.3% for the V-V pair in males. The decreasing order of pattern frequency among the five categories of finger combinations for W-W is IV-IV>I-I > V-V>II-II> III-III, in males. Similarly for females with a very slight difference is IV-IV (77.0%)> V-V (55.4%)> I-I (55.2%)> II-II (29.5%) > III-III (14.5%) respectively.

The maximum frequency of UL-UL, 65.2% for the III-III pair, was followed by 42.6% for the V-V pair in males among the five pairs of fingers. However, for females the frequencies are slightly different in UL-UL combination, III-III (72.6%)> I-I (38.8%) > II-II (37.7%) > V-V (30.8%) > IV-IV (16.2%).

Table 2. Pattern combinations (in %) between the pairs of homologous fingers

Pairs of				Pattern combination						
fingers	A-A	LR-LR	UL-UL	W-W	A-LR	A-LU	A-W	LR-LU	LR-W	LU-W
					Males					
I-I	0.0	0.0	39.4	52.7	0.0	0.0	0.0	0.0	0.0	8.0
II-II	2.3	5.3	35.7	32.2	0.0	4.1	1.2	5.8	0.6	12.9
III-III	0.6	0.0	65.2	21.9	0.0	0.0	0.0	1.1	0.0	11.2
IV-IV	0.5	0.5	19.8	67.7	0.0	0.5	0.0	1.0	0.5	9.4
V-V	0.0	0.0	42.6	46.3	0.0	0.0	0.0	0.0	0.0	11.1
Total	0.7	1.1	40.3	44.7	0.0	0.9	0.2	1.5	0.2	10.4
					Females					
I-I	0.0	0.0	38.8	55.2	0.0	0.0	0.0	0.0	0.0	6.0
II-II	1.6	0.0	37.7	29.5	1.6	4.9	0.0	1.6	3.3	19.7
III-III	0.0	0.0	72.6	14.5	0.0	1.6	0.0	0.0	1.6	9.7
IV-IV	0.0	1.4	16.2	77.0	0.0	0.0	1.4	1.4	1.4	1.4
V-V	0.0	0.0	30.8	55.4	0.0	0.0	0.0	0.0	0.0	13.8
Total	0.3	0.3	38.3	47.7	0.3	1.2	0.3	0.6	1.2	9.7

Table 3 presents the frequency of individuals with monomorphic hands, i.e. bearing the same pattern on all the ten fingers. The highest frequency was found (male 54.6%, female 65.8%) for the combination UL+W out of 15 combinations. The pattern W (9.3%), UL (4.6%) and UL+ RL (3.1%) are found in males, whereas in females they were 4.1%, 1.4% and 1.4% respectively. Pattern W shows a higher frequency (male – 9.3%, female 4.1%) compared with UL (male – 4.6%, female 1.4%).

Table 3. Frequency of pattern type combinations on the ten fingers in males and females

Pattern	Males		Females	
	N.	%	N.	%
A only	–	–	–	–
RL only	–	–	–	–
UL only	9	4.6	1	1.4
W only	18	9.3	3	4.1
A + RL	–	–	–	–
A + UL	7	3.6	1	1.4
A + W	–	–	–	–
RL + UL	6	3.1	1	1.4
RL + W	–	–	–	–
UL + W	106	54.6	48	65.8
A + RL + UL	1	0.5	–	–
A + RL + W	–	–	–	–
A + UL + W	11	5.7	6	8.2
RL+UL+ W	29	14.9	12	16.4
A + RL + UL + W	7	3.6	1	1.4
Total	194	100.0	73	100.0

Palmar patterns

The occurrence of patterns represented in the terms of the pattern present and absent in five palmar configurational areas is shown in Table 4. [Please, put **Table 4** here] A general trend of a rich frequency at the same pattern was present in males on both palms IV (30.3%) > Th/I (15.3%) > III (8.0%) >II (2.6%) > Hyp (1.5%), whereas in females it was present on IV (46.2%) > Th/I (16.8%) > III (6.5%) > II (3.2%) > Hyp (0.0%) respectively. The poorer patterns are on the Hypothenar and the second interdigital areas in both sexes. The frequencies of the same pattern are higher in most of the areas compared with the different types of the pattern. The bilateral symmetry of the presence/absence of the pattern is more pronounced both in males and females, in the Hypothenar (male 94.5%, female 96.8%) and the II interdigital areas (male 95.6%, female 94.7%). The presence of the palmar pattern only on the left or the right hands varies in different palmar areas in both sexes.

Table 4. Percent distribution of palmar patterns in males (M) and females (F)

On both palms:					Interdigital					
	Hypothenar		Thenar		II		III		IV	
	M	F	M	F	M	F	M	F	M	F
Absent	94.2	96.8	62.0	54.7	93.4	91.5	63.5	66.7	41.6	26.9
Present	1.5	0.0	15.3	16.8	2.6	3.2	8.0	6.5	30.3	46.2
Same pattern	0.4	0.0	10.6	13.7	2.2	3.2	8.0	5.4	28.1	40.9
Different pattern	1.1	0.0	4.7	3.2	0.4	0.0	0.0	1.1	2.2	5.4
Bilateral symmetry	94.5	96.8	72.6	68.4	95.6	94.7	71.5	72.0	69.7	67.7
Pattern only on:										
Left palm	4.0	3.2	9.5	9.5	1.5	2.1	4.0	2.2	21.9	20.4
Right palm	0.4	0.0	13.1	18.9	2.6	3.2	24.5	24.7	6.2	6.5

Sex comparisons

Finger pattern frequencies between sexes show little variations, a significant sex difference is found only for the 3rd finger (0.023) out of all digits and thus shows homogeneity in nature (Table 5). Out of five digital pairs of pattern combinations, only II-II pair is significantly (0.041) different between sexes. Finger patterns and palmar patterns exhibit almost similar variation, out of five palmar areas, only IV (0.025) area is significantly different. The remaining Hypothenar and Thenar/I areas are homogeneously distributed in both sexes.

Table 5. Sex comparisons by χ^2 test of finger and palmar patterns

Variables	d.f.	χ^2	p
Finger pattern			
L I	1	0.76	0.383
II	3	0.36	0.948
III	3	2.94	0.401
IV	3	3.97	0.265
V	2	3.74	0.154
All	3	0.95	0.801
R I	2	2.95	0.229
II	3	9.51	**0.023***
III	3	3.42	0.331
IV	3	3.80	0.284
V	2	0.57	0.752
All	3	0.34	0.952
10 Fingers	3	0.13	0.968
Pattern combination			
I-I	2	1.3	0.522
II-II	9	17.5	**0.041***
III-III	6	10.3	0.113
IV-IV	8	10.1	0.258
V-V	2	3.4	0.830
Pattern comb. 10 fingers	8	9.20	0.326
Palmar patterns			
Hyp	3	3.32	0.348
Th-I	3	1.28	0.729
II	3	1.71	0.635
III	3	0.69	0.876
IV	3	9.31	**0.025***

* Marked differences are significant when $p < 0.05$.

DISCUSSION

From the above presentation, it appears that there is a homogeneous distribution of pattern types regarding the fingers and the palmar configurational areas between sexes and between the right and the left sides with a very little variation. Therefore, a trend of similarity is observed in these areas. The Bedouin population is characterized by

having high frequencies of whorls, compared to loops and low frequencies of arches and radial loops those are almost uniformly distributed on all the fingers and in both sexes (W>UL>RL>A). The decreasing order from finger to finger is for W: IV>I>V>II>III; for UL: III>V>II>I>IV; and for A: II>III>IV respectively. These findings are not corroborated by earlier studies in different non-tribal populations: Micle and Kobyliansky (1987) in Yemenite Jews; Micle & Kobyliansky (1988) among North African Jews; Arrieta et al. (1991) among Basque from Salazar valley; Crawford and Duggirala (1992) among Eskimo and Amerindians; Dittmar (1994) among Chilean Aymara Indians; Sivakova et al. (1995) among North Slovakia Isolates; Karmakar et al. (2002), and Sengupta and Karmakar (2003) among Indian populations; Karmakar et al. (2007) among the Chuvashian population of Russia; Karmakar et al. (2010) among Turkmenian populations. The above studies observed the decreasing order of digital pattern frequency is UL> W>A >RL and decreases from finger to finger mostly in the following order V>III>I>IV>II. Holt (1968) stated that certain patterns tend to occur more frequently on some digits than on others, which seem to be constant for any population. Roberts (1982) concluded that qualitative dermatoglyphic traits are a complex outcome of the developmental process in which individual digits of the same genetic fields occur but at different locations. The dermatoglyphics of the Muzeina Bedouin tribe, biologically a small isolated consanguineous population, is perhaps expected to have some differences in the digital pattern expression than in other non-inbreeding/non-tribal populations. Our results are similar with the earlier well-known hypothesis (Cummins and Midlo 1961) that the highest frequency of W is a common characteristic feature of tribal populations, which indicates a simpler genetic basis due to the consanguinity/isolation of the Muzeina Bedouin tribe. Similarly, for pattern combinations, the highest occurrence of W-W (67.7%) was followed by the UL-UL (65.2%) combination for five categories of finger combinations.

The present finding (W>UL>RL>A) is in full conformity to the general trends observed earlier on tribal populations (Cummins and Midlo 1961, Malhotra 1974, Kumar and Ramchandraiah 1991, Deka et al 1991, Sengupta and Karmakar 2004). The dermatoglyphic features are in close conformity to the other nomadic/tribal populations perhaps due to isolated tribes are small in numerical strength and thus the scope

for the founder effect and the random genetic drift are also greater among them. Malhotra et al. (1980) explained that such frequency of patterns among the Nandiwallas are due to the genetic drift and the similar evolutionary forces acting in the same direction among the nomads were responsible for the observed similarity of dermatoglyphic features among them.

A general trend of a rich frequency of palmar pattern present in IV interdigital area on both palms in both sexes is similar with the earlier studies in different populations (Karmakar et al 2002, 2007, 2010). The finger and palmar patterns show homogeneity in nature except the 3rd finger and the 4th palmar area, which have a significant sex difference. The present results are not exactly similar with our previous studies on other populations – Chuvashian (Karmakar et al 2007), Indians (Karmakar et al 2002), Turkmenians (Karmakar et al 2010), perhaps due to a major ethnic difference and the inbreeding level in the Muzeina tribe.

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VARIATIONS IN SOME ANTHROPOMETRICAL PARAMETERS OF THE WOMEN WITH THE DIFFERENT IRIS COLOR IN LATVIA

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ABSTRACT

The aim of this study was to confirm the variations between the women with the different iris color in their anthropometrical parameters. The sample of subjects consisted of 873 women from Latvia. Twelve anthropometrical measurements were taken, all of which covered the area of the longitudinal, transversal, circular dimensionality and the subcutaneous fatty tissue. The differences between the anthropometrical parameters were determined by means of the t-test for independent samples.

In 51.8% the iris color was blue as the most prevalent color, and in 7.1% the color was brown as the least prevalent color. In many of the parameters were found intra-group differences, which signaled that the group was heterogeneous in terms of the evaluated characteristics. The results showed that there were some statistically significant differences of the anthropometrical parameters between the women with the different iris color. Further studies are recommended to investigate the probable associations.

Key words: *anthropometrics, women, iris color, variations, comparison*

INTRODUCTION

Population variations in the body size represent one of the most important parameters in the study of the ongoing evolution of man [1, 2]. There is considerable evidence indicating that the human variation in the body size is the result of the interaction of environmental and genetic factors at both the developmental and adult stages [16].

The eye color or, more accurately, the iris color is one of the most obvious physical characteristics of a person. Pigmentation, including the eye color, is one of the major racial and diagnostic features in anthropological studies and it is used to characterize differences between populations. The studies carried out by certain authors have shown that there may be some associations between the eye color and the constitutional type [3, 5]. The study of the normal pigmentation variation in humans is more recent and has been investigated by anthropologists, medical scientists and by researchers for the prediction of visible phenotypes to be used as an investigative tool [7].

The aim of the study was to confirm some variations between the women with the different iris color in their anthropometrical parameters.

MATERIAL AND METHODS

A total of 873 women took part in the data collection between 2001 and 2005 in Latvia. All of them were healthy at the time of investigation.

The body height was used to evaluate longitudinal dimensionality. For all the women the body weight was measured. The following parameters were used to evaluate the transversal dimensionality: biacromial width, the waist width and the bicristal width. The following parameters were used to evaluate the subcutaneous fatty tissue: biceps and triceps skinfolds, subscapular and suprailiac skinfolds. The chest, waist and hip circumferences were used to evaluate circular dimensionality.

All the anthropometrical measurements were carried out according to the methodological recommendations by R. Martin and K. Saller [13]. The anthropometrical measurements were measured by the author of this study together with the medical nurses of the anthropology unit of the Institute of Anatomy and Anthropology (IAA). The Swiss company`s "Siber-Hegner and Co" anthropometric set, the skinfold caliper, the steel measuring tape and the same electronic weight scales were used during the investigation.

The visual assessment of the eye color was carried out using the traditional scale of Martin, based on 16 ocular prostheses. The iris color was determined according to the Martin/Schulz`s table of the iris-color. The four-category grading system (groups of the iris color) was used to determine the variations in some anthropometrical parameters: blue (group I), grey (group II), green (group III) and brown (group IV).

The data were entered into the SPSS Statistics program 17.0. The basic descriptive parameters were calculated for all the results: the arithmetic means, the standard deviation, the minimum value (min), the maximum value (max) and the range. The differences between the anthropometrical measurements of the women were examined using the t-test for independent samples. The level of significance was defined as $p<0.05$; $p<0.01$ and $p<0.001$.

RESULTS

The sample of the subjects consisted of 873 women. The mean age of the participants was 28.78±13.76 years. In 51.8% the iris color was blue as the most prevalent color, and in 7.1% the color was brown as the least prevalent color. The prevalence of grey and green iris color was 16.2% and 25.0%, respectively. The descriptive parameters for anthropometric characteristics of women with the different iris color were presented in Table 1 and Table 2.

The differences between the mean values for the anthropometrical parameters were shown in Table 3. The results for the mean body height for the women showed that the difference between the shortest (164.34±7.34 cm for the brown iris color or group IV) and the tallest body height (165.53±6.36 cm for the grey iris color or group II) was 1.19 cm. The differences between the minimum and the maximum values for the anthropometric measures were in accordance with the displayed differences in the body height. The greatest difference between the lowest (62.63±11.78 kg for the green iris color or group III) and the highest (65.81±14.75 kg for the brown iris color or group IV) the mean value for the body weight was 3.18 kg. The results showed that no statistically significant differences were noted for the mean values of both mentioned anthropometrical parameters in the compared iris color groups.

Table 1. The descriptive parameters for anthropometric characteristics of the women with the blue and grey iris color

I group – blue iris color (n=452)	Range	M	SD	min	max
Body height, cm	32.80	165.31	6.59	149.50	182.30
Body weight, kg	66.30	62.93	11.80	40.30	106.60
Biacromial width, cm	11.40	35.49	1.84	28.50	39.90
Waist width, cm	17.90	26.08	2.41	20.50	38.40
Bicristal width, cm	18.90	29.27	2.70	22.00	40.90
Chest circumference, cm	65.80	85.30	7.87	57.00	122.80
Waist circumference, cm	66.00	70.59	10.47	52.50	118.50
Hip circumference, cm	61.10	95.58	9.39	77.00	138.10
Biceps skinfold, mm	20.00	7.24	2.76	2.00	22.00
Triceps skinfold, mm	27.40	13.09	3.90	3.40	30.80
Subscapular skinfold, mm	29.60	14.09	5.87	4.80	34.40
Suprailiac skinfold, mm	31.20	14.70	5.71	3.80	35.00
II group – grey iris color (n=141)	Range	M	SD	min	max
Body height, cm	34.50	165.53	6.36	147.50	182.00
Body weight, kg	65.30	64.93	12.83	45.40	110.70
Biacromial width, cm	11.80	35.86	2.00	28.50	40.30
Waist width, cm	15.90	26.99	2.83	22.10	38.00
Bicristal width, cm	17.40	30.45	3.18	25.50	42.90
Chest circumference, cm	42.40	87.61	8.51	73.10	115.50
Waist circumference, cm	51.00	73.38	11.39	56.00	107.00
Hip circumference, cm	80.00	97.63	10.54	57.00	137.00
Biceps skinfold, mm	18.70	7.43	3.06	2.60	21.30
Triceps skinfold, mm	25.30	13.30	4.64	5.00	30.30
Subscapular skinfold, mm	29.20	14.61	6.09	4.80	34.00
Suprailiac skinfold, mm	32.40	15.14	6.20	5.00	37.40

n – number of women; M – mean; SD – standard deviation; min – minimum; max – maximum

Table 2. The descriptive parameters for anthropometric characteristics of the women with the green and brown iris color

III group – green iris color (n=218)	Range	M	SD	min	max
Body height, cm	35.80	164.62	6.62	145.30	181.10
Body weight, kg	79.10	62.63	11.78	41.00	120.10
Biacromial width, cm	23.70	35.71	2.23	29.70	53.40
Waist width, cm	16.70	26.39	2.53	20.90	37.60
Bicristal width, cm	16.50	29.67	2.83	24.80	41.30
Chest circumference, cm	43.00	86.27	7.65	74.20	117.20
Waist circumference, cm	64.30	72.32	11.32	56.00	120.30
Hip circumference, cm	52.20	96.48	9.61	81.00	133.20
Biceps skinfold, mm	19.00	7.39	2.93	2.00	21.00
Triceps skinfold, mm	27.20	12.81	4.07	2.80	30.00
Subscapular skinfold, mm	30.20	14.27	5.50	4.80	35.00
Suprailiac skinfold, mm	32.00	14.63	5.66	4.00	36.00
IV group – brown iris color (n=62)	Range	M	SD	Min	max
Body height, cm	29.90	164.34	7.34	150.70	180.60
Body weight, kg	83.10	65.81	14.75	41.00	124.10
Biacromial width, cm	10.40	35.91	1.76	29.10	39.50
Waist width, cm	12.20	26.66	2.40	21.40	33.60
Bicristal width, cm	12.90	29.87	2.66	23.90	36.80
Chest circumference, cm	51.80	88.06	9.41	74.20	126.00
Waist circumference, cm	80.00	75.56	14.72	56.00	136.00
Hip circumference, cm	64.30	98.25	11.21	81.50	145.80
Biceps skinfold, mm	19.80	8.15	3.76	3.40	23.20
Triceps skinfold, mm	16.00	13.28	3.86	5.20	21.20
Subscapular skinfold, mm	27.20	15.31	5.88	6.80	34.00
Suprailiac skinfold, mm	27.60	16.37	6.98	7.00	34.60

n – number of women; M – mean; SD – standard deviation; min – minimum; max – maximum

Table 3. The t-test for the mean anthropometrical parameters of the women with the different iris color

Mean anthropometrical value	Blue I (n=452)	Grey II (n=141)	Green III (n=218)	Brown IV (n=62)	I/II		I/III		I/IV		II/III		II/IV		III/IV	
					t-value	p	t -value	p	t -value	p	t -value	p	t -value	p	t -value	p
Body height, cm	165.31	165.53	164.62	164.34	–0.34	0.737	1.28	0.202	1.08	0.280	1.29	0.199	1.17	0.243	0.29	0.773
Body weight, kg	62.93	64.93	62.63	65.81	–1.72	0.085	0.30	0.763	–1.75	0.081	1.74	0.082	–0.43	0.667	–1.77	0.078
Biacromial width, cm	35.49	35.86	35.71	35.91	–2.03	0.043	–1.36	0.173	–1.68	0.094	0.63	0.529	–0.16	0.872	–0.63	0.530
Waist width, cm	26.08	26.99	26.39	26.66	–3.78	0.000	–1.57	0.118	–1.80	0.073	2.10	0.037	0.81	0.422	–0.75	0.455
Bicristal width, cm	29.27	30.45	29.67	29.87	–4.37	0.000	–1.78	0.076	–1.66	0.097	2.45	0.015	1.26	0.210	–0.51	0.612
Chest circum-ference, cm	85.30	87.61	86.27	88.06	–2.99	0.003	–1.51	0.131	–2.53	0.012	1.55	0.122	–0.34	0.737	–1.54	0.124
Waist circum-ference, cm	70.59	73.38	72.32	75.56	–2.70	0.007	–1.95	0.052	–3.32	0.001	0.86	0.388	–1.15	0.254	–1.85	0.065
Hip circum-ference, cm	95.58	97.63	96.48	98.25	–2.19	0.029	–1.15	0.251	–2.05	0.041	1.06	0.288	–0.38	0.705	–1.23	0.220
Biceps skinfold, mm	7.24	7.43	7.39	8.15	–0.70	0.485	–0.65	0.514	–2.31	0.021	0.12	0.902	–1.43	0.154	–1.68	0.094
Triceps skinfold, mm	13.09	13.30	12.81	13.28	–0.54	0.590	0.84	0.399	–0.37	0.709	1.05	0.294	0.02	0.982	–0.82	0.415
Subscapular skinfold, mm	14.09	14.61	14.27	15.31	–0.92	0.360	–0.39	0.695	–1.54	0.124	0.54	0.587	–0.76	0.446	–1.29	0.197
Suprailiac skinfold, mm	14.70	15.14	14.63	16.37	–0.79	0.430	0.14	0.885	–2.10	0.037	0.81	0.421	–1.25	0.214	–2.02	0.044

n – number of women; p – statistically significance

The values for the mean parameters for the transversal dimensionality of the skeleton for the women indicated that there were small numerical differences between the lowest and the highest mean values for all the measured parameters. The maximum mean value for the biacromial width (35.91±1.76 cm) was noted for the women with the brown iris color or in group IV. In group II or for the women with grey iris color were the highest mean values for the waist width and the bicristal width (26.99±2.83 cm and 30.45±3.18 cm). Statistically significant differences were found between the mean values of the waist width and the bicristal width for the women with the blue and grey (group I and group II) iris color ($p<0.001$).

The studied variables for circular dimensionality for the women indicated a different range. The minimum mean values for the chest, the waist and the hip circumferences (85.30±7.87 cm; 70.59±10.47 cm and 95.58±9.39 cm) were noted for the women with the blue iris color or in group I. The maximum mean values of the mentioned parameters (88.06±9.41 cm; 75.56±14.72 cm and 98.25±11.21 cm) were indicated for the women with the brown iris color or in group IV. The differences were statistically significant between both compared groups ($p<0.05$ and $p<0.001$).

The differences between the minimum and the maximum values for skinfolds indicated great ranges for the measured points. Great intra-group differences were noted. The minimum mean values for biceps skinfold (7.24±2.76 mm) and the subscapular skinfold (14.09±5.87 mm) were found for the women with the blue iris color or in group I, but the maximum mean values of these parameters (8.15±3.76 mm and 15.31±5.88 mm) were noted for the women with the brown iris color or in group IV. Statistically significant differences between the mean values of the mentioned groups were described only for the biceps skinfold ($p<0.05$).

In group III or for the women with the green iris color the minimum mean values for triceps and suprailiac skinfolds (12.81±4.07 mm and 14.63±5.66 mm) were noted. The maximum mean value for the triceps skinfold (13.30±4.64 mm) was indicated for the women with the grey iris color or in group II, but the maximum mean value for the suprailiac skinfold (16.37±6.98 mm) was found for the women with the brown iris color or in group IV. The difference between the minimum and the

maximum values for the suprailiac skinfold was statistically significant ($p<0.05$).

DISCUSSION

Eyes need not necessarily be regarded only as the physiological devices of sight, as the organs that receive information from the outside of the organism. Eyes certainly represent the structures that offer information about the present and the future behaviour [11]. Compared to the eyes of our closest relatives, human eyes are somewhat unusual in both their color and shape. There is some evidence of a relationship between the iris color and a variety of other factors. The eye color is also suspected for its role as a possible medicinal prognostic factor.

The study of the human iris color as a physical trait is based on the developmental biology, morphology, chemistry and the genetic determinants of the structure known as the iris [8]. The iris is a small connective tissue and the muscular structure of around 12 mm in diameter with a central opening called the pupil. It controls the amount of light entering the eye which is focused by the lens onto the retina so as to provide the sense of vision. It contracts in bright light making the pupil smaller and dilates in dark conditions making the pupil larger, which together with the source of the incident light can influence the perception of an individual's eye color and the iris pattern. In the brown iris there is an abundance of melanocytes and melanin in the anterior border layer and the stroma whereas in the blue iris these layers contain very little melanin [9].

The iris color can provide different information about an individual. These visible characteristics, which are generally called the texture of the iris, are unique to each subject. In addition to the changes with pathological conditions, the color of the iris can be a particularly useful indicator. The measurement of the iris color and its changes can be of great importance.

The color of the iris is affected by genetic and racial factors, and suggested as an independent factor in ocular conditions [6, 15]. This report is one of the few reports in the world in which the distribution of the iris color is studied in a population; nonetheless, the use of different definitions and measurement techniques for the iris colors makes

comparisons with other reports difficult [14]. Variations may be due to genetic or environmental differences, or simply to chance. The presence of these differences dictates that the description of a single individual is not sufficient to describe an entire species' morphology, ecology, development or anything else. Instead, the description of many individuals taken together defines a range of the variation that encompasses the species. The variation in morphology between individuals is the most obvious kind of variation.

Determining the association between the iris color and different eye conditions can help us use the iris color as a predictive factor in some ocular variables and conditions [4]. The human iris has many other characteristic patterns that are not measured through an assessment of eye color and these will also be under strong genetic influence [10, 12], but remain to be fully investigated. For example, although the eye color is assumed to be fixed for adult life, there can be changes as individual ages or changes in disease states. The iris color can be affected by a variety of ocular disorders. It is suspected that the iris color may not remain constant throughout life.

On the basis of the results we can conclude that in Latvia the most prevalent color of the iris for women was blue. There were statistically significant differences between the women with the different iris color in some studied anthropometric parameters. In group II or for the women with the grey iris color statistically highest mean values were for waist width and bicristal width than the mean values of mentioned parameters for the women with the blue iris color or in group I. All the studied mean values for circular dimensionality were statistically significantly higher for the women with the brown iris color or in group IV than the mean values of circumferences for the women with the blue iris color or in group I.

It would be of interest to continue such a study to document the variations of some anthropometrical parameters of the women with the different iris color related to their geographical origin in Latvia.

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SEVENTY YEARS OF THE ANTHROPOLOGY SECTION OF THE ESTONIAN NATURALISTS' SOCIETY

(PART III)

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Part I of the current article, published in issue XVIII of *Papers on Anthropology*, gave a short characterization of the Estonian Naturalists' Society and viewed the emergence of its specialized subsidiary units. It described in detail, the foundation of the Anthropology Section in 1939 and the preceding period, 1853–1939, in the Society, assessed from the viewpoint of anthropology. Thereafter, the most essential facts were presented as excerpts from the annual reports of the Anthropology Section until the year 1994 (incl.) [12].

After the demise of Prof. J. Aul at the end of August of that year, the Section was headed by L. Heapost PhD who did it until April 2004.

The activities of the Anthropology Section in this period, presenting excerpts from the Section's annual reports, were dealt with in the second part of the article, which was published in the previous, XIXth issue of *Papers on Anthropology* [13].

To the meeting of the Anthropology Section on 21 April 2004, L. Heapost PhD, who had been Chair of the Section for nearly ten years, had submitted an application for resignation from the duties of the Chair of the Anthropology Section of the Estonian Naturalists' Society. After the approval of her application, the new chair was elected. Prof. H. Kaarma proposed that G. Veldre PhD, who had been secretary of the Section from 1996, should be elected as new Chair of the Anthropology Section. No other candidates were nominated. By open ballot, G. Veldre was unanimously elected Chair of the Anthropology Section. The meeting was attended by six members of the Section (L. Heapost, H. Kaarma, J. Kasmel, M. Lintsi, L. Saluste and G. Veldre) [1, 6].

The third part of the article will cover the events at the Anthropology Section under Gudrun Veldre's supervision until April 2009 when 70 years passed from the foundation of the Anthropology Section.

On 22 October 2004 the Anthropology Section held a research paper presentation meeting, Juhan Aul's Day, in the building of the Estonian Naturalists' Society. The meeting was dedicated to Juhan Aul's 107th birth anniversary and the 65th anniversary of the Anthropology Section.

The opening address was delivered by M. Sammul DBiol, President of the Estonian Naturalists' Society. Presentations were made by: J. Kasmel – *On the beginning of anthropological research of Estonians*; E. Jalakas (Estonian Academy of Security Sciences) – *Relations between body build characteristics and physical abilities in students of the Estonian Academy of Security Sciences*; G. Veldre – *On the prospects of somatology when the Anthropology Section has reached its 65th year*; M. Saava (Estonian Institute of Cardiology) – *On studies of the aged population in Tallinn*.

Prof. H. Kaarma presented the new issue of the collection of articles *Papers on Anthropology XIII,* which had come out by J. Aul's birth anniversary. The collection included articles by Anthropology Section members L. Heapost, H. Kaarma, J. Limbo, M. Lintsi, and G. Veldre.

At the end of the meeting, a discussion arose about further trends in the work of the Section and about publicizing anthropological research, including the necessity for Internet and popular-scientific publications. President of the Estonian Naturalists' Society proposed that publications of the Society should be more actively used for issuing anthropological research papers.

The meeting had 20 participants.

Participation in international events:

1. At the conference dedicated to the 425th anniversary of Vilnius University, *200 years of Lithuanian anthropology: modern trends, history, relation to medical practice and humanities* in Vilnius from 27–30 October 2004 presentations were made by:

R. Allmäe – *Cremations in Western Estonia in V–XIII cc*;

L. Heapost – *The population of South-East corner of Estonia at the end of the iron age and in the middle ages;*

J. Limbo – *Dental enamel hypoplasia in the Pada cemetery (XII–XIII century).*

2. Section members L. Heapost and G. Veldre participated in the international conference dedicated to the 75th birth anniversary of Academician V. P. Alekseev, *Human ecology and demography in the past and at present*, in Moscow from 14–17 November 2004. Because of organizational and information exchange problems, the main organizers of the conference had not received the abstracts of presentations from many participants, including the members of our Section, and, therefore, their presentations had been left out of the very dense programme of the conference.

From this conference, our Section members brought along a great number of anthropological research papers published in Moscow in recent years, including *Horizons of Anthropology* (Moscow, 2003) which includes L. Heapost's, H. Kaarma's and G. Veldre's articles based on their presentations at the eponymous international conference in 1994 [1, 6].

In 2005 the Anthropology Section held three meetings, one of them was a research paper presentation meeting:

The meeting that took place in the rotunda of the Old Anatomical Theatre (38 Lossi Street in Tartu) at 17:15 on 27 April had 11 participants: L. Heapost, H. Kaarma, J. Kasmel, L. Kiisk, M. Laurits, J. Limbo, M. Lintsi, V. Loolaid, L. Saluste, G. Veldre and I. Õunapuu. The meeting was chaired by G. Veldre.

There were the following items on the agenda: 1. Future prospects of the Anthropology Section – good ideas and concrete proposals were expected from all participants. 2. Prof. H. Kaarma – *On organizing the work of the Anthropology Section at the international conference on the 200th anniversary of the Old Anatomical Theatre* (23–25 September). 3. Any other business.

At 15:00 on 20 October a research paper presentation meeting, Juhan Aul's Day was held in the building of the Estonian Naturalists' Society. The meeting was dedicated to Juhan Aul's 108th birth anniversary.

The opening address was delivered by M. Sammul DBiol, President of the Estonian Naturalists' Society. Presentations were made by: J. Kasmel – *On anthropological research in Estonia, Latvia and Lithuania from 1918 to 1940*; Ü. Kirss – *On predicting the weight and height of children aged up to two years*; M. Lintsi – *Impressions from the German anthropological congress in Munich in 2005*; M. Toomsalu –

Impressions from the jubilee conference of the Old Anatomical Theatre in 2005 in Tartu.

Prof. H. Kaarma presented the XIVth issue of the collection *Papers on Anthropology* which was the bulkiest of all published until then, containing 34 articles on 394 pages. Six articles had been written by the members of the Anthropology Section.

The meeting had 22 participants.

At 16:15 on 1 December, the meeting summarizing the activities of the Anthropology Section in 2005 was held in Room 301 at Vanemuise 46.

The meeting had 9 participants: L. Heapost, H. Kaarma, J. Kasmel, M. Laurits, J. Limbo, M. Lintsi, L. Saluste, G. Veldre and I. Õunapuu.

The agenda included: 1. Short summaries by Anthropology Section members on their main activities in 2005. The presenters were: H. Kaarma (*On the situation of the Anthropometric Register*); L. Heapost (*On the conference in Pskov and on paleodemography*); J. Limbo (*On using the enamel defects or hypoplasia caused by childhood metabolic stress in anthropology – The case of the Pada skeletal series*); L. Saluste and G. Veldre (*Impressions from the conference in Minsk*). 2. Planning the work of the Anthropology Section for 2006 3. Any other business.

That year, the Anthropology section also presented a project for anthropological research of the inhabitants of Pärnu in 2006.

On 22 September, during the events of the Car-free Day organized by the Tartu Environmental Education Centre, M. Lintsi and G. Veldre participated in the work of the Health Corner in Küüni Street in Tartu. They measured people's height and body mass, calculated their body mass index and body fat content and measured their blood pressure.

Participation in international events:

1. Oral presentations were made at the international theoretical and practical conference *Genetic and morphological markers in anthropology, criminalistics and medicine* in Minsk from 15–17 June 2005: L. Heapost – *Variability of Estonians' height from the 12th to the 20th century*; L. Saluste – *Comparison of standards of height, weight and BMI for adult populations of Estonia and Belarus*; G. Veldre – *Somatotypes and arterial blood pressure in Estonian children aged 12–15 years*.

L. Heapost's presentation attracted great attention at the plenary session of the conference. The texts of the presentations will be published in the conference collection.

2. The international conference *Tissue Biology* dedicated to the 200th anniversary of the Old Anatomical Theatre in Tartu from 23–25 September 2005, also had an anthropology section (in Biomedicum, Ravila 19–1024 on 24 September). There were participants from Estonia, Latvia, Lithuania, Hungary and Germany. Nine presentations were made, among them four presentations by the members of the Anthropology Section of the Estonian Naturalists' Society.

The conference considered it necessary to organize such conferences regularly (biannually in different Baltic states) [2, 7].

In 2006 the Anthropology Section held four meetings, two of them were research paper presentation meetings.

At the meeting of the Anthropology Section of the Estonian Naturalists' Society in Room 301 at 46 Vanemuise Street at 13:15 on 25 January, guest speaker Prof V. Tillmann delivered the lecture *How does the child grow? Different aspects of short-term growth*. The presenter was asked a number of questions and the lecture was followed by a lively discussion around the coffee table. The meeting had 14 participants.

At 14:00 on 13 March, the exhibition *Leiu Heapost 70* was opened at F. Puksoo Gallery in Tartu University Library. The exhibition was supported by the Estonian Cultural Endowment. The opening of the exhibition was followed by a research paper presentation meeting in the building of the Estonian Naturalists' Society (2 Struve Street).

At the opening of the exhibition M. Viikmaa made a presentation on L. Heapost's works.

In the building of the Estonian Naturalists' Society the following presentations were made: J. Kasmel – *Graduation theses on Estonian school students physical development supervised by Prof. Juhan Aul;* J. Limbo – *Dental pathologies in pit grave Pada cemetery and votic grave Jõuga cemetery in Northeast Estonia*; R. Allmäe – *On funeral traditions in Läänemaa and Setumaa Counties*.

There were 34 people listening to the presentations. Ten more people participated at the opening of the exhibition.

At 15:00 on 20 October, the Anthropology Section of the Estonian Naturalists' Society and the Centre for Physical Anthropolgy at the University of Tartu arranged a research paper presentation meeting, Juhan Aul Day, in the building of the Estonian Naturalists' Society. The meeting was dedicated to Juhal Aul's 109th birth anniversary. The meeting had 27 participants.

The collection *Papers on Anthropology XV* had come out*;* it included 27 articles on 320 pages; among them seven articles were by members of the Estonian Naturalists' Society.

The Section meeting at 16:00 on 18 December on the premises of the Centre for Physical Anthropology in the Old Anatomical Theatre (38 Lossi Street in Tartu) discussed the events of the bygone year and the plans for the beginning year. The meeting had eight participants.

It was decided to hold the first meeting of the Section in 2007 in the third week of January.

At the interdisciplinary conference of medical anthropology *MEDICA III: The alien becomes familiar* in the hall of the Estonian Literary Museum on 10–11 May, Jaan Kasmel made two oral presentations; both co-authored by T. Kasmel – *On studies of Estonian school students' physical development in the town of Tartu and the districts of Tartu and Elva (now Tartu County) from 1956–1958* and *On folk medicine at Baltic conferences of history of science from 1958–1959*.

At the conference *Tartu University History Museum 30* from 5–6 December, J. Kasmel made three oral presentations.

During the events of the Car-free Day organized by Tartu Environmental Education Centre on 22 September, L. Saluste and G. Veldre participated in the work of the Health Corner in Küüni Street in Tartu. They measured people's height and body mass, calculated their body mass index and body fat content and measured their blood pressure.

Participation in international events:

1. At the *XIII Congress on Nutrition and Metabolism in Renal Disease* in Merida (Mexico) from 28 February to 4 March, L. Kiisk made the presentation *Nutritional Status in Kidney Transplant Patients* (co-authors M. Lintsi, S. Mesikepp, E. Seppet, L. Saluste, Ü. Pechter and M. Ots).

2. At the IVth international conference of students *Проблемы культурогенеза и древней истории Восточной Европы и Сибири* (*Problems of genesis of culture and ancient history of Eastern Europe*

and Siberia) in St. Petersburg from 27–30 April J. Limbo made the presentation *Dental caries in pit grave Pada cemetery (12th –13th cc.) and votic grave Jõuga cemetery (12th – 16th cc.) in Northeast Estonia.*

3. At the 2nd international conference *Актуальные проблемы спортивной морфологии и интегративной антропологии* (*Topical problems of sports morphology and integrative anthropology*) at Moscow University from 29–30 May L. Saluste participated with the poster presentation *Сравнительный анализ телосложения 50-летних мужчин сельской местности о. Хийумаа (Эстония) и Гресторпа (Швеция)* (*Comparative analysis of body build of 50-year-old men in a rural area in Hiiumaa Island (Estonia) and Grästorp (Sweden)*) (co-authors G. Veldre, J. Peterson, M. Lintsi, H. Kaarma, M. Aunapuu, A. Arend, T. Hedner, M. Viigimaa).

4. At the 15th Congress of the European Anthropological Association in Budapest from 31 August to 3 September, *Man and Environment: Trends and Challenges in Anthropology,* G. Veldre made the oral presentation *Blood pressure differences in adolescents with various body build*; J. Limbo made the poster presentation *Dental pathologies from two skeletal populations of different burial customs from the end of Iron Age / Early Medieval in Northeast Estonia,* and R. Allmäe (co-authors L. Maldre and M. Aun) made the poster presentation *Iron Age cremations in South-Eastern Estonia*.

5. J. Kasmel participated at the XXII Baltic Conference on the History of Science in Vilnius and Kaunas from 5–6 October where he was engaged in eight oral presentations [3, 8].

In 2007 the Anthropology Section celebrated several major anniversaries.

On 23 April, in cooperation with the Institute of History at Tallinn University and the non-profit association Archaeology Centre a Research Day and exhibition *Karin Mark 85. 55 years of anthropology at the Institute of History* was organized.

The selection of exhibits and arrangement of the exhibition was led by Anthropology Section members L. Heapost, R. Allmäe and J. Limbo-Simovart.

At the Research Day presentations were made by L. Heapost – *On K. Mark's role in Estonian anthropology* and J. Limbo-Simovart *On*

odontology at the Institute of History now and its further trends of development.

The Research Day had more than 35 participants.

From 15–16 October, the Centre for Physical Anthropology and the Institute of Anatomy at the University of Tartu arranged an international anthropological conference dedicated to the 110th birth anniversary of Juhan Aul (1897–1994).

For the time of the conference, the major part of Karin Mark's 85th birth anniversary exhibition was brought from the History Institute of Tallinn University to the Old Anatomical Theatre in Tartu where it was put up next to the Medical Collections of the Faculty of Medicine.

With the help of the working team of the Medical Collections of the Faculty of Medicine (M. Toomsalu and others), exhibits were also displayed in honour of Prof. J. Aul's 110th birth anniversary.

At the opening session of the conference at 10:00 on 15 October presentations were made by three members of the Anthropology Section: J. Kasmel – *An overview of Juhan Aul's biography*, L. Heapost – *Memories about working with Juhan Aul*, and G. Veldre – *Assessment of physique peculiarities of the human body.*

At the afternoon session of 15 October at the Old Anatomical Theatre, M. Lintsi made the presentation *Foundation of the Anthropology Section of the Estonian Naturalists Society*.

Together with conference guests, a wreath was taken to the grave of Juhan Aul, the founder of the Anthropology Section of the Estonian Naturalists' Society, at Raadi cemetery.

Approximately 40 people participated in the work of the conference.

By the beginning of the conference, the collection *Papers on Anthropology XVI* came out; it included 26 articles on 292 pages; among them five articles by members of the Estonian Naturalists' Society.

After Internet correspondence, the Section members decided to hold the meeting summarizing the activities of the Anthropology Section in 2007, which was planned for December, early in the following year.

At 16:00 on 18 December a Christmas gathering was arranged on the premises of the Centre for Physical Anthropology in the Old Anatomical Theatre (38 Lossi Street in Tartu). It was attended by five members of the Section.

At the seminar *Interdisciplinary approaches to studying past events* at Waide motel (near Elva in Tartu County) from 16–17 December 2007, R. Allmäe and J. Limbo-Simovart made a joint presentation

Methods of physical anthropology in interpreting skeletal populations (the case of St. John's Church in Pärnu).

Participation in international events:

A – symposiums, conferences, congresses:

1. At the international symposium Rank, gender and society around the Baltic 400–1400 in Kuressaare from 23–27 May, J. Limbo-Simovart made the presentation Sex differences in Late Iron Age Northeast Estonia as indicated in dental pathologies and enamel hypoplasia.

2. On 2 June G. Veldre made an oral presentation as an invited guest at the 6th scientific conference *Advances in the assessment of physical development disorders* in Warsaw – *Estonian Experiences in Assessment of Physique Peculiarities in Adolescents.*

3. At the international theoretical and practical conference *Актуальные проблемы физической и социокультурной антропологии* (*Topical questions of physical and sociocultural anthropology*) in Minsk from 19–21 June, oral presentations were made by L. Heapost – *Этнокультурный фон антропологического формирования юго-восточных эстонцев в 11–15 вв* (*Ethnocultural background to the anthropological formation of Northeast Estonians in 11th–15th centuries*) and G. Veldre – *Blood pressure differences in Estonians with various body build – some preliminary data of HYPEST study.*

L. Saluste (co-authors H. Kaarma, M. Lintsi, S. Koskel, A. Arend) made a poster presentation (in Russian) – *Estonian national norms of height, weight and body mass index for men and women aged 20–70 years*.

4. At the conference of Russia's ethnologists and anthropologists in Saransk from 9–14 July, L. Heapost made the presentation *On the ethnocultural background of the anthropological formation of SE Estonians in the 11th–15th cc.*

5. At the international conference in Vilnius, *Anthropology and Medical Practice,* G. Veldre made the oral presentation *Bivariate body height-weight classification, a useful tool in systematization and analysis of medical data.*

6. At the 4th Conference of Baltic Morphologists in Riga from 19–20 November, members of the Anthropology Section made two oral presentations: H. Kaarma, co-authors L. Saluste, G. Veldre, E.–M. Tiit – *Prospects for studies of body build and nutritional habits in Estonia*; J. Kasmel, co-author T. Kasmel – *On the beginning of systematic*

anthropological research at the University of Tartu (the former Imperial University of Dorpat).

J. Kasmel also made a poster presentation, co-author T. Kasmel – *Professor Juhan Aul as an anthropologist (for the 110th birth anniversary of Prof. J. Aul).*

B – upgrading of qualification, advanced training courses:

1. On 17 January R. Allmäe and J. Limbo-Simovart visited the departments of archaeology and forensic medicine at *Museovirasto* (Finland's National Board of Antiquities) and the University of Helsinki where they acquainted themselves with preservation of osteological and archaeological collections, excavation methods of mass graves and forensic medical methods in osteology.

2. From 6–17 August R. Allmäe studied the methods of determination of osteological age and paleodemographic analysis at the Summer University in Odense which was arranged in cooperation between the Max Planck Institute for Demographic Research (MPIDR) and Department of Anthropology (ADBOU), (Institute of Forensic Medicine, University of Southern Denmark, Odense).

3. From 17–28 September R. Allmäe and J. Limbo-Simovart participated in organizing the short course *Bioarchaeology – opportunities for interpretation of life and lifestyle of the people of the past* at the Department of Archaeology at Tallinn University. The international short course was arranged in cooperation with the Universities of Tartu (M. Konsa, V. Lang) and Vilnius (R. Jankauskas) [4, 9].

In 2008 the Anthropology Section held three meetings, two of which were research paper presentation meetings. For the first time, one of the meetings was held using the mediation of the Internet (Skype).

By 16:00 on 15 February, eight members of the Anthropology Section residing in Tartu had gathered into one of the buildings of Tartu University Hospital (Room 208 at 8 Puusepa Street in Tartu). Simultaneously, three members of the Section residing in Tallinn had gathered into J. Limbo-Simovart's office at the Department of Archaeobiology and Ancient Technology at the Institute of History at Tallinn University (at 6 Rüütli Street in Tallinn). The reason for such a gathering was the decision of the Section to use Skype for holding its

meeting. This made it possible to hold the meeting without some members of the Section travelling from Tallinn to Tartu.

The agenda of the meeting was as follows: summary of the activities of the Anthropology Section in 2007 (after the introduction by Chair of the Section G. Veldre, all participants took the floor); election of the board of the Anthropology Section (according to the Section's rules of procedure); discussion of the question whether the rules of procedure of the Anthropology Section should be changed considering the further activities of the Section; drawing up the work schedule of the Anthropology Section for 2008; any other business.

According to the election results, no changes were made in the board. The meeting found that it was possible to continue the Section's activities without changing its rules of procedure; still there should be more variety in the activities of the Section so that they would include more activities stipulated by the rules of procedure.

On 24 March, in cooperation with the Institute of History at Tallinn University and the non-profit association Archaeology Centre, the Anthropology Section arranged the Research Day dedicated to Karin Mark's 86th birth anniversary *Physical anthropology. The second Research Day at the Institute of History* (in Room 307 at 10 Rüütli Street in Tallinn).

From the five presentations on the agenda tree were made by Anthropology Section members: L. Heapost – *Analysis of the osteological material of Võllamägi mass grave* (6 Ravi Street in Tallinn); R. Allmäe. *Methods of physical anthropology in interpreting skeletal populations (the case of St. John's Church in Pärnu)*; J. Limbo-Simovart – *Methods of physical anthropology in interpreting skeletal populations (the case of St. John's Church in Pärnu). Odontological analysis.*

The presentations were followed by an excursion to the Archaeology Museum of the Institute of History and a discussion around the coffee table. The Research Day had 13 participants.

At 15:00 on 16 October a research paper presentation meeting, Juhan Aul Day, was held in the building of the Estonian Naturalists' Society. The meeting was dedicated to Juhan Aul's 111th birth anniversary.

The opening address was delivered by T. Viik, President of the Estonian Naturalists' Society. Presentations were made by J. Kasmel – *15 years of the Centre for Physical Anthropology*; M. Lintsi – *Heino*

Tiik 75; M. Toomsalu – *Medical collections as communicators of health awareness.*

Prof. H. Kaarma presented the collection *Papers on Anthropology XVII,* which contained 25 articles on 339 pages. Among them, there were eight articles by the members of the Anthropology Section:

The meeting had 23 participants.

At 15:00 on 17 December, a Christmas gathering was held on the premises of the Centre for Physical Anthropology in the Old Anatomical Theatre (38 Lossi Street in Tartu). It was attended by six members of the Section.

On 26 September at the opening conference of the series of lectures *Pärnu throughout centuries*, R. Allmäe and J. Limbo-Simovart made a joint presentation *Pärnu garrison – its origin and living conditions*.

Participation in international events:

1. At the 16th Congress of the European Anthropological Association in Odense (Denmark) from 28–31 August, R. Allmäe made the presentation *Pärnu garrison and its varied origin* and was the co-author of the presentation made by R. Jankauskas and G. Gerhards – *Bio-archaeology – research and public presentation in the Baltic states*. J. Limbo-Simovart made the presentation *Dental enamel hypoplasia in the 17th–18th cc. town population from Pärnu, Estonia*. The abstracts of presentations were published.

2. At the conference of the Association for Environmental Archaeology (AEA) in Arhus (Denmark) from 12–14 September, R. Allmäe made the presentation *Role of fire in burial customs on the basis of two Iron Age burial places in Estonia***.** The abstract of this presentation was also published.

3. At the national conference in Russia *Адаптация как фактор формирования антропологического своеобразия* (*Adaptation as a factor in formation of anthropological peculiarities*) in Moscow from 8–9 December, J. Limbo-Simovart made the presentation *Dental pathologies and stressmarkers in the Iron Age End/Early Medieval Estonia* and L. Heapost **–** *Изменчивость цвета глаз и волос у финно-угорских народов* (*Variation of eye and hair colour in Finno-Ugric peoples*).

The abstracts of both presentations were published in the conference collection [5, 10].

In 2009 the Anthropology Section celebrated its 70th anniversary with several events during the whole year.

On 23 March, the Anthropology Section, in cooperation with the Institute of History at Tallinn University, arranged a Research Day dedicated to Karin Mark's 87th birth anniversary (in the large hall at 6 Rüütli Street). Presentations were made by M. Viikmaa – *On determination of sex in animals* and P. Hõrak – *Sexual selection in humans*.

The Research Day had approximately 30 participants [11].

As the Anthropology Section had its 70th anniversary at the end of April 2009, and the report of the Section's activities for that year does not include any more events during the first four months of the year, the overview of the activities of the Anthropology Section during its seventy years has reached its end.

An overview of events at the Anthropology Section until the end of 2009 will be presented in the future together with its activities in the following years.

We are thankful to Chair of the Anthrology Section Gudrun Veldre DBiol who kindly allowed us to use the reports of the activities of the Anthropology Section from 2004–2008 for writing the third part of this article.

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THE RESEARCH OF PHYSICAL CONDITION, PHYSICAL ACTIVITY AND NUTRITION OF TEACHER EDUCATION STUDENTS

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ABSTRACT

The goal of the research is to identify and assess parameters of physical development, actual nutrition, energy expenditure and energy requirement of the young adult males. The research was conducted in 2008–2010 and students (n=84) of biology, physical education and psychology specialties at Vilnius Pedagogical University participated in it. Height and weight measurements were taken by electronic medical scales with the height measuring equipment. We analysed an average energy value of a daily food ration; the intake of carbohydrates, protein, and fat; physical activity and energy requirement by using 7-day nutrition and physical activity diaries filled out by the students participating in the research (20.35±0.99 years old). The body weight of the majority of the young adult males was normal (69.05 per cent), 26.19 per cent of them had the body weight above the normal range, and the body weight of 4.76 per cent of the students was insufficient. Physical activity ratio of the future teachers is 1.79±0.382. An average energy value of nutrition ration corresponds to energy expenditure ($p=0.684$) but is lower than energy requirement ($p<0.001$). The ratio of the main nutrients (protein, fat, and carbohydrates) in the nutrition of the young adult males is unbalanced; 27.38 per cent of them consume too much protein, and 1.19 per cent exceeds the recommended daily intake of protein more than twice. According to our data, only 7.14 per cent of the students get enough fat, 36.91 per cent of them consume too much fat, and 55.95 per cent of the respondents get too little fat although close to the norm. Our research demonstrated that there is a deficiency of carbohydrate in a daily ration food of 95.24 per cent of the young adult males, and the carbohydrate content in the nutrition of 34.52 per cent of the young men is more than twice lower than the norm. In comparison with the individual norms of these nutrients (considering the respondent's body

weight, age, and physical activity), protein intake of the young adult males is too high ($p<0.001$), the received fat content corresponds to the recommended intake ($p=0.663$), and there is carbohydrate deficiency in a daily food ration ($p<0.001$).

Key words: *students, physical condition, physical activity, nutrition.*

INTRODUCTION

There are certain peculiarities of physical condition and the related lifestyle at every stage of human life. Both in Lithuania and in other countries of the world, the conducted thorough and comprehensive researches of physical condition in children and teenagers are especially important for the further human development. However, after the teenage stage a person enters the stage of youth, and that is like a transition to the mature stage of human life. The lifestyle for most young adults changes: they become more self-dependent, have to change the rhythm of daily activities and their physical activity. At this period of life it is especially important to maintain and improve their physical condition, the habits of rational nutrition, as well as work and leisure regime, because all that makes an essential base for good health, physical and mental efficiency, spiritual comfort and self-confidence. A period of university studies is one of the most important personality development stages related to learning when a person is under constant stress, and that requires great willpower and emotional efforts. Unfortunately, students lack time for eating; therefore, their nutrition regime and health get worse. This is proved by a number of researches carried out at higher education institutions both in Lithuania and in other European countries [3, 6, 8, 15, 18]. There are numerous scientific studies in social and biomedical areas aimed at the assessment of social development, professional readiness, health, physical development data of different groups of young adults [1, 2, 7, 11, 13, 15, 17]. Despite the fact that the published data quite thoroughly analyse the situation of young adults, each conducted research, however, provides additional information and reflects the specificity of a certain region, some social group or a stage of life which blend into a general context of researches and are important for the development of the society and for the future.

According to the data of the World Health Organization (WHO), 40–60 per cent of the human health depends on the lifestyle, 30–40 per cent is impacted by the environment, 10–15 per cent is inherited, and only 8–10 per cent of health depends on health care [19]. At birth, every person acquires a certain health potential which he strengthens throughout his entire life or may also weaken it. A person himself is responsible for his own health and has to take care of it, to strengthen and cherish it. In order to maintain good health and not to deteriorate the quality of life, it is necessary to follow certain principles of holistic lifestyle: rational nutrition, the enhancement of physical and mental powers, physical activity, and the refusal of harmful habits. It is also necessary to select food individually, to use more functional foods. Optimum movement is a precondition to live a full-fledged life at all stages of human life. According to the WHO data, already on the second day after the decrease in physical activity, mental activity reduces up to 50 per cent, the concentration of attention declines, nervous tension increases, etc. [19].

MATERIAL AND METHODS

The students (n=84) of biology, physical education and psychology took part in the research. Their average age was 20.35±0.99 years old. The total sample of the research was divided into two groups: the students whose physical activity ratio was from 1.2 to 1.5 (low and moderate physical activity) belonged to the first group, and the second group consisted of the students whose physical activity ratio was from 2 to 2.7 (high and very high physical activity). Electronic medical scales SECA 704 with the height measuring equipment SECA 220 were used to measure the physical development parameters. To study actual nutrition and physical activity, we used 7-day nutrition and physical activity diaries filled out by each student participating in the research in accordance with the consumed quantity of food and the size of portions as well as the energy expenditure for daily activities. To determine food portions, we used *A Photographic Atlas of Food Products and Food Portion Sizes* (2007). The quantities of protein, fat, and carbohydrates, as well as the food energy value, were determined referring to food composition tables [5]. Energy expenditure was assessed according to the tables of energy expenditure in different types of activities of *The Basics of Sports Medicine*. Having analysed a 7-day nutrition ration (the

composition of food and meals, the nutritional and energy value) and physical activity, an average daily energy value of a nutrition ration (kcal), energy expenditure (kcal), a physical activity ratio, and an individual energy requirement were calculated. A physical activity ratio was determined by the recommendations of the World Health Organization: 1.2 means very low physical activity; 1.3 – low physical activity; 1.5 – moderate physical activity; 2.0 – high physical activity; 2.7 – very high physical activity. The daily energy requirement (DER) was calculated according to the formulae: DER=BMR×PAR (BMR – basal metabolic rate; PAR – physical activity ratio), BMR=65.4+(13.7×W)+(5.0×H)–(6.8×A) (W – body weight, kg; H – height, cm; A – age in years). The statistical analysis was performed using the STATISTICA 6.0 software package. Statistical significance was determined by applying the Student's t-test and the Kruskal-Wallis test criteria of independent samples.

RESULTS

According to the data of the specialists consistently researching actual nutrition and the lifestyle of the Lithuanian population, the recent changes in the body weight of the Lithuanian population are adverse to their health: the number of people, having insufficient or excess body weight, has been increasing [4, 9]. It is known that physiological functions of the people having insufficient body weight may be impaired, and obese people are at risk to get non-infectious diseases (WHO). The average values of physical development of the students involved in our research: height – 183.26 ± 7.378 cm, body weight – 79.19 ± 11.402 kg, BMI – 23.53 ± 2.664 kg/m^2 (Table 1). The body mass index (BMI) of a majority (69.05 per cent) of future teachers is within the normal range, while BMI of 26.19 per cent of the students is above the normal range (Figure 1). This is an on-going research [10, 12] supplemented with an additional number of respondents, new methodologies, and data interpretations.

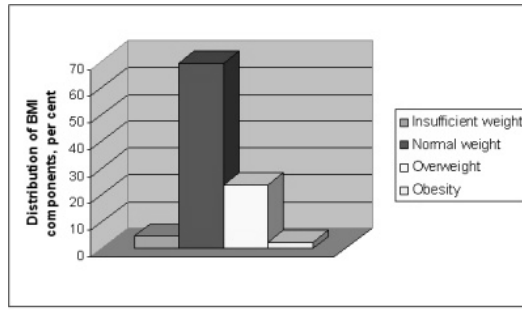

Figure1. Physical development data of the young adult males.

An average energy value of food is 2466.95 ± 812.753 kcal: fat – 912.72 kcal (103.51 g), carbohydrates – 1142.54 kcal (287.93 g), and protein – 411.69 kcal (101.22 g). The daily average energy expenditure – 2591.69 ± 671.561, the average of physical activity ratio – 1.79 ± 0.382, according to which the daily energy requirement is 3457.13 ± 834.319 kcal (Table 1, Table 2).

Table 1. Physical development, the energy value of the food ration, energy expenditure and energy requirement of the young adult males

Young adult males, n=84	*Height, cm*	*Weight, kg*	*BMI, kg/m²*	*Food energy value, kcal*	*Physical activity*		*Daily energy requirement, kcal*
					Daily energy expenditure, kcal	*Ratio*	
Average	183.26	79.19	23.53	2 466.95	2 591.69	1.79	3 457.13
Standard deviation	7.38	11.40	2.66	812.75	671.56	0,38	834.32
Maximum value	199.00	123.00	32.35	5 359.20	4 366.00	2.70	6 046.38
Minimum value	167.00	56.00	18.21	673.70	1 222.14	1.20	1 867.92
Median	182.00	78.50	23.13	2 360.48	2 606.00	2.00	3 499.23

One of the principles of healthy lifestyle is rational nutrition and consistent maintenance of the proportion of the nutrients having energy value. According to physiological nutrient and energy norms that ensure physiological needs of the individual, protein must make up 10–15 per

cent, fat – 28–30 per cent, and carbohydrates – 55–62 per cent of the energy value of the daily food ration (Order No. 510 of the Minister of Health of the Republic of Lithuania of 25 November 1999). Referring to the data obtained during our research, the ratio of energetic nutrients in the diet of the students is very diverse (Table 2; Figure 2, 3 and 4). In the daily nutrition of all (100 per cent) the researched young adult males the ratio of the main nutrients (protein, fat, and carbohydrates) is unbalanced.

Table 2. The energy value of the main nutrients in the daily food ration of the young adult males

Main nutrients	*Quantity (g) M±SD*	*Energy value (kcal) M±SD*	*Energy value (proc.) M±SD*
Fat	103.51±46.880	912.72±392.435	36.62±9.994
Carbohydrates	287.93±113.343	1 142.54±450.465	46.20±9.469
Protein	101.22±37.849	411.69±157.487	17.18±6.259

Protein is important for the different physiological functions of the body; however, as a source of energy it makes up the smallest part, and the body will use protein energy only in case of the shortage of carbohydrates; 27.38 per cent of the young adult males consume too much protein, and 1.19 per cent of them exceed the recommended protein intake more than twice because eggs, meat dishes and leguminous vegetables constitute a significant part in their daily food ration. Fat is the energy substance which is hard to expend; however, fat deficiency can slow down its metabolism in the body. Our research demonstrates that only 7.14 per cent of the students get the sufficient quantity of fat, 36.91 per cent of them consume too much, and 55.95 per cent of the respondents receive too little fat although close to the norm (Figure 3). Carbohydrates are the main and most easily accessible source of energy. Moreover, other components important for physiological processes enter the body together with carbohydrates: minerals, vitamins, fibre, and water. Fibre and water give a sense of satiety, but their energy value is low. This is particularly important for the overweight or obese people. The research showed that 95.24 per cent of the students lack carbohydrates in their daily food ration, and the quantity of carbohydrates consumed by 34.52 per cent of the young adult males is more than twice lower than the norm. Having compared

the quantities of the main nutrients in the daily food ration with the individual norm of these nutrients (taking into account the respondent's body weight, age and physical activity), we determined that the protein intake is too high ($p = 0.0003$) (Figure 2.), the fat intake meets the recommended rate ($p = 0.663$) (Figure 3), and there is carbohydrate deficiency in nutrition ($p = 0.000$) (Figure 4).

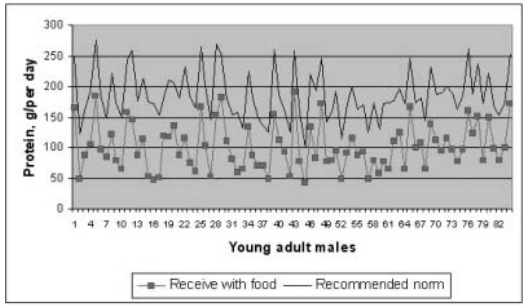

Figure 2. The quantity of protein in the daily food intake of the young adult males compared with the recommended norm, $p<0.005$.

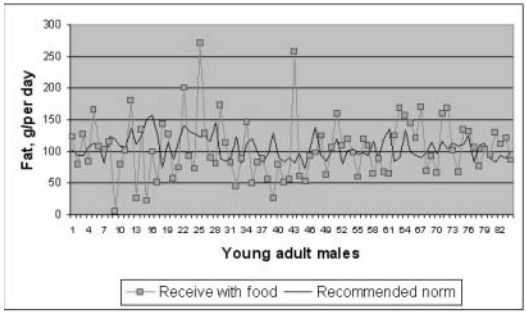

Figure 3. The quantity of fat in the daily food intake of the young adult males compared with the recommended norm, $p>0.05$.

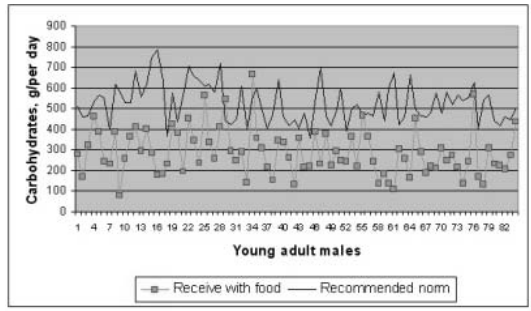

Figure 4. The quantity of carbohydrates in the daily food intake of the young adult males compared with the recommended norm, $p<0.005$.

The assessment of physical activity data demonstrates that a majority of future teachers (58.33 per cent) are of high and very high physical activity (2–2.7), while 15.48 per cent (1.2 to 1.5) of them are of low and very low physical activity (Figure 5).

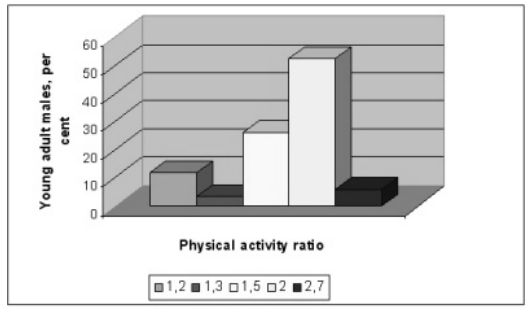

Figure 5. Distribution of the physical activity ratio of the young adult males.

In the average daily food ration of both trial groups (according to the students' physical activity), the quantities of protein, fat, and carbohydrates (kcal) were similar ($p> 0.05$). The activities of physically active students are basically related to sports, and their nutrition is dominated by poultry and fish products, various kinds of porridge, chocolate, nuts, raisins. The students whose physical activity is low and moderate eat more junk food: pizzas, kebabs, kybyns, chips, sandwiches, etc. The sources of fat are basically the same: oil, butter and

other food products containing fat: sour cream, mayonnaise, fatty meat; however, fat reserves of the young adult men who actively go in for sports are supplemented with eggs, milk, and chocolate. In the food ration of the students whose physical activity is high and very high the quantity of carbohydrates (per cent) is closer to the WHO recommended norms; however, there is no essential difference between the two trial groups ($p > 0.05$) (Figure 6).

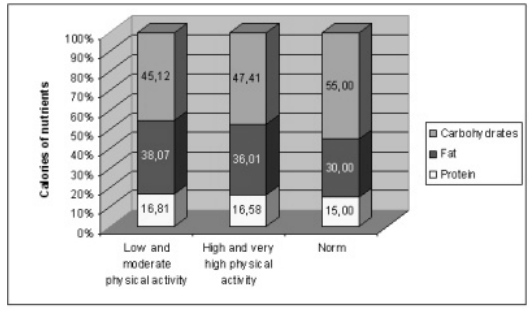

Figure 6. A part of energy value (per cent) in a daily food ration of the young adult males of different physical activity and the WHO recommended norm.

The data of the research demonstrate that according to the results of physical development and physical activity, the quantity of energy in the daily food ration of the young adult males is insufficient. The students whose physical activity is high and very high receive less energy with food than they should get in accordance with their physical activity and basal energy metabolism ($p = 0.000$) (Figure 8). Energy value of the food consumed by the students of low and moderate physical activity is also lower than the energy requirement ($p = 0.000$) (Figure 9). The energy expenditure compared with the energy requirement is also lower in both groups ($p < 0.001$) (Figure 8, 9).

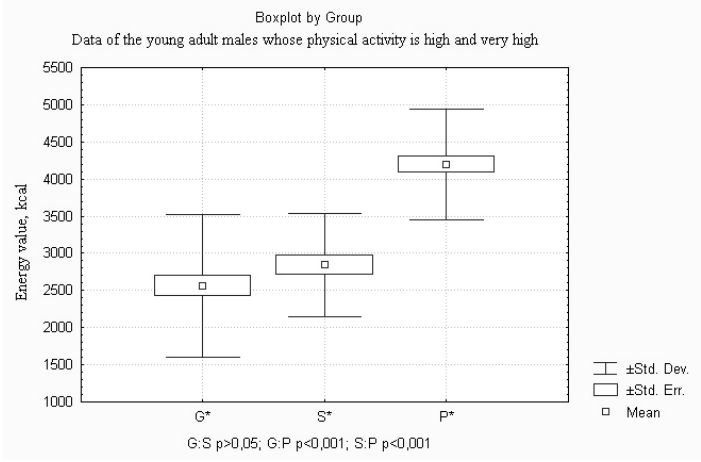

Figure 7. The comparison of the energy value of food, energy expenditure and energy requirement of the young adult males whose physical activity is high and very high (G* – energy value of food (received energy); S* – energy expenditure; P* – energy requirement).

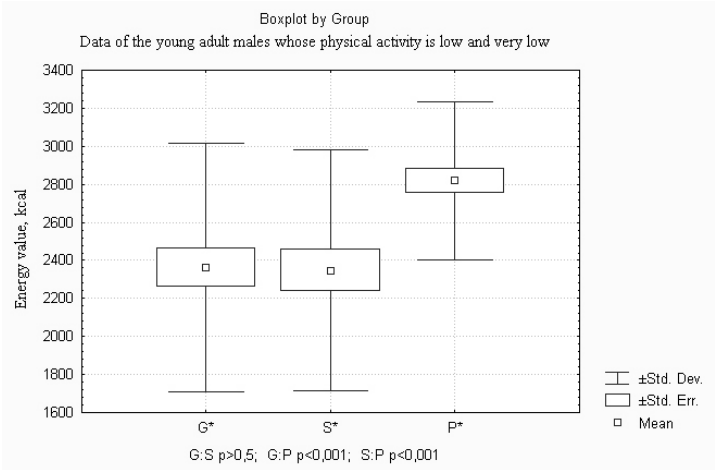

Figure 8. The comparison of the energy value of food, energy expenditure and energy requirement of the young adult males whose physical activity is moderate and low (G* – energy value of food (received energy); S* – energy expenditure; P* – energy requirement).

DISCUSSION

According to the data obtained in 1952–1962, the average height of the male students (21–30 years old) of Kaunas higher schools was 173.82 cm, and the average body weight was 68. 2 kg [16]. Therefore, after four decades young adult males are taller by 9.44 cm and heavier by10.99 kg. Compared with the body mass index of the first-year students of the Western Region higher schools of the United States of America [17], the data of our research demonstrate that 0.76 per cent of the young adult males have insufficient body weight, while a number of overweight students is bigger by 9.81, and a number of obese young men is smaller by 3.62 per cent. The energy value of food ration of the U.S. students, whose body mass index is less than 25 [17], was by 192.3 kcal higher compared with our respondents; meanwhile, the energy value of the food consumed by those, whose BMI is above the normal range (≥ 25), was less by 23.84 kcal. The daily energy value of the food ration of the students participating in the research is lower by 139.05 kcal in comparison with the value of the food ration of the Lithuanian men but by 188.59 kcal higher compared with the Estonian men and lower by 116.05 kcal compared with the Latvian men [1]. However, compared with the data of high-performance sportsmen [13], our researched students get even 2 019.05 kcal less energy with food.

In the average daily food ration of future teachers fat made up 36.6 ± 10 per cent (Table 2), and according to the data of J. A. Abaravicius (2008), fat made up by 8.8 per cent more in the nutrition of the Lithuanian men (25–64 years old) and by 6.1 per cent more in the food of the Latvian men but only by 0.1 per cent less in the food ration of the Estonian men. Future teachers receive relatively little carbohydrates – 46.2 ± 9.5 per cent while in the food ration of the Lithuanian men carbohydrates constitute even a smaller part i. e. 39.4 ± 12.3 per cent. The protein intake of the Lithuanian men corresponded to physiological nutrition standards, and according to our obtained results, the protein norm is exceeded by 2.18 per cent, but 58.33 per cent of the young adult males take active part in sports. Having compared the distribution of the quantity of main nutrients in the food ration of the young adult males with the data of the athletes, active in more endurance requiring kinds of sports [13], our researched students use less of these nutrients: the difference in protein intake makes 46.82 g, in fat – 88.55 g, and in carbohydrates – 164.69 g. The quantitative ratio of protein, fat and

carbohydrates in the actual food ration of sportsmen is 1:1,3:3,1 [13], and this ratio in our researched group is 1:1,03:2,74. Both studies show carbohydrate deficiency in the nutrition of the young adult males.

The development of nutrition researches in Lithuania reveals essential changes in the consumption of energy nutrients: in 1933, in the nutrition of Lithuanian farmers protein made 11 per cent of the daily food intake, fat – 17 per cent, and carbohydrates – 70 per cent. The researches of lifestyle and actual nutritional of adult Lithuanians conducted by the National Nutrition Centre in 1997–1998 show that the nutrition of adult Lithuanians has notably changed: the fat intake has significantly increased while the carbohydrate intake has decreased, a lot of cholesterol which unfavourably affects health has appeared in food, nutrition has become irrational and unbalanced [14]. Our researches as well as the researches carried out by other authors only confirm that [1, 13, 15].

According to the data of J. A. Abaravicius (2008), the energy expenditure of the people in most Central and Eastern European countries, including Lithuania, has been constantly decreasing. The research data demonstrate that low physical activity is typical of the respondents of the Baltic countries, but 16.6 per cent of the Lithuanian men are active in sports more frequently. The physical activity of the researched young adult males is high (physical activity ratio is 1.79 ± 0.38).

CONCLUSIONS

1. The body weight of the majority of the young adult males is normal (69.05 per cent), 4.76 per cent of them have insufficient body weight, and the body weight of 26.19 per cent of the young adult males is above the normal range.
2. The ratio of the main nutrients, i.e. protein, fat, and carbohydrates, in the food ration of the young adult males is unbalanced.
3. The young adult males consume too much protein ($p < 0.001$), the fat intake meets the recommended norm ($p = 0.663$); however, there is carbohydrate deficiency in nutrition ($p < 0.001$).
4. The energy value of the daily ration corresponds to the energy expenditure ($p = 0.684$) but it is lower than the daily energy requirement ($p < 0.001$).

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THE ANALYSIS OF UNDIAGNOSED MALIGNANCIES

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ABSTRACT

Among the pathology methods autopsy is also a valuable tool in evaluating diagnostic accuracy in oncology. Malignancies are undiagnosed due to the lack of some laboratory investigations, the absence of specific symptoms of cancers and the delayed treatment of them.

The aim is t*o analyze* the *reasons and* the *structure of* undiagnosed *malignancies in order to* review the failure of their detection during the person's life and the hyper diagnosis of tumours in *some hospitals of Riga (*Latvia) *in adults and children.*

Key words: *undiagnosed malignancies, diagnostic errors in children, adults*

MATERIAL AND METHODS

We have analyzed 62 adult and 11 children cases with undiagnosed and misdiagnosed malignancies from the Pathology Center, the Riga Eastern Clinical University Hospital and the Pathology Bureau, the Children's Clinical University Hospital. *The records of age, sex, location,* the *size of the tumor,* the *pathological diagnosis, complications and* the *evaluation of some clinical and laboratory data from* the *autopsy protocols and* the *clinical epicrisis were used. Morphological examinations were carried out with routine methods and with immunohistochemical reactions for the detection of CK* $_{AE\ 1/3,}$ *CD 20, CD 3, WT1, vimentin and muscle antigens. The results were statistically evaluated using* the *Excel programmer.*

Undiagnosed cancers in adults mostly were located in gastrointestinal system (62%), haematopoetic organs (14%) and lungs (6%). In

children during their life the diagnostics of the tumour was not carried out in hospital in the cases of soft tissue tumours (28%), leukemia and lymphoma (27%) and renal neoplasms (18%).

Many of the protocols which included the clinical epicrisis showed that many symptoms suggesting malignancy were often missed and most commonly in our analyzed cases they were anemia, weight loss and pain symptoms.

The hyperdiagnosis of tumours was also proved in children and adults. The clinical presentation of the malignancy was misdiagnosed for a benign illness of the same organ.

The patient's delay in seeking medical help is also responsible for the late diagnosis of malignancies and therefore the screening of the population at risk must be introduced in Latvia on the primary level of health care for children and adults.

The most common reasons of discrepancy between the clinical and pathological diagnose was a short time of hospitalization (20%), insufficient examination of the patient (19%), objective diagnostic difficulties of tumours (17%).

INTRODUCTION

Cancer has been one of the leading causes of death in the 21st century. Cancers are misdiagnosed due to insensible results, poor laboratory investigations, delayed treatment and lack of medical care [4]. The failure to diagnose occurs in many cases due to the lack or mild symptoms in malignancies. Some neoplasm's symptoms often do not start until the disease has reached an advanced stage [5].

According to the recent studies in the United States and Europe around 12% of all the cases of cancer are misdiagnosed [9, 17]. The inability to identify a primary site of cancer poses many challenges. The primary site of cancer usually dictates the treatment, the expected outcome, and the overall prognosis.

The delayed treatment, the failure of diagnose at the beginning of neoplasm increases the mortality rate due to the oncology process [15, 20]. The early detection of cancer is vital for successful treatment, higher survival rates, and decreased medical costs. When cancer is diagnosed after the disease has progressed, more drastic forms of treatment are required. Intense cancer treatments (such as higher doses

of radiation and chemotherapy) are not only painful and debilitating but cause added medical expenses [1, 13]. The early diagnosis of cancer leads to a higher curative rate and a survival rate [16, 18].

The aim is to *analyze* the *reasons and* the *structure of misdiagnosed cases of malignancies in order to* review the failure of diagnosing malignant illnesses and the hyper diagnosis of malignancies in *some hospitals of Riga in* Latvia *in adults and children.*

MATERIAL AND METHODS

In order to review the rate and the occurrence of undiagnosed malignancies the research was carried out in the Pathology Center, the Riga Eastern Clinical University Hospital and in the Children's Clinical University Hospital.

From 2,481 autopsies made in the *Pathology Center (Riga) in the years 2004–2010 in adults 62 cases with* the *discrepancy of clinical and post mortem diagnosis in oncology were found. But in* the *Children's Hospital from 1,705 post mortem examinations 11 cases were detected with undiagnosed tumours in the clinical departments. The recordings of age, sex, location,* the *size of the tumor,* the *pathological diagnosis, complications and* the *evaluation of some clinical and laboratory data from* the *clinical epicrisis were used. Morphological examinations were carried out with routine methods and with immunohistochemical reactions for the detection of CK* $_{AE\ 1/3,}$ *CD 20, CD 3, WT1, vimentin and muscle antigens. The results were statistically evaluated using* the *Excel programmer.*

RESULTS

The analysis of 62 cases of adults and 12 children's cases of clinically undiagnosed or misdiagnosed oncology diseases was done. In adults from 62 cases in 53 persons tumours were revealed only at autopsy. The remaining 9 cases were hyper the diagnosis of cancer but the actual cause of death was another disease missed during clinical diagnosis. Of the undiagnosed cancers most commonly they were located in the gastrointestinal system, representing 62% of all the undiagnosed malignancies, in the haematopoetic system-12, 9%, but others in some

cases were in lungs, the urogenital system, soft tissues and the endocrine system (Figure 1).

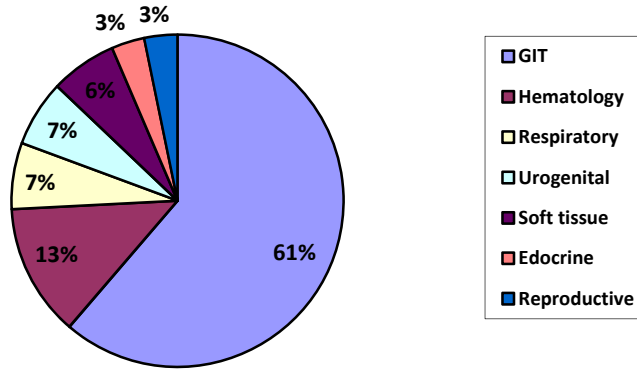

Figure 1. Undiagnosed malignancies of adults in the hospitals of Riga.

In 17 of the undiagnosed cancer cases, the diagnosis of the primary location of the cancer was incorrect with the mistaken clinical diagnosis that of malignancy from another location, which in reality were metastases, not the primary tumor. Distant metastases were recognized and diagnosed as the main clinical disease in 13 cases.

In autopsies we have already found dissemination of cancer without symptoms at the primary site but with clinical symptoms arising at the site of metastases.

Many of undiagnosed cases had a complication diagnosed as the main disease. Metastasis is one of the main complications of cancer in our analyzed group of patients. Symptoms, signs or another illness were diagnosed as the main disease in 25 cases. Clinical doctors, instead of malignancies, have put the diagnosis of: the coronary heart disease and atherosclerosis (9 cases), Jaundice (6 cases), acute abdomen (3 cases), bleeding (2 case), pneumonia (3 case) and anemia (2 cases). Many of the protocols which included the clinical epicrisis showed that many symptoms suggesting malignancy were often missed, e.g. severe anemia (14 cases), weight loss (8) and pain symptoms (5) were some of the signs and symptoms from the clinical epicrisis or the post mortem report which are associated with malignancies; 13 out of 30 cases of gastro-intestinal system neoplasm and 5 out of 7 hematological malignancies

had anemia fixed in the clinical epicrisis. Unexplained weight loss was mentioned in 8 of these misdiagnosed cases.

Of the 30 undiagnosed tumors which were located in the gastro-intestinal system, 8 were detected in the stomach, 7 – in the pancreas, 6 – in the large intestines, 6 – in the liver and the biliary tract, 1 – in the small intestines and 2 – in the esophagus.

Obvious presentations of malignancy were missed in some cases-(e.g. in 4 cases of undiagnosed liver cancer, the patient presented with an enlarged liver weighing 2.7 kg during autopsy instead of 1.5 kg); 5 cases of the missed diagnosis of pancreatic head tumors were diagnosed simply as "jaundice".

The number of undiagnosed cases of hematological neoplasms was 7 in total; 4 of the cases had obvious laboratory data suggesting an ongoing malignant process in the organism. (e.g. thrombocytopenia 66 000, hemoglobin 8.8, erythrocytes 3.7 which supports the presence of anemia).

Enlarged lymph nodes and spleen (5 cases) should have suggested the physician at least to consider the diagnosis of hematological malignancy.

Histological variations of cancers were mainly different subtypes of adenocarcinomas and three cases were neuroendocrine tumors. The average mortality age for females was 68.4 but for males – 61.1 years.

The situation with discrepancies between clinical and post mortem diagnosis in children is a little bit different. Mainly undiagnosed malignancies were found in the soft tissues (rhabdomyosarcoma, extraskeletal osteosarcoma) and kidneys (rhabdoid tumour, mezoblastic adenoma). Patients with renal tumours were treated from another kidney pathology in the hospitals of small towns; 27% of undiagnosed malignancies were leukemia, mainly acute ones and lymphomas at the average age of 7.6 years, Instead of hematological diseases clinical doctors diagnosed : meningococcal infection, nephroblastoma and the "enlarged kidney".

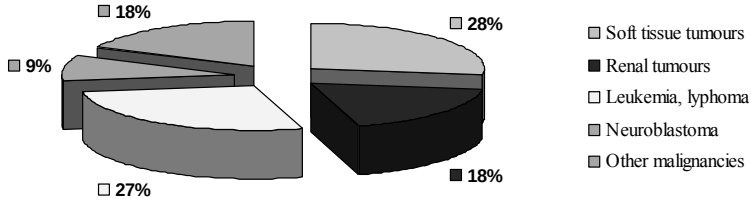

Figure 2. Undiagnosed malignancies in children (years 2004–2010 in Riga).

Another group of our analyzed cases constituted the hyper diagnosis or the misdiagnosis of malignancies.

In 14 adults and 1 child in clinical departments "malignancy" was diagnosed but the true pathological diagnosis was different non-oncological pathology (Table 1). The true cause of death in these cases was a benign illness of the same organ, e.g., cholangiocarcinoma was diagnosed instead of the gallbladder stone disease. But in an 8-month-old girl brain tumour was misdiagnosed with the malformation of venous vessels and intracerebral bleeding. In all these cases there was too late hospitalization of the patient.

Table 1. Misdiagnosed malignancy cases in adults and children.

Clinical diagnosis.	Number of cases	Post mortem diagnosis
Gastric cancinoma	4	Decompensated micronodular liver cirrhosis
Malignant process of mediastinum	1	Decompensated micronodular liver cirrhosis
Cholangiocarcinoma	2	Gall stone disease
Neoplastic syndrome	2	Atherosclerosis with intestinal gangrene.
Gastric carcinoma	1	Anorexia nervosa
Lung cancer	3	Lobar pneumonia.
Thyroid cancer	1	Lobular pneumonia.
Brain tumor (8-month-old girl)	1	Malformation of brain vessels with cerebral bleeding

DISCUSSION

In Latvia, like in most of countries of the world, mortality from malignancies is on the second place after cardiac and vessel pathologies [14]. Regardless of the highly developed diagnostic techniques and medical health care systems, malignancies are commonly misdiagnosed and undiagnosed throughout the world and through centuries [3, 6, 8]. It was revealed that many symptoms such as pain, anemia and weight loss were among the leading symptoms that were misdiagnosed in these cases. Compared with other researches carried out in different regions of the world, these symptoms tend to play the leading role in most of the undiagnosed malignancy cases [2, 11, 12]. The bigest number of undiagnosed malignancies is from the gastro-intestinal system (62%). This situation tends to appear similar when compared with the research carried in Brazil, Japan and Poland [2, 16, 21]. American researchers show that 2% of malignancies are of unknown primary origin. Misinterpretation of symptoms and signs contributes most to the undiagnosed malignancies [1, 18]. This can be due to the lack of adequate diagnostic techniques [10] or the lack of medical care. But in our analyzed cases patients are looking for medical help at the last stages of tumours or even die at home without any medical aid. In the last years physicians pay more and more attention to nonspecific symptoms of malignancies and one of them is thrombosis and thrombembolism [7, 8, 19]. In our adult cases in one third part of persons had myocardial infarction-also as a manifestation of thrombosis in coronary arteries.

Very important criteria for autopsy discrepancies are described by Goldman L. et al. [4]:

1. Class I – the missed major diagnosis with the potential adverse impact on the survival and that would have changed management.
2. Class II – the missed major diagnosis with no potential impact on survival and that would have not changed therapy
3. Class III – the missed minor diagnosis related to the terminal disease but not related to the cause of death.
4. Class IV – the other missed minor diagnoses.

Our research proved that in 22.5% of adults' outcome of the disease could be changed by clinical doctors. But in paediatric cases practically all the patients were not curable at the time of hospitalization (class II).

Decreased autopsy rates in countries, also in Latvia, should also be taken into consideration as not always the real reason of death can be detected by clinical practitioners.

CONCLUSIONS

1. Most commonly undiagnosed tumors were in the gastro-intestinal system at the stage $T_4N_2M_1$ and histologically they were adeno-carcinomas in the patients with a short time of hospitalization.
2. Signs and symptoms such as weight loss, anemia and pain which were associated with malignancies were not taken into consideration by clinical doctors and the diagnosis of malignant tumour was missed and therefore everyone needs the improved screening and monitoring of oncology cases.
3. Despite high technologies of diagnostic equipment the discrepancy of clinical and pathological diagnoses are still present in the oncology cases and the most common reasons of it is a short time of hospitalization, the insufficient examination of the patient, the wrong formation of medical documentation and also the patient's delay in seeking medical help.

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SEXUAL DIMORPHISM OF PELVIC MORPHOLOGY VARIATION IN LIVE HUMANS

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ABSTRACT

This study represents the comparison of the morphology variation in different planes of female and male pelvis. Anthropological literature represents two different views of the pelvic morphology variation. On the one hand, the variation is considered lower in females than in males. On the other hand, some empirical findings demonstrated no differences in the pelvic morphology variation between sexes. Moreover, some measures of female pelvis demonstrate higher coefficients of variation. Taking into account that previous findings were based on linear measures, it seems important to analyze pelvic proportions and the variation of the inlet, middle, and outlet planes.

The study was based on the retrospective pelvimetry of three-dimensional computer tomography of 176 live males and 212 females. Anterioposterior diameters and transverse diameters were measured in four planes, and their ratios were calculated in order to evaluate proportions and variances. The Levene's test for the equality of variances was used to evaluate the observed variation in male and female pelvis morphology.

The results confirmed well established sexual dimorphism in the pelvic linear measures – the anteroposterior diameters of the midplane and the outlet. In addition, this study identified higher variation in the transverse diameter of the inlet in females. The proportions demonstrated no differences in variation in all the planes but the midplane including bispinous diameter ($F=11.34$; $p<.01$). It seems important that the female pelvis cavity demonstrated lower variance than the male pelvis cavity in the midplane including bispinous. This finding supports the view of selective pressure on the female pelvis and its intensity in the midplane.

Key words: *pelvic morphology, sexual dimorphism, pelvimetry.*

INTRODUCTION

Evolutionarily, the human pelvis has adapted to two processes which changed the morphology of its cavity. Primarily, the human pelvis was shaped by the erect posture and the bipedal locomotion [1]. It became more triangular because of the increased transverse diameter of the inlet, widening and the enlargement of the sacrum, and the shortening of the pubic symphisis. Secondarily, the female pelvis was exposed to an additional selective pressure of the obstetric difficulties caused by the encephalization of a newborn [1, 7, 10]. As a result, the shape of the female pelvic cavity became shorter and more cylindrical. The observed pelvic sexual dimorphism is genetically determined and modulated by the steroid influence on the pelvic growth [10]. An additional impact on the pelvic shape is related to the early physical work and early pregnancy [1].

Anthropological literature represents two different views of the pelvic morphology variation. Some authors considered that an additional evolutionary pressure on the female pelvis results in the lower variation in its morphology in comparison to the male pelvis [6]. Tague [9], in its turn, has found no differences in the variation between males and females. Moreover, some pelvic dimensions demonstrated higher variation in females. It should be noted that these conclusions were based on the variances of the linear measures made on skeletal collections. The aim of this study was to analyze the differences of live humans' pelvic morphology variation in males and females with focusing on the proportions of lesser pelvic planes.

It is possible to analyze at last the four planes: the inlet, two midplanes, and the outlet. The inlet plane includes the linea terminalis. The first midplane is formed by the midpoint of pubic simphysis, two acetabulum centers, and a joint between the second and the third sacral vertebras. It is the wider plane of the pelvic cavity. The second midplane is formed by the lower border of pubic simphysis, two ishial spines, and a joint between the fourth and the fifth sacral vertebras. It is the shorter plane of the pelvic cavity. The outlet plane is formed by the lower border of pubic simphysis, pubic rami, ischial tuberosities and the tip of coccyx.

It should be noted that the indexes of proportions – the ratios of linear pelvic measures – are not widely applied in the research on sexual dimorphism. Taking into account that the human pelvis' shape can be

represented as a closed ring with a cylindrical cavity, the analysis of pelvic proportions can be helpful in the description of pelvic planes and in the comparisons of pelvic shapes without analyzing other general anthropometric measures (e.g. height). Research on the variation of these proportions can adds to understanding of pelvic morphology in females and males. Three-dimensional pelvimetry in live humans, in its turn, can provide good visualization of bones and a high accuracy of measures [2].

The research question was: What are the differences in the variation of proportions of the inlet, middle, and outlet pelvic planes and the corresponding linear pelvic measures?

MATERIAL AND METHODS

The study was based on the archive data of the Department of Radiology, "Gaiļezers" Hospital, Latvia. The measures were based on the pelvic images performed in the period from October 2009 to November 2010. Archive data were available according to legal requirements. The research sample included 122 females aged from 18 to 84 (the mean age=48.1, SD=18.3 years) and 176 males aged from 18 to 82 (the mean age=43.6, SD=16.1). Exclusion criteria were bones' fractures, osteoporosis, scoliosis, and polytraumas.

Three-dimensional multiplanar reconstruction was performed on 1.25 mm slices. The anteroposterior diameter and the transverse diameter of each plane were detected.

(1) Diameters of the inlet: the anteroposterior diameter – the distance between the posterosuperior border of the pubic simphysis and the promontory of the sacrum; the transverse diameter – the maximum distance between iliopectineal lines;

(2) Diameters of the midplane including biacetabular: the anteroposterior diameter – the distance between the posterior midpoint of the pubic simphysis and the border of the second and the third sacral vertebrae; the transverse diameter (biacetabular) – the distance between the middle of acetabulums;

(3) Diameter of the midplane including bispinous: the anteroposterior diameter – the distance between the lower border of pubic simphysis and anterior fourth and fifth sacral vertebrae; the transverse diameter (bispinous) – the lowest distance between two ischial spines;

(4) Diameters of the outlet: the anteroposterior diameter – the distance between the lower border of pubic simphysis and the tip of coccyx; the transverse diameter (bituberous) – the maximum distance between the two internal points of ischial tuberosities.

Indexes of proportions in each plane were calculated as the ratio of the anteroposterior and the transverse diameter. The data analysis was performed using PASW 18.0 program in order to compute descriptive statistics, the Levene's test for the equality of variance, and the *t*-test.

RESULTS

Table 1 demonstrates the descriptive statistics of linear measures, the indexes of pelvic planes, and the inferential statistics concerning the comparison of means and the variances of these measures. All the linear measures of the lesser pelvis represent sexual dimorphism with higher means in females than in males. The most visible differences are observed on bispinous ($t=20.85$, $p<.001$) and bituberous ($t=20.72$, $p<.001$). According to the Levene's test for equality of variances, the variances of linear pelvic measures statistically differ in three dimensions: the transverse diameter of inlet ($F=5.54$, $p<.01$), the anteroposterior diameter of midplane ($F=4.82$, $p<.01$), and the anteroposterior diameter of outlet ($F=17.33$, $p<.001$). For all of them the variance is higher in females than in males.

Table 1. Summary of descriptive and inferential statistics for pelvic dimensions and their variance

	Males (n=176)		Females (n=212)		Levene's test for equality of variances	t-test
Measures	X	SD	X	SD	F	t
Inlet diameters						
Anteroposterior	119.23	10.16	124.18	10.26	0.08	5.39***
Transverse	126.82	7.49	135.08	8.49	5.34*	10.44***
Midplane 1 diameters						
Anteroposterior	127.78	8.98	131.46	9.84	1.26	3.87***
Biacetabular	113.82	7.21	122.23	8.33	2.61	10.63***
Midplane 2 diameters						
Anteroposterior	116.48	7.49	122.52	8.74	4.82*	7.22***
Bispinous	93.64	8.64	112.31	9.27	2.01	20.85***
Outlet diameters						
Anteroposterior	95.97	7.38	99.69	9.78	17.33***	4.22***
Bituberous	103.48	9.33	124.18	10.39	2.30	20.72***
Indexes of proportion						
Inlet	0.94	0.08	0.93	0.10	3.37	–1.54
Midplane1	1.13	0.10	1.08	0.10	0.07	–4.67***
Midplane2	1.25	0.14	1.10	0.10	11.34**	–12.72***
Outlet	0.94	0.11	0.81	0.11	0.02	–11.24***

*** – $p < .001$. ** – $p < .01$.

Indexes of proportions demonstrate that there are no significant differences in the proportions of the inlet plane. The comparison demonstrates that the transverse diameter is higher than the anteroposterior diameter for both sexes. There are also no differences in variance in this plane.

In the midplane, including biacetabular, proportion indexes statistically differ for both sexes ($t=–4.67$, $p<.001$) without differences in their variance. The index is greater than 1.00 in females and males. It

means that the anteroposterior diameter is greater than the transverse diameter. The female pelvic cavity is wider and rounder because the index is closer to 1.00 than in males.

In the midplane including bispinous, indexes are statistically higher for males and females ($t=-12.72$, $p<.001$). This plane demonstrates also statistically significant differences in variance ($F=11.34$, $p<.01$). The index of this plane is greater than 1.00 for both sexes. The anteroposterior diameter is greater than the transverse diameter. This plane has also a rounder shape with a wider cavity and a lower variation in the proportion in females.

The outlet indexes are statistically different ($t=-11.24$, $p<.001$) without differences in variance. The outlet is wider in females. At the same time, the index is less than 1.00, and the transverse diameter is wider than the anteroposterior diameter for both sexes.

DISCUSSION

In general, the results of this study demonstrate significant differences in the variation of linear pelvic measures and their proportions in females and males. The higher variation was detected on the anteroposterior diameter in the midplane of the pelvic cavity including bispinous. At the same time, the proportion in this plane is less variable in females than in males. Higher variation was detected also on the variation in the transverse diameter of the inlet plane and in the anteroposterior diameter of the outlet plane without significant differences in the variation of proportions of these planes.

These findings need to be discussed by taking into account the findings on variation in the anteroposterior diameter of the midplane and the outlet of the female pelvis presented by Tague [9]. In accordance with Tague, higher variability in lower pelvic planes is determined by the effect of relaxin secreted during pregnancy that increases the mobility of pelvic joints. During delivery sacral nutation, the anteroposterior diameter of the outlet can increase by 10 to 20 mm [3].

In line with Tague's findings, the present study demonstrates that the anteroposterior diameters of the midplane and the outlet are more variable in females than in males. In addition, higher variability in the transverse diameter of the inlet was found. This is a new finding that was not observed in previous studies [6, 9, 10]. The observed

differences in the variance of the transverse diameter can be explained by a hormonal effect at the end of pregnancy, which leads to softening of the pubic simphysis. This process allows the pubic bones to move apart 1 cm that increases pelvic diameters [5]. It is possible to consider the effect of relaxin as being the principal determinant to higher variability in lower pelvic planes as also in the inlet.

One more explanation is related to differences in materials under investigation. This study was based on pelvimetry in live humans, but Tague's and Meindl's studies [9, 6] were based on the measurements of skeletal collections without a compensation of pelvic joints (e.g. simphyseal disk).

Another explanation of higher variations in linear measures in females addresses to the human birth mechanism. During delivery the sagital suture of the fetal head is placed in the transverse diameter of the inlet and the anteroposterior diameter of the outlet. It is possible to suppose that this process has an impact on the higher variation in these dimensions. This point requests an additional research because the findings demonstrated higher sexual dimorphism in the anteroposterior diameter of the inlet, the transverse diameter of the midplane, and the transverse diameter of the outlet, which are related to a biparietal deformation during delivery [4].

According to Schultz [7], the pressure of selection on the female pelvis is related to the process of fetal encephalization, which impacted the inlet and the midplane of pelvis. The present study also confirms differences in proportions of pelvic planes. It seems important that the most visible sexual dimorphism is observed in the midplane, whereas the inlet proportions do not differ in males and females. This finding is in accordance with Tague [10], who classified the anteroposterior and the transverse diameters of the inlet as being nondimorfic.

In the present study, the midplane, including biacetabular, demonstrates no differences in the sense of variation. In comparison to bispinous, the biacetabular diameter demonstrates a lower variability in both sexes [9]. This tendency is interpreted as the adaptation to the erectal posture when the weight is distributed along the alae of sacrum and through the ischial tuberosites towards the acetabulum [5]. Therefore, the main adaptation in the human pelvis in this dimension is related to the erect posture and bipedality.

Concerning the midplane including bispinous, the most important finding represents the lower variation of its proportion in females than in males. The observed variation of this index is lower despite the higher variation of the anteroposterior diameter in females than in males. It is possible to suppose that the linear measures of this plane have higher correlation in females (i.e. the higher anteroposterior diameter of midplane is related to the higher bispinous diameter). The mean index of the proportion is 1.08. Therefore, the shape of female pelvis in this plane is near to round. In males, the anteroposterior diameter is less related to the bispinous diameter that results in the higher variation in the shape of the pelvic cavity. According to the evolutionary biology, there is an inverse relationship between the intensity of stabilizing selection and the variance in phenotypes (and genotypes) within a population [8]. Lower variation in the morphology of the midplane – the narrowest plane of the female pelvis – confirms higher selective pressure addressing to the process of human birth.

To sum up, this study demonstrates significant sexual differences in the variability of pelvic dimensions. The suggested focusing on the indexes of proportions was useful in the identification of the differences in the midplane. These results support the view on the intensity of the selection intensity as inversely related to phenotypic variability.

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DISTRIBUTION OF NANOPARTICLES IN THE PREGNANT RAT: THE MORPHOLOGIC AND SPECTROSCOPIC STUDY

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ABSTRACT

The nanoparticles (NP) applications in industry and biomedicine are growing despite of superficial understanding about the mechanisms of NP biological interactions and the possible toxicity. The beneficial NP applications in medicine, as well as potential adverse effects, first of all depend on the NP accumulation and localization in the organism. The human in pre-natal stages is more sensitive to toxic materials than the adult organism and, therefore, it is physiologically protected from harmful agents by the selective transport across the placental barrier. However, there is a lack of studies on the NP penetration through this barrier and the accumulation in foetus.

In this study fluorescence spectroscopy and confocal microscopy methods were employed to investigate the distribution of polyethylene glycol (PEG) coated semiconductor quantum dots (QD) in the pregnant rat model. The main results indicate that QD are systemically distributed in the body and can be found in all the investigated organs, including placenta and uterus 3 h after the intraperitoneal injection. However, QD could not be detected in the foetal tissues (embryo, yolk sac placenta, umbilical cord). The study shows that QD mainly accumulate in the maternal blood sinuses of the placenta and the QD passage to the foetus is prevented by the placental barrier.

***Key words*:** *nanoparticles, fluorescence, imaging, placenta, barrier*.

INTRODUCTION

Nanoparticles (NP) are organic and inorganic substances in the size range of 1–100 nm and humans have been exposed to such particles throughout their history. However, the industrial era dramatically changed sources, doses, and the types of NP. Nanotechnology is a rapidly developing field leading to an increase of engineered NP with conceptually new physical and chemical properties, which might induce novel effects in biological systems [6, 7]. The number of commercial NP-based products in food, cosmetics and medicine is expanding [1, 15]. This phenomena raises concerns about NP accumulation, long-term retention in organism and the forthcoming toxic effects.

Quantum dots (QD) are semiconductor NP in the size of 2–10 nm and they have superior optical properties when compared with organic dyes. QD have broad absorption spectra, bright photoluminescence (PL), they are photostable and can be easily chemically modified for biological functionalization. It was shown that QD can be toxic *in vitro* and *in vivo* and the adverse effects depends on QD physicochemical and environmental factors [3, 7, 10]. However, the biological interaction of NP mainly depend on their size and surface coating [4, 13] and these properties can be easily tuned in the case of QD. It makes QD a perfect model of NP to investigate the fundamental mechanisms of the NP biodistribution and physiological effects.

The studies of the NP penetration through the placental barrier are important because of few aspects. Firstly, is the evaluation of possible embryotoxicity and teratogenicity of nanoagents. Secondly, the NP which do not pass to the foetus could be used as the drug delivery platform during pregnancy to avoid the effect on embryogenesis [11]. Finally, the specialized NP which permeate the placental barrier could be used for embryo-targeted prenatal diagnostics and therapy. By now there are only few in vivo studies on the NP accumulation in foetus and there is a lack of knowledge of general patterns of the NP transport across the maternal-foetal barrier.

In this study we used the methods of fluorescence spectroscopy and confocal microscopy to investigate the accumulation of CdSe/ZnS PEG coated QD in the tissues of the pregnant rat and to evaluate their penetration across the placental barrier to the foetus.

MATERIALS AND METHODS

Animal breading

Albino *Wistar* rats (9–11 weeks old) were obtained from the State Research Institute Center of Innovative Medicine (Vilnius, Lithuania). Animals were housed under the conditions of constant temperature, humidity and the standard light /dark cycle (12 h/ 12 h). Food and fresh drinking water were available *ad libitum.* The study was approved by the Lithuanian Animal Care and Use Committee (No 0019; 2001–2005).

After being acclimated for at least 7 days, female rats were mated overnight with the males of the same strain. Vaginal smears from each female rat were collected and subjected to microscopic examination on the following morning in order to determine the oestrous cycle and the presence of sperm. The day of sperm detection in vaginal smears was designated as day 0 of gestation.

The CdSe/ZnS quantum dots coated with polyethylen glycol (PEG) were used for experiments (Qtracker-655, non-functionalized, Invitrogen Inc.). The stock solution was diluted up to 0.8 μM in saline and injected intraperitoneally on the day 18 of gestation. The animals of the control group were injected with pure saline.

QD pharmacokinetics measurements

The 50 μl blood samples were punctuated from the tail vein before (control sample) and after the QD injection (t: 0.5 – 24 h). In total 4 animals were used for the experiments. The blood was instantly mixed with 50 μl heparin solution to prevent coagulation. The solution was diluted up to 1.5 ml with saline and centrifuged for 10 min at 1500 rcf. The supernatant was used for fluorescence spectroscopy. The relative QD concentration in the blood plasma was assessed by subtracting the autofluorescence spectra and evaluating the PL intensity at the peak. The data from different experiments (n=4) was normalized to the maximum intensity and averaged. The averages were fitted using the biphasic dose response model (1):

$$y(x) = A_1 / (1 + 10^{((b_1 - x) * h_1)}) + A_2 / (1 + 10^{((b_2 - x) * h_2)}), \quad (1)$$

x – independent variable (time), $A_{1,2}$, $b_{1,2}$, $h_{1,2}$ – fitting parameters.

Fluorescence spectroscopy

3 h after injection all the rats were subjected to the Caesarean section in the state of neuroleptoanalgesia (Calipsol 0.5 ml per one rat) on day 18 [8]. The internal organs, including uterus with the formed embryos, were removed. The excised organs were washed in saline and drained.

The fluorescence spectra were measured using the Varian Cary Eclipse spectrometer coupled with the fiber optics module. The blood solution samples were measured using the standard 1 cm plastic cuvettes. The excitation light of 480 nm was used.

Sample preparation and fluorescence microscopy

The internal organs including uterus, placenta and embryo were sectioned using the cryomicrotome setup. The section slides were divided in two groups: untreated and stained with haematoxylin/eosin (HE). The analysis was performed using the Nikon Eclipse TE-2000 fluorescence microscope with C1 confocal scanning system (objectives x10/0.7 and x60/1.4 Plan Apo VC oil). The argon ion laser was used for the excitation light of 457 nm and 488 nm.

RESULTS

Fluorescence spectroscopy

After the intaperitoneal injection, the QD presence in the blood plasma was observed after 15 minutes by the means of fluorescence microscopy. The QD concentration reached its maximum after ~2.5 h and started to decrease afterwards (Figure 1.). After 24 h QD could not be detected in the blood plasma indicating their clearance from blood and/or degradation. The increase is associated to the QD absorption from the intraperitoneal space via the lymphatic or blood vessels. The QD decrease is thought to be mainly determined by the uptake of the reticuloendothelial system and accumulation in tissues [4].

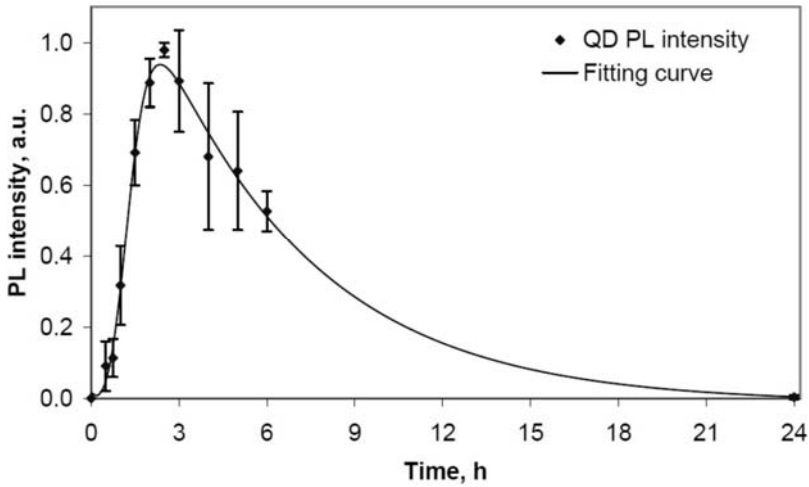

Figure 1. The kinetics of QD concentration in the blood plasma of the pregnant rat after the intraperitoneal injection. The data are represented as the mean ± standard deviation from n= 4 animals.

The QD incubation interval of 3 h hours was selected for the evaluation of QD distribution in tissues as it represents the maximum likelihood to detect QD by means of fluorescence techniques. QD accumulation in tissues was evaluated by means of fluorescence spectroscopy. The characteristic QD PL was seen in the spectra of all the investigated organs, including liver, lungs, muscle, heart, thymus, etc. These results indicate that QD were distributed systemically due to blood circulation.

QD could also be detected in the uterus and placenta tissues (Figure 2.). However QD PL was not registered in the embryonic tissues: embryo, yolk sac placenta and umbilical cord. The fluorescence of the yolk sac placenta surrounding the embryo is mainly addressed to the PL peak at 619 nm which is attributed to the endogenous porphyrins (Figure 3.). The porphyrin accumulation in the pregnant rat was observed earlier [5].

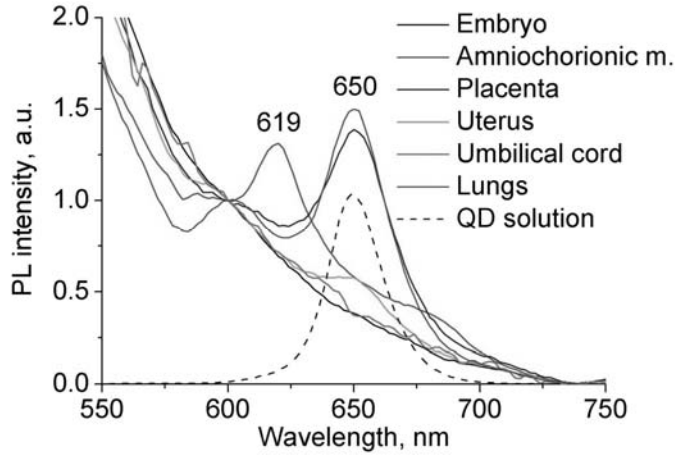

Figure 2. The fluorescence spectra of rat tissues indicating the presence of QD in the maternal tissues (uterus, placenta) 3 h after the QD injection.

Microscopic analysis of tissue samples

The cryotome dissection was used to prepare tissue slides for the microscopy analysis. The prepared samples were described by histological examination using the standard HE staining technique under low magnification (Figure 3). In the sample of placenta the characteristic structures of decidua, junctional zone and labyrinthine zone layers are easily identified.

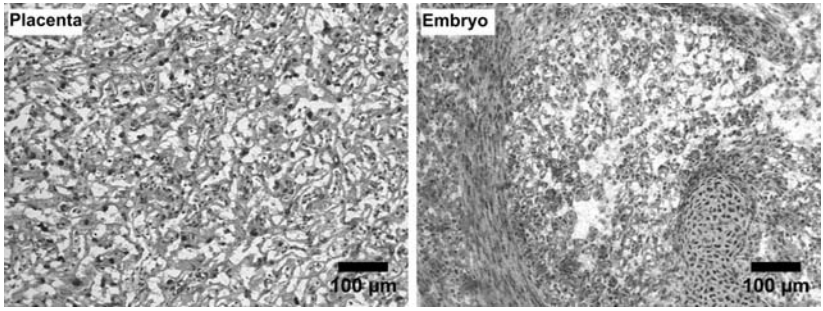

Figure 3. The H&E stained cryosections of the placenta (the labyrinthine zone) and the embryo, objective x10/0,25.

The unstained slides were used to investigate the QD localization. The confocal microscope coupled with a spectral detector enables the discrimination of the QD PL from the tissue autofluorescence with high spectral resolution. This technique revealed that QD are accumulated in the labyrinthine zone of the placenta (Figure 4). QD appeared distributed in the samples not homogenously, but patterned, indicating QD accumulation in the areas with a lower autofluorescence background (represented green). In the labyrinthine zone the maternal blood sinuses lack of endogenous fluorophores which are more abundant in the connective foetal tissue, therefore maternal blood results in lower fluorescence intensity and darker areas in the image when compared with foetal tissue. In this way, QD are mostly distributed in the maternal blood sinuses.

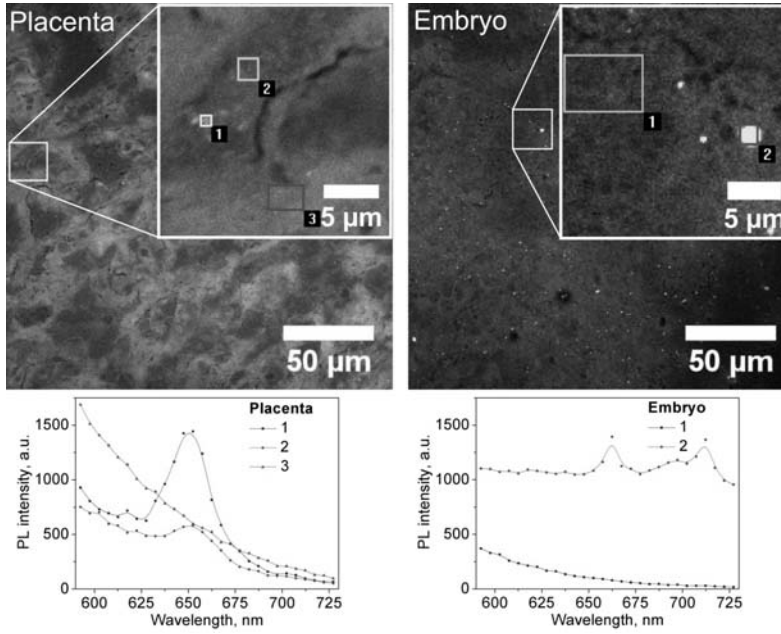

Figure 4. Confocal microscopy image of the rat placenta (labyrinthine zone) and the embryo tissue slides 3 h after QD injection. The patterned red distribution of QD PL (at 650 nm) in the placenta is mainly attributed to the tn the maternal blood sinuses of the placenta. The characteristic QD PL was not registered in the embryo sample, objective x60/1,4.

The examination of the embryo tissues showed no appearance of QD PL in the samples. Some yellowish fluorescent endogenous fluorophores were seen, but the PL spectra were distinct from the QD PL spectra found in the placenta. These results confirm the spectroscopic findings.

DISCUSSION

By now there are only few *in vivo* studies on nanoparticles (NP) penetration through the placental barrier and the forthcoming accumulation in the foetus. Recently Chu M et al. investigated the transplacental transport of thiol-capped CdTe QD in mice [3]. Twenty-four hour after intravenous administration the highest QD accumulation was registered in liver and spleen, while the cadmium concentration in the foetus reached only 0.6% of the injected dose. The fluorescence microscopy technique was unable to detect QD in the embryonic tissues. The surface stabilization with polyethylene glycol (PEG) or SiO_2 decreased QD uptake to the puppies. The embryotoxic effects of QD were found to be dependent on the cadmium dose in the foetus. However, the authors indicate that the performed mass spectrometry assay was not able to discriminate between QD and free cadmium ions, therefore it is not possible to say if QD penetrated the placental barrier or they were degraded in the maternal organism and the formed cadmium ions were transported to the foetus [3].

Another study reported pregnancy complications in mice induced by the silica and titanium dioxide (TiO_2) nanoparticles [15]. The whole-body optical imaging analysis showed the accumulation of fluorescently labelled 70 nm size silica and 143 nm size TiO_2 NP in placenta 24 hours after intravenous NP injection. However the most intense fluorescence for all NP was observed in the liver. Electron microscopy revealed that NP were localized in the trophoblast cells of the placenta, as well as in the foetal liver and foetal brain tissues. The quantitative analysis was not performed. However, the silica NP of bigger size – 300 nm or 1000 nm – were not observed in the placenta or foetuses. The NP induced adverse effects on the embryo development such as growth inhibition, resorptions, placental dysfunction and other functional changes. The observed physiological changes were related to the NP penetration across the barrier and they were not induced using the 300 nm and 1000 nm NP [15].

The lack of *in vivo* studies on NP transplacental passage and the disambiguous results of different groups raise the need for additional investigations on physiological NP effects to embryogenesis. Our results show that fluorescence spectroscopy and confocal microscopy methods can be used to investigate the accumulation and pharmacokinetics of fluorescent nanoparticles in the experimental animals. The main findings indicate that 3 hours after intraperitoneal QD injection they are systemically distributed throughout the organism with blood circulation and can be found in all the organs, including the uterus and the placenta. High QD concentration in blood resulted in the QD appearance in the maternal sinuses of the placenta. However, they were not detected in the foetal tissues using fluorescence spectroscopy and microscopy techniques. It shows that the QD penetration across the placental barrier is highly limited. According to *ex vivo* studies, the dendrimers appear in the foetal compartment already 15 min after the NP addition to the maternal perfusate [11] and their concentration keeps rising up to 6 h. The concentration of polystyrene NP in the foetal compartment was saturated after 3 h of perfusion [14]. These data indicate that the period of 3 h used in our experiments should be sufficient for QD to penetrate across the placental barrier.

It was also shown that the PEG coated gold NP accumulated in the placenta without passing to the foetal tissue [12]. Different *ex vivo* studies on human models showed that the NP penetration through the placental barrier highly depends on the size and the surface coating of the NP [11, 12, 14]. We used PEG coated QD which increases the overall size of the nanoparticles and minimizes the interactions with biomolecules. The increase in size reduces the passive passage of NP through the biological barriers [2] and the NP interactions with proteins are essential for NP cellular adhesion and the forthcoming endocytosis by the trophoblast cells [9, 13]. Therefore the PEG coating could result in the lower QD transplacental passage.

It is worth mentioning, that the QD PL intensity remained stable during fluorescence imaging which is not characteristic of the conventional organic dyes used for fluorescence microscopy. The effect of photo bleaching is one of the main limitations in fluorescence-based methods and it can be overcome using the semiconductor QD.

For conclusion, PEG coated CdSe/ZnS quantum dots distributed systemically in the body of the pregnant rat 3 h after the intraperitoneal

injection and accumulated in all the investigated organs, including the uterus and the placenta. Fluorescence microscopy revealed that QD mainly accumulate in the maternal blood sinuses of the placenta, but they were not found in the foetal tissues. The fluorescence-based investigations show that the NP passage to the foetus is prevented by the placental barrier. Further studies using the particles of different sizes and materials are naturally needed in order to conclusively evaluate the mechanisms of the NP transplacental transport and to extract the knowledge on possible adverse or beneficial effects of the NP application in medicine.

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EVALUATION OF THE CHICKEN EMBRYO CHORIOALLANTOIC MEMBRANE MODEL FOR LARYNGEAL TUMOR TRANSPLANTATION

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ABSTRACT

The laryngeal squamous cell carcinoma is the second common malignant tumor of the respiratory tract and together with recurrent respiratory papillomas represents the most common tumors of the larynx. Many experimental models are used to study the morphology of malignant tumors. The chicken chorioallantoic membrane (CAM) model is one of them. The CAM has all the nutrition needed for the piece of the transplanted tumor to survive. The aim of this study was to investigate whether the laryngeal papilloma and the laryngeal squamous cell carcinoma tissues transplanted on the chick CAM survive with their main histological features, and to determine the morphological changes of the CAM with different transplants. For the preparation of the CAM, fertilized hen eggs were put into an incubator for 3 days. Then the windows in the shell were opened. The fresh samples of tumors were transplanted on the CAM on the 7th day of incubation. After 3 days after transplantation the CAM with onplants were excised and fixed in the 10% formalin solution. Morphological changes in the control CAM and in the CAM with tumor onplants were observed using the digital camera on the OLYMPUS microscope. The results showed that the CAM with the laryngeal squamous cell carcinoma onplant was distinctly thicker than that of the control group and than the CAM with the papilloma onplant; the chorionic epithelium was thickened and appeared stratified of up to 5–6 layers and in some locations squamous keratinized; the mesenchymal cells were densely arranged under the tumor transplants. We observed that morphological changes in the thickness of the CAM and the chorionic epithelium were more obvious in the CAM under the

carcinoma transplants. After 72 hours of the tumor tissue transfer onto the membrane, the tumor cells retained their vitality and also their influence on the CAM tissues could be observed.

***Key words*:** *chorioallantoic membrane, chicken embryo, laryngeal squamous cell carcinoma, laryngeal papilloma.*

INTRODUCTION

Recurrent respiratory papillomas (RRP) represent the most common benign tumor of the larynx, in adults it constitutes about 10% of all the laryngeal tumors or about 87% of all the benign laryngeal tumors [19]. RRP manifest as mucosal, exophytic and benign neoplasms, usually consisting of irregular, multiple and cauliflower-like clusters, which have an intact basement membrane [11, 19]. The patients with RRP experience a different course of the disease. In some cases after the first presentation papillomatosis never recurs. Sometimes it presents with a mild course that recurs rarely. Others experience a severe disease causing outspread papillomas into the trachea and lungs with a possible lethal outcome [3, 9]. Moreover, RRP are associated with some risk (3–12%) of malignant transformation [19]. It is known that human papilloma virus type 11-positive patients show a more frequent incidence of malignisation. The majority of the malignant lesions are laryngeal squamous cell carcinomas (LSCC) [3, 11].

Laryngeal cancer is the second most common cancer of the respiratory tract with an estimated incidence rate of 5.1/100,000 in males worldwide in the year 2008 [15]. The behavior of the tumor and the survival rate in LSSC patients are different. The 5-year overall survival ranges from 0 to 100%, depending on the T- and N- category, the management approach, the tumor location and comorbidities. It is discussed what conditions, tumor characteristics or certain treatment approaches are responsible for the higher survival rates [15].

Consequently, different research models and *in vivo* assays for studying the behavior of laryngeal papilloma and laryngeal cancer tumors are performed. The classical assays for studying angiogenesis *in vivo* include the rabbit ear chamber, the mouse dorsal skin and the air sac, the chicken embryo chorioallantoic membrane (CAM), the iris and the avascular cornea of the rodent eye and the zebrafish [14]. The most

preferable these days are cottontail rabbit and nude mice models to investigate laryngeal papilloma and laryngeal cancer tumors [4, 5].

Nevertheless, the chicken embryo CAM is appropriate and a well-established model to study the behavior of different tumors [1, 2, 8], laryngeal as well. It offers the advantage of being a well vascularized and easily accessible medium, which serves as a gas and nourishment exchange surface. The main advantage of the CAM is quick, economical and a good matrix for the screening of the tumor growth [2, 14, 18]. The system of the CAM provides the cells with an approximately physiological support of nutrients, cytokines, hormones and vascularization as the natural tissue site [1, 8].

The aim of this study was to investigate whether the laryngeal papilloma and laryngeal squamous cell carcinoma tissues transplanted on the chick CAM survive with their main histological features, and to determine the morphological changes of the CAM with different transplants.

MATERIALS AND METHODS

Tissue samples. Fresh laryngeal papillomas (two cases) and two LSCC tissue samples were obtained from the operated patients in the Lithuanian University of Health Sciences Kaunas Clinic. These patients had clinical, histological and/or radiological diagnosis of laryngeal papillomatosis or LSSC. The fresh tumor tissue samples were carried to the laboratory in the isotonic saline solution. They were transplanted onto the chicken CAM in the period of 160 to 168 hours of the egg incubation within 45–60 minutes after the samples were obtained.

Chorioallantoic membrane model

Fertilized hen eggs (*Cobb-500*) were obtained from local the hatchery (Dovainoniu paukstynas, Lithuania) and kept in an incubator at 37.7ºC and 59–60% humidity, with continuous ventilation and while being rotated to and fro. On the third embryonic day (approximately 72 hours of incubation) 20 eggs' shells for each experimental line were sterilized with the 70% ethanol solution and the air chamber was punctured. After drilling the shells with a high speed drill above the yolk with embryo, the oval windows of about 1 cm^2 on the top of the shells were opened and covered with a transparent sterile tape to prevent dehydration and to

permit the observation of embryos. Then eggs were placed back into the incubator without rotation. On the 7th day of incubation the tumor tissue obtained directly from the operation theatre was sliced into approximately 1x2x2 mm pieces and each piece of tumor was transplanted onto the CAM, which was gently traumatized by laying a sterile strip onto the surface of the epithelium and then removing it immediately. After 72 hours after transplantation 2 to 4 eggs were opened. Embryos, if alive, were live-fixed in the 4% formalin solution. The CAMs with the adhering tumor were excised and fixed in the 10% formalin. The eggs incubated under the same conditions and the embryos processed according to the same protocol but without tumor onplants served as controls. After fixation a piece of the CAM with the tumor tissue was cut and embedded into paraffin, sliced 5μm thick and stained with hematoxylin and eosin.

Histological slides were evaluated histologically, and the morphometrical determination of changes in the CAM and the chorionic epithelium thickness was performed using the digital camera on the OLYMPUS microscope and the CellSense Dimentions softwear.

Statistical analysis

Data are given as means ±SD and were analyzed with the MS Excell and SPSS software. The normal distribution of parametrical variables was tested using the Student's *t* test. Results were considered significant at $p<0.05$.

RESULTS

Different types of laryngeal tumors were tested on the chicken CAM. The tumor tissue vitality was evaluated in the histological slides by observing the cells with nuclei and the appearance of the cytoplasm. After 72 hours of the tumor tissue transfer onto the membrane, the tumor cells retained their vitality and also their influence on the CAM tissues could be observed.

Tumor tissue histology

The papilloma consisted of multiple fragile clumps, with a thin and well-vascularized connective tissue core, a thick stratified squamous

epithelium and a continuous basement membrane. The underlying connective tissue had several mononuclear cells. We observed the mentioned structures in the tumor onplants after 72 hours of transplantation, but the mononuclear cells were located among the epithelial cells, close to the CAM and the tissue sample interface. Some loss of intercellular junctions in the papilloma epithelium, which may also be responsible for the tissue fragility, was observed as well. The fragility of the papilloma tissue could cause the detachment of the onplant from the CAM thus papilloma tissue pieces did not adhere to the CAM several times and were lost during the experiments.

The carcinomas consisted of the solid pieces of polymorphous atypical squamous epithelial cells with a large nucleus, prominent one or several nucleoli, abundant acidophilic cytoplasm. The transferred carcinoma tissue samples on the CAM never flowed away and firmly adhered to the membrane.

Morphological characteristics of CAM

The CAM under the onplanted carcinoma tissue was thickened due to the thickening of the chorionic epithelium and the mesenchymal layer under it. The chorionic epithelium was thickened and it appeared stratified of up to 5–6 layers and in some locations squamous keratinized (Figure 1 a, b). The thickness of the chorionic epithelium under carcinoma and papilloma onplants was 43.5±20.2 μm and 15.3±9.7 μm, in the neighboring sites – 30.9±12.7 μm and 8.9±2.6 μm, respectively; and even in distant from tumor sites the chorionic epithelium differed from the control CAM epithelium ($p<0.005$). In our investigation the control CAM epithelium thickness on day 10 was 5.14±0.79μm.

The thickness of the CAM under the tumor onplants of carcinoma and papilloma was 696.6±92.9 μm and 50.9±3.9 μm, at the neighboring sites 429.1±125.4 μm and 51.7±7.1 μm, respectively, $p<0.005$. The thickness of the CAM under the papilloma onplants did not differ significantly from the control CAM, but they were thicker in the neighboring and distant sites from the onplants ($p<0.005$, Figure 2).

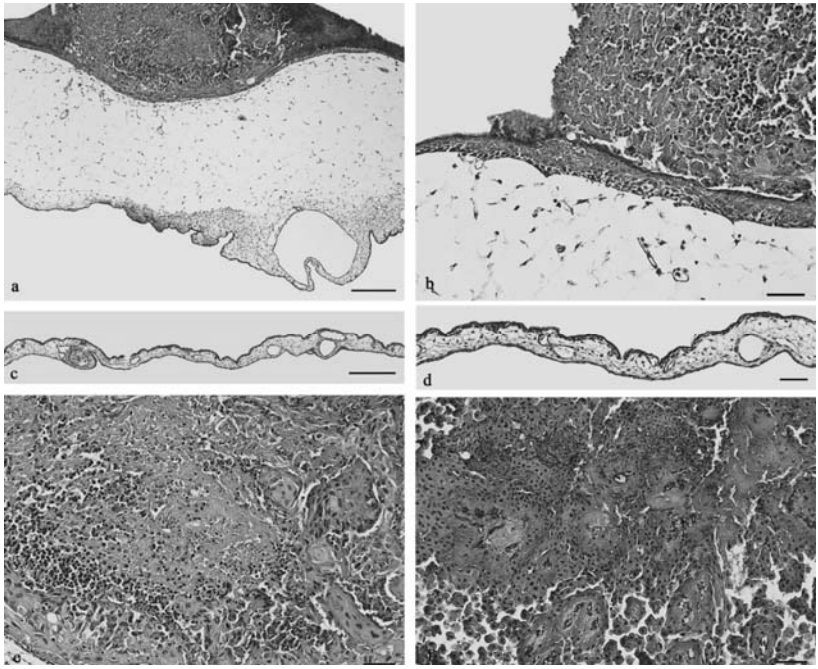

Figure 1. Densely arranged mesenchymal cells (a) and the thickened chorionic epithelium (b) of the CAM with LSCC onplant. The control CAM and its epithelium (c, d). Cells of LSCC (e) and papilloma (f) posses nuclei and vary in size and shape, i.e., retained their vitality after 72 hours of transplantation. a, c – scale bar 200 μm, original magnification 4x; b, d, e, f – scale bar 50 μm, original magnification 10x.

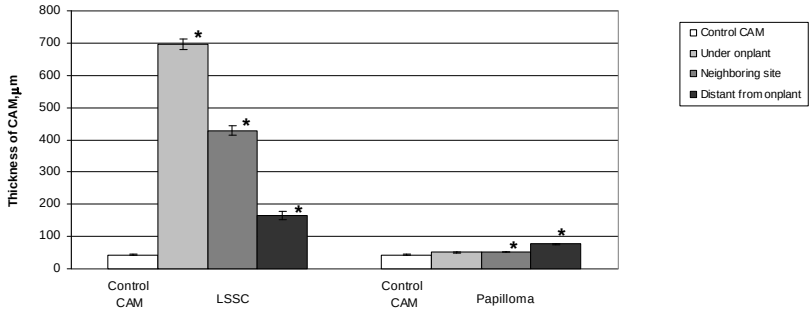

Figure 2. The thickness of the chorioallantoic membrane (CAM) with the onplants of laryngeal squamous cell carcinoma (LSCC) and papilloma (*$p<0.005$ compared to the control CAM).

The capillary network under the chorionic epithelium in the onplanted tumor region (both carcinomas and papillomas) was very rarely distributed, but in the distant sites it appeared normally dense. The large vessels appeared similarly distributed in the experimental and the control CAMs.

We noticed the difference in the arrangement of the CAM mesenchymal cells under the tumor onplants. The mesenchymal cells were densely arranged in the CAM situated under the transplanted papilloma tissue and in the neighboring sites, but in the distant locations did not differ from the control CAM mesenchyme density. Under the carcinoma onplants the mesenchyme was both loosely and densely arranged (see Figure 1a), but in distant from tumor sites it did not differ from the control.

DISCUSSION

A biological model that reflects the physiologic conditions of a solid tumor is needed for effective investigations in the cancer research. Three-dimensional in vitro models include cultured biopsies, and multilayer cultures such as cell clusters and spheroids [10].

The chick chorioallantoic membrane is a very simple extraembryonic membrane which consists of the chorionic epithelium (of ectodermal origin), the mesenchymal layer with capillaries and larger blood vessels

and the allantoic epithelium (of endodermal origin), facing the allantois cavity. By the 10th day of incubation, the CAM comprises the fully developed capillary plexus. Having a rich capillary network just under the chorionic epithelium surface, it provides the developing embryo with oxygen and calcium, and also has been employed as a model to characterize tumor growth because it can provide the transferred cells or onplanted tumor pieces necessary nutrients. The advantages of the chick embryo CAM model include the facts that: embryos can be obtained as pathogen free; until the 18th day of the incubation of tumor with host tissue is not complicated by the reactions of the host's immune system; the ability of tumor cells to traverse the chorionic epithelium and establish contact with the mesoderm beneath can be used as a convenient and easily scored end point for invasion [1].

In this series of experiments we tested a possibility to use a chicken CAM model for laryngeal tumor investigation. The results of this experiment show that both papilloma and carcinoma tumors may survive on the CAM and have an influence on the CAM itself (see Figure 1 e, f). The cells from tumor cell lines are easily transfected to the CAM, but their characteristics as tissue components are already changed, as they lack their natural interaction with one another and with other tissue components. We transplanted the pieces of tumors and observed their vitality after three days of transplantation. This shows that the CAM is able to provide enough nutrition to the tumor tissue that it remains alive. It is known that the local infiltration of the normal tissue by tumor cells and their dissemination to distant sites involve migration through the stroma of the connective tissue as well as the penetration of natural barriers, such as the basement membrane [13]. P.B. Amstrong (1982) noticed that the untraumatized chorionic epithelium is a nearly impenetrable barrier to the cells of invasive tumor lines [1]. Therefore it is very important if the CAM is damaged or not. The papilloma pieces had a tendency not to adhere to the CAM, while the pieces of carcinoma merely adhered to the CAM surface and by 72 hours already performed the influence on the CAM and induced morphological changes. A. Moscona (1959) described keratogenic metaplasia in the chorionic epithelium, which manifested itself by the alteration into the squamous stratified highly keratinized epithelium [12], but this metaplasia was due to the environmental exposure. We observed the keratogenic metaplasia in the chorionic epithelium just

beneath the onplanted carcinoma, but not the papilloma tissue pieces, and this was not observed in the control membranes or distant from the carcinoma onplant sites.

We observed the increased density of mesenchymal cells in the CAM below the tumor onplants. This may indicate that the growth stimuli, coming from the transplanted tumor tissue, can induce the proliferation and accumulation (or grouping) of mesenchymal cells. Although the thickness of the CAM was not much increased in the papilloma experiment, the increased density of mesenchymal cells was observed nearly in the whole thickness of the CAM under the papilloma onplant. We suppose that the increased density of mesenchyme cells and the thickening of the CAM and of the chorionic epithelium is the result of the CAM response to the factors coming from the onplanted tumors; and the differences in the changes of the CAMs with different tumors may depend upon the different behavior of benign and malignant tumors. Further more, each case is unique, and often the laryngeal cancer of the same differentiation and stage takes a completely different clinical progress route. That is why we could observe the different thickness of the CAM and the chorionic epithelium in carcinoma and papilloma experiments. Further investigations have to be performed to determine the factors which are derived from different tumors and their significance for the thickening of the CAM itself and for the thickening of the chorionic epithelium.

The CAM is a highly vascular extraembryonic membrane, which functions as an oxygen and calcium supplying structure; in the chicken embryo it initially appears on day 5 of incubation. From days 5 to 10, the CAM vessels progressively differentiate into capillaries, arterioles, and venules. The future capillaries' cells migrate to a position beneath the ectodermal layer of the CAM (the chorionic epithelium) and form a dense plexus of small vessels. On about day 7, the capillaries begin to migrate outward between the ectodermal cells [7]. According to the data of B.E. Dunn et al, on 10 days of incubation an electron-lucent squamous cell layer covers much of the chorionic epithelium. Intraepithelial capillaries are separated from the chorionic surface by a relatively thick (>5–μm) cytoplasmic layer [**6**].

Vascular network density in the CAM chorionic epithelium remains unchanged during the incubation. We did not notice an evident increase in the major or minor blood vessel density in the CAM in proximity of

the transplants – neither the papilloma, nor the LSCC tumor. On the contrary – this kind of carcinoma might have suppressed the appearance of new capillaries under it in the chorionic epithelium during the first 3 days of transplantation. This may also depend upon the tumor tissue expression of certain genes, which influence the new blood vessel formation and different types of cancers may express different factors; e.g. glioblastoma C6 line cells placed on the CAM in only one day attracted a dense network of blood vessels (our unpublished observation) as well as glioblastoma tissue pieces' onplants induced angiogenesis in the CAM [2].

The results of this investigation allow us to continue the research of different laryngeal tumors and to compare their invasiveness, their developmental behavior in longer experiments, the further influence on the changes of the CAM and its blood vessels. Perhaps it is not worth to prove once again that the intact epithelium is an impenetrable barrier for tumor invasion [1]. As not all papillomas gain a developmental character of a malignant tumor, it would be interesting to investigate the behavior of papilloma tumors and their invasiveness on the CAM, as well as to determine their relation to papilloma virus types, which may be responsible for recurrent laryngeal papillomatosis.

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THE TOOTH SIZE IN THE END OF THE ESTONIAN IRON AGE

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ABSTRACT

Mesiodistal (MD) and buccolingual (BL) crown diameters of all observable permanent teeth were measured in four skeleton series from the Iron End Estonia, total of 254 individuals. Teeth sizes in the End of the Estonian Iron Age were typical of Northern Caucasoids who are mesodontic. All the teeth in the observed group were larger than in the historical skulls from Southern Lithuania and smaller than in historical skulls from Northern Finland. As for adult individuals, the sex was determined and teeth measures were registered separately in men and women. Differences between men and women were calculated. All the men had teeth bigger than women and although these differences were moderate, most of them were statistically significant. Most dimorphic teeth were upper canines (difference between male and female BL – 8.3%, VL – 6.4%) and the discriminant analysis based on upper canine tooth measures enabled correctly classify 88.2% of women and 73.6% of men. Least dimorphic were upper and lower incisors, which did not differ between men and women.

Key words: *the End of the Estonian Iron Age, tooth size, odontometry, sex differences.*

INTRODUCTION

Teeth size (length and width) are most often registered anthropological measures [15]. Teeth are simple to measure with high reliability and data are easy to process because it is distinctive to each population that both men's and women's teeth dimensions are subject to normal

distribution, as it is also common to all adult anatomical measures within one certain population [14].

Archaeological bone collections usually differ from this ideal population with normal distribution, because of the subjects' small number and uncertain derivation.

Teeth have a special meaning in archaeology due to the reason of them being the strongest bone structures left after death and decay. It is also important that once fully formed, teeth's size and form will not change during the lifetime and therefore it is possible to measure and compare all the fully formed teeth crowns. It gives a chance to study teeth morphology in the groups where the age of individuals varies.

It is common in archaeology to use teeth measures to determine teeth size differences between men and women to establish the sex of individuals [5, 17, 18]. The determination of sex on the basis of teeth measures is also used in today's forensic medicine [4], it is possible due to the fact that teeth measures are larger for men than women in all human populations [4, 5, 12, 14, 15, 18]. Also in archaeological bone collections bilateral asymmetry of dental dimensions is measured to estimate the stress level in different populations [14, 15, 16, 22].

Teeth sizes are also measured to compare differences between populations. In some cases it is possible to identify differences between the populations so precisely that it enables to determine belonging of single individuals to different human populations [19]. However, it is usually impossible to distinguish close human populations based on tooth size, because although tooth measures are population specific and under strict genetical control [9], the final size of teeth is determined by many genes and in addition to heritability the environment is also important in the formation of final size of a tooth [9, 14, 15]. The tooth size reflects a complex interaction between a variety of genetic and environmental factors during the morphogenesis [16] and has a continuous range of variations among individuals and between populations. Still, as separate human populations have different teeth sizes and no matter if the cause to that is the environment or genes, it will enable to use odontometry to differentiate human populations by teeth metrical features [6, 13, 15].

Teeth measures have also been changing throughout the time in addition to genetic and environmental affects. It is mostly described by

the teeth size reduction, for example, as it is clearly been observed in Europe since Upper Paleolithic to the modern times [3, 11].

Although it is determined that teeth features are different comparing separate populations, there is also possible the inner population differentiation of groups by measuring the teeth. For groups being men and women. The teeth size is bigger for men in all the population, only the extent of difference varies.

The aim of the current work is to describe the teeth measures of skeletal series from the End of the Iron Age and compare it to neighbouring historical skeletal series. The aim is also to find differences in teeth sizes between men and women, the extent of these differences, and if it is possible to distinguish men and women based on these differences.

MATERIAL AND METHODS

For all the studied permanent teeth, the maximal length or buccolingual (BL) diameter and maximal width or mesiodistal (MD) diameter was measured in four skeletal series (Pada, Jõuga, Karja, Viira) from the End of the Estonian Iron Age (11^{th}–14^{th} cc) [14, 25].

It was possible to measure the permanent teeth of 253 subjects. Teeth were measured with a sliding calliper with the accuracy of 0.1 mm. The teeth with fractured or extremely worn crowns, strong caries or dental calculus, were excluded from the study. Medium sizes of antimeres were used, and only then the individual had one tooth present from two antimeres, the remaining tooth measures were used.

The sex of adult subjects, 192 all together, was determined using conventional skeletal-based methods [7,23]. Teeth sizes were separately registered for 104 men and for 88 women. The percentage of the size difference of men and women was registered.

Both the robustness index (tooth area mm^2) – BL(mm) x MD(mm) and crown module m_{cor}= (BL_{cor} + MD_{cor})/ 2 [14, 25] were used to describe the teeth overall size. The crown index I_{cor} = (BL_{cor} /MD_{cor}) x 100 [14, 25] was used to describe the shape of molars. For comparison skeletal series from Lithuania, Estonia and Finland were used [2, 5, 10, 21, 24].

Statistically significant differences between men and women were found using the Student's t-test. Differences with $P<0.05$ were

considered statistically significant. The discriminant analysis was used to find out if differences in the tooth size can be used for sex determination. The statistical package SPSS was used for data processing.

RESULTS

The descriptive statistics of crown diameters are reported in Table 1. Both mesiodistal and buccolingual measures in maxilla and mandible correspond to the formula $M^1>M^2>M^3$. The molars crown module, reflecting the size of molars crown, was on upper molars $m_{cor}M^{(1-3)}$ = 10.35.

The index showing molars features was as follows:

upper molars $I_{cor}M^1$= 110, $I_{cor}M^2$= 117, $I_{cor}M^3$= 119

lower molars $I_{cor}M_1$ = 92,7, $I_{cor}M_2$ = 94,3, $I_{cor}M_3$ = 92.3

All the teeth sizes there were larger for men than women with the exception of the width of upper incisors and both the width and length of lower incisors. The difference for all the teeth was bigger on the upper jaw. Although the percentage of difference is small, it is still statistically significant. The biggest difference can be seen comparing the width of upper canines of men and women (BL) – 8.3%, t-test $p<0.01$. By using the discriminant analysis with these teeth it was possible to correctly identify 73.6% men and 88.2% women (Table 2).

DISCUSSION

The general pattern in teeth sizes, distinctive to all human groups, are also noted in the skeletal series from the Estonian Iron Age. BL measures are growing distal direction for the upper teeth and from MD diameters of the upper jaw it is the highest for the molars and the lowest for premolars. The lower jaw has similar BL and MD sizes for teeth [14].

Distinctive to Europeans are the crown indexes for lower and upper molars [25].

Table 1. Mesiodistal (MD) and buccolingual (BL) diameters of teeth in the total sample of the End of the Estonian Iron Age, and in men and women

		Total			Male			Female			Diff. Betw. M & W	
Upper jaw		**N**	**X**	**Std**	**N**	**X**	**Std**	**N**	**X**	**Std**	**%**	p
I1	**BL**	108	6.9	0.50	39	6.9	0.45	32	6.9	0.60	0	
	MD	85	8.4	0.59	32	8.5	0.08	23	8.2	0.14	3.5	
I2	**BL**	108	6.1	0.35	41	6.1	0.30	35	6.1	0.36	0	
	MD	86	6.5	0.49	33	6.5	0.45	25	6.4	0.55	1.5	
C	**BL**	126	8.2	0.56	61	8.5	0.47	41	7.8	0.45	8.3	**
	MD	112	7.6	0.45	53	7.8	0.42	38	7.3	0.35	6.4	**
P1	**BL**	107	8.9	0.56	52	9.0	0.48	36	8.7	0.62	3.3	*
	MD	109	6.7	0.38	54	6.8	0.35	36	6.5	0.38	4.4	*
P2	**BL**	103	9.0	0.61	48	9.2	0.58	37	8.9	0.52	3.3	*
	MD	102	6.4	0.41	49	6.5	0.39	36	6.3	0.38	3.1	
M1	**BL**	135	11.3	0.54	46	11.5	0.49	34	11.0	0.52	4.3	**
	MD	135	10.3	0.58	43	10.4	0.58	37	9.9	0.55	4.8	**
M2	**BL**	142	11.2	0.63	56	11.4	0.56	50	10.9	0.49	4.4	**
	MD	145	9.6	0.57	58	9.7	0.53	52	9.4	0.48	3.1	**
M3	**BL**	90	10.7	0.80	46	11.0	0.66	38	10.3	0.85	6.3	**
	MD	88	9.0	0.65	46	9.1	0.64	38	8.8	0.65	3.3	*
Lower jaw												
I1	**BL**	103	5.7	0.41	34	5.8	0.06	28	5.7	0.10	0	
	MD	66	5.3	0.36	16	5.3	0.44	11	5.2	0.38	1.9	
I2	**BL**	107	6.0	0.32	41	6.1	0.35	30	6.0	0.29	0	
	MD	90	5.8	0.38	31	5.8	0.35	24	5.8	0.42	0	
C	**BL**	121	7.6	0.55	59	7.8	0.43	35	7.2	0.48	7.7	**
	MD	112	6.8	0.47	52	6.9	0.5	33	6.4	0.5	7.2	**
P1	**BL**	111	7.6	0.55	54	7.7	0.48	38	7.5	0.52	2.6	*
	MD	115	6.7	0.39	56	6.8	0.38	40	6.6	0.4	2.9	*
P2	**BL**	104	8.0	0.55	53	8.1	0.77	38	8.0	0.87	1.2	
	MD	106	6.7	0.49	54	6.8	0.6	38	6.6	0.9	2.9	
M1	**BL**	117	10.2	0.53	41	10.3	0.49	24	10	0.56	2.9	*
	MD	114	11.0	0.59	39	11.0	0.65	22	10.6	0.49	3.6	*
M2	**BL**	102	9.9	0.51	49	10.1	0.48	32	9.6	0.39	4.9	**
	MD	103	10.5	0.59	47	10.6	0.55	47	10.6	0.55	2.7	*
M3	**BL**	74	9.6	0.65	44	9.8	0.58	28	9.2	0.46	6.1	**
	MD	78	10.4	0.75	48	10.6	0.64	28	10.1	0.84	4.7	*

MD – Mesiodistal and BL – buccolingual diameters (in mm), M – the mean values and STD – standard devations.

Differences between men and women – T-test, value of p (statistical significance of differences) * – $p \leq 0.05$; ** – $p \leq 0.01$

Table 2. The summary of statistics of canonical discriminant functions for tooth size differences between male and female. The most dimorphic teeth BL and MD diameters

Tooth	Sex	Wilks' Lambda	X^2 for covariance homogenity	Can. correlation	% corr. classified
Upper canine	M	0.556	49.27	0.666	73.6
	F				88.2
Lower canine	M	0.603	40.47	0.630	82.7
	F				80.6
Upper M2	M	0.775	25.49	0.474	69.8
	F				80.0
Upper M1	M	0.792	16.29	0.456	70.7
	F				68.8
Lower M2	M	0.791	17.31	0.457	68.9
	F				68.8

The crown module for upper molars stays between the range of 10.2–10.49, they can therefore beheld as mesodontic, the latter is distinctive to the present day Northern-Caucasoids [25]. Southern-Europeans have the smallest teeth or are microdontic in the present day, most macrodontic are equatorial groups, native Americans and arctic Mongoloids [13, 25]. Between these two groups, most of the Asian groups, sub-Saharan Africans but also Northern-Europeans are positioned mesodonts [13, 25].

Most varied teeth are usually the third molars [18, 21]. The same is also true for the group examined by us. Mandible sizes were more variable than maxilla sizes.

Subjects from the Estonian Iron Age have all quite similar sized teeth compared to skeletal series from neighbouring areas. All the teeth are smaller only in the first millennium Lithuanian comprehensive series. Figure 1 shows the robust indexes of teeth from the upper jaw which represent the overall size of the teeth from the neighbouring skeletal series. Size relations on the lower jaw are rather similar.

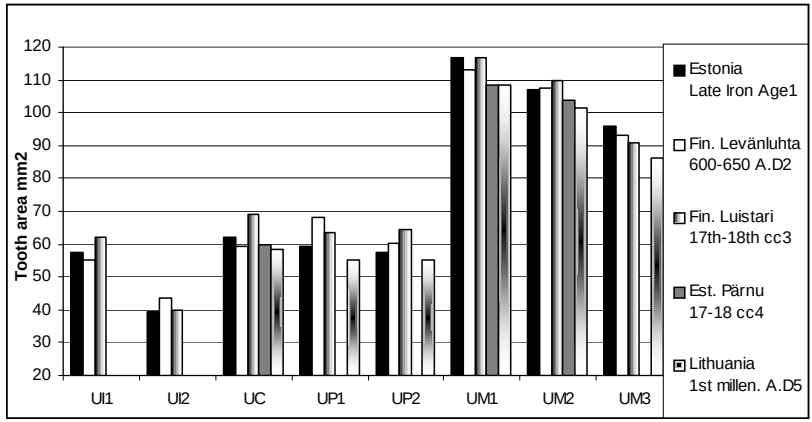

Figure 1. Tooth sizes (tooth area – BLxMD mm^2) in the upper jaw in different skeleton series compared to the End of the Estonian Iron Age (2 – Formisto 1993, 3 – Salo 2005, 4 – Allmäe, Limbo 2008, Papreckiene, Cesnys 1983).

The size of the teeth has been in continuous reduction since Pleistocene [3, 11, 13, 15, 25]. The cause for this has been a natural selection. The smaller teeth indicate the adaptation with the decreased energy demand and with smaller jaws and the smaller body size [15]. It is also thought that the increased population density causes teeth reduction [13]. The Probable Mutation Effect proposed by Brace and colleagues suggests that the development of sophisticated food preparation techniques and pottery removed the selective pressure being in favour of larger teeth and as the size became selectively insignificant, it allowed the reduction through the accumulated effect of random mutations [8, 13, 14]. Therefore the populations with a longer tradition of food preparation should show larger reduction in the teeth size [13]. The latter have been considered to be the cause why the east and the west of Eurasia have smaller teeth sizes compared to the rest of the world [13]. While the so-called meat eating populations have preserved larger teeth [24], the teeth sizes of early modern skeletal series from Pärnu, the town from Estonia, show negative secular changes in teeth sizes, both for men and women from the town of 16^{th} –17^{th} centuries, had smaller teeth than in the End of the Iron Age [2].

At the same time it is noted that the teeth sizes remaining the same [5] or even increase [15] in same areas over the time. Temporal increase in the tooth size is possibly reflecting improvements in the nutritional status [15]. The teeth size increase has been noticed in Europe after the Middle-Age in the regions with substantial nutrition changes [11]. But in addition to that, environmental factors have caused the teeth size increase. It has also been assumed that changes in the genetic constitution played its role [8].

The men's teeth are larger than the women's almost in all the human populations. The tooth size dimorphism differs from population to population, both in the percentage of dimorphism and in dimorphism patterning [12]. It has been noticed that some present day human populations have few dental dimensions statistically significantly lager in women than in men [4].

Humans have reduction not only in the teeth overall sizes but also the reduction of sexual dimorphism starting from Pleistocene, the greatest dimorphism staying in canines dimensions like in other primates [11]. Sexual dimorphism extends 3–9% in the canine size for modern day humans [15], it is especially larger for the upper canine BL sizes. BL sizes tend to be more affected by dimorphism than MD dimensions altogether [1]. The least difference can be seen in the dimensions of incisores of men and women. The same applies to the Estonian skeletal series form the End of the Iron Age.

The discriminate analysis is the most frequently used method for the determination of the subjects' sex by teeth dimensions. The correct classification has been made in over 90% of cases with the determination by different teeth dimensions [5, 15, 18]. The results of the analysis are population specific and tooth dimensions and discriminant formulas that are applicable in one population can not be used in others. The dimensions of canines [5, 17, 24], especially BL dimensions [1], are most preferable data for the discriminant analysis. Although the best results for the studied series were not achieved by using only the BL dimensions of canines but the BL and the MD dimensions of the upper canines (Table 2).

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CORRELATSION BETWEEN ANTHROPOMETRICAL VARIABLES AND BODY SURFACE AREA

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ABSTRACT

The goal of the present study was to investigate correlation between the body surface area created by various formulas and other anthropometric measurements.

The subjects of the present investigation were 17-year-old conscripts of the town of Tartu and Tartu County.

In all of them height, weight, 33 anthropometric variables and 12 skinfolds were measured. The measurements were made according to the recommendations of Martin (Knussmann, 1988).

The body surface area was calculated by five different formulas.

There was significant correlation between the body surface area and the other anthropometric variables.

***Key words**: correlation analysis, anthropometrical variables, body surface area.*

INTRODUCTION

In the second half of the 20th century anthropologists from Estonia Tiik [1] and Kaarma [2, 3] in their studies were interested in applying the correlation analysis in physical anthropology. It was shown that there is significant correlation between the weight and the other anthropometrical variables and also between the height and the others anthropometrical variables [1, 2]. In this situation Kaarma made an

essential novelty corollary and named the height and weight as leading variables among all the investigated anthropometrical variables. In the studies of Kaarma also the body surface are was used, but there we did not find any investigations of the correlation between the body surface area and the other anthropometrical variables.

The goal of the present study was to investigate the correlation between the body surface area and the others anthropometrical variables.

The second goal of the study was to investigate the difference of the mean results of the body surface area calculated by various formulas in 17-year-old conscripts.

MATERIAL AND METHODS

The subjects of the present study were 739 seventeen-year-old conscripts from the town of Tartu and the Tartu County. Measurements were taken of each subject in all 47 anthropometric variables. Total body weight was measured with Soehnle digital scale with precision of 0.05 kg. During the anthropometric investigation the rules of Martin (Knussmann 1988) [4] were followed. Height measurements included eight variables: height, suprasternale height, processus xiphoideus height, umbilical height, symphyseal height, acromiale height and height of anterior superior iliac spine.

Breadth and depth measurements were as follows: biacromiale breadth, chest breadth and depth, waist breadth, bicristal diameter, elbow breath, wrist, femur and bimalleolar breadth. Abdomen depth was measured between umbilicus and processus spinosus columnae vertebralis lumbalis on horizontal plane.

Circumferences were as follows: chest, waist, neck, hip, arm relaxed and arm flexed and tensed, forearm, wrist, upper thigh, calf and minimum ancle circumference. Pelvis circumference was measured laterally at the level of the iliac crests. Midthigh was measured in the middle of distance between spina iliaca anterior superior and upper crest of patella. Head circumference was measured superior to the eyebrow line and encompassing the occipital protuberance. Skinfolds were measured as follows: chin, chest, midaxillary, suprailiac, supraspinale (the fold was picked up three-four centimeters above the anterior superior iliac spine on a diagonal line going downwards and inwards), subscapular, abdominal, biceps and triceps, femoral, calf and dorsal

surface of right hand. In skinfolds measuring recommendations of Lohman et al. [5] and Heyward and Stolarzcyk [6] were also followed.

All anthropometrical variables were measured on the right side.

Sternal length was calculated as suprasternale height minus processus xiphoideus height.

Abdominal length was derived as processus xiphoideus height minus symphyseal height.

Trunk length was calculated as suprasternale height minus symphyseal height. Upper limb length was calculated as acromiale height minus dactylion height.

Lower limb length was calculated as sum of the heights of anterior superior iliac spine and symphyseal height

For predicting the body surface area several different formulas are recommended.

In 1916 Du Bois and Du Bois [7] measured in nine individuals the body surface area directly using molds. From these results they generated a formula to predict body surface are using height and weight alone.

We used the following variant of the formula BSA (m^2) = 0.007184 x height (cm)$^{0.725}$ x weight (kg)$^{0.425}$.

The second formula was generated by Haycock [8]: BSA (m^2) = 0.024265 x height (cm)$^{0.3964}$ x weight (kg)$^{0.5378}$.

The third formula was produced by Gehan and George [9]: BSA (m^2) = 0.0235 x height (cm) $^{0.42246}$ x weight (kg) $^{0.51456}$.

The fourth formula was calculated by Boyd [10]: BSA (m^2) = 0.0003207 x height (cm) $^{0.3}$ x weight (grams) $^{(0.7285 - (0.0188 \times \mathrm{LOG}\ (\mathrm{grams}))}$.

The fifth formula was recommended by Mosteller [11, 12]: BSA (m^2) = ([Height (cm) x Weight (kg)]/ 3600)$^{0.5.}$

The data were processed by the SAS for Windows version 6.12 software. The level of significance was set at $p < 0.05$.

RESULTS

The results are presented in Tables 1 and 2.

Table 1. Correlations between anthropometrical variables data and body surface area calculated by five authors formulas of 17-year-old conscripts

No	Variable	Dubois and Dubois	Hay-cock	Gehan and George	Boyd	Mos-teller
1.	weight (kg)	956	987	985	991	980
	height and segments (cm)					
2.	height	684	572	584	549	608
3.	sternum length	315	296	298	290	302
4.	abdomen length	236	200	204	192	211
5.	trunk length	529	474	480	461	491
6.	upper limb length	579	459	492	481	524
7.	lower limb length	556	454	508	432	486
	breadths and depths (cm)					
8.	biacromial breadth	664	642	645	636	650
9.	chest breadth	647	672	670	676	666
10.	waist breadth	688	737	733	744	724
11.	bicristal breadth	566	562	562	558	564
12.	chest depth	652	682	680	686	674
13.	abdomen depth	654	717	711	727	700
14.	femur breadth	535	543	543	543	542
15.	ancle breadth	523	504	506	498	510
16.	elbow breadth	524	516	518	513	519
17.	wrist breadth	458	440	442	436	447
	circumferences (cm)					
18.	head circumference	563	556	557	554	559
19.	minimal neck circumference	752	793	790	799	783
20.	chest circumference	818	865	861	872	853
21.	waist circumference	770	834	828	844	817
22.	pelvis circumference	806	855	850	862	842
23.	hip circumference	846	882	879	887	873
24.	proximal thigh circumference	838	889	884	898	876
25.	midthigh circumference	771	816	812	823	804
26.	calf circumference	777	822	818	829	810
27.	ancle circumference	691	723	721	728	715
28.	arm circumference	771	835	829	846	818

29.	forearm circumference	739	785	781	793	774
30.	wrist circumference	713	736	734	739	731
	skinfolds (mm)					
31.	chin skinfold	510	570	564	580	554
32.	chest skinfold	598	661	654	670	644
33.	midaxillary skinfold	647	717	710	728	698
34.	suprailiac skinfold	645	737	731	746	720
35.	supraspinale skinfold	614	678	672	688	661
36.	abdominal skinfold	653	718	712	728	700
37.	subscapular skinfold	652	718	711	728	700
38.	biceps skinfold	510	567	561	576	551
39.	triceps skinfold	638	698	692	708	681
40.	thigh skinfold	616	669	664	677	654
41.	calf skinfold	610	661	656	668	647
	indices					
42.	body mass index	759	844	836	859	821

In Table 1 the correlations between the weight and the body surface area are given, they are very strong. The correlations between the height and the body surface area are a little weaker. All correlations are significant.

Table 2. Mean and SD of body surface area calculated by five authors formulas of 17-year-old conscripts

No.	Formula	Mean ± SD m^2	Difference significance – p
1.	Du Bois and Du Bois	1,866±0.16	
2.	by Haycock	1.848±0.02	0.396
3.	by Gehan and George	1.837±0.17	0.500
4.	by Boyd	1.847±0.18	0.499
5.	by Mosteller	1.852±0.18	0.436

In Table 2 the mean and SD values in m^2, which are calculated by five author's formulas are given. Comparing these results, using the paired sample t-test, there was no significant difference ($p>0.05$).

DISCUSSION

The present investigation showed that in the material of the 17-year-old conscripts of the town of Tartu and the Tartu County there are really the correlations between the body surface areas calculated by five different formulas and other anthropometrical variables of the body. Thus it is demonstrated, that not only the height and the weight and the body mass index, as it was shown our previous study[13], but also the body surface area calculated by height and weight is well correlated with other anthropometric variables of the body in the 17-year-old conscripts.

The body surface area is used for the adjustment of the drug dose [14, 15] and of the dose of dialysis in children and adolescents [16].

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BODY COMPOSITION AND THE SOMATOTYPE OF EUROPEAN TOP ROLLER SPEED SKATERS

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ABSTRACT

The purpose of this study was to examine the differences between the body types of roller speed skaters. We started from the question of whether there is a roller speed skating type and which may be the most important constitutional conditions.

To this end, the authors of this work made use of the constitution typologies of Conrad, Knußmann, Tittel and Wutscherk, Parnell, Heath and Carter, and the proportion figures. The skin fold thickness measurement, the bioelectrical impedance analysis, the active substance-body and body-mass index were additionally involved in determining body composition.

A total of 45 people were surveyed: the most successful male and female European roller speed skaters. Of these, 22 were sprinters and 23 long-distance speed skaters. The average age of the sprinters was 22.5 years (sd = 3.5 years) and long-distance speed skaters 22.3 years (sd = 2.6 years). For comparison, 49 students with an average age of 20.5 years (sd = 3.5) were used as control group.

According to the typology of Knußmann, roller speed skaters may be classified as leptomorph. The students in the control groups and the sprinters tend to a larger and heavier body growth on average than the long-distance speed skaters.

From the gathered data an athletic body weight stands out significantly ($p \leq 0.001$) lighter in speed skaters compared to the control groups. Within the study group the long-distance speed skaters show the lowest body weights, followed by the sprinters and the control groups.

The present study suggests that a European roller speed skater of the elite class is built smaller, more athletic and lighter than the comparison subjects of the collective. But, of course, there will always be found the

athletes who do not comply with the found body data, yet they are successful in European or World Championships. Based on the collected data, there are clues to a particular type of constitution in roller speed skating.

Key words: *Sports Anthropology, Sports Anthropometry, Inline Speed Skating, Roller Speed Skating, Speed skating, Body Composition, Body Fat.*

INTRODUCTION

Roller skating is a popular trend sport worldwide. Derived from the ice skates, roller skates have spread all over the world in the nineties. Roller speed skating, a form of roller sports, is a popular as well as a competitive sport. Every year, European and World Championships are being conducted in this sport, and the titles are redistributed every time. But what makes a roller speed skater successful? What are the best conditions to maximize individual performance? Is there a relationship between athletic performance and physique? Top performances in sports can only be provided if the athlete is able to achieve the physical, mental and social conditions and combine them with advanced training.

The central question of this study is to what extent there is a roller speed skating type and which might be the important constitutional requirements for such a type. Furthermore, the question will be expanded to see if there are type-dependent differences within the disciplines of competition.

Not only the results could be of great importance for talent scouting, they could also make an important contribution to the optimization of training management and planning. Individual deficits in body composition could be identified and treated.

METHODS

For this study sports anthropometrical data of the most successful European roller speed skaters were collected. For this purpose, 45-line speed skaters (m = 26, f = 19) were compared with 49 subjects in the control group (m = 28, f = 21). The speed skaters were subdivided by successes in short or long-distance, long-distance skaters or sprinters.

The entire collective consists of 94 participants, divided into 54 male and 40 female. The youngest respondent was 18 years old and the oldest was 32 years old. The average age was 21 years. All the subjects volunteered for the anthropometric study and presented their data anonymously for this work.

The anthropometric study took place at the European championships in Speed Skating, July 29 through August 8, 2009 in Ostend, Belgium. All the male and female roller speed skaters were exclusively national team athletes from Europe. The fact that all the athletes were in a competition period ensured that they were in the best possible training conditions. Only the data of the best European skaters (Top 8) was used. Among the subjects, there were multiple European and World Champions. Depending on the discipline and success, the athletes were divided into long-distance skaters or sprinters.

ANTHROPOMETRIC MEASURES

The anthropometry data in this work correspond to the international standard [10, 11] and were completed by the authors of this study. The provisions of body weight and bioelectrical impedance analysis (BIA) of the subjects were measured on a calibrated scale. Heights and arm span were measured with the anthropometrical measurements. Length and width dimensions were measured with the small and large calipers. To determine the body circumferences, a commercially available tape measure was used. The skinfolds were measured with a caliper of the company Siber Hegner. The results were statistically checked by means of ANOVA.

Knußmann [8], with the help of the discriminant analysis, developed an objective method of determining body types for the leptomorph-pyknomorph primary set of variations of Conrad. The exactly defined landmarks after Knussmann [8] were conduced as the foundation of the examined and calculated measures.

Conrad developed a checkerboard-like coordinate system in which he contrasts the typology of a hyper sculptor (athletics) with the hypoplastic (asthenic). He combines the hypoplastic-hyperplastic variation series with the leptomorph-pyknomorph set of variations [14, 15].

Parnell [12, 13] and Heath and Carter [6, 7], developed a three digit index, with which they exactly describe the somatotypes. They divide the human physique into three body types: ektomorph, mesomorph and endomorph, each of which individually is divided into seven different degrees of severity. The first number of the triple describes the fat factor (endomorphy) of the subjects. It is calculated by Parnell [12, 13] from the sum of skinfolds (mm) from HFF triceps, subscapular-HFF, HFF-suprailiacal and age. The second figure of the triple shows the combined muscle and roughness factor (mesomorphy) and the third a factor of linearity (ektomorphy).

RESULTS

The observations of the body data of all the tested groups are summarized in Table 1. The long distance roller speed skaters show the smallest percentage of body fat (BIA, Caliper), height, mass, BMI and Broca Index data of all participants for gender specific and investigation-specific data. The control groups and the sprinters tend on average to a larger and heavier body growth than the long distance skaters.

According to Knußmann the body typognose of the male and female roller speed skaters can be classified as leptomorph (Figure 1, 2). It can be assumed that the long-distance skaters tend to leptomorphy more than the control group. In the second set of variations (macrosomia / microsomia) all the subjects show some macro values. It seems that the long-distance skaters tend to be the smallest subjects, while the sprinters appear slightly larger. The largest bodies tend to have the people of the control group as they have the highest data on average. The factor category of all the subjects affects the first set of variations (leptomorphy / pyknomorphy) very significantly and to the second set of variations (microsomia / macrosomia) highly significant.

According to the constitution typology of Conrad all male and female long-distance skaters in Figure 5 are exclusively on the leptomorph side. The athletes tend to the metro-dimensional leptomorph direction.

Table 1. Results of body composition data of all the examined groups

	Long Distance male	Short Distance male	Control group male	Long Distance female	Short Distance female	Control group female	p
(n)	12	14	28	11	8	21	n.s.
Age (yrs)	21.7 (2.3)	23.0 (3.9)	21.3 (3.8)	23.0 (2.7)	21.6 (2.6)	19.5 (2.9)	n.s.
Height (cm)	174.8 (5.6)	177.3 (5.9)	180.4 (5.9)	159.9 (3.9)	166.0 (5.0)	169.6 (7.0)	n.s.
Mass (kg)	68.9 (6.3)	73.2 (7.5)	75.7 (10.0)	52.8 (4.8)	58.3 (2.1)	64.8 (13.1)	≤ 0.001
BIA-Fat (%)	8.0 (1.9)	8.1 (3.0)	15.3 (6.9)	17.7 (4.0)	20.8 (2.3)	24.5 (5.7)	n.s.
Caliper-Fat (%)	11.0 (2.1)	9.7 (2.0)	17.8 (6.5)	17.5 (2.2)	17.6 (1.0)	24.8 (4.9)	n.s.
Broca Index	92.0 (5.4)	94.7 (6.1)	94.6 (14.2)	98.0 (7.5)	98.5 (6.1)	103.2 (15.2)	n.s.
BMI (kg/m²)	22.5 (1.3)	23.2 (1.5)	23.3 (3.4)	20.6 (1.5)	21.2 (1.0)	22.4 (3.4)	n.s.

The results of this work show that the somatocharts that were found have similar results according to Parnell or Heath and Carter (Figure 3, 4). The results by Heath and Carter show that the male speed skaters tend more to the ecto-mesomorphic somatotype. The female roller speed skaters are in the central area, with a slight tendency to the endo-mesomorphic direction. It turns out that women are generally more endomorph and less mesomorph than men.

Compared to the younger people of the control group, the considerations of the typology of Conrad and the somatocharts of Parnell, and Heath and Carter showed no significant differences on average, but they tended strongly in the direction of the typognose by Knußmann.

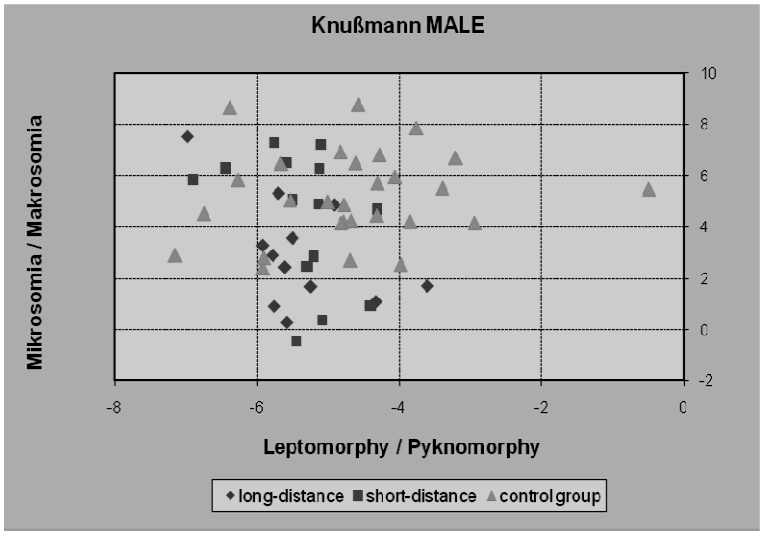

Figure 1. System of the male constitution types after Knußmann.

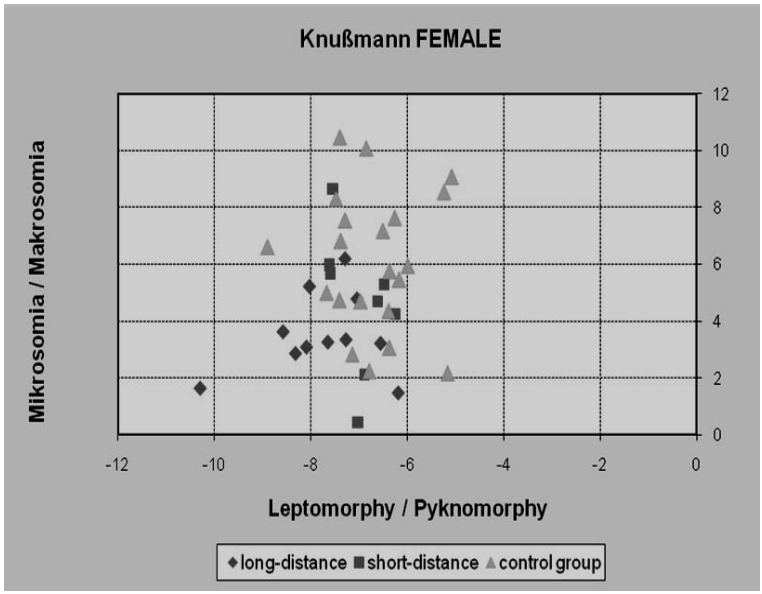

Figure 2. System of the female constitution types after Knußmann.

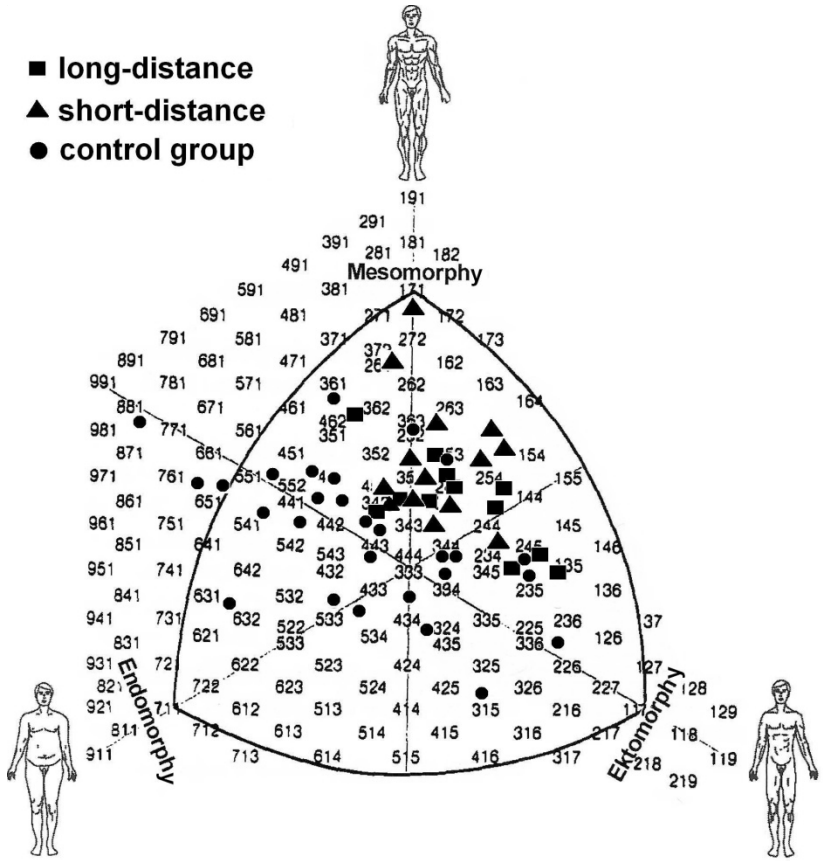

Figure 3. Somatochart after Heath and Carter of the male group.

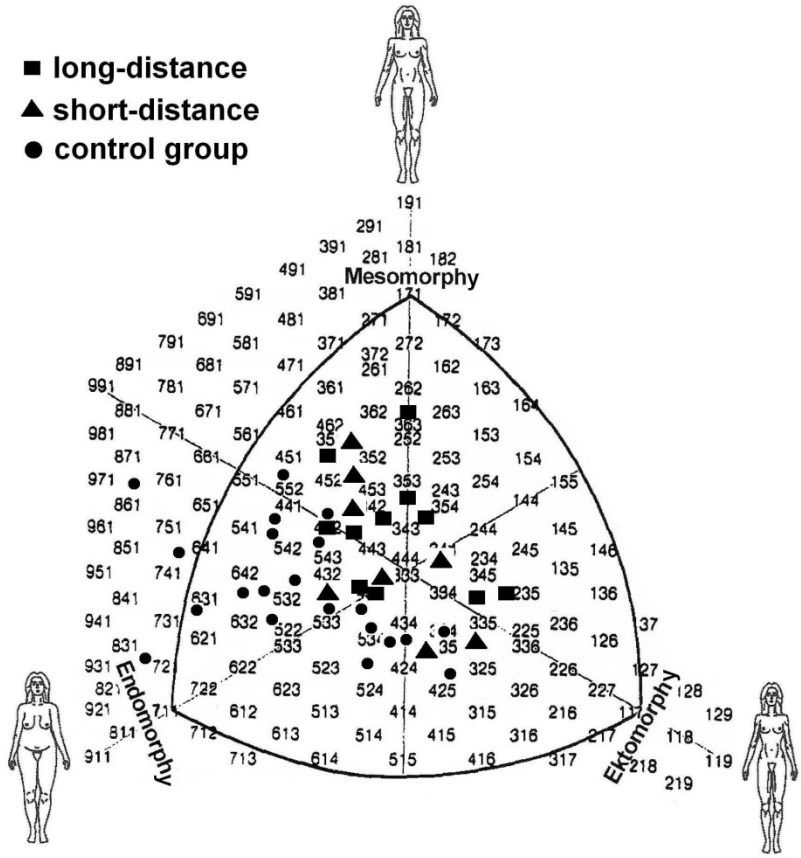

Figure 4. Somatochart after Heath and Carter of the female group.

A = long-distance male **D =** long-distance female
B = short-distance male **E =** short-distance female
C = control group male **F =** control group female

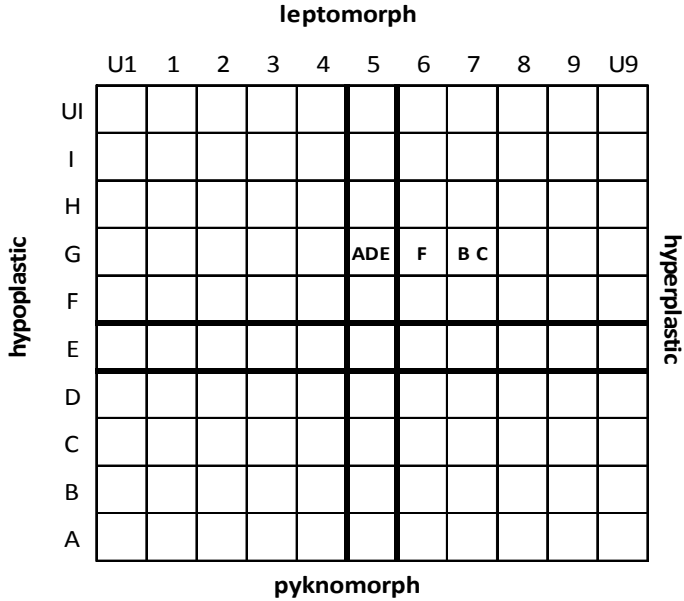

Figure 5. Chessboard pattern graphic after CONRAD with the average of both genders and disciplines.

DISCUSSION

The results of body height show a tendency to the effect that the most successful European roller speed skaters in 2009, are smaller on average than the control groups and the men larger than the women. The average height is 174.8 cm in the male long-distance skaters, 177.3 cm in the sprinters and 180.4 cm in the control group. Among the female long-distance skaters women have an average height of 159.9 cm, the sprinters are slightly larger with 166 cm and the subjects of the control group are with 169.6 cm the largest. The results are not significant

($p > 0.05$), but it is possible that the average European roller speed skater tends to be smaller than the normal European citizen.

Highly significant differences in body weight were displayed ($p \leq 0.001$) among the various categories. The male and female long-distance skaters show the lowest weight, followed by the sprinters and then the subjects in the control groups. In male subjects the long-distance skaters weigh 68.9kg on average, the sprinters 73.2 kg and the control group 75.7 kg. In the female category the long-distance skaters are the lightest with 52.8 kg on average, followed by the sprinters with 58.3 kg. The subjects in the control group have the highest body weight 64.8 kg. The men are heavier and taller on average than the women.

Among the speed skaters the long-distance athletes tend to have the lowest height and weight. This may be related to the requirements of the athlete. A long-distance skater who is to contest in an endurance distance on a 200 m track has to be able to move as economically as possible. The centrifugal forces in a curve are higher on the body the heavier the athlete is.

The larger and heavier the athlete, the more weight he has to move and the more force he has to raise per push. In the sum of a distance a large and heavy speed skater has to spend more energy than a smaller and lighter athlete. Bernhard & Jung [1] confirm the assumption that for the same physical strain a large runner requires up to 25 per cent more energy than a smaller one. If we transfer this to roller speed skating, a larger athlete has to train more for the same amount of power than a smaller one. Otherwise his competitive performance might very likely be lower.

A short statured roller speed skater has agility benefits in the speed of movement and action. Among the speed skaters it is extremely important that they will not only adapt to situations quickly and react to them, but also that they can perform quickly. On the other hand, a taller speed skater may have benefits on a road course. Because of the longer legs the athlete can increase his phase of pushing. Consequently, he does not have to take as many steps as a small speed skater and would thus save energy.

Because of the results of the fat data (BIA, caliper), it is to assume that the strength training important for the speed skating sprinter leads to adaptation with a reduction of subcutaneous fat and increased muscle

mass [18]. For the long-distance skaters the slightly higher fraction of fat needs not be a performance-limiting reason.

Like a sprinter, a long-distance skater has to be able to move quickly, but because of the longer race distance he has more time to get to high speeds. A sprinter has to reach his top speed in no time [2], so too much subcutaneous fat would be dead weight and thus counter-productive in both categories. Possibly because of a lack of movement and fitness, the people in the control groups show higher data, although there are exceptions.

Among the female subjects both categories of women speed skaters show almost identical fat values on average. This suggests that the female roller speed skaters show no difference in fat content and possibly in the requirements.

By the results after Knußmann it can be assumed that the speed skaters tend more to leptomorphy than the control group. The roller speed skater appears rather thin and athletic, which helps him to meet his requirements. The long-distance skaters seem to tend to be the smallest subjects on average (3.0), while the sprinters appear slightly larger (4.3). The male control group tends to have the largest body, with the highest average (5.2). It can be assumed that the speed skater group tends to a thinner and smaller physique compared to the control group.

The female athletes present themselves through their superleptomorph and ultra macrosomic data as athletic, thin and small people, compared to the control group. Here it is clear that in roller speed skating the proportion and size factor plays a role and the athletic training of body shapes has an important meaning for the speed skating performance.

The results according to Heath and Carter support these statements. The requirement of all the speed skaters is characterized by the increased physical strain and sports specific movements in the lower extremities [3, 4]. The average data of mesomorphy of the roller speed skaters reflects the tendency for higher muscle mass. The slight tendency to ektomorphy clearly shows that the skaters, in contrast to endomorphy, are rather narrow and slender.

For men it seems to be important to have a strong and powerful but also light body. Although power increases with additional muscle mass, so does the body weight. The male speed skaters weigh 71.2 kg on average. The results show that the male speed skater tends more to the

ecto-mesomorphic somatotyp. A highly trained mesomorphy appears to be less limiting for sprinters than for long-distance skaters.

For the long-distance skaters it is important both to be light for the endurance, as well as possessing enough power and speed for intermediate and final sprints. For the required pace hardness in international competitions it is crucial to meet these requirements. The body constitutions may limit these benefits, so that no large and heavy long-distance skaters can successfully establish themselves among the international leaders.

The average of both male speed skating categories is located in the ecto-mesomorphic range. The female roller speed skaters tend to have a slight tendency in the endo-mesomorphic direction. It is not safe, however, to determine a specific somatotype with them, because the female athletes, as opposed to men, are distributed among all the areas of the somatocharts.

From this it can be interpreted that for women the human physique needs not be a limiting factor for the success in roller speed skating. Obviously, a higher portion of subcutaneous fat is not giving them any substantial disadvantage. It turns out that women are generally more endomorph and less mesomorph than men. The reason for this, according to Gualdi Russo et al. [5], lies in the female genetics, which by nature are directed to gender specific functions. Furthermore, the endomorphy data correlate negatively with physical fitness [7]. This might also be the cause for the data of the control groups.

By results according to CONRAD, all the long-distance skaters and even the female sprinters are mainly on the leptomorphic side. The long-distance skaters show up as lean, muscular types of medium height. These constitutional features are probably also related to the requirements in the category of athletes. For a speed skater, it is important not to have a high body weight, because he often has to race long distances and in the curves of a track he will be confronted with the law of centrifugal force [9]. Radius, speed and weight determine the centrifugal force of the curve of routing. The small, slim and light body structure meets the requirements of the long-distance skater and proves to be an advantage in practice and competition. The requirements of good female long-distance skaters seem to coincide with that of the sprinters. Most female athletes take part in both competitions, in the short as well as in the long distances.

With the same leptomorphy shape, the male speed skating sprinters and the control group are both in the average of an increasingly hyperplastic type. It is expected that the athlete is built a little stronger and taller on average. Body mass and the size do not seem to be as limiting under competitive conditions and training requirements as with the long-distance skaters. Nevertheless, the sprinters have to move more mass in competition or training, but in a shorter distance and exposure time.

Comparing the male study groups among themselves, it is striking that the groups do not differ greatly on average. The sprinters are in the same coordinate field (G 7) with the control group and next to them are the long-distance skaters (G 5). The speed skaters have manifested no significant differences in the coordinate field. In this study sample according to CONRAD no clear type of a speed skater can be revealed.

As a result of the present study it can be assumed that a European roller speed skater of the elite class is built smaller, more athletic and lighter than the comparison subjects of the collective. Based on the collected data, there are clues to a particular type of constitution in roller speed skating.

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IMMUNOHISTOCHEMICAL DISTRIBUTION OF IGF-1, bFGF AND THEIR RECEPTORS IN DECIDUAL, EMBRYONIC AND TUBAL HUMAN PREGNANCY TISSUE

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ABSTRACT

Embryo implantation is a complicated process involving mother and *conceptus* cells differentiation, proliferation and invasion that are essential for successful pregnancy. Almost every cell in the human body is affected by IGF-1 that is one of the most potent natural activators of cell growth and multiplication, and also a potent inhibitor of the programmed cell death. bFGF, acting via its receptor FGFR1, is one of the factors involved in mediating the angiogenesis, proteolysis and apoptosis during the implantation. OBJECTIVE: To establish pregnancy inducted difference of appearance of bFGF, IGF-1 and their receptors in the embryonic, decidual and tubal tissue. STUDY DESIGN: In this study 14 tubal pregnancy and 10 decidual tissue samples were evaluated immunohistochemically in order to define the distribution of bFGF, FGFR1, IGF-1, IGF-1R. RESULTS: The Mann-Whitney U test was used as appropriate for the evaluation of significant differences. FGFR1 appearance dominated on bFGF in the decidual ($z=2.539$, $p=0.01$), tubal ($z=2.539$, $p=0.01$) and embryonic ($z=2.539$, $p=0.01$) tissue. IGF-1 and IGF-1R appearance in the decidual, tubal and embryonic tissue was not statistically different. It was the same as IGF-1 and IGF-1R expression in gravid endometrium, but in the ectopic implantation site IGF-1R was particularly absent ($z=1.935$, $p=0.05$), only mesothelium and some epithelial cells stained. CONCLUSION: IGF-1, IGF-1R, bFGF and FGFR1 are widely appearing growth factors in actively developing and

differentiating of the human embryonic tissue during the first trimester. Both endometrial and fallopian tube tissues express more FGFR1 than bFGF that testify the stimulation of compensatory adaptation of the organ during pregnancy. IGF-1 and IGF-1R richly appear in gravid endometrium. IGF-1 is widely distributed in both the mother and the embryo tissues but only some of them are IGF-1R marked in a case of ectopic pregnancy. The deficit of IGF-1R in the fallopian tube might be a result of cell growth restriction and the impaired process of trophoblast invasion.

Key words: *growth factors, implantation, tubal pregnancy, immuno-histochemistry.*

INTRODUCTION

Embryo implantation is a complicated process involving mother and *conceptus* cells differentiation, proliferation and invasion that are essential for successful pregnancy. Only 25 to 30% of conceptions result in a live birth [22]. A significant proportion of pregnancy loss is caused by embryo chromosomal disorders, so more than 50% of the first trimester spontaneous aborts are aneuploid [6, 13, 18]. Embryo implantation failure due to impaired uterine receptivity took place in up to 30% of early pregnancy loss [7]. However, 1–2% of embryo implantations in the world are ectopic [9, 27]. The research of the abnormally implanted embryo had shown no karyotype changes [8, 10], so molecular signalling at the time of blastocyst nidation could probably be the key to the explanation of normal and abnormal implantation [1].

The insulin like growth factor (IGF-1) and the basic fibroblast growth factor (bFGF) are found during pre-implantation and implantation in uterus [3] in addition, these factors and their receptors regulate cellular events of the early embryonic period. As growth factors orchestrate cell growth, differentiation and proliferation during embryogenesis, the aim of our study was to define the appearance of IGF-1, bFGF and their receptors in uterine pregnancy, tubal pregnancy and embryonic tissues.

MATERIAL AND METHODS

The study was performed with the permission of the Ethics Committee of Riga Stradins University (18.12.2007). Human oviduct parts were obtained from 14 patients of Riga 1st Hospital, who had undergone salpingoectomy for tubal pregnancy with informed consent. The human embryo and the gravid endometrium tissue were obtained from 10 patients who had unplanned pregnancy termination in the Riga Medical Centre "Elite" with informed consent. Tissue samples were taken from January 2007 to January 2008. Age, parity, the contraception method, pelvic inflammatory and sexually transmitted diseases episodes, the partner count had been carried out for all the patients.

The tissue samples were fixed in 2% formaldehyde and 0.2% picric acid mixture with 0.1 M phosphate buffer (ph 7.2). Then the samples were rinsed in the thyroid buffer containing 10% sucrose and embedded in paraffin. The tissues were cut into 6-μm-thick sections and were dewaxed with toluene and rehydrated through a graded ethanol series. The sections were stained with haematoxylin and eosin (H&E) using standard procedures to obtain a review picture of the slide.

We used the biotin–streptavidin method for the determination of the basic fibroblast growth factor (FGF basic rabbit polyclonal to bFGF (ab16828), dilution 1:200, *Abcam,* UK); the fibroblast growth factor receptor 1 (FGFR1 rabbit polyclonal to FGFR1 (ab10646), dilution 1:100, *Abcam* UK); insulin-like growth factor 1 (IGF-I goat polyclonal to IGF-1 (MAB291), dilution 1:100, *R&D systems*, Germany); the insulin-like growth factor 1 receptor (IGF-IR mouse monoclonal to IGF-1R (AF-305-NA), dilution 1:100, *RnD Systems*, Germany.

At least five microscopic fields (X200) were analyzed using the microscope Leica DM RB (Leica Microsystems, Germany).

The distribution of these factors was detected semi-quantitatively (0/– occasional positive structure in the visual field, + few positive structure in the visual field, ++ moderate number of positive structure in the visual field, +++ numerous positive structure in the visual field. The data were analyzed by the nonparametric rank analysis with SPSS Statistic 17 software. A Mann-Whitney U test was used as appropriate for the evaluation of significant differences. A p-value <0.05 was considered as statistically significant.

RESULTS

The average patient's age in the tubal pregnancy group was 29.6 years (23–43). This pregnancy was the first for 3 of them (25%). n=6 patients (50%) had a previously documented pelvic inflammatory disease (PID) episode and n=1 had undergone right sided salpingectomy due to the previous ectopic pregnancy. Only one of them used the intrauterine device (IUD) for contraception. The number of partners and the legal abortion count were not significantly different. The average patient's age in the uterine pregnancy termination group was 30.9 years (23–43). This pregnancy was current for all the patients. None of the patients had any documented PID episode or tubal surgery. None of the patients used contraception.

Routine haematoxylin and eosin slides showed tubal mucosal edema. It was typical to see the proliferation of epitheliocytes, the infiltration of lymphocytes and leukocytes as well as capillary stasis. Only the embryonic structure found in any case of tubal pregnancy were chorionic villi binding to the fallopian tube structures or to germ membranes (yolk sac as well). No specific finding, despite decidual endometrium and trophoblast tissue, was seen in the routine uterine pregnancy slides.

The immunohistochemical analysis of tubal and pregnant endometrium tissues resulted in the different appearance of the recurred growth factors (Figure 1).

IGF-1 was widely distributed in the fallopian tube epithelium (Figure 2) but IGF-1R focally stained the apical surfaces of tubal epitheliocytes (Figure3). Despite, epithelium IGR-1R stained only mesothelium and was absent in chorionic structures. Cytotrophoblast, sincytiotrophoblast and extraembryonic mesenchymal cells contained IGF-1 (Figure 4). Peripheral trophoblast focally contained IGF-1 positive cells. IGF-1 as well stained macrophages and neutrophils. Endometrium showed numerous IGF-1R cells, but IGF appeared in epithelium moderately. Trophoblasts contained moderate numbers of IGF-1 cells in a case of uterine pregnancy (Figure 5). Peripheral trophoblast widely expressed both IGF-1 and IGF-1R. Separate connective tissue cells in endometrium demonstrated IGF-1 and IGF-1R immunoreactivity. IGF-1 was widely distributed in both mother and embryo tissues but IGF-1R only in some of them. IGF-1 and IGF-1R appeared in embryonic respiratory, duodenal and mesonephric epithelia (Figure 6, 7). Embryonic *hepar*

contained focally a moderate number of IGF-1 and IGF-1R positive cells. Mesenchymal cells around the future skeleton and *perichondrium* moderatly demonstrated IGF-1 (Figure 8) and IGF-1R positive cells.

FGFR1 appeared both in tubal and decidual tissue. Numerous positive structures were seen in tubal and endometrial epithelia (Figure 9). *Citolemmae* of muscle cells, endotheliocytes, nerve fibers and peripheral trophoblast cells demonstrated immunoreactivity for FGFR1 in both implantation sites. A moderate number of FGFR1 positive structures in extraembrional mesenchyma, citotrophoblast and syncitiotrophoblast appeared (Figure 10). Tubal and endometrial epithelium contained moderately bFGF (Figure 11). A few bFGF positive cells were seen in tubal and endometrial stroma (Figure 12)

The numerous distribution of FGFR1 had been established in sklerogenic mesenchyma, perichondrium, in the proliferating cartilage area and in degenerating *chorda dorsalis* of human embryo. The largest part of the muscle, nerve fibers's plasmolemmae and endotheliocytes contained FGFR1. FGFR1 immunoreactivity concentrated in the spinal ganglions. The epitheliums of skin and its appendages tongue and salivary glands have been positive for FGFR1. The mesothelium of pleura and pericardium has contained FGFR1. Myocardium has been weakly positive FGFR1. bFGF have been shown the same distribution in embryonic tissues as FGFR1 but moderately.

The Mann-Whitney U test was used as appropriate for the evaluation of significant differences. FGFR1 appearance dominated on bFGF in the decidual ($z=2.539$, $p=0.01$), the tubal ($z=2.539$, $p=0.01$) and the embryonic ($z=2.539$, $p=0.01$ tissue. IGF-1 and IGF-1R appearance in the decidual, the tubal and the embryonic tissue was not statistically different. It was the same as IGF-1 and IGF-1R expression in gravid endometrium, but in the ectopic implantation site IGF-1R was particularly absent ($z=1.935$, $p=0.05$), stained only mesothelium and some epithelial cells.

Table 1. The distribution of bFGF, FGFR1, IGF-1 and IGF-1R in tissue

Structure/ Factor	***FGFR***	***bFGF***	***IGF1R***	***IGF-1***	***FGFR***	***bFGF***	***IGF1R***	***IGF-1***
	Tubal pregnancy tissue				Uterine pregnancy tissue			
Epithelium	+++	+	+	+++	+++	++	+++	+++
Myocytes	+++	+	0/–	0/–	+++	+	0/–	0/–
Endo-theliocytes	++	+	0/–	0/–	++	+	0/	0/–
Nerve fibers	++	+	0/–	0/–	++	+	0/	0/–
Mesothelium	++	+	++	++	none	none	none	none
Extra-embryonic mesenchyma	+++	0/–	0/–	0/–	+++	0/–	0/	0/–
Cytotro-phoblast	++	0/–	0/–	+++	++	0/–	0/	+
Sincytiotro-phoblasts	+++	0/–	0/–	+++	++	0/–	0/	++
Peripheral trophoblast	+	+	0/–	++	+	+	+++	++
Macrophages	0/–	+	0/–	+	+	+	++	+
Neutrophils	0/–	+	0/–	+	+	+	++	+

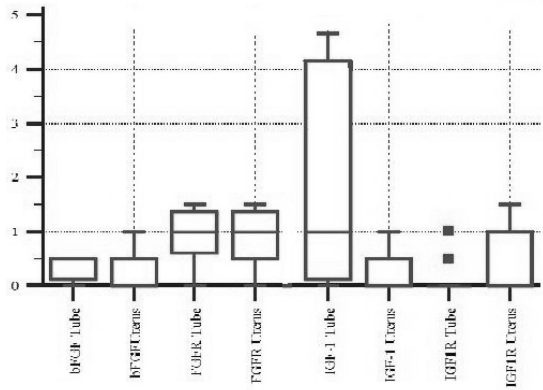

Figure 1. Immunohistochemical distribution of bFGF, FGFR1, IGF-1, IGF-1R in tubal and pregnant endometrium tissues.

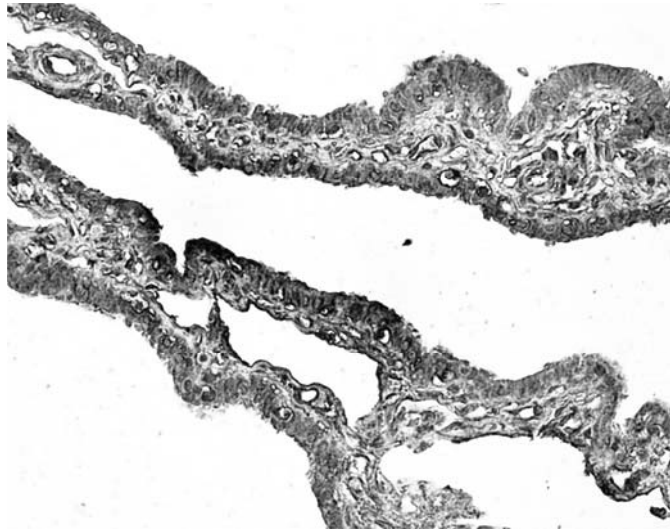

Figure 2. IGF-1 was widely distributed in fallopian tube epithelium. IMH IGF-1 250X.

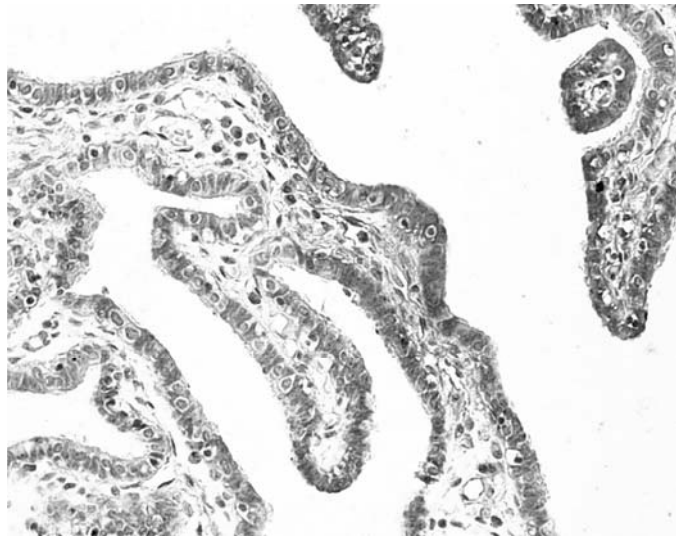

Figure 3. IGF-1R focally stained the apical surfaces of tubal epitheliocytes. IMH IGF-1R 250X.

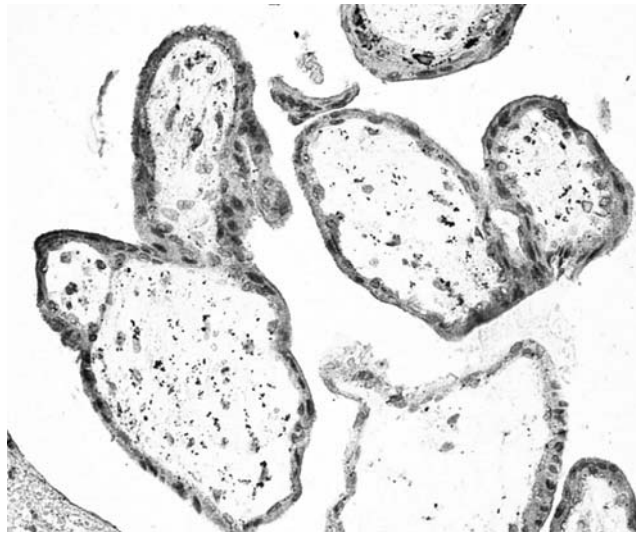

Figure 4. Cytotrophoblast, sincytiotrophoblast and extraembryonic mesenchymal cells moderately contained IGF-1. IMH IGF-1 400X.

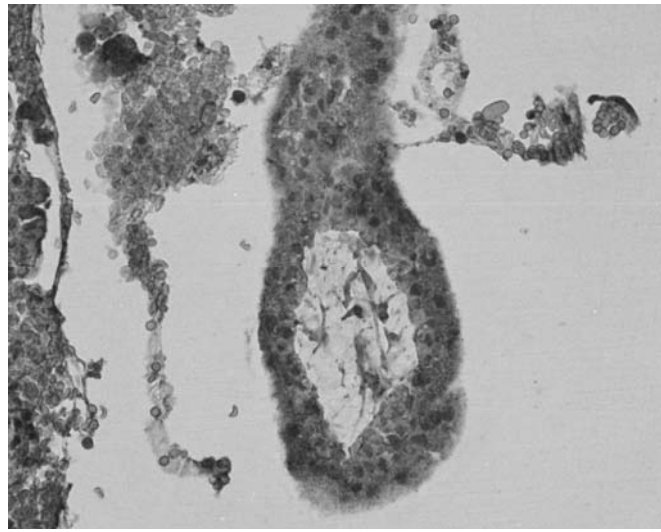

Figure 5. Trophoblasts contained moderate numbers of IGF-1 cells in a case of uterine pregnancy. IMH IGF-1 400X.

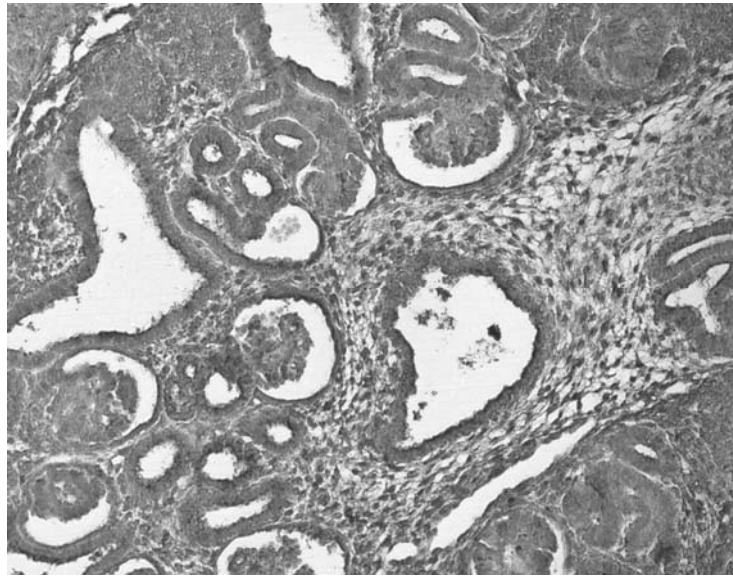

Figure 6. IGF-1 in embryonic kidney. IMH IGF-1 400X.

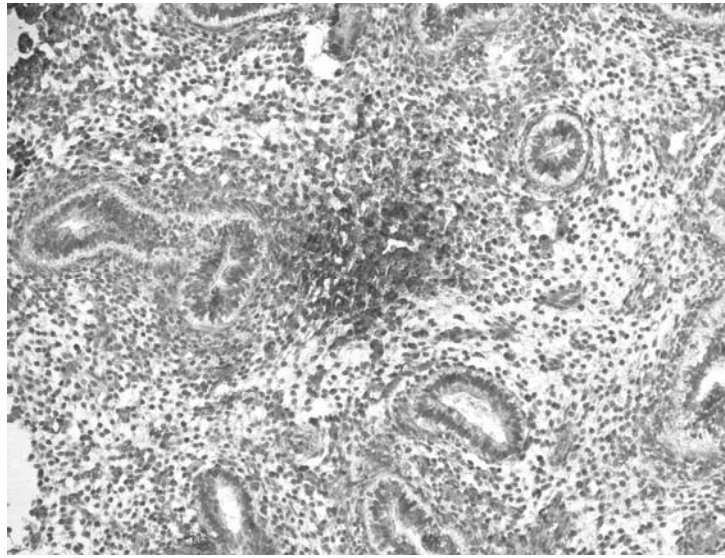

Figure 7. IGF-1 in embryonic lungs. IMH IGF-1 400X.

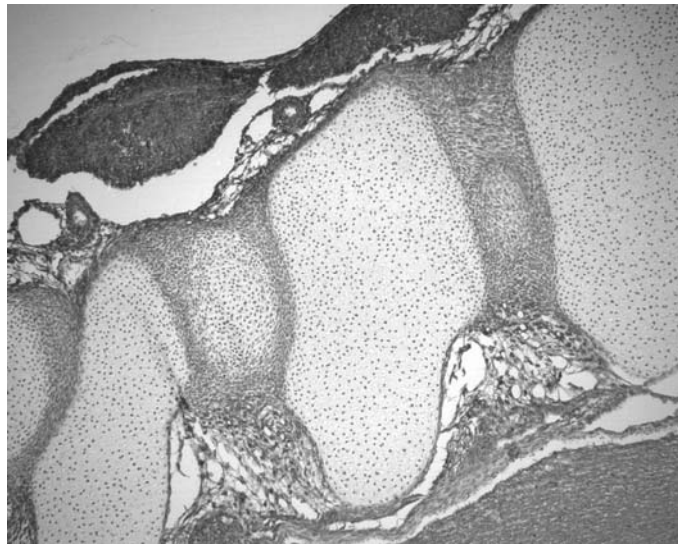

Figure 8. Mesenchymal cells around the future skeleton and *perichondrium* moderatly demonstrated IGF-1. IMH IGF-1 400X.

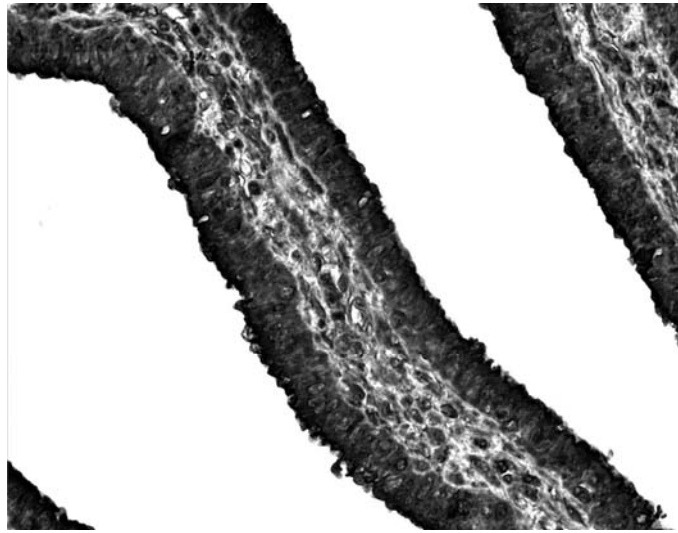

Figure 9. Numerous FGFR-1 positive structures were seen in tubal epithelium. FGFR1 IMH 400X.

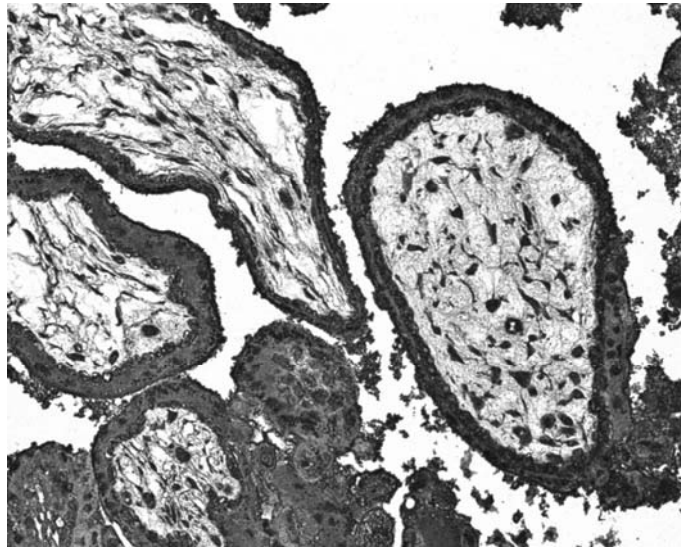

Figure 10. FGFR1 in extraembrional mesenchyma, citotrophoblast and syncitiotrophoblast. FGFR1 IMH 400X.

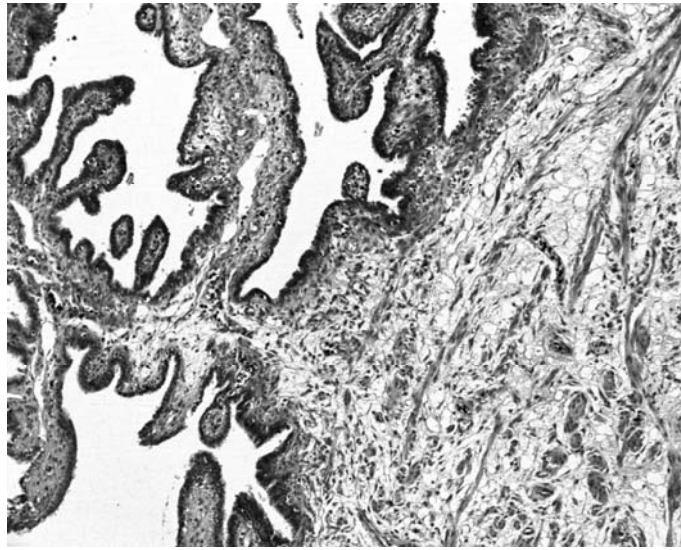

Figure 11. Tubal epithelium moderately contained bFGF. bFGF IMH 250X.

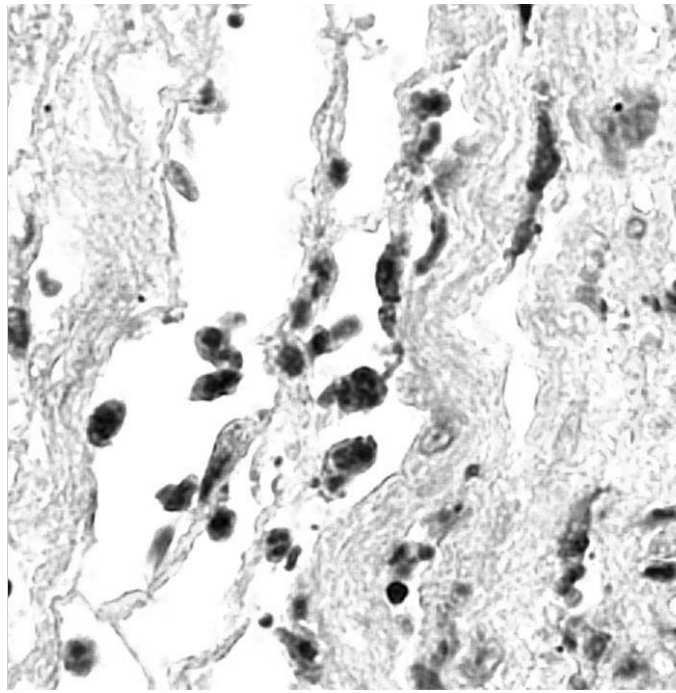

Figure 12. A few bFGF positive cells were seen in tubal stroma. bFGF IMH 400X.

DISCUSSION

bFGF is able to accumulate in the nucleolus where it stimulates ribosomal protein transcription [4]. bFGF is the first factor inducting mesodermal differentiation in *vivo* un *in vitro* and is abundant in the cells of mesenchimal and neuroectodermal origin [31]. The embryonic digestive tract, lungs, kidney, salivary and sebaceous glands, striated and smooth muscles express bFGF [11]. bFGF is one of the factors involved in mediating the angiogenesis, proteolysis and apoptosis during the implantation [21, 33]. bFGF appears in the maternal circulation during pregnancy, with peak values late in the 2nd trimester. The levels of bFGF in the maternal serum correlate positively with the fetal size both in the 2nd trimester and at term [14].

The fibroblast growth factor receptor 1 (FGFR1) is the most sensitive bFGF receptor [26]. The widespread distribution of FGFR1 in multiple mature organ systems suggests an important functional role in the normal human adult tissue [16]. It could be found in the membranes of the most anchorage dependent cells also around and in their nuclei [12, 28]. FGFR1 is a widely expressed membrane receptor of developing of human tissues, including neurons, vascular basement membranes, skin, and bone growth plates. Our previous data showed FGFR1 participation in the regulation of the human embryonic tissue formation [20]. Our findings demonstrated that the tubal, decidual and *conceptus* tissue contained more FGFR1 than bFGF. We speculate that the excess of the receptor is due to the compensatory adaptation of the organ to the gestation process.

Almost every cell in the human body is affected by IGF-1 which is one of the most potent natural activators of cell growth and the multiplication and a potent inhibitor of the programmed cell death [32, 5]. In addition to the insulin-like effects, IGF-1 can also regulate cell growth and development, especially in nerve cells, as well as the cellular DNA synthesis [25, 29]. Overexpression of IGF-1R in cancer cells results in increased invasion and *vice versa* [31]. IGF-1 and its receptor (IGF-1R) are essential for embryo growth and survival. Endometrial decidual cells express IGF-1 gene in the implantation site [19], but IGF-1 gene null mice have been shown to be infertile [2]. Maternal IGF-I stimulates fetal growth by activating the placental transport of nutrients to fetus [17]. In a case of fetus's growth retardation, IGF-1 compensatory increasing had been established [23, 15]. Peripheral trophoblast cells of preeclamptic women demonstrated increased immunoreactivity as well [24]. We discovered IGF-1 and IGF-1R in gravid endometrium. The fallopian tube epithelium, trophoblast and connective tissue cells demonstrated a positive reaction for IGF-1 in our study, but IGF-1R in these structures was particularly absent. This finding may indicate the possible restriction of the cell's growth and the restriction of trophoblast invasion in the tissues affected by ectopic pregnancy. Abundant distribution of IGF-1, bFGF and their receptors in the first trimester embryo developing organ systems suggests their participation in human embryogenesis.

CONCLUSIONS

1. IGF-1, IGF-1R, bFGF and FGFR1 are widely appearing growth factors in the actively developing and differentiating human embryonic tissue in the first trimester.
2. Both endometrial and fallopian tube tissues express more FGFR1 than bFGF that testifies the stimulation of the compensatory adaptation of the organ during pregnancy.
3. IGF-1 and IGF-1R richly appears in gravid endometrium.
4. IGF-1 is widely distributed in both mother and embryo tissues but IGF-1R marked only some of them in a case of ectopic pregnancy. The deficit of IGF-1R in the fallopian tube might be a result of cell growth restriction and the impaired process of trophoblast invasion.

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DISTRIBUTION OF HUMAN B-DEFENSIN 2, TNF-ALPHA, IL-1 ALPHA, IL-6 AND IL-8 IN PSORIATIC SKIN

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ABSTRACT

Psoriasis is a chronic, inflammatory, proliferative condition of the skin, clinically characterized by red, scaly plaques. Psoriasis has a large and heterogenous genetic and immunologic background with the dysregulation of the host defense system. The human keratinocytes obtained from psoriasis lesions are a very rich source to various antimicrobial peptides. Both Th 17 and Th 1 pathways play a role in the pathogenesis of psoriasis. Our aim of the study was to evaluate the expression of human beta defensin 2 and TNF alpha in correlation with interleukins 1 alpha, 6 and 8 in the skin biopsies of psoriatic lesions.

We evaluated 14 *Psoriasis vulgaris* patients' skin samples. Skin biopsies were obtained using a routine punch method. All the tissue specimens were stained with hematoxylin and eosin and by immunohistochemistry for human beta defensin 2, TNF-alpha, IL-1 alpha, IL-6 and IL-8. We graded the intensity of staining semiquantitatively.

We observed intraepithelial lymphocytes, marked the diffuse intradermal infiltrates of inflammatory cells, the inflammatory cells in the hair follicle, surrounding sweat glands and subepithelial blood vessels. Defensin-containing and TNF-alpha positive cells were found in all the skin samples: defensin-containing cells varied from few to abundant positive structures in the visual field and TNF-alpha positive cells varied from few to numerous positive structures in the visual field. IL-1 alpha expressed poorly, while IL-6 positive cells were found in the range from few positive to the abundance of positive structures in the visual field and IL-8 positive structures varied from numerous positive structures to the abundance of positive structures in the visual field.

We conclude that IL-6, IL-8 and TNF-alpha are most common cytokines for psoriatic skin lesions. A moderate number of structures expresses the antimicrobial protein defensin in the psoriatic skin.

***Key words*:** *antimicrobial peptides; cytokines; human keratinocytes; psoriasis.*

INTRODUCTION

Psoriasis is a chronic, inflammatory, proliferative and enormously variable in the extent, duration and periodicity condition of the skin, in which both genetic and environmental factors play an important role. Psoriatic skin lesions are characterized by red, scaly, sharply demarcated and indurated plaques [5]. The classical histological picture of psoriasis includes parakeratosis with neutrophils, a thin granular layer, acanthosis, focal spongiosis, increased mitotic figures, dilated blood vessels in papillary dermis and the perivascular infiltrate of lymphocytes in the early disease course. At a later stage also elongated rete ridges and absent granular layer can be found. Although psoriasis has many histological features, the only truly diagnostic criteria are Munro micro-abscesses and the spongiform pustules of Kogoj [10].

The most common clinical variant *Psoriasis vulgaris* affects 85 to 90% of all the psoriasis patients and is most studied in scientific researches. Psoriasis has a large and heterogenous genetic and immunologic background with the dysregulation of the innate immune system [11]. The human keratinocytes obtained from psoriasis lesions are a very rich source to various antimicrobial peptides such as human alpha and beta defensins, cathelicidin LL-37, psoriasin. The role of the ultraviolet B (UVB) irradiation of vitamin D has been studied and was found to be an important part in the activation of antimicrobial immunity, particularly cathelicidin pathway [1]. T helper (Th) 17 cells have a conclusive role in the host defense and any alterations in its pathway can lead to the inflammatory conditions of the skin such as psoriasis. While interleukin-6 (IL-6) is responsible for the initial Th17 cell differentiation, interleukin-1 (IL-1) possibly finalizes the differentiation [3, 13]. Another key regulator of the innate immune system is tumor necrosis factor (TNF) alpha, which takes part in Th1 cell pathway and has industrially designed antagonists as therapeutic agents [12].

Our aim of the study was to evaluate the expression of human beta defensin 2 (HBD-2) and TNF alpha in correlation with interleukins 1 alpha, 6 and 8 in skin biopsies of psoriatic lesions.

MATERIAL AND METHODS

Patients

Patient selection criteria were created to exclude possible affecting side factors. Our target patient was at least 18 years old, suffering from psoriasis at least 6 weeks, having visible characteristic psoriatic eruptions in typical localization sites, without a fierce tan. All the patients were diagnosed by a dermatologist. We selected fourteen psoriasis patients with the histologically confirmed diagnosis of *Psoriasis vulgaris*. Skin biopsies were obtained using the routine 3 mm punch biopsy method and local lidocaine anesthesia. At the time of biopsy all the patients were off any topical or systemic psoriasis medication for at least one month.

The study was approved by the Ethics Committee at Riga Stradins University, the permit issued on 10 September 2009.

Methods

Skin biopsies were first fixed in the Stefanini's solution, dehydrated and embedded in paraffin. Further, four micrometer thick sections were prepared and stained routinely with hematoxylin and eosin.

The immunohistochemical method (IMH). Human beta defensin 2 (cat No AF 2758, LOT VJU015051, obtained from the goat, 1:100 dilution, R&D Systems, Germany), TNF-alpha (code ab 6671, obtained from the rabbit, 1:100 dilution, Abcam, Cambridge, UK), IL-1 alpha (B7: sc-9983, obtained from the mouse, 1:50 dilution, Santa Cruz Biotechnology, Inc., USA), IL-6 (NYRhIL6: sc-73319, obtained from the mouse, 1:50 dilution, Santa Cruz Biotechnology, Inc., USA) and IL-8 (C-19: sc-1269, obtained from the goat, 1:50 dilution, Santa Cruz Biotechnology, Inc., USA) were used by biotin – streptavidin IMH (Hsu et al., 1981).

Our findings were illustrated using Leica DC 300F camera and the image processing and analysis software Image Pro Plus.

The intensity of immunostaining was graded semiquantitatively:

few positive structures in the visual field were labelled with +,
a moderate number of positive structures in the visual field was labeled with ++,
numerous positive structures in the visual field were labeled with +++,
and the abundance of positive structures in the visual field was marked with ++++.

RESULTS

Intraepithelial lymphocytes and the marked diffuse intradermal infiltrates of the inflammatory cells were observed in all the patients (Figure 1). Similarly infiltration of inflammatory cells were also found in the hair follicle, the surrounding sweat glands and the subepithelial blood vessels. Arteriole sclerosis and sweat gland cell vacuolization were detected, as well as the Munro microabscesses were observed.

Defensin-containing cells varied from few (+) to abundant (++++) positive structures in the visual field (Figure 2). Particular increase of defensin-positive structures was observed in the sites of well defined inflammation.

TNF-alpha positive cells were found in all the skin samples – mainly subepithelium and their number varied from few (+) to numerous (+++) positive structures in the visual field (Figure 3).

IL-1 alpha findings were poor and varied from negative (–) to few (+) positive structures in the visual field (Figure 4), meanwhile IL-6 and IL-8 both showed explicit expression. IL-6 positive cells were found in the range from few (+) positive to abundance (++++) of positive structures in the visual field (Figure 5). IL-8 positive structures mostly varied from numerous (+++) positive structures to abundance (++++) of positive structures in the visual field. IL-8 expressed in epidermis and the connective tissue, inflammatory infiltrates, the hair follicle external root sheet and around blood vessels (Figure 6–8). All the results are summarized in Table 1.

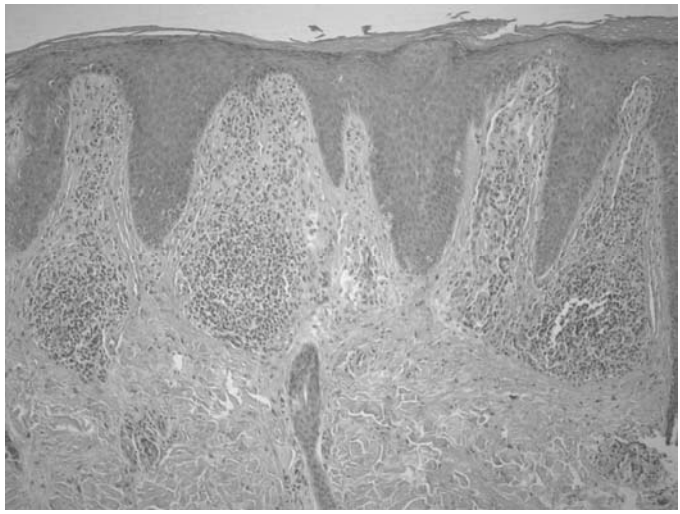

Figure 1. Explicit inflammatory cell infiltrates in the dermal layer of the skin. Hematoxylin and eosin, X 100.

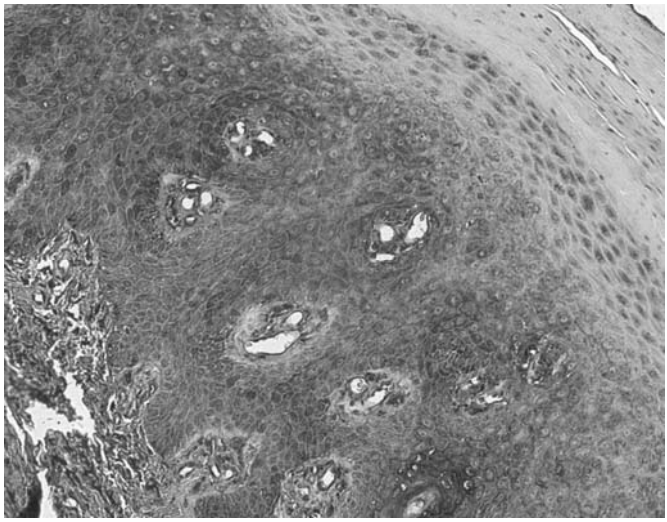

Figure 2. Abundance of defensin-containing cells in the psoriatic skin lesion with a marked border between *stratum spinosum* and *stratum granulosum*. Human beta defensin 2 IMH, X 200.

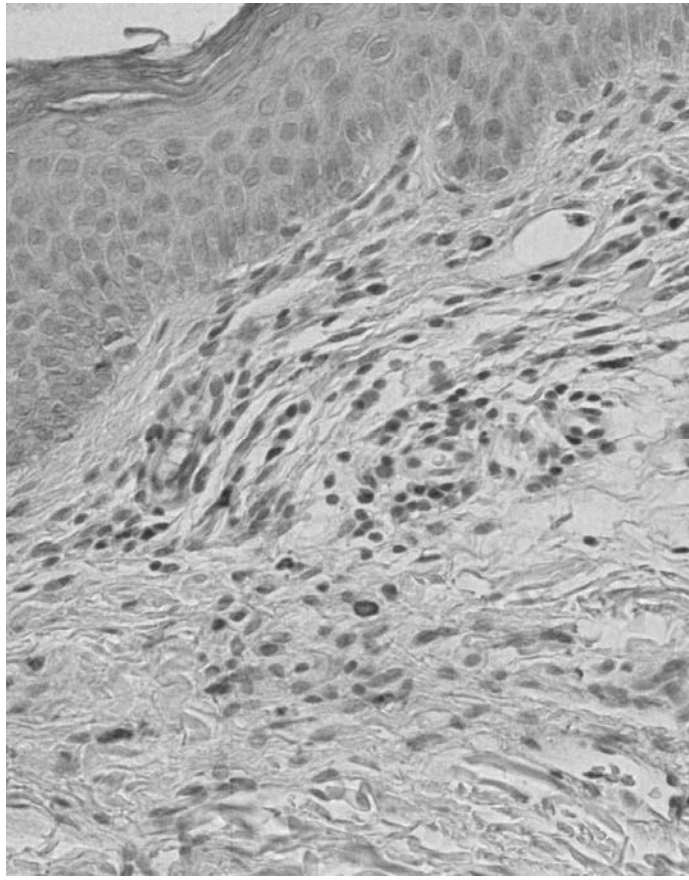

Figure 3. Numerous TNF-alpha positive structures in subepithelium. TNF-alpha IMH, X 400.

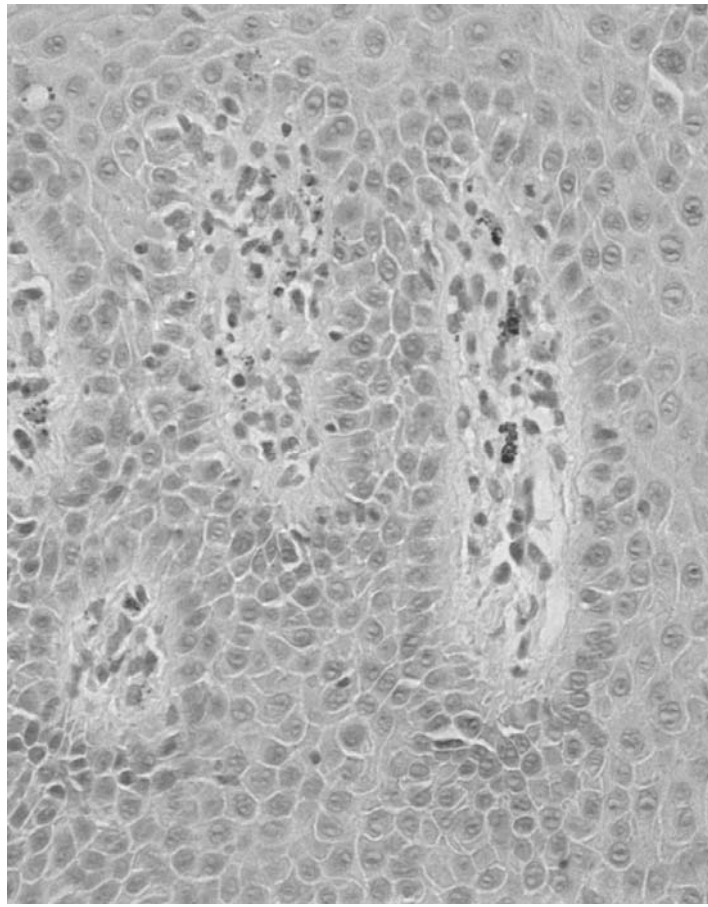

Figure 4. Sparse IL-1 alpha positive cells in the psoriasis patient's skin. IL-1 alpha IMH, X 400.

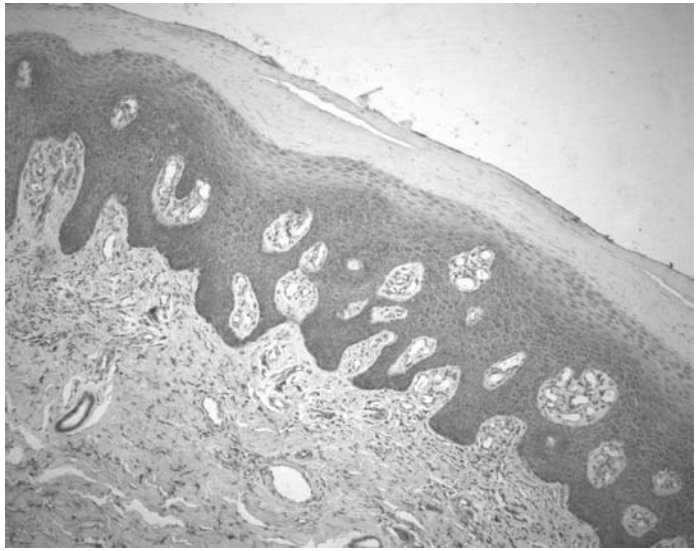

Figure 5. Pronounced expression of IL-6 in the skin from a psoriasis patient with a long progress of the disease. IL-6 IMH, X 100.

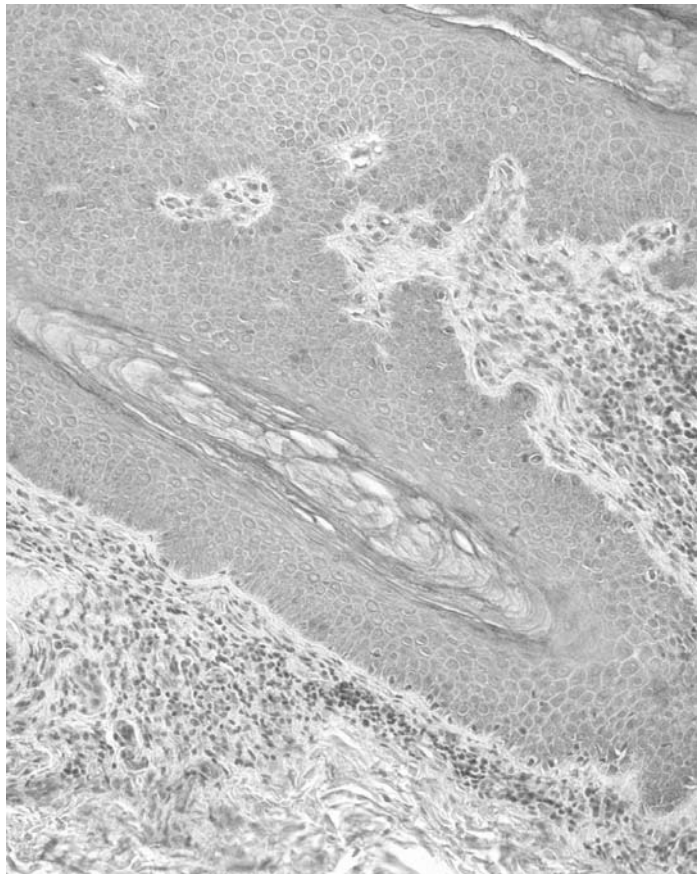

Figure 6. Even distribution of IL-8 through epidermal layer of the psoriatic skin. IL-8 IMH, X 200.

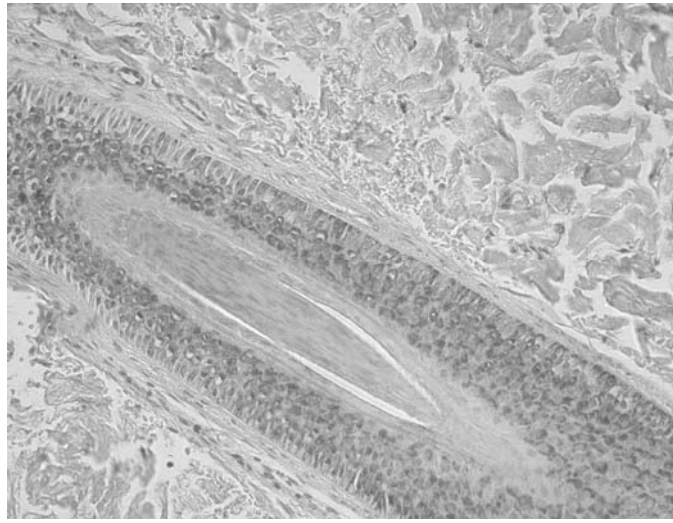

Figure 7. Marked expression of IL-8 in the external root sheath of the hair follicle. IL-8 IMH, X 200.

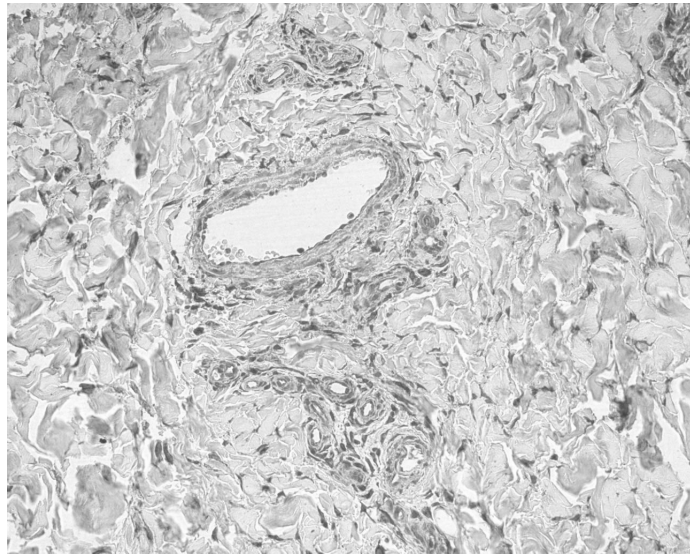

Figure 8. IL-8 positive cells surrounding dermal blood vessels. IL-8 IMH, X 200.

Table 1. Semiquantitatively evaluated expression of HBD2, TNF-alpha, IL-1 alpha, IL-6 and IL-8 positive structures in psoriasis lesions

Patient	Human beta defensin 2	TNF-alpha	IL-1 alpha	IL-6	IL-8
No. 1	++	+	–	+++	++
No. 2	+++	+++	+	+++	+++
No. 3	+++	+++	–	+++	++++
No. 4	+++	+++	+	+++	++++
No. 5	+++	+++	+	++	++++
No. 6	++	+	+	++	++++
No. 7	++++	++	–	++++	++++
No. 8	++	++	+	++	+++
No. 9	+	+++	+	++	+++
No. 10	+	++	–	++	+++
No. 11	+	++	+	++	++++
No. 12	+	+++	–	+	+++
No. 13	+	++	–	++	++++
No. 14	+	++	–	++	+++

To obtain statistical data we used non-parametric statistics and the Spearman's rank correlation coefficient was calculated. A correlation between defensin and IL-1 alpha or TNF-alpha and IL-1 alpha was not obtained. We found statistically significant correlation between human beta defensin 2 and IL-6 – the Spearman's rank correlation coefficient was 0.7745. Between defensin and IL-8 the Spearman's rank correlation coefficient was 0.3629 (weak), while between TNF-alpha and IL-6 (0.0054) and TNF-alpha and IL-8 (0.1123) statistically we could not find a relevant correlation.

DISCUSSION

This study reflects the complicated nature of psoriasis. The information obtained from our study can help fill gaps in the still very incompletely understood pathogenesis of classic psoriasis.

Antimicrobial peptides have been evaluated in many studies regarding various skin conditions. Skin antimicrobial peptides include an enormous group of various host defense agents, such as dermcidin, secreted by eccrine sweat glands, psoriasin S100A7, RNase 7 and

RNases from eosinophils, cathepsin G, bactericidal/permeability increasing protein and related proteins, the secretory leukocyte protease inhibitor, elafin, and trappin-2, eppin, antimicrobial sperm proteins, histatins, platelet microbicidal proteins, kinocidins [14]. In our study we chose to evaluate human beta defensin 2 in the tissue and it expressed in all the skin samples with more pronounced distribution in inflammatory areas. The recent novel study has found a close relation between human beta defensin 2 and inflammatory cytokines in the serum of patients with psoriasis. Human beta defensin 2 acted as a stimulator by enhancing interferon gamma, TNF-alpha, IL-10, IL-1 beta, IL-6 and IL-22 production and regulator by suppressing IL-17 or stimulating IL-10 production [8].

The role of TNF-alpha in the pathogenesis of psoriasis is substantial and TNF-alpha inhibitors have been used in the therapy of many inflammatory diseases including psoriasis. Therefore we chose to include TNF-alpha in our study. Distribution in tissue samples varied mostly between a moderate number of positive structures and numerous positive structures in the visual field and was found to be more expressed than human beta defensin 2 even in the skin with week findings of defensin.

We evaluated the expression of IL-1 alpha, IL-6 and IL-8. IL-1 alpha in our patients' skin varied from a completely negative finding to few positive structures. In contrast several other studies have found the overexpression of IL-1 family cytokines and even the new signaling system in psoriasis [6, 7]. IL-6 and IL-8 both were found to be positive in our skin tissue and it accords with previously known data as these interleukins have been found in high levels in active psoriasis patients, both skin and serum samples. The serum level of IL-6 has also been described as a good predictor for the successful topical therapy outcome as it prevents from immunosupression [2, 4, 9].

We conclude:

1. IL-6, IL-8 and TNF-alpha are most common cytokines for psoriatic skin lesions.
2. A moderate number of structures expresses the antimicrobial protein defensin in the psoriatic skin.

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TROPHININ AND INTEGRIN β_3 EXPRESSION IN THE HUMAN ENDOMETRIUM. A PILOT STUDY

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ABSTRACT

The study involved ten patients with tubal factor infertility. Patients (Group I, age 28–34 years, n=5; Group II, age 35 – 40 years, n=5) underwent endometrial biopsy on postovulatory days 7–10 of the natural menstrual cycle. Endometrial biopsies were studied by histological methods, presence of trophinin and integrin β_3 in the endometrial surface epithelium and in the glandular epithelium was assessed by immunohistochemistry.

Low levels of β_3 integrin and trophinin were estimated in the endometrial epithelial cells in most patients, particularly in Group II, which reflects reduced endometrial capacity for adhesion and points to problems with the implantation of the embryo.

Key words: *endometrium, infertility, immunohistochemistry, trophinin, integrin β_3.*

INTRODUCTION

Infertility is a condition that affects a couple and is defined as the lack of conception after an arbitrary period of 12 months with regular sexual intercourse and without using any contraception. Female infertility is mainly caused by tubal factor infertility, impaired endometrial function and endocrine dysfunctions [3]. During the last decades, more attention has been paid to different immunohistochemical studies of the endometrium, which should contribute to figuring out the specific roles and locations of epithelial cell proteins, essential for implantation.

Trophinin is one of the membrane proteins involved in the implantation process. Trophinin mediates apical cell adhesion and is expressed during formation of the placenta in trophoblast and at the implantation site on the endometrial luminal epithelium at the time of embryo implantation. Trophinin and trophinin-associated cytoplasmic proteins tastin and bystin are expressed on the human placenta during the early stages of pregnancy and can only be found in the placenta at the beginning of the first trimester. The role of trophinin in the implantation process diverges significantly among species. Trophinin probably does not have a crucial role in embryo implantation and placental development in the mouse because trophinin gene knock-out mice are still fertile [11].

Trophinin, tastin, and bystin form a unique complex mediating apical cell adhesion between human trophoblasts and endometrial epithelial cells. Trophinin proteins have been found in the cytoplasm of syncytiotrophoblast of chorionic villi and in decidualized endometrial stroma [12]. Trophinin mediates adhesion of syncytiotrophoblastic cells to the apical plasma membranes. Trophinin is not a typical membrane protein. The N-terminal region contains about 70×10^6 amino acid residues, is hydrophilic and is predicted to be localized in the cytoplasm. Resulting from that, this region is identifiable for the region-specific antibodies only if the cells are permeabilized. The rest of the trophinin molecule, including the C-terminal region, consists of decapeptide repetitions from which three are relatively hydrophobic and three relatively hydrophilic, indicating a true membrane protein. The three relatively hydrophilic regions are predicted to be expressed on the cell surface.

Implantation of the trophoblast during normal pregnancy is a strictly regulated and controlled process. However, the processes of trophoblastic invasion are similar to those of malignant tumour metastasis, as both processes are often accompanied by aggressive cell proliferation, host cell destruction, and angiogenesis [13, 16].

Trophinin is a molecule associated with implantation of the human embryo. Finding out its specific roles could contribute to a better understanding of placentar processes. Human endometrium tightly regulates expression of trophinin, which is expressed only within a restricted region of the apical side of luminal endometrial epithelium at a time coincident with the “window of implantation” (WOI). During early stages of pregnancy trophinin, tastin and bystin are strongly

expressed in the human placenta, especially in the trophoblast of chorionic villi and in the endometrial glandular epithelium, particularly at the utero-placental interface. In ectopic pregnancies (intrinsic for humans), trophinin is expressed in the trophoblast as well as in the epithelium of the fallopian tube. Studies suggest that trophinin plays an important role in human embryo implantation, including the pathogenesis of ectopic pregnancy [12].

Integrins form a class of cell surface proteins, which mediate cell-cell and cell-extracellular matrix attachment [4, 15]. Integrins, heterodimeric transmembrane extracellular matrix receptors, are important in implantation of an embryo in the stage of adhesion. Integrins function as receptors between molecules of extracellular matrix and adhesion molecules of cells [15]. Nine different α and β subunits are known to exist in human endometrium. By combining with one another, they may carry out different functions in the epithelium [5]. During the WOI, presence of integrins $\alpha_1\beta_1$, α $4\beta_1$ ja α $v\beta_3$ has been found in the human Fallopian tube epithelium [17]. Studies have shown that integrin β_3 exists in both the Fallopian tube epithelium and endometrial epithelium; it is up-regulated during receptivity period of the endometrium. Disturbances in the expression of integrin β_3 have been found in the case of female infertility and disorders of the luteal phase [6, 7].

In this study we focused on immunohistochemical analysis of trophinin and integrin β_3 protein expression in the endometrium of TFI patients.

MATERIAL AND METHODS

Endometrial biopsies of ten patients in reproductive age were collected in Nova Vita Clinic (Tallinn, Estonia). The biopsy was taken under general anaesthesia between the 7^{th} and 10^{th} post-ovulation day in the natural menstrual cycle which corresponded to the endometrium implantation phase, i.e. the 21^{st} to 24^{th} cycle day. The age of Goup I patients was under 34 years (n=5; mean age 30.0±0.7); the age of Group II patients was higher that 34 years (n=5; mean age 37.2±1.0). Endometrial biopsies were obtained from informed women in a protocol approved by the Ethics Review Committee on Human Research of the University of Tartu.

Patients were numbered consequently according to their enrollment into the study.

Serum progesterone levels were normal in all patients except one patient (no 1) of Group II with very low levels.

Histology

The specimens for light microscopy were fixed in 10% buffered formalin and embedded in paraffin with a vacuum processor Tissue-Tek® VIP™ 5 Jr (Sakura, USA). Specimens were cut with microtome Ergostar HM 200 (Microm, Germany) at three-μm thickness and stained using hematoxylin-eosin and van Gieson methods for general orientation to sections. Slides were observed and photographed by a Zeiss Axiophot-2 microscope (Zeiss, Germany).

Immunohistochemistry

3 μm thick paraffin sections mounted on poly-L-lysine coated SuperFrost slides (Menzel-Gläser, Germany) were deparaffinized and rehydrated. Peroxidase activity was removed by 0.6% H_2O_2 (Merck, Germany) in methanol (Merck, Germany). Then sections were washed in tap water and in PBS (pH=7.4; Gibco, Invitrogen, USA) for 10 min, treated with normal 1.5% goat serum (Gibco, Invitrogen Corporation, USA) for 30 min at room temperature and incubated with the first antibody [trophinin *(Abcam, UK)* diluted 1:500; integrin β_3 *(CD61, Dako)* diluted 1:250] overnight at 4°C in the humidity chamber. In the next day, sections were washed in PBS and incubated with the universal secondary antibody (VECTASTAIN ABC Universal Kit, Burlingame, USA) for 30 min at room temperature. Sections were washed with PBS and peroxidatic activity was detected with DAB (Vector, USA) applied for 5 min at room temperature. Then sections were rinsed, counter-stained with haematoxylin, dehydrated and mounted with DPX (Fluka, Switzerland). Trophinin and integrin β_3 labeling was expressed by a subjective scale ranging from 0 to 4 (0 – no reaction; 1 – minimal reaction; 2 – weak reaction; 3 – moderate reaction; 4 – strong reaction). Two independent observers in a blinded fashion performed the evaluation. Immunohistochemical negative controls were performed by omitting primary antibody.

Statistics

To compare results of immunohistochemical studies of group I and II, Mann-Whitney U-test was applied.

RESULTS

Histology

The histological studies showed the normal structure of the endometrial columnar epithelium in eight patients' biopsy. In two patients' biopsies (Group II) the endometrial columnar epithelium was changed – the cells were low with irregular shape. Connective tissue stroma in all patients' biopsies was, as a rule, rich in cells.

Immunohistochemistry

Immunohistochemical staining with trophinin showed a weak reaction (grade 2) in two patients' luminal epithelium (Figure 1) and a minimal reaction (grade 1) in four patients' luminal epithelium and two patients' glandular epithelium (Table 1). In Group II (patients older than 34 years), trophinin level was lower than in Group I patients, but the difference was not statistically significant. In Group I (patients younger than 34 years), integrin β_3 expression in the epithelium of uterine glands was high in one patient (no 7, Table 1, Figure 2). In other patients results varied from minimal to moderate reaction (Table 1), but in Group II (patients older than 34 years) integrin level was significantly lower compared to younger patients (Group I).

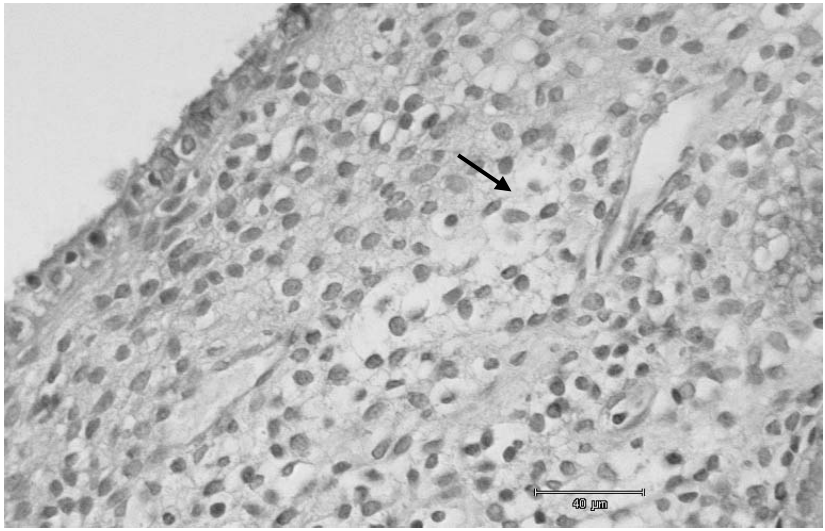

Figure 1. Minimal positive reaction to trophinin in the endometrial luminal epithelium (arrow). DAB + haematoxylin.

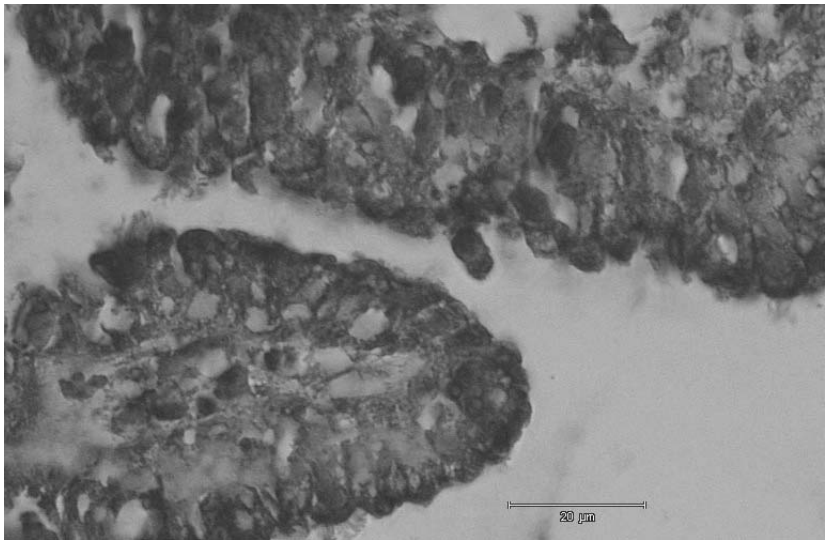

Figure 2. Strong integrin β_3 immunohistochemical reaction in the endometrial glandular epithelium. DAB+hemalaun.

Table 1. Results of immunohistochemical study

Group I	Integrin β_3		Trophinin	
	EL	EG	EL	EG
2	1	2	2	1
4	2	1	0	0
5	1	1	1	0
6	2	3	0	0
7	2	4	2	1
x±SEM	**1,6±0,3**	**2,2±0,6**	**1±0,5**	**0,4±0,3**
Group II				
1	0	1	1	0
3	1	0	0	0
8	1	1	0	0
9	0	0	1	0
10	0	0	1	0
x±SEM	**0,4±03***	**0,4±0,3***	**0,6±0,3**	**0**

EL – luminal epithelium; EG – glandular epithelium;
0 – no reaction; 1 – minimal reaction; 2 – weak reaction; 3 – moderate reaction; 4 – strong reaction
*P<0.005 Group I vs Group II

DISCUSSION

Endometrial maturation is a complex physiological process where tissue remodelling results in permissive environment for embryo invasion. Endometrium goes through proliferative, secretory and menstrual phases during spontaneous menstrual cycle, and histological changes of endometrium are strongly regulated by ovarian steroid hormones. According to literature, approximately 70 million couples suffer from infertility over the world, and only half of them are seeking infertility medical care [1]. In female infertility the main reasons are changes in the functional reproductive organs [5, 18], which can originate from hormonal problems as well as from different reproductive organ pathologies (e.g. ovarian pathologies, endometriosis, uterine pathology). Successful implantation of the blastocyst requires a receptive endometrium. The process is completed during the second embryonic week [5]. During the last decades, more attention has been paid to various immunohistochemical studies [10], which should contribute to finding out the

specific roles and locations of different epithelial cell proteins essential for implantation. During the initial phase of implantation, fetal trophoblast cells invade and migrate into the maternal decidua [19], during which molecular bonds form between trophoblast and the epithelial cells via L-selectine. Also a molecular complex of trophinin, tastin, and bystin comprising a complex mediating cell adhesion between trophoblast and endometrial epithelial cells is considered important [16]. Trophinin is a complex membranous protein a part of which is expressed on the cell surface. In complex with trophinin, cytoplasmatic proteins tastin and bystin regulate the activity of trophinin (which in turn regulates activity of cellular adhesion). In our immunohistochemical study, the assessed level of trophinin in the patients' biopsies of luminal epithelium was low or absent, in particular in Group II with patients aged over 35 years (Table 1), which could serve as a potential factor promoting infertility. Identified low levels of trophinin or the lack of the protein on the patient's luminal and glandular epithelium acknowledges limited capability for adhesion resulting in impaired implantation.

At blastocyst implantation, an important role is played by integrins which function as receptors between molecules of extracellular matrix and adhesion molecules [15]. Luminal epithelial cells express several integrins, but during WOI increased expression of $\alpha v\beta_3$ integrin has been reported [8]. In addition to endometrial cells, expression of integrins is noted in embryo trophoblast [21]. It has also been shown that the expression of endometrial $\alpha v\beta_3$ integrin correlates with the pregnancy outcome of IVF [5]. On the other hand, in a study where expression of integrins in the endometrium was compared between fertile women of the control group with women who had undergone IVF procedures, no differences were found in the integrin expression between these two groups [20]. At the same time, when β_3 integrin endometrial expression was compared between fertile and infertile women, a lower level of integrin expression was found in the endometrial glandular epithelium of infertile women but not in the surface epithelium [2]. In our study strong β_3 integrin staining in the glandular epithelium was seen in one patient only (no 7), and moderate staining in another patient (no 6) of Group I, while in surface epithelium only minimal or weak staining was found (Table 1). In Group II (patients aged over 35 years), β_3 integrin staining was weak or missed entirely (Table 1), which once more reflects problems with implantation

of an embryo in these patients, particularly in patients of advancing age. The age of patients has been found to play an important role in the outcome of IVF procedures. For example in a Singapore study, fertilization rates of 151 patients who underwent IVF procedures were 50.9% in 34-year-old or younger women, 49.3% in the group of women aged 35–39 years, and 37.9% in women aged 40 years or older. The pregnancy rates were 43.2%, 32.7%, and 14.3%, respectively [9]. Similar age-dependent results have also been obtained in another investigation where pregnancy rates of patients undergoing assisted reproduction declined from 48.8% in women aged less than 30 years to 13.6% in women aged over 42 years [14] and in a retrospective study based on 230 patients where the effect of age on success in women undergoing in vitro fertilization was assessed. Patients were divided into two groups with age either under or over 35 years and the study demonstrated a decline in pregnancy rate from 35.17% in women younger than 35 years to 17.93% in women older than 35 years. It has been found that the impaired implantation efficiency seen in older women is apparently independent of the magnitude of their stimulation response. "Oocyte factors" are felt to be primarily responsible; however, some available data suggest that uterine factors, e.g. diminished endometrial receptivity, may also play a role [14].

Formation of endometrium is a complex physiological process in which receptivity for embryonic implantation is established. Although individual morphological and molecular markers involved in the implantation process are well known and thoroughly studied, a complex overview of the significance of different markers and the dynamics of the process on the whole is still lacking. We think that the studies of the endometrium need an integral approach to the subject, involving different morphological methods e.g. histology, immunohistochemistry, gene- and ultrastructure studies, forming a whole picture of the processes in question.

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BONE MORPHOGENETIC PROTEINS AS REGULATORS OF NEURAL TUBE DEVELOPMENT

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INTRODUCTION

Bone morphogenetic proteins (BMP-s) belong to the transforming growth factor (TGF) β superfamily that mediates a multitude of developmental processes in various tissues. In recent years the roles of BMP-s in embryonic development and cellular functions have been extensively studied. The important roles of BMP signalling in the development of nervous system, heart, cartilage and bone have been demonstrated by studies of transgenic and knockout mice as well as of animals and humans with naturally occurring mutations in BMPs and related genes [5]. The central nervous system (CNS) is highly regionalized along both its anterposterior and dorsoventral axes [27]. Anterioposterior regionalization of the CNS is clearly evident from the localization of cerebral cortex at the anterior end, spatially and functionally distinct areas that arise within forebrain, midbrain and hindbrain, and spinal cord at the posterior end. Similary, different neurons and neural structures originate at specific dorsoventral positions of the neural tube. Within the ectoderm, BMP activity has been shown to inhibit neural development, promote epidermal differentiation and influence the specification of dorsal neurons and neural crest [1].

In this review we highlight the temporal and spatial influence of BMP signalling from the earliest step of neural induction to neural tube patterning when the forebrain, midbrain, hindbrain, and the spinal cord at the posterior end are formed.

GENERAL FUNCTIONS AND CLASSIFICATION OF BMP-s

Bone morphogenetic proteins, now widely known for their involvement in many biological processes, were first examined for their ability to induce cartilage and bone formation, and this was the reason for assigning this group of proteins their names. BMP-s were first described by Dr. Marshall Urist in 1965 [39]. Four proteins were initially identified, and one of them, BMP-1 is a metalloproteinase [23]. The other three (BMP-2, -3 and -4) are members of the transforming growth factor β (TGFβ) superfamily and they mediate a multitude of developmental processes in various tissues. Subsequently, molecular cloning studies have identified more than 20 members of the BMP subgroup in the TGFβ family, from various species [23]. As shown in Table 1, several BMP-s possess monikers owing to the diversity of their effects observed in multiple species and tissues [34]. Because BMP-s have been isolated from different species, several of the BMP-s have alternate names that are often used interchangeably [28]. For instance, BMP-7 is OP-1, BMP-11 is GDF-8 and etc. Although it is not known whether all of the members of this subgroup are involved in bone differentiation, they control a wide range of biological processes in various cell types. This class of proteins has been shown to have roles in cellular lineage commitment, differentiation, proliferation, patterning, morphogenesis, cellular maintenance and apoptosis [34]. There are also increasing evidence that secreted signalling molecules of the BMP superfamily play versatile roles in many aspects of embryonic development, including neurogenesis [14].

Table 1. Functions of bone mophogenetic proteins

BMP	Functions
BMP-1	Induces bone and cartilage. Involved in dorsoventral patterning.
BMP-2	Induces osteoblast differentiation and osteogenesis. Involved in cardiac cell differentiation, epithelial to mesenchymal transition, dorsoventral patterning and craniofacial development.
BMP-3 (osteogenin/GDF-10)	It is an osteogenesis inhibitor, it negatively regulates bone density. Involved in dorsoventral patterning.
BMP-4	Involved in bone mineralisation, muscle development, formation of teeth, limbs, lungs and eyes. Also involved in dorsoventral patterning and craniofacial development.
BMP-5	Induces bone and cartilage development. Involved in formation of liver, lung and optic nerve.
BMP-6	Induces osteogenic markers in mesenchymal stem cells. Involved in joint integrity.
BMP-7 (OP-1)	Involved in the transformation of mesenchymal cells into bone and cartilage. Also involved in kidney, bladder, brain and and craniofacial development.
BMP-8 (OP-2/3)	Induces bone and cartilage development. Involved in craniofacial development.
BMP-9	Involved in chondrogenesis, hepatogenesis and nervous system development.
BMP-10	Involved in the trabeculation of embryonic heart.
BMP-11 (GDF-8)	Involved in the mesodermal patterning and nervous system development.
BMP-12 (GDF-7/CDMP-3)	Induces joint morphogenesis. Facilitates growth of ligament.
BMP-13 (GDF-6/CDMP-2)	Induces joint morphogenesis. Facilitates growth of ligament.
BMP-14	Involved in chondrogenesis, limbs development and fracture healing. Facilitates growth of tendon.
BMP-15	Involved in the oocyte and follicular development.

BMP-s INFLUENCE THE PROCESS OF NEURULATION

Neurulation is the process of forming the neural tube, which will become the brain and spinal cord [30]. The embryonic precursor of the neural tube is the neural plate, or neuroepithelium, a thickened region of ectoderm on the dorsal surface of the early embryo [9]. The neural plate is subsequently converted into the neural tube by a two-stage process. Primary neurulation gives rise to the neural tube that will later develop into the brain and most of the spinal cord. This process involves shaping and folding of the neuroepithelium, with formation of neural folds that undergo fusion in the midline to generate the neural tube and create two continuous epithelial layers: surface ectoderm on the outside and the inner neural tube [10]. Secondary neurulation leads to the formation of the neural tube in the caudal sacral and coccygeal regions [15]. In the neural tissue BMP signalling is essential for many steps of neural development. BMP-s act at different stages of neural development and in different regions of the CNS to regulate cell fate, proliferation and differentation. The first step is an early embryonic cell fate decision that determines whether cells will form neural or non-neural ectoderm [26]. The CNS initially develops from the dorsomedial region of the embryonic ectoderm, a process that requires the active repression of BMP signals. Although BMP activity must be inhibited to allow initial neural fate determination, it is clear that BMP signalling positively regulates CNS development at later stages of development [33]. Once the neural tissue is established, BMP signalling has a positive influence on the regulation of dorsal neural cell type formation [26].

BMP SIGNALLING INHIBITION ACTIVATES NEURAL INDUCTION

During neural induction the embryonic neural plate is specified and set aside from other parts of the ectoderm. Several recent studies have shown that BMP-s are expressed in the ectoderm surrounding the entire neural plate [4], domains where phosphorylated Smad-I is also detected, indicative of active BMP signalling [13]. A widespread molecular explanation is the “default model” of neural induction, which proposes that high BMP activity defines epidermis, while absence of BMP specifies the neural plate [32]. It has also been proposed that intermediate concentrations specify the border of the neural plate, including

the region fated to give rise to placodes and neural crest [40]. BMP causes the inwards displacement of the border (narrowing the neural plate), while antagonists cause the reverse [32]. There is no doubt that the modulation of BMP activity (including the control of Smad I) is crucially important for neural development to occur normally [22]. BMP signalling apparently needs to be inhibited at least three times during early development to generate a normal neural plate [32]. The first inhibition takes place at very early stages of development, the second at the mid-/late gastrula stage and the third at the late gastrula/early neurula stage. These inhibitions establish the initial dorsoventral polarity of the embryo and also establish the differential competence of different ectodermal regions to respond to later signals. Chordin and noggin are probably the most important BMP antagonists in these developmental stages. These BMP activity repressions (in particular BMP-4, which acts as an epidermal inducer) led researchers to construct the "default model" of neural induction, which proposes that cells within the ectoderm have an autonomus tendency to differentiate into neural tissue [16]. These neural inducers induce only forebrain and midbrain types of tissues. Induction of caudal neural tube structures, hindbrain and spinal cord, depends on two other secreted proteins, Wnt-s and FGF-s. In addition, RA (retionic acid) appears to play a role in organizing the cranial-caudal axis [38, 40].

BMP-s PROMOTE AND INFLUENCE NEURAL CREST CELLS

The role of BMP signalling in neural crest induction is tightly linked to the induction of the neural plate [17]. After gastrulation, neural crest cells are specified at the border of the neural plate and the non-neural ectoderm. The periferial nervous system arises from neural crest and placodal cells derived from neural plate border cells. Neural crest cells, which contribute to a vast array of cell types are generated along the entire rostrocaudal neuroaxis where the neural plate border cells generate placodal but no neural crest cells [29]. The ability to segregate and migrate away from the neuroepithelium is one of the unique features of the developing neural crest, and for many years, the neural crest has been the preferred model to analyze the molecular basis of cell migration in normal and also pathological situations [35]. Induction of neural crest cells requires an interaction at the junctional border of the

neural plate and surface ectoderm. Intermediate concentration of BMP-s is established at this boundary compared to neural plate cells that are exposed to very low levels of BMP-s and surface ectoderm cells that are exposed to very high levels. The proteins chordin, noggin and follistatin regulate these concentrations by acting as BMP inhibitors [17]. The intermediate concentration of BMP-s, together with Wnt proteins, induce Pax genes and other transcription factors that "specify" the neural plate border. We have a unique opportunity to examine a substantial number of human embryos at the stages of neural tube formation. Figure 1 shows intermediate expression of BMP-4 in neural crest cells during the development of the neural tube. It still remains unclear whether BMP and other proteins (Wnt, SHH) signals act in parallel or have separate roles during the initial induction of neural crest cells [29].

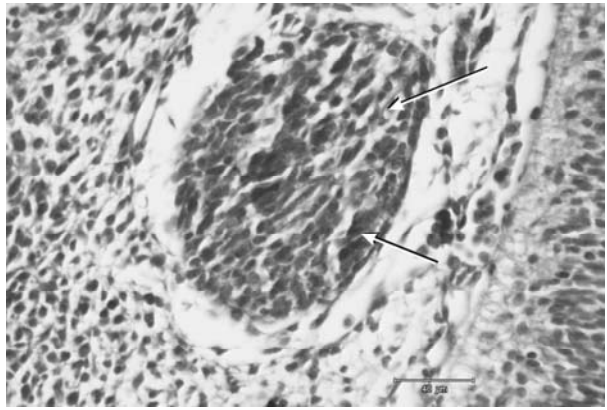

Figure 1. BMP-4 expression in the neural crest cells during the developement of the neural tube. Arrows indicate intesive immunostaining of neural crest cells (Carnegie stage 16).

ROLE OF BMP-s IN DORSAL-VENTRAL PATTERNING OF THE NEURAL TUBE

Members of the BMP family of signalling proteins are involved in embryonic dorsoventral patterning in both vertebrates and invertebrates [2]. A widely accepted model of dorsoventral patterning postulates that a morphogenetic BMP activity gradient patterns cell fates of all of the

dorso-ventral axis. It has been suggested that members of the TGFβ superfamily, including several of the BMP-s, play a similar role in the dorsal neural tube [36]. The specification of dorsal neuronal cell fates appears to depend on a cascade of inductive signals initiated by cells of epidermal ectoderm that flank the neural plate and propagated by roof plate cells within the neural tube [24, 7]. BMP proteins have a prominent role in mediating these dorsalizing signals. Targeted mutations of BMP signalling components in the mouse also indicate a role for BMP signalling in dorsoventral patterning in mammals [37]. Our work with human embryos points to the considerable expression of BMP-2 and BMP-4 in the dorsal part of the developing neural tube (Figure 2). Thus, the results of BMP-2 and BMP-4 expression in the human developing neural tube are generally in concordance with the data obtained from animal studies supporting the idea that BMP-s are key regulators in the differentiation of the neural tissue, especially in the dorsal part of the neural tube. Additional signals, including members of the Wnt and FGF families, may also contribute to the proliferation and differentiation of dorsal neuronal cell types.

The level of BMP signalling may be modulated by a diverse group of proteins that emanate from the dorsal side of the embryo and inhibit BMP activity. Chordin, noggin and follistatin can all bind directly to BMP-s and overexpression studies have demonstrated that these proteins dorsalise embryos through the inhibition of BMP activity [11]. Unlike BMP-2 and BMP-4, noggin and chordin may diffuse within the embryo and so a gradient of BMP activity could be established through widespread production of BMP-s whose signalling activity is modulated by the activity of BMP antagonists diffusing from the dorsal side of the embryo [21] and thus a gradient of BMP-dependent positional information extending throughout the entire neural and non-neural ectoderm is formed [1]. Figure 3 demonstrates BMP-4 expression in the non-neural ectoderm during the development of the neural tube of human embryos.

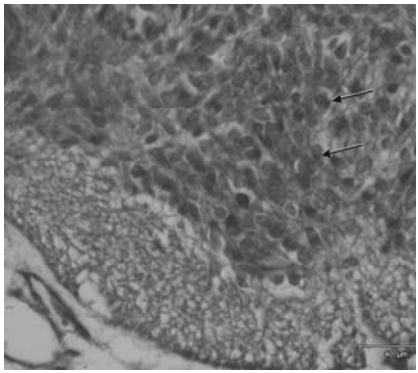

Figure 2. BMP-4 expression in the dorsal part of the developing neural tube (Carnegie stage 14).

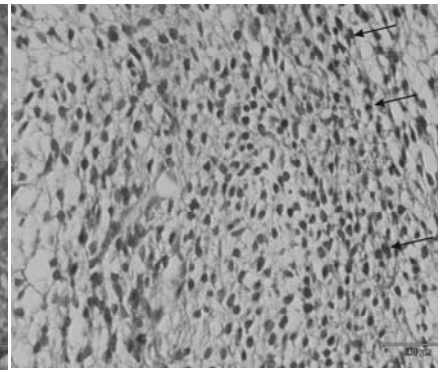

Figure 3. BMP-4 expression in the non-neural ectoderm during the development of the neural tube (Carnegie stage 18).

BMP-s SIGNALLING AND ESTABLISHMENT OF THE SPINAL CORD

After neural tube closure the most anterior end of the neural tube gives rise to the forebrain consisting of the telencephalon and the diencephalon, while the posterior regions form the midbrain, the hindbrain and the spinal cord [31]. As the caudal neural tube closes, roof plate progenitors differentiate into mature roof plate cells, which occupy the dorsal midline region. Roof plate cells act as a dorsal midline organizing center controlling numerous aspects of dorsal spinal cord development [8]. The first evidence for a role of roof plate in dorsal spinal cord patterning came from chick and mice studies [5, 6], and nowadays the dorsal patterning mechanisms are best described in the developing spinal cord. Several members of the BMP family are specially expressed in the roof plate during neural tube development at the dorsal interneuron formation in both mouse and chick. Expression of BMP-s also appeared in the roof plate of the developing spinal cord of human embryos (Figures 4, 5). In addition to the BMP-s expression of the roof plate, significant expression of BMP-s in non-neural ectoderm and neural crest cells was evident (Figure 4).

The dorsoventral patterning is dictated by SHH emananting from ventral regions of the neural tube and BMP-s and Wnt-s originating from the dorsal position. In addition, the members of the FGF family have a specific role in the control of proliferation and patterning of neural progenitors [38]. Several lines of evidence indicate that BMP signalling contributes to the patterning of dorsoventral axis of the neural tube and is required for the correct specification of cell types in dorsal regions [41]. In addition, BMP-s antagonise SHH activity in the neural tube and influence the proliferation of neural progenitors. Importantly, the effects of BMP-s on cell proliferation in the neural tube may be mediated by Wnt signalling and conversely, Wnt signalling can transcriptionally be induced by BMP-s [18]. The molecular mechanism of this cross-inhibition remains to be elucidated. These findings have led researchers to propose that growth in the dorsal spinal cord is regulated by a balance between Wnt and BMP signalling.

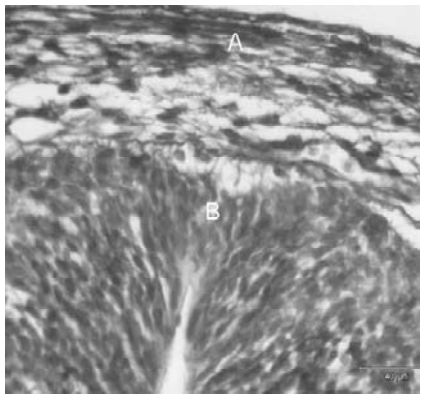

Figure 4. BMP-4 expression in a) non-neural ectoderm b) roof plate c) neural crest cells in developing spinal cord (Carnegie stage 14).

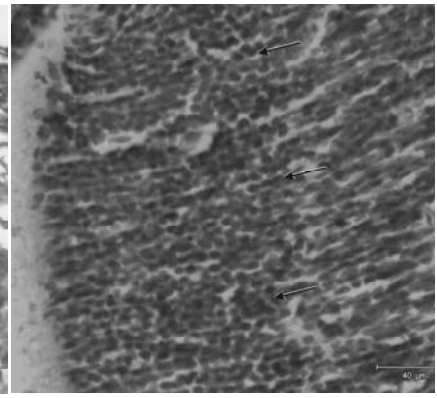

Figure 5. BMP-2 expression in the roof plate of developing spinal cord (Carnegie stage 16).

CONCLUDING REMARKS

In embryonic development BMP-s, specially BMP-2 and BMP-4, are critical signalling molecules required for the early differentiation of the embryo and establishing the dorsoventral axis. The specification of

neuronal subtypes becomes evident with the appearance of distinct cell types at defined positions along the dorsoventral axis of the neural tube [19]. The dorsoventral patterning of the neural tube is dictated by sonic hedgehog (SHH) emanating from ventral regions of the neural tube and BMP-s and Wnt-s originating from dorsal portions [3]. BMP and SHH signals appear to have opponent and antagonistic functions in the control of cell fate along the dorsoventral axis of the neural tube [12]. Together the data support the hypothesis that polypeptide growth factors of the TGF play key roles in the development of the neural tube at the initial stages of neurogenesis [20]. In addition, the members of the TGF family have specific roles in the control of proliferation and patterning of neural progenitors [38].

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DENTAL DISEASE IN A 17TH–18TH CENTURY GERMAN COMMUNITY IN JELGAVA, LATVIA

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ABSTRACT

Aims: To determine the frequency and distribution of dental caries, periapical lesions, the periodontal disease, ante-mortem tooth loss and enamel hypoplasia in a high status, urban post-medieval population from the Duchy of Courland and Semigallia, and to compare these rates with those obtained from contemporary populations from urban and rural Latvian cemeteries.

Materials: The sample analysed consisted of the dental remains of 108 individuals (39 male, 42 female and 27 non-adults) excavated from the Jelgava Holy Trinity Church cemetery in Latvia. A total of 1,233 teeth and 1,853 alveoli were examined.

Results: The frequency of the observed conditions in this population was overall high but not anomalous for the post-medieval period in Latvia. The differences between the age and the sex groups when comparing the number of individuals affected were not significant. The number of teeth and/or alveoli affected by caries, the periodontal disease and the ante-mortem tooth loss proved to be significantly higher in females than males in both age groups and in total. The prevalence of enamel hypoplasia was high in both sex groups.

Conclusions: The overall high rates of destructive dental diseases in this population were linked to the diet high in soft carbohydrates and refined sugars. The significant differences between the number of teeth and alveoli in male and female dentitions affected by caries, the periodontal disease and the ante-mortem tooth loss were linked to a differential diet, as well as high fertility demands and differences in the composition of male and female saliva. The large number of the adult individuals affected by enamel hypoplasia proved that most of the population was subject to severe metabolic stress episodes during

childhood, but that many children were likely to survive these hardships into adulthood. The comparison with other contemporary populations proved that all the Holy Trinity Church cemetery population had an equally high prevalence of dental diseases, especially with regards to other urban populations.

Key words*: skeletal remains, diet, status, childhood stress.*

INTRODUCTION

This study was initiated after recent excavations in the Jelgava Holy Trinity Church in Latvia, which provided a unique opportunity to look into the health status of a wealthy German community of the 17th and 18th centuries. Although it is acknowledged that the German population in Latvia was socially and economically advantaged over the native Latvian population [12: 234; 58: 7], the German society was stratified itself, and the period between the 17th and 18th centuries was politically complicated. Moreover, a high social status does not necessarily mean better health due to cultural differences, or during hardships such as wars, famines or epidemics [8; 31: 76; 47].

The dental disease was chosen as the main focus of this study because it is a useful tool for looking into many aspects of past populations including their diet, hygiene and social status, as well as the childhood stress [36: 261]. Teeth generally survive well in a wide variety of archaeological contexts, as opposed to other elements of the skeleton. As a result, the dental disease can be studied when other skeletal data are poor or not available. This enables comparisons among and between skeletal populations, provided that similar recording methods have been used and that the data are presented in an appropriate way [ibid: 273].

Consequently, three main aims have been set up for this study: 1) To record and assess the prevalence rates of dental diseases in the Holy Trinity Church cemetery population (HTCCP), 2) to compare these rates with those obtained from four broadly contemporary populations from urban and rural Latvian cemeteries, and 3) to find similarities and differences in the patterns of dental diseases between the HTCCP and the other four cemeteries.

MATERIALS

During the summer of 2009 and the autumn of 2010, one hundred and eight individuals were excavated from the inside of the Jelgava Holy Trinity Church during reconstruction work. The church was built for the German community of Jelgava in 1615, and used as their final resting place until 1780, when it was forbidden by law to bury people inside churches [**57].** Due to the restricted space the burials had to be laid on top of each other over time, and three layers of burials could be distinguished during the excavation [40].

Apart from the in-situ burials, a large number of isolated human bones were found, for which the MNI (Minimum Number of Individuals) was calculated as 320. This indicates that older burials were often destroyed by later ones. On the other hand, all the burials of the same layer followed a set order of rows [ibid.].

With regards to the preservation of the skeletal material, it was very well preserved, with most individuals having the cranial and post-cranial skeleton present. In total, there were 39 male, 42 female and 27 non-adult individuals in this skeletal collection.

For comparative analysis, the data on dental caries, periapical lesions and the ante-mortem tooth loss were compiled from the excavated cemeteries of Ventspils [15], Valmiera [56], Madona and Cesvaine [16]. Thanks to similar recording methods, the available data could be reliably compared. Where possible, the comparison of dental pathologies was carried out in the age and the sex groups.

Historical background

Between the 17th and 18th centuries Latvia was involved in wars between Sweden, Russia and Poland, each country wanted a share of land and accordingly, the control over trade routes [11; 12]. In this period, the city of Jelgava was the capital of the Duchy of Courland and Semigallia, which stretched from the southern-most part of Latvia across the whole south-western border to the Baltic coastline. This meant that the Duchy had access to all major international land and sea trade routes [11: 53]. The general population of Jelgava in this period was stratified according to the family income [42: 13]. It is believed that most people, buried in the Jelgava Holy Trinity Church cemetery, were wealthy citizens from the families rich enough to afford to bury their relatives within the church, which was essentially a paid service [56: 40].

Throughout the existence of the Duchy, every generation experienced war and subsequently, famines and plagues [11: 226]. These disasters particularly struck the inhabitants of Jelgava, as the city was a major political centre. Whenever the Duchy could not avoid being involved in war, Jelgava with the Duke's seat was the main target [ibid: 96]. Moreover, the population of the city was severely affected by the famine in 1697 and the plague shortly after [ibid: 146]. Jelgava allegedly lost 1,316 people just in one year during the Great Plague of 1710, which came amid yet another war (The Great Northern War (1700–1721)) [ibid: 191], leaving only about one third of the Jelgava population alive [28: 16].

METHODS

To estimate the age in adult individuals, a series of widely used methods were applied [2; 4; 26; 27; 34; 35; 41]. For non-adult individuals, mainly tooth formation and eruption methods were used [52: Tables 5.24–26, figs. 5.77–78] in combination with the epiphyseal fusion and long bone measurements where teeth were not available for study [14; 51]. Sex estimates in adult individuals were based on the subjective assessment of the morphology of the pelvis and the skull [5: 16–38].

For the observation of the dental disease, all the individuals with the present mandible and/or maxilla (even partly preserved), and at least one observable tooth/alveolus, were included in the analysis. The analysis was entirely macroscopic. The calculations and the analyses for each condition in adults were carried out by age and sex groups, dividing the individuals in those aged 20 – 40 (including two female individuals aged between 18 and 20 years), and above 40 years. Non-adult individuals age groups were not used due to the scarcity of pathological lesions.

For the recording of caries, mainly the methods developed by Hillson [21] and Lukacs [36] were used. Caries was identified as present only if there was a visible lytic lesion penetrating the tooth crown or root, and recorded as present or absent. In juveniles, all erupting and erupted teeth were observed. Periapical lesions were recorded as present only if they were associated with the apices of one or more roots, Lukacs [36: 271] and Ogden [46: 297]. No distinction between granuloma, cyst and chronic abscess was attempted [ibid.] because it was beyond the scope of this analysis. In juveniles, all the alveoli with partially of fully

erupted teeth were observed. The periodontal disease was recorded as present or absent [3: 155]. In juveniles, only the alveoli with fully erupted, in-situ deciduous or permanent teeth were observed. The ante-mortem tooth loss was recorded as present if there were signs of remodelling, or if the sockets were completely remodelled. Calculus was recorded as present or absent and by its location above or below the gingival line (sub- or supragingival) [3; 22]. In juveniles, all the fully erupted teeth were observed. Enamel hypoplasia was recorded as present only if the hypoplastic defect was clearly visible without magnification. To record enamel hypoplasia, more than one tooth of any type had to be affected, to avoid recording the enamel defects caused by trauma. The defects were recorded according to their type (vertical or horizontal, groove or pit [5; 49]. No calculations of the age of formation were attempted due to the difficult interpretation of the measurements [24: 174]. In juveniles, all the teeth with fully formed crowns (including loose unerupted teeth) were observed. For statistically significant differences between the affected individuals and the number of affected teeth/alveoli in the age and the sex groups, the Chi-square and Fisher exact tests were used. The tests were performed using SigmaPlot, version 11.0, level of confidence $p<0.05$. The calculations for the prevalence of each condition were carried out both by observable individual count (crude prevalence) and by tooth/socket count (true prevalence). Prevalence rates were calculated based on the observable individuals and the observable teeth/alveoli for each dental disease.

RESULTS

It was possible to estimate sex in all the adult individuals in this population, and the non-adult individual from burial 17 (17–19 years old) could be reliably sexed as male. From the 108 excavated individuals, 31 male, 35 female and 19 non-adult individuals at least one dental disease for including could be observed in the analysis. Apart from the dental disease, the tooth wear, typical of regular clay-pipe smoking, visible as a distinctive foramen affecting the upper and lower second incisors and canines, was present in two male individuals (burials 26, aged 25–30 years and 59, 50+ years old).

Table 1. Crude prevalence of dental diseases in the Holy Trinity Church cemetery adult population (individual count)

Age (yrs)	Males	%	Females	%
Dental caries	(n/N)		(n/N)	
20–40	5/13	38.5	12/16	75.0
40+	10/15	66.7	11/16	68.8
Total	15/28	53.6	23/32	71.9
Periapical lesions				
20–40	2/13	15.4	9/16	56.3
40+	6/18	33.3	6/17	35.3
Total	8/31	25.8	15/33	45.5
Periodontal disease				
20–40	9/13	69.2	11/14	78.6
40+	15/15	100.0	17/17	100.0
Total	24/28	85.7	28/31	90.3
*AMTL**				
20–40	5/11	45.5	10/14	71.4
40+	15/18	83.3	18/19	94.7
Total	20/29	69.0	28/33	84.8
Calculus				
20–40	12/12	100.0	11/13	84.6
40+	13/13	100.0	12/12	100.0
Total	25/25	100.0	23/25	92.8
Enamel hypoplasia				
20–40	12/13	92.3	11/14	78.6
40+	12/14	85.7	10/12	83.3
Total	24/27	88.9	21/26	80.8

n – number of individuals with dental disease; N – number of individuals with observable teeth/alveoli; * – ante-mortem tooth loss

Regarding the dental disease, the prevalence of caries in adult individuals was high, especially among females in both age groups (Table 1). In total, 15 of 28 male and 23 of 32 female individuals were affected (53.6% and 71.9% respectively). The disease was more prevalent only in older male individuals, affecting fewer females in the older age group. Although considerably more females than males were affected by caries in the younger age group (75.0% and 38.5% respectively), this difference, based on crude prevalence calculations,

was not statistically significant (χ^2=2.585, p=0.108). The number of carious teeth (true prevalence), however, was significantly higher in females than males in both age groups as well as in total (for all the calculated values based on true prevalence rates see Table 2). The number of carious teeth in male individuals significantly increased with age (from 3.3% to 8.1%). In females, the number of affected teeth reduced with age by 0.7 percent, but so did the number of observable teeth (Figure 1).

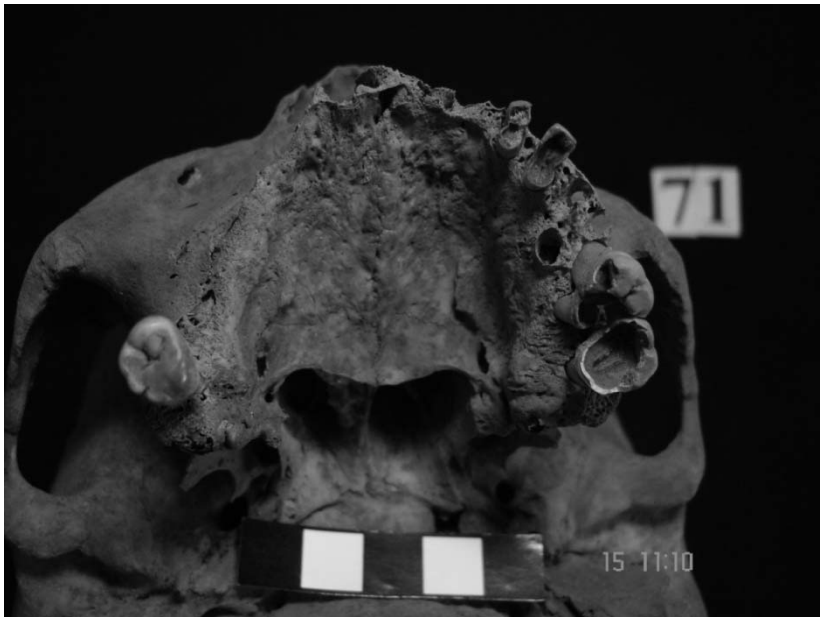

Figure 1. Gross caries and ante-mortem tooth loss in the maxilla of a female individual 50–60 years old (burial 71).

In non-adult individuals, caries affected two of 11 observable children (18.2%, burials 84 (12–13 years old) and 92 (2–2.5 years old), both had two lesions) and four of 128 observable teeth (3.1%).

Periapical lesions affected eight of 31 (25.8%) male and 15 of 33 (45.5%) female individuals (Table 1). There were significantly more teeth affected in female than male dentitions in the younger age group (Table 2). In 11 observable juveniles, only one had the lesion (9.1%,

burial 17) and accordingly, one of 128 tooth positions was affected (0.8%)).

The analysis of the periodontal disease revealed a surprisingly high frequency of this condition in both adult age and sex groups, although it increased with age (Table 1). Based on crude prevalence calculations, in male individuals the increase with age was statistically significant ($p=0.035$), but not so in female individuals ($p=0.081$). The number of affected alveoli significantly increased with age in both sex groups (Table 2). The disease also affected significantly more alveoli in female than male dentitions in both age groups and in total (Table 2). In most of the adult individuals, the periodontal disease first affected the posterior teeth and advanced to affect the anterior teeth with increasing age. The condition was not observed in juveniles (nine individuals and 81 teeth in alveoli were examined).

The ante-mortem tooth loss was only present in adult individuals and the prevalence slightly increased with age in both sex groups. In total, 20 of 29 male (69.0%) and 28 of 33 female (84.8%) individuals were affected. As with caries and the periodontal disease, the number of the lost teeth differed significantly between males and females (Table 2), although the number of individuals affected was relatively similar (Table 1). There were significantly more teeth affected in females than in males in both age groups and in total, significantly more teeth were lost in older individuals in both sex groups (Table 2).

The supra-gingival calculus was present in almost every adult individual (48 of 50 (96.0%) of the observed male and female individuals (Table 1). The number of affected teeth significantly increased with age in both sex groups, and females had significantly less teeth with calculus deposits than males in both age groups and in total (Table 2).

The sub-gingival calculus was present in three of 25 (12.0%) male and nine of 25 (36.0%) female individuals, which all had the periodontal disease ranging from moderate to considerable. All the male individuals with the sub-gingival calculus were older than 40 years, but among female individuals two were aged between 30 and 40 years, and the other seven were older than 40 years.

In juveniles, the supra-gingival calculus was recorded in 3 of 9 observable individuals (33.3%) and 42 of 93 teeth (45.2%). No sub-gingival calculus deposits were present.

Table 2. True prevalence of dental diseases in the Holy Trinity Church cemetery adult population (tooth count), including chi square values*

Age (yrs)	Males	%	χ^2/p values (male age groups)	Females	%	χ^2/p values (female age groups)	χ^2/p values (age groups+totals between sex groups)
	n/N			n/N			
Dental caries							
20–40	11/334	3.3		40/253	15.8		**26.873/<0.001**
40+	23/284	8.1	**5.924/0.015**	31/205	15.1	0.00527/ 0.942	**5.285/0.022**
Total	34/618	5.5		71/458	15.5		**28.750/ <0.001**
Periapical lesions							
20–40	4/386	1.0		18/432	4.2		**6.484/0.011**
40+	10/490	2.0	0.820/0.365	11/545	2.0	3.152/0.076	0.0381/0.845
Total	14/876	1.6		29/977	3.0		3.244/ 0.072
Periodontal disease							
20–40	87/334	26.0		140/250	56.0		**52.730/<0.001**
40+	212/332	63.9	**94.678/<0.001**	167/206	81.1	**31.132/<0.001**	**17.273/<0.001**
Total	299/666	44.9		307/456	67.3		**53.923/<0.001**
*AMTL***							
20–40	20/386	5.2		49/432	11.3		**9.237/0.002**
40+	109/490	22.2	**48.713/<0.001**	247/545	45.3	**130.144/<0.001**	**59.871/<0.001**
Total	129/876	14.7		296/977	30.3		**62.477/<0.001**

Calculus							
20–40	293/333	88.0		174/241	72.2		**21.952/<0.001**
40+	268/268	100.0	**32.581/<0.001**	178/200	89.0	**18.123/<0.001**	**28.527/<0.001**
Total	561/601	93.3		352/441	79.8		**41.661/<0.001**
Enamel hypoplasia							
20–40	97/327	29.7		65/234	27.8		0.153/0.695
40+	58/238	24.4	1.682/0.195	70/171	40.9	**7.117/ 0.008**	**11.941/ <0.001**
Total	155/565	27.4		135/405	33.3		3.641/0.056

*values in **bold** are statistically significant; n – number of teeth with dental disease; N – number of observable teeth/alveoli; ** – ante-mortem tooth loss

Enamel hypoplasia had a high prevalence in the adult population in both sex groups (Table 1). Overall, there was a slightly higher frequency of defects in males than in females, 20 of 27 male (74.1%) and 17 of 28 female (60.7%) individuals had linear defects which appeared as grooves or, less common, as pits (Figure 2). Seven male and 11 female individuals had no enamel defects. With regards to the number of affected teeth, there were no significant differences between the sex groups in total, but significantly more teeth were affected in older females than in younger females and older males (Table 2).

After observing 19 juvenile dentitions and 157 deciduous and permanent teeth, no hypoplasic defects were found (0.0%).

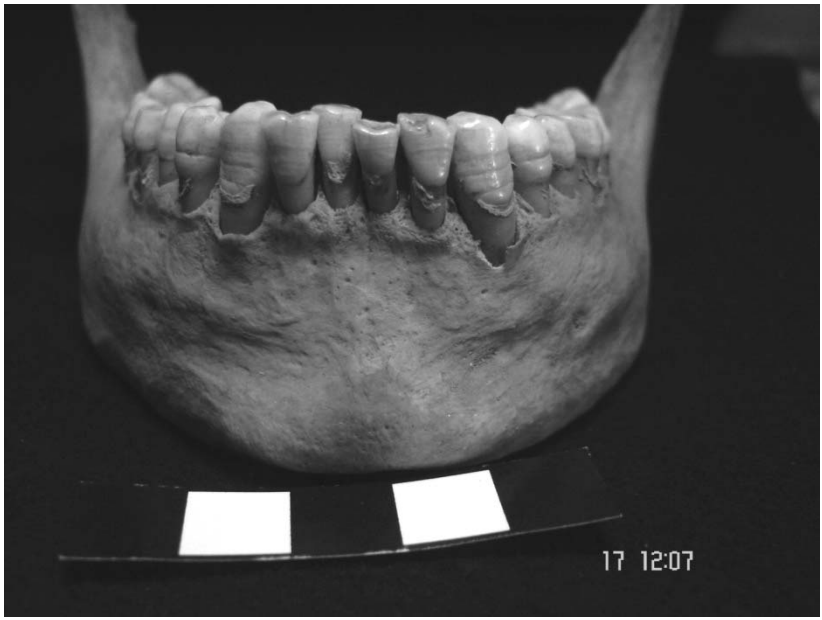

Figure 2. Gross linear enamel hypoplasia in the form of grooves affecting the anterior and posterior mandibular teeth in a male individual aged 35–40 years (burial 23).

DISCUSSION

The results of the dental analysis proved that the prevalence of the observed diseases and conditions in the HTCCP was high. First of all, caries affected more than half of the adult population. It is known that one of the most influential factors in the development of caries is the diet comprising soft, sticky foods and refined sugar [7; 24: 291; 32: 179; 43; 44; 45]. In many, but not all the populations, older individuals and females in general are more likely to have the destructive lesions [8; 29; 31: 72; 32; 37; 38; 48; 50; 54]. Higher caries rates in females have most commonly been explained by a differential diet [8] and increased fertility demands [1; 32; 37; 38]. Both explanations are possible in the HTCCP. Firstly, it has recently been proven that the increase of oestrogen levels in female saliva during pregnancy can be responsible for higher rates of caries in this sex group [39: 547]. Accordingly, if women in the HTCCP had frequent pregnancies, this would inevitably result in more carious teeth. Secondly, historical sources suggest that the bread consumed by the upper classes in Latvia was made of finely ground pure grain and was quite soft [10]. Moreover, from the end of the 17th century sugar was imported from the Duke's own colonies in the Southern Atlantic Ocean [11: 61], and it would have been available for those who could afford to buy it. While the presence of carious foods in the diet was responsible for the overall high caries rates in the HTCCP, it could be that the diet of females was slightly softer and with higher amounts of refined sugar. Likewise, it has to be taken into account that the saliva flow rate is lower in females, thus also slowing the rate of food residue clearance from teeth and the restoration of the protective qualities of saliva after meals [38: 905; 39: 545–6].

The relative scarcity of carious lesions in juvenile individuals might be due to the fact that a number of partly or completely erupted teeth were lost post-mortem, reducing the number of observable teeth. It is also important to remember that caries is a disease that slowly progresses with age, and that probably the length of time carbohydrates were consumed in this population was too short for most of the children to develop carious lesions. Alternatively, the prevalence of the disease in children could have been very low due to a differential diet, especially with regards to the amount of refined sugar in the diet.

The observation of periapical lesions in the HTCCP proved that in most cases, they occurred in response to carious teeth. The caries-

induced infection of the pulp is known to be one of the most common reasons for the development of these lesions [22: 284; 23: 322]. The fact that young females in the HTCCP had more carious teeth than young males might explain the significant difference in the number of periapical lesions between the sexes in the younger age group. The presence of carious roots in both males and females, with the crowns lost to gross caries, indicate that in Jelgava it was not a common practice to extract teeth, although it is believed that the procedure was practised around the world since ancient times [23: 323; 53: 237].

The observation of the periodontal disease revealed markedly high rates in the Holy Trinity Church cemetery adult population. It is thought that the alveolar bone loss, which is the main consequence of the periodontal disease, is most likely caused by bacterial infection on the gingiva (the soft tissue surrounding the teeth) [20: 225–226; 31: 78]. On the other hand, while the mildest form of the periodontal disease, gingivitis, is a very common condition in living populations [53: 239], it only progresses into the destructive periodontal disease when the existing micro-flora of the mouth starts to change due to other processes affecting the mouth [6: 242].

The onset of the disease could also depend on the individual rate of caries and poor oral hygiene [31: 78]. As is the case with dental caries, the periodontal disease has also a higher prevalence in the populations which consume softer foods and thus have lower attrition rates [ibid: 80]. Accordingly, it is possible that the rates of caries combined with a soft diet and poor oral hygiene were the main contributing factors to the periodontal disease in this population. In addition, the nutritional status, as well as diseases such as scurvy could have affected its frequency [ibid.].

The relatively high prevalence of the ante-mortem tooth loss in the HTCCP is not surprising taking into account the equally high prevalence of other destructive dental diseases discussed above, which all can eventually lead to the tooth loss during life [31: 77; 46: 288]. Likewise, the significantly higher number of lost teeth in female dentitions is consistent with the higher rates of teeth and alveoli affected by caries and the periodontal disease in this sex group.

The almost 100% prevalence of the supra-gingival calculus in the Holy Trinity Church cemetery adult population is not unusual when compared to other archaeological populations [3: 160]. Its presence on

the teeth is commonly linked to the level of oral hygiene in the population, although the calculus formation also depends on other factors such as the diet and inheritance [24: 289; 33]. It is believed that the diet high in protein can result in more severe deposits [ibid.]. The high overall prevalence of the supra-gingival calculus in the HTCCP could indicate poor oral hygiene. The significant differences in the number of affected teeth between age groups in male and female individuals suggest a gradual increase in the deposits with age. Significant differences in the number of teeth with calculus deposits between males and females in both age groups and in total could be linked to the presence of more dietary proteins in the diet of male individuals.

The sub-gingival calculus, on the other hand, is only associated with the periodontal disease [23: 312], and this was also the case in the HTCCP. As the periodontal disease was somewhat more prevalent in females, the higher number of individuals with sub-gingival calculus in this sex group is not surprising.

Finally, the adult population of the Holy Trinity Church cemetery had high frequencies of enamel defects. Enamel defects only occur in early childhood during the process of tooth crown formation [17: 64]. It has been suggested that the disrupted enamel formation on the tooth crown can be either hereditary, traumatic, or caused by a systemic metabolic stress such as nutritional deficiencies and/or diseases [18: 280; 31: 44–45]. In living populations, the formation of dental enamel defects depends on a variety of factors including environmental conditions, the time period in question, the level of development of the country, and the ethnicity of the affected individuals [9: 85; 13; 19; 25]. In most archaeological populations, however, where the defects affect numerous teeth in the same individual, they are believed to have resulted from a systemic metabolic stress [9: 81; 18: 281; 30: 547]. With regards to the HTCCP, this is consistent with the historical data about the frequent wars and subsequent outbreaks of plague and possibly other infectious diseases that this population endured. Moreover, during country-wide famines, it is possible that there was no, or very little, food available in the city of Jelgava. It has been reported that the children born during, or shortly after a major famine develop enamel defects [31: 46], and this might have been the case for at least some individuals in the HTCCP. The significant differences in the number of affected teeth between older male and female individuals might suggest that during certain periods such as wars boys received better care than girls, taking

into account that many of the excavated individuals might represent the same generation [40]. To better explain the differences, however, the defects would have to be analysed and compared according to the affected tooth types in each individual, as it is known that some teeth are more susceptible to the formation of defects than others [9: 84; 19: 11].

With regards to the absence of enamel defects in the non-adult population, it might be possible that the defects on deciduous teeth would be visible with magnification. As a large number of juvenile teeth were lost post-mortem, it is possible that the teeth with enamel defects were among those lost. Alternatively, it has been demonstrated that enamel defects occur in the children who were healthy enough to actually survive childhood stress episodes [55: 355]. The high frequency of enamel defects in the adult population of the Holy Trinity Church cemetery indicates a relatively good chance of survival during hardships. The lack of enamel defects in the non-adult population might therefore represent the weaker individuals who died before enamel disruption became observable on their dentition.

The prevalence of dental caries, periapical lesions and the ante-mortem tooth loss in the HTCCP was compared to two rural low status (Madona and Cesvaine [16]) and two small town moderate status (Ventspils [15] and Valmiera [56]) populations (Table 3). There were no data on periapical lesions for the urban Valmiera population, and no data by the age group in the rural Madona and Cesvaine populations.

Table 3. Dental disease by age and sex groups in broadly contemporary Latvian cemetery populations (crude prevalence/individual count)

	Dental caries	%	*Periapical lesion*	%	*AMTL**	%
	n/N		n/N		n/N	
HTCCP						
Males						
20–40	5/13	38.5	2/13	15.4	5/11	45.5
40+	10/15	66.7	6/18	33.3	15/18	83.3
Total	15/28	53.6	8/31	25.8	20/29	69.0
Females						
20–40	12/16	75.0	9/16	56.3	10/14	71.4
40+	11/16	68.8	6/17	35.3	18/19	94.7
Total	23/32	71.9	15/33	45.5	28/33	84.8
Ventspils**						
Males						
20–40	4/10	40.0	3/10	30.0	3/10	30.0
40+	12/15	80.0	8/18	44.4	8/13	72.2
Total	16/25	64.0	11/28	39.3	16/23	57.1
Females						
20–40	4/7	57.1	4/9	44.4	5/9	55.6
40+	6/10	60.0	6/11	55.5	9/11	81.8
Total	10/17	58.8	10/20	50.0	14/20	70.0
Valmiera**						
Males			*No data*			
20–40	2/4	50.0			1/4	25.0
40+	1/7	14.3			7/10	70.0
Total	3/11	27.3			8/14	57.1
Females			*No data*			
20–40	4/5	80.0			0/4	0.0
40+	5/7	71.4			4/7	57.1
Total	9/12	75.0			4/11	36.3
Madona[1]						
Males (Total)[2]	6/8	75.0	1/7	14.3	9/14	64.2
Females (Total)[2]	7/9	77.8	1/7	14.3	7/13	53.8
Cesvaine[1]						
Males (Total)[2]	4/14	28.5	5/21	23.8	12/23	52.2
Females (Total)[2]	4/11	36.4	8/19	42.1	13/19	68.1

n – number of individuals with dental disease; N – number of individuals with observable teeth/alveoli;

* – ante-mortem tooth loss; ** – urban moderate status; [1] – rural low status; [2] – data by age group not available

The comparison revealed that the HTCCP had the highest rates of caries among the observed urban populations (63.3% against Ventspils (61.9%) and Valmiera (52.1%)), whilst rural Madona and Cesvaine revealed the highest and the lowest caries rates among all the comparative populations (76.4% and 32.0% respectively). In the HTCCP, the rates of the ante-mortem tooth loss were the highest overall (77.4% against urban Ventspils (69%) and Valmiera (48%) and rural Madona and Cesvaine (both 59.0%)). The rates of periapical lesions in the HTCCP were the second highest after Ventspils (35.9% and 43.0% respectively). In rural Madona the rates were the lowest overall (14.2%), while in rural Cesvaine the rates of periapical lesions were very similar to caries rates in this population (32.5%). No patterns in the observed dental disease rates when comparing urban versus rural or high status versus low status populations could be distinguished. Dental disease rates in the HTCCP and urban Ventspils, which was a major port, were similar regardless of status differences. This pattern, especially with regards to caries, could partly be due to the availability of refined sugar within the Duchy. For a more reliable explanation, however, more detailed information on the dental status in the Ventspils population would be necessary.

Valmiera, Madona and Cesvaine, on the other hand, were all under the rule of the Polish-Lithuanian Commonwealth. It is possible that the factors such as cultural beliefs, fertility demands, availability of foods and the general quality of life differed substantially in the regions ruled by different political powers, and that these differences are at least partly responsible for the lack of dental disease patterns in the compared populations. On the other hand, the substantial differences between the neighbouring rural Madona and Cesvaine populations might be due to the quality of life that different landlords in the same region provided to their subjects. More data on the dental status from these populations, such as tooth wear and the number of affected teeth would be necessary for a more informed interpretation.

With regards to the differences between age and sex groups, while some similarities between populations could be observed, there were also exceptions. For example, the caries rates proved to be higher in female individuals in almost all of the observed populations except urban Ventspils where the rates in female individuals were 5.2% lower than in male individuals (Table 3). Likewise, the caries rates increased

by age in male individuals and decreased in female individuals only in the HTCCP and Ventspils population, but decreased in both sex groups in the Valmiera population.

The rates of periapical lesions in the HTCCP were lower than in the Ventspils population and higher than in the observed rural populations. Female individuals were affected more frequently than male individuals in all the populations except rural Madona, were the frequency was identical in both sex groups. The frequency of lesions in the female individuals of Ventspils population decreased with age, a trend that was not observed in the HTCCP.

Finally, the frequency of ante-mortem tooth loss proved to be higher in female individuals not only in the HTCCP but also in the Ventspils and Cesvaine populations. In Valmiera and Madona, however, it was higher in male individuals. In fact, the difference of ante-mortem tooth loss between the female individuals in the HTCCP and Valmiera population proved to be statistically significant ($p=0.004$). It is important to remember that both populations had also very high caries rates. Although such data were not available for the Valmiera population, it can be hypothesised that the severity of carious lesions, as well as the periodontal disease, was different between these populations. It has to be noted that the ante-mortem tooth loss was the only condition that increased with age in both sex groups in all the three urban populations for which the data were available. The dental wear typical of pipe-clay smoking, however, was only recorded in the HTCCP, and this could indicate that unlike sugar, tobacco was a commodity affordable exclusively to higher social classes at the time.

CONCLUSION

It can be concluded that the observed high prevalence of dental caries, periapical lesions, the periodontal disease and the ante-mortem tooth loss in the Holy Trinity Church cemetery adult population is linked to a diet comprising soft carbohydrates and refined sugar. The scarcity of dental diseases in the non-adult population, especially with regards to caries, is indicative of a differential diet for children or the slow progression of the disease with increasing age. The higher number of carious teeth in females might be linked to frequent pregnancies and the differences in saliva composition and flow rate. Alternatively, a softer

diet could also have been responsible for the higher frequency of destructive dental diseases in this sex group. The possibility of differential diet between males and females was also supported by the higher number of teeth with calculus deposits in male dentitions.

The large number of adult individuals affected by enamel hypoplasia was suggestive of a short, but severe systemic metabolic stress during childhood, which could be linked to the problematic political and socio-economic situation in the city of Jelgava and the outbreaks of epidemics. Importantly, the presence of these enamel defects in the HTCCP indicated that the high status of these individuals did not save them from the hardships experienced by the entire society. Nevertheless, their presence also indicated the population's ability to survive these hardships beyond childhood. The significant differences observed in the number of affected teeth between males and females in the older age group ask for a more detailed analysis of the types of teeth affected, as this type of analysis could provide more clues to differential care for boys and girls during certain periods of time.

The inter-population comparison of dental caries, periapical lesions and the ante-mortem tooth loss revealed that no overall pattern based on the status or environment was observable. For all the compared dental diseases and conditions the prevalence of the affected male and female individuals in both age groups differed between the populations. The only exception was the ante-mortem tooth loss, which increasingly affected older individuals in both sex groups in all the populations. It seemed that overall the prevalence of dental diseases in the HTCCP was moderate, especially with regards to the two urban populations.

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ASSESSMENT OF THE PHYSICAL ACTIVITY LEVEL FOR THE STAFF MILITARY PERSONNEL

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ABSTRACT

Sport and physical activity is an important and compulsory part for the people who connect their lives with the military system. The military speciality and the quality of execution of the service duties and tasks depends on the fitness level and physical preparedness. We have provided the assessment of physical activity for the staff personnel that is involved in the administration staff work. Otherwise each military officer should be ready to fulfil orders and tasks. Effective and active military service is impossible without an appropriate level of physical preparedness. We have provided the questionnaire for staff officers in 2009–2010 (n=52). The questionnaire embraced all kinds of physical activities during the daytime. We have received information about sports and physical activity during the service time and free time, the received data about the participation in morning physical activities, the use of the stairs (to go upstairs and downstairs), the execution of physical exercises during the working hours and sports activities after working hours and in the weekend, taking part in the sport events, physical work at home. We have the data of the self-assessment concerning overweight, smoking and alcohol using, the regular regime (sleeping hours and the diet). We have included the questions concerning health problems (the cardiovascular disease, the gastrointestinal disease, the respiratory disease, trauma and ect) and the duration of medical incapability (days per year). We have calculated the points of the physical activity questionnaire. After that we divided the respondents into four groups (lower, moderate, good and high) according to the results of the level of physical activity. The assessments of the physical activity level allow correlating the life style, and the support and keep physical preparedness and develop physical abilities of the staff personnel.

***Key words*:** *Physical activity, military personnel.*

INTRODUCTION

Sport and physical activity is an important and compulsory part of the military personnel life. The military speciality and the quality of execution the service duties and tasks depends on the fitness level and physical preparedness [6, 8, 9]. We have provided the assessment of physical activity for the staff personnel that is involved in the administration, the staff work. Otherwise a military officer should be ready to fulfil the orders, tasks. The execution of service duties could not be possible without the appropriate level of physical preparedness [12, 13, 14]. Regular physical exercises have health benefits in relation to cardiovascular and metabolic diseases [1, 10, 11]. It is very important to motivate individuals to be active that allows keeping physical preparedness on the appropriate level and developing physical abilities [2, 4, 5, 6, 7].

MATERIAL AND METHODS

We have provided the questionnaire for the staff officers in 2009 (n=22) and 2010 (n=30). The staff officers aged from 28 years to 37 years.

The questionnaire includes the positions that allow us to collect the information about sport and physical activity during the service time and after it [3]. The questionnaire embraced all the spectrum of the day's physical activities: morning physical activities, the use of the stairs (to go upstairs and downstairs), physical exercise during the working hours, sports activities after working hours, and in the weekend, participation in the sport events, physical work at home, the self-assessment concerning overweight, smoking and alcohol using, regular sleeping hours and the diet. Regular physical activities have beneficial effects in general and particularly in relation to cardiovascular and metabolic diseases. We included the questions related to health problems (cardiovascular, respiratory, gastrointestinal diseases, trauma and ect) and the duration of medical incapability (days per year). The data of the questionnaire were evaluated according to the scale (in points) and calculated. Those allow us to divide respondents into groups according to the levels of physical activity (low, moderate, and good, high).

RESULTS AND DISCUSSION

The questionnaire contains about eleven positions, each one gets evaluation in points. We have calculated the total daily physical activity level (account of points) and received results. The respondents are divided into four groups according to the level of physical activity: low, moderate, good, high. According to the questionnaire results the low level of physical activity in 2009–2010 was in 23.3% –31.8% of respondents, the moderate level of physical activity in 20–45.4%, the good level of physical activity in 22.7% – 23.3% of respondents, the high level of physical activity in 33.3% of the respondents in 2010 and no one in 2009 (Figure 1).

Morning physical activities have favourable influence upon the working capacities. Respondents' answers have shown that 13.6% respondents in 2009 and 13.3% in 2010 have morning physical exercises. There were 23.3% of respondents in 2010 and two times more respondents (54.5%) in 2009 that did not at all make morning physical activities (Figure 2).

In the modern society when the distance between the office and the living apartment is large, we would like to get information about sports (physical) activities of respondents during the week, during the weekend, and about the duration and frequency of sport activities. All the respondents have physical activities (Figure 3) during the week time or in the weekend. The majority of respondents have regular physical activities during the week (after service) or in the weekend 31.8% (2009) and 60% (2010). Physical activities during the service time have regularly 31.8% (2009) and 43.3% (2010) of respondents. The duration of sport exercises was variable from 2 hours to 8 hours per week. There are about 6.6% of respondents who spend up to 2 hours for physical activities in the week, 10% of respondents spend more than 10 hrs for sport activities, the majority of respondents spend for sport activities about 3–6 hours per week. We have asked the questions concerning the respondents' attitude to using the elevator or stairs. The results of the questionnaire showed that 68.2% (2009) and 63.3% (2010) of respondents regularly used the stairs. Only 3.3% in 2010 and 18.2% of the respondents in 2009 do not like stairs and use the elevator where it is possible.

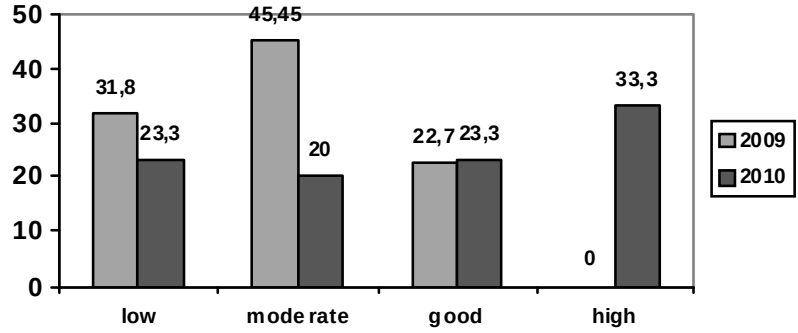

Figure1. Distribution of respondents according to the physical activity level (%).

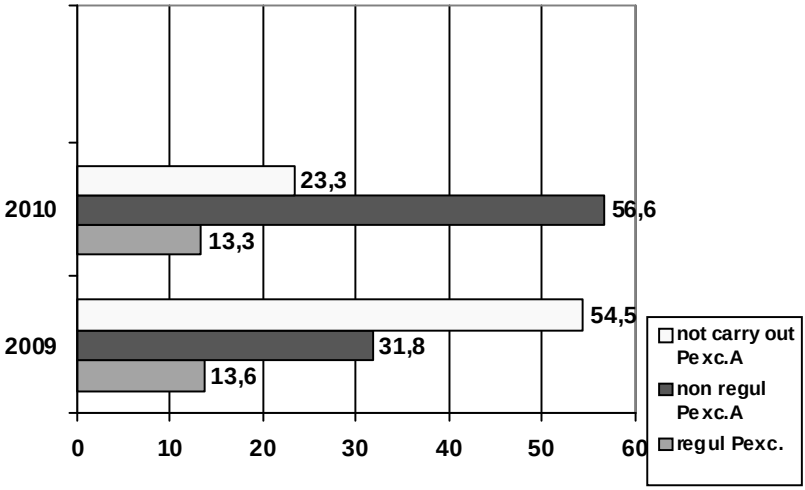

Figure 2. Respondent's attitude to morning physical exercises (%).

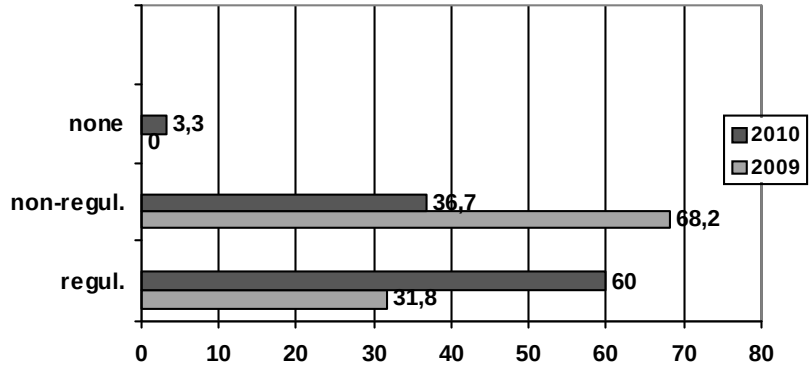

Figure 3. Physical activities of respondents in week/weekend (%).

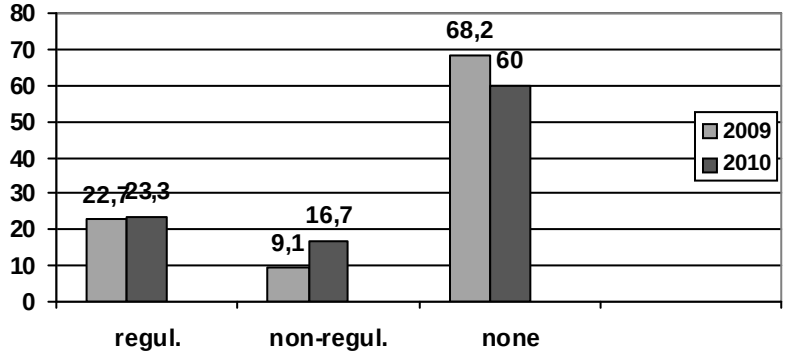

Figure 4. Speadwide of smoking in respondent groups(%).

The negative impact on the health and well-being is exercised by bad habits (smoking, using of strong alcohol, ignoring the regular meals and sleeping insufficient). We have asked questions about these problems in the questionnaire and receioved such feedback. Regular smokers in the staff personnel 22.7% in 2009 and in 23.3% of respondents 2010; 4.5% of respondents (2009) and 20% (2010) did not use alcohol at all; 13.6% of respondents (2009) and 16.6% (2010) have less than 6 hours of sleeping time.

We have received the information that about 77.3–80% of respondents get medical illness certificate for 5 days per year. The reasons of incapacity were related to health problems such as trauma in 77.3–80% cases, respiratory diseases- in 22.7% –33.3% cases, skeleton and muscular diseases in 18.2% cases, cardiovascular diseases in 3.3% cases.

During the daily activities individuals have physical activities outside the service, it could be organized at the home, in the garden, outside the house ect. We asked the question about the respondents' attitude and participation in these activities. We have received positive answers (confirming participation in home physical activities) from 36.4% (2009) and 46.7% (2010) respondents; 59.1% (2009) and 53.3% (2010) have no regular physical activities at home, but 4.5% (2009) of respondents did not take part in physical activities at home at all.

In the modern society when the distance between the office and the living apartment is large, people use transport (individual or public) for travelling from home to office. Walking as a physical activity is not widely spread. We ask a question about the regular daily walking to the office and back home. We received such data: 54.5% of respondents in 2009 and 36.7% of respondents in 2010 did not walk from home to office and back. They use any kind of transportation. But 54.5% (2009) of respondents and 36.7% (2010) of respondents regularly walk to office.

CONCLUSION

1. The low level of physical activity indicated the problems to pass annual physical tests and the problems of physical fitness. The respondents in that group can get health problems overload, the harmful effect of physical load during service or mission. The lower level of physical activity according to the questionnaire was fixed for 31.8% staff officers in 2009 and 23.3% of staff officers in 2010. The moderate level of physical activity was determined for 45.5% of respondents in 2009 and 20% of respondents in 2010, the good level of physical activity characterizes 22.7% (2009) and 23.3% (2010). The high level of physical activity was recognized for 33.3% of respondents in 2010, but in 2009 there was no person with a high level of physical activity.

2. The respondents preferred the physical activities during the week/weekend regularly 31.8% in 2009 and 60% in 2010; 68.17% of respondents in 2009 and 36.7% of respondents in 2010 have no regular physical activities. The personnel have the motivation to be active and to keep physical preparedness.
3. Regular morning physical activities characterize 13.3% of respondents (2010) and 54.5% in 2009; 13.6–23.3% of respondents did not spend the time for morning physical activities.
4. 22.7% of respondents in 2009 and 23.3% of respondents in 2010 were smokers. Smoking has a negative impact on health, on physical readiness;. 60–68.2% of respondents do not smoke at all.
5. Self-estimation revealed that overweight problems were fixed for about 13.3% of the respondents in 2010 and 63.6% in 2009. The most spread healthy problems were connected to the respiratory system disease and to trauma.

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ALTERATIONS OF CERVICAL VERTEBRAE IN TWO INDIVIDUALS FROM THE LATE ANTIQUTY NECROPOLIS FROM THE "BIG MOUND" NEAR CABYLE, BULGARIA

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ABSTRACT

The anthropological examination of two skeletons, of individuals identified as males, at 30–40 and 60–65 years, respectively, excavated from the grave complexes of the Necropolis of Big Mound, Cabyle, dated in the late 4th century AD, revealed abnormalities in the cervical section of the vertebral column, in C 1st and 2nd. In the first case (grave N 2,), neural arches of C1 and C2 are fused together by two clearly visible bony bridges at the dorsal side of neural arches. In the second case (grave N 3) both vertebrae are changed, with the dens axis strongly bended to the left, the articulation surfaces with the first vertebra are on different geometrical surfaces, the left one being on a higher position and bended at approximately 90°. The form the first cervical vertebra is adequate to the changes of the second vertebra. A lack of lesions, characteristic of the trauma or the infection is observed. The case from grave N 2 appears easy to be explained with inborn anomaly, as the Clippel-Fiel syndrome. The appearance of fusion, realized by clearly distinguishable bony bridges and clearly divided vertebrae from each other, instead of characteristic of the inborn condition, close, undivided position of neural arches with unclear outlines, the result from abnormality in embryonic development makes it also possible to have the interpretation as a survived trauma in the region. The changes of cervical vertebrae from the individual from grave N 3, could be interpreted as the development in the course of ossification of the centre of the dens axis and these both as halves of atlas during infancy.

***Key words*:** C1–C2 abnormalities, Late Antiquity, Bulgaria

INTRODUCTION

The necropolis, having series of graves, excavated on the "Big Mound" near the ancient town of Cabyle, after the researchers of the archeological site [8] had left, is dated in the late 4th century AD. The excavations uncovered nine graves, containing more complete skeletons presenting bones *in situ* in anatomical order from whole/part of the skeleton [8] from eight individuals. There were found also traces from disturbed graves, including many single fragments from human bones from the fill of graves or from the terrain.

MATERIAL AND METHODS

For the anthropological identification of skeletal remains were used the methods, described in Acsádi & Némeskeri [1] for the gender determination after the assessment of features on pelvic and cranial bones, with the priority of data from pelvic bones. For the inclusion of more information were used also the measurements of long bones as diameters of femoral, humeral and radial heads, the bicondylar breadth of femora and humeri and the length of clavicle compared to the tables of Pearson and Thieme in Bass [3], Kühl [4], and Alekseev [6]. The age at death of the adult individuals was assessed according to the methods for scoring the pubic bone symphyseal surface relief following Todd in Schwartz [5] and the cranial suture closure following Simpson-Olivier in Alekseev-Debets [7].

In the material five males, two in the age group of adults, 20–40 years at death, two matures, 40–60 years, one senile, of 60–65 years and four females, two in the age group of adults, 20–40 years and two matures, 40–60 years were recognized. After single fragments were determined, the remains of more than 19 individuals, from which more than nine children in the age group *Infants I*, 0–7 years of age, and ten individuals with finished skeletal development, over 18–20 years of age.

In the anthropological examination of two skeletons from the grave complexes, dated in the late 4th century AD, were found abnormalities in the cervical section of the vertebral column. In the first case, the individual from grave N 2, square J/-1, neural arches of two cervical vertebrae are fused together (Figure 1: 1–3). The fragments are identified as the posterior arch and the right lateral mass of C1 and the respective posterior arch and the right lateral mass of C2, fused dorsally

with two bony bridges. The bridges between the affected vertebrae develop from the inside of the arches and are clearly divided and defined. The excavated material did not present the whole cervical section of the vertebral column, but from a fragment it appears that the following C3 shows a developed bony reaction from arthritis on the right superior articular facet, correspondent to similar changes on the right inferior articular facet of the C2 (Figure 1: 4–5).

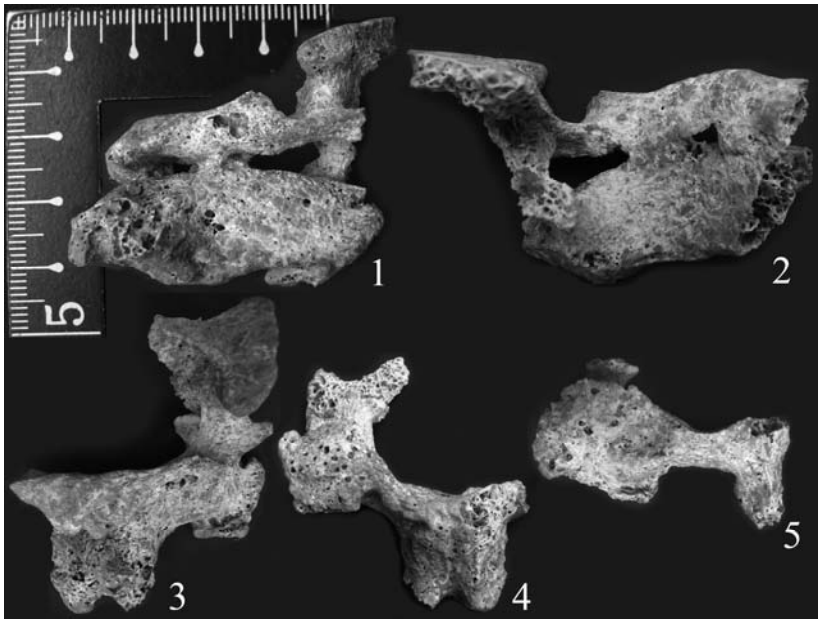

Figure 1. Grave N 2, square J/-1, the Big Mound, Cabyle. Fragments from C1-C3. 1. Fragment from C1-C2 posterior view. 2. Fragment from C1-C2, view from the vertebral foramen. 3. Fragment from C1-C2 proximal view. 4. Fragment from C1-C2 distal view. 5. Fragment, lateral right proximal articular surface of C3.

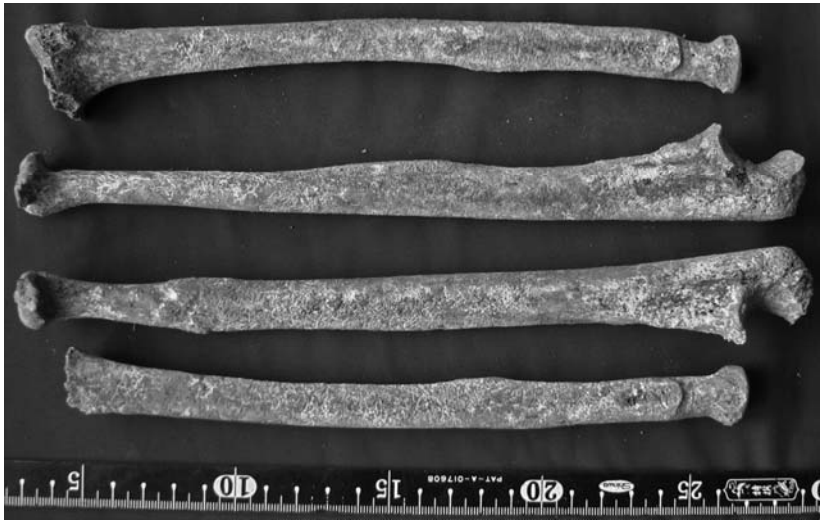

Figure 2. Grave N 2, square J/-1, the Big Mound, Cabyle. Radiuses and ulnae.

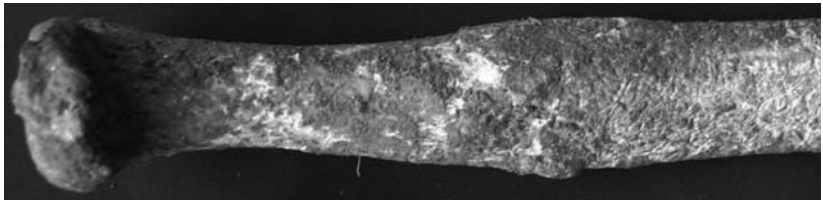

Figure 3. Grave N 2, square J/-1, the Big Mound, Cabyle. Detail, distal part of radius with traces of trauma.

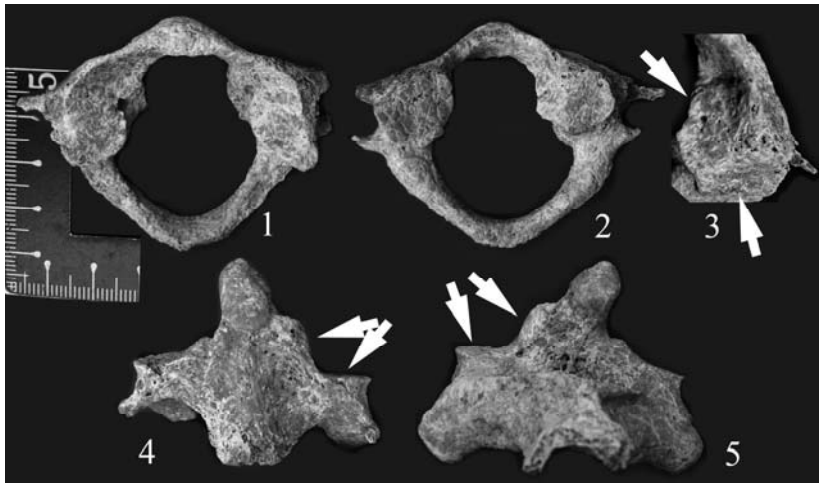

Figure 4. Grave N 3, square J/-1, the Big Mound, Cabyle. 1. C1 proximal view. 2. C1 distal view. 3. Detail, the left inferior articulation surface of C1 arrows to surfaces of changed articulation facet. 4. C2 anterior view, arrows to the surfaces of the changed articulation facet. 5. C2 posterior view, arrows to the surfaces of the changed articulation facet.

The skeletal material from the individual found in grave N 2, square J/-1 was highly destructed and incomplete. The skull was presented with fragments from the cranial vault, including the frontal bone, with the supraorbital region, the fragments from both parietals, from the occipital bone (from the squama and the basilar part), fragments from both temporal bones. From the face skeleton both zygomatic bones and the fragment from the right part of maxilla were present. Mandible allowed reconstruction. Dentition was fragmentarily preserved. From the bones of the postcranial skeleton both humeral bones, radiuses, ulnae and the right clavicle were complete or fragmentarily presented accompanied with the incomplete number of carpals, metacarpals and phalanges of hands. From both scapulae only fragments, both presenting glenoid fossa and acromion were found. From the vertebral column four cervical and four thoracic vertebrae were found. A fragment from the manubrium of sternum was also present.

With no preserved pelvic bones, the gender of the individual from grave N 2 square J/-1 was ascertained as a male after the descriptive

features of the cranial fragments as the moderately developed relief of *glabella* region, the oval shaped superior orbital margin, the developed temporal relief over the external auditory meatus and on the mastoid processes and massiveness and the relief of mandible were fixed. The values of the vertical diameter of the humeral head and the bicondilar breadth were more close to the mean for the male gender after the used tables. After the cranial sutures obliteration, the age of this individual was estimated 60–65 years, the age group of senile. Fragments from dentition showed advanced teeth loss – ascertained at least one incisor, two premolars and five molars lost ante mortem. Alveolar processes show the traces from the advanced periodontal disease. The bones from upper limbs and the vertebral column showed advanced degenerative changes on the articulation surfaces. On the material from this individual the presence of a trauma lesion on the distal third part of the diaphysis of the left ulna was found (Figures 2, 3). The correspondent part of the left radius was not affected from a trauma, but suffered from the advanced arthrosis in the wrist joint (Figure 2).

The second case of the abnormal anatomical form of the cervical vertebrae was registered on C1 and C2 of the individual from grave N 3, square J/-1. In this skeleton the dens axis of C2 is strongly bended to the left, the vertebral body appears changed from the normal anatomical form. Its articulation surfaces with C1 lie in different geometrical surfaces, a part of the left one being bended at approximately 90° with one surface remaining with proximal orientation, on a higher position and the other part, perpendicular, with the lateral orientation, the right lateral articulation surface, respectively, remains on a lower position (Figure 4: 4–5). The form of the C1 is adequate to the changes of the C2, being bended according to the uneven positions of the articulation surfaces of the vertebral body of the second vertebra (Figure 4: 1–3). Its left distal articulation surface shows the form respective to the geometry of the articulation of the C2 (Figure 4:3). On both, C1 and C2 are not ascertained lesions, which can be interpreted as traces from the fracture or the infectious process, neither posttraumatic nor from general infection. The articulation between the dens axis and the atlas suffered from strong degenerative changes, pronounced on both bones. In this case the C3 and C4 are present and do not show changes, including the signs for the development of the degenerative joint disease.

The skeletal material from the individual from grave N 3, square J/-1 was preserved in a better condition. Again, the skull was fragmentary

with parts from the frontal bone, with the supraorbital region, fragments from both parietal and temporal bones and the occipital squama. From the face both zygomatic bones and fragments from the alveolar processes of maxilla and mandible with partially preserved dentition were present. The postcranial skeleton was represented by both humeral bones, radiuses, ulnae, femurs, the right tibia and the left clavicle were fragmentarily presented accompanied with the incomplete number of carpals, metacarpals, tarsals, metatarsals and the phalanges of hands and feet. From both scapulae only fragments with preserved glenoid fossa and acromion were found, from pelvic bones fragments, including both pubic bones were found, on both pelvic bones a greater sciatic foramen is preserved. From the vertebral column four cervical, 11 thoracic and all five lumbar vertebrae were found. A big portion of sacrum was also preserved, allowing the reconstruction of the form and dimensions. Numerous fragments of ribs were also present.

During the anthropological examination the gender of this individual was ascertained as a male, after some features of the observed pelvic fragments, as the form of a greater sciatic foramen and the reconstructed pubic angle. Some of the observed features on cranial fragments, as the developed relief of the *glabella* region, the developed relief of supraorbital arches, the oval form of the upper margin of both orbits, the developed temporal relief over the external auditory meatus, the lateral tubercle of both zygomatic bones, the massive mandible, with the developed relief on the angle led to this identification. Characteristic of the male gender were the taken measurements of the bones of limbs. The age at death of this individual was estimated 30–40 years after the obliteration of cranial sutures. The relief of the pubic bones and the auricular surfaces on the iliac bones was difficult for accurate assessment, because of its post mortal destruction, but it appeared as a characteristic for the determined age. Dentition of this individual was with one fragmentarily preserved incisor, two canines, five premolars and eight molars. From the present teeth, the upper second left molar was affected by caries, the reduced alveolar process and the shallow alveolus on the place of the lost upper right first molar pointed to its destruction from the carious process and even the loss shortly ante mortem. Alveolar processes showed the traces of the periodontal disease. A line of enamel hypoplasia could be correlated to the second year of individual development. On orbits the lack or *cribra orbitalia*

was ascertained. On lumbar vertebrae changes caused by spondylosis were observed. Traces from initial arthrosis were found on both glenoid fossae, on humeral trochlea at both sides and on the olecranon of both ulnae.

RESULTS AND DISCUSSION

Both finds present controversial aspects of interpretation. The case from grave N 2, square J/-1 appears easy to be explained with inborn anomaly of the vertebral column, as the Clippel-Fiel syndrome. Embarrassing in this interpretation is the appearance of fusion, realized by bony bridges, clearly visible and distinguishable from each other and from neural arches, clearly divided as well from each other, instead of the characteristic for the inborn condition close, undivided position of the fused parts of bones as a result of the failure of formation of fissures between them in the development as described by Barnes [2]. Possible explanation of the abnormality in this case could be as well a survived trauma in the cervical section of the vertebral column. A trauma, observed on the left ulna can not be related with the changes in cervical vertebrae, but could be regarded as a circumstantial evidence for the risks in the life of this individual.

The cervical vertebrae from the individual from grave N 3, square J/-1 showing no lesions, which can be undoubtedly interpreted as traumatic, or bone reaction characteristic of infectious condition, do not show abnormality, registered on the anthropological material as an inborn condition as well. The changes, visible on the find, could be interpreted as developed in the course of ossification of the posterior arch and the lateral masses of C1 and the ossification of dens axis, the body and the dorsal arch of C2 during infancy, from the early stage of the process, with fusing of the arch and the body of C2 at 3 years, the simultaneous ossification of the posterior arch of C1 between 3–4 years and the fusion of the anterior arch of C1 with the lateral masses at 5–9 years as described in Swartz [5]. The normal ossification of C1 and C2 could have been disturbed by a trauma, and the survival of the individual at that age. It could also have been caused by a specific activity, which could have overloaded the section of the vertebral column disturbing the normal morphology of C1-C2.

Both findings from the cervical section of both individuals could be regarded as a result from conditions during infancy of specific hazards of everyday life of the population or the specific activity of individuals at that age. The rest of the skeletons of the series, which are poorly preserved, did not show such changes in the cervical section of the vertebral column or traces of trauma.

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CHRONIC ALCOHOL ABUSE IS IMPLICATED IN THE OXIDATIVE STRESS AND THE CHANGES IN THE NEUROTROPHIC FACTOR RECEPTOR EXPRESSION IN THE HUMAN CNS

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ABSTRACT

Alcohol abuse and alcoholism induce brain damage, and, in some cases, neurodegeneration. The pathogenesis of the alcohol-induced injury of the CNS is a complex process in which oxidative stress plays an essential role. Alcohol increases the formation of reactive oxygen species and affects the antioxidant defense system of the brain. It is well known that oxidative stress induces apoptosis in neurons, as well as in other cell types. Neurotropins and their receptors have a crucial role in neural regulation. The aim of this study is an overall analysis of the CNS neuronal and glial cell death and the oxidative status by the use of immunohistochemical methods.

Three different CNS regions – cortical, subventricular and basal ganglia were analyzed in the autopsy samples obtained from 10 chronic alcohol abused patients. The immunohistochemical detection of oxidative damage was performed using anti-Cu/Zn SOD monoclonal antibody, neural activity – anti-NGFR ($p75^{NTR}$) antibody, apoptosis – the TUNEL reaction. Both, quantitative and semiquantitative estimations were used for the evaluation of results. The subventricular zone was characterized by a negative (75%) and a moderate (25%) astroglial SOD1 expression, the basal ganglia region – by strong (43%), moderate (43%), and low (14%) neuronal and moderate (71%) and low (29%) astroglial SOD1 expression, whereas, the cortex – by strong (33%) and moderate (66%) neuronal and moderate (67%), low (17%) and negative (17%) astroglial expression. The SOD1 expression was not detected in oligodendroglia and ependymocytes. Brain regions showed variability in the apoptotic cell death rates. Neuronal TUNEL-positive staining in

basal ganglia was higher than in the cerebral cortex. TUNEL-positive astrocytes were detected in the white matter, more frequently in the basal ganglia region when compared to the cortex. The apoptosis marker was nearly absent in ependymal and oligodendroglial cells. The rate of TUNEL-positive cortex endothelial cells was detected at 7.9% level in the case of chronic alcohol abuse. Neuronal processes showed heterogeneous NGFR expression: in the cortex, basal ganglia and the subventricular zone (negative/low), whereas the subcortex and the white matter – moderate and moderate/strong, accordingly. Alcohol-induced CNS vulnerability is related to the increase in oxidative stress; furthermore, it suggests an increased risk of neurodegeneration for neuronal and glial cells.

***Key words*:** *CNS, chronic alcoholism, oxidative stress, apoptosis.*

INTRODUCTION

Experimental and post-mortem studies indicate that the most probable risk factor which induces global changes in brain morphology, is toxicity, including the chronic use of alcohol [5, 17, 19]. The basal ganglia provide a variety of functions in addition to the regulation of motor activity. The dysfunction of alcohol-abuse affected neuronal and glial cells gives rise to neurodegeneration in the basal ganglia. It has been reported that protective mechanisms, including neuronal loss, are involved in neurodegenerative disorders [9]. The programmed cell death is essential for normal tissue homeostasis. However, a chronic exposure to alcohol is the cause of increasing the amount of apoptotic cells in the striatum. Alcohol-induced oxidative stress plays a role in the pathogenesis of an injury in the central nervous system (CNS) [15]. A number of studies provided data on the changes of the nerve tissue response to the oxidative stress due to alcohol abuse. The importance of Cu/Zn superoxide dismutase (SOD1) in the CNS is confirmed by numerous findings of a protective action of this enzyme against brain injury and neuronal death [13, 10]. Recent studies [11, 14] have reported controversial results on human $p75^{NTR}$, also known as the low affinity nerve growth factor receptor (NGFR). It has been found to increase or inhibit the axonal growth, reduce or promote the neuronal cell death, and is necessary or not required for the inhibition of neuronal regeneration. Results on neurotrophins expression in different CNS

regions are greatly varying, experimental evidences suggest that alcohol exposure affects the expression of receptors for neurotrophic factors and impairs the production and the release of growth factors [6, 8]. The subventricular zone in the wall of the lateral ventricle is of particular interest because neurogenesis persists there during adulthood [20]. The mechanisms underlying alcohol-induced changes are unclear. The goal of the present study was to investigate alcohol-initiated morphological changes in the autopsy brain tissue by the use of immunohistochemical methods.

MATERIAL AND METHODS

The autopsy brain tissue was obtained from 10 chronic alcohol users with postmortem interval <48 h, aged 33 to 59 years according to the criteria for alcoholic cases [7]. The postmortem tissue was fixed in formalin and embedded in paraffin for routine histology. Three different CNS regions – cortical, subventricular and the basal ganglia were analyzed and compared with the brain tissue from the control non-alcoholic group. Immunohistochemical reactions were performed at sections using anti-Cu/Zn SOD monoclonal antibody (1:50, Novocastra) and anti-NGFR monoclonal antibody (1:50, DakoCytomation), apoptosis was detected by terminal deoxynucleotidyl transferase-mediated biotin-dUTP nick end labeling (TUNEL) reaction (Roche). A semiquantitative scale was used to estimate the degree of SOD1 and NGFR expression. The immunostaining was scored as follows: strongly positive, 3; moderate positive, 2; low positive, 1; and negative, 0. The percentage of TUNEL-positive cells was quantitated by counting TUNEL positive and negative cells in 10 random microscope fields. All the tissue sections were analyzed using a Leica microscope (x400). Study procedures were conducted in accordance with the rules of the Ethical Committee.

RESULTS

Semiquantitative evaluation revealed the following percentages of low, moderate and strong expression of SOD1: 14% low, 43% moderate, 43% strong in the basal ganglia neurons; 29% low and 71% moderate in the basal ganglia astroglial cells (Figure 1), whereas the cortex was

characterized by moderate (67%) and strong (33%) neuronal SOD1 expression; moderate (66%), low (17%) and negative (17%) astroglial SOD1 expression. In comparison, the neurons of the control group and, particularly, astrocytes showed a low SOD1 expression. The white matter showed low (50%) and moderate (50%) astroglial SOD1 expression, and moderate SOD1 expression in neuronal processes (Figure 2), whereas the control group showed negative or occasionally low astroglial and neuronal processes SOD1 expression. The subventricular zone showed negative (75%) and moderate (25%) astroglial SOD1 expression, whereas the control group showed scattered SOD1 positive astrocytes. Negative SOD1 expression is revealed in ependimocytes and oligodendrocytes (Table 1).

Neuronal TUNEL-positive staining in the basal ganglia (Figure 3) was higher than in the cerebral cortex. TUNEL-positive astrocytes were detected in the white matter, more frequently in the basal ganglia region than in the cortex. TUNEL-positive staining in the brain tissue in the cases of chronic alcoholism compared with the control group was significantly higher. Apoptosis was not detected in oligodendroglia (Figure 4) and was almost zero in the ependyma. The apoptosis of endothelial cells was also detected within the vascular beds. The percentage of TUNEL-positive cortex endothelial cells varied from 2.88% up to 10.46% in the cases of chronic alcoholism.

Neuronal processes showed heterogeneous NGFR expression in the cortex, subcortex and basal ganglia regions (Table 2). The axons of the basal ganglia neurons demonstrated negative or low NGFR expression, whereas the white matter showed moderate or strong expression (Figure 5). The cortex showed negative or low, whereas the subcortex – moderate NGFR expression (Figure 6). NGFR expression in the subventricular zone was negative or low.

Table 1. Occurrence and distribution of SOD1 expression in alcohol users

	Subventricular zone			Subcortex						Cortex		
				Basal ganglia			White matter					
Nr	epen-dyma	pro-cesses	astro-	asto-	oligo-	neuro-	pro-cesses	astro-	oligo-	astro-	oligo-	neuro-
1				2	0	3	1/2	0/1	0			
2				2	0	3	1/2	0/1	0			
3				2	0	2	2	2	0	2	0	2/3
4				2	0	3	1	0/1	0			
5							0/1	2	0	2	0	2/3
6	0	0	0	0/1	0	2	3	0/1	0	0/1	0	1/2
7	0	2	2				1	0/1	0	0	0	1/2
8	0	0	0				1/2	1/2	0	1/2	0	1/2
9	0/1	1/2	0				1/2	1/2	0	2	0	1/2
10				2	0	1/2	2/3	2	0			

Table 2. Occurrence and distribution of NGFR expresssion in alcohol users

Nr	Basal ganglia	White matter/ basal ganglia	Subventricular area	Cortex	White matter/subcortex
1	1	3			
2	1	2/3			
3				0/1	0/1
4	0/1	2/3			
5			0	0	2/3
6			0	0	3
7		1/2	0	0/1	1
8	0	1/2	1		
9				0	2
10	1	3	0/1		

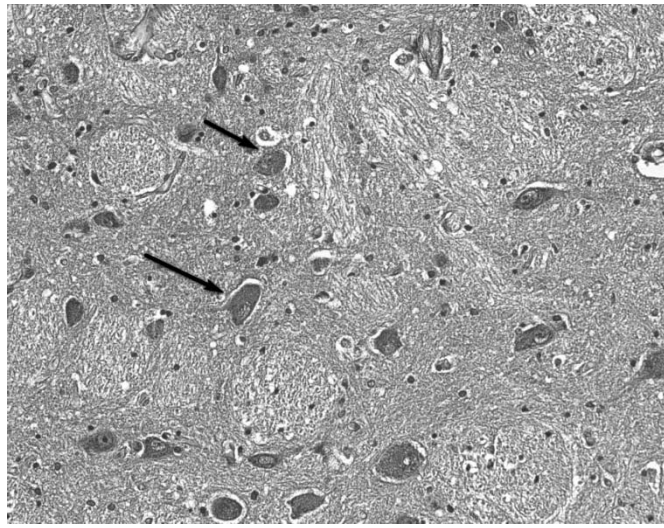

Figure 1. SOD1 immunopositive neurons (black arrows) in the basal ganglia region. Original magnification x250.

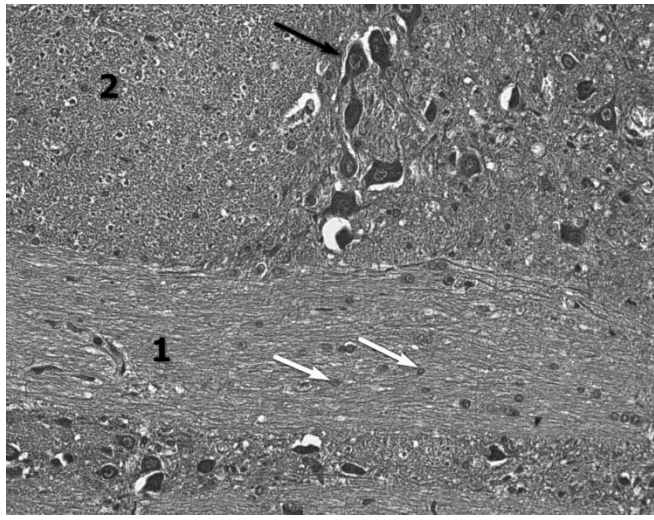

Figure 2. SOD1 immunonegative oligidendroglia (white arrows) in the white matter (1), SOD1 immunopositive neurons (black arrow) in the cortex (2). Original magnification x250.

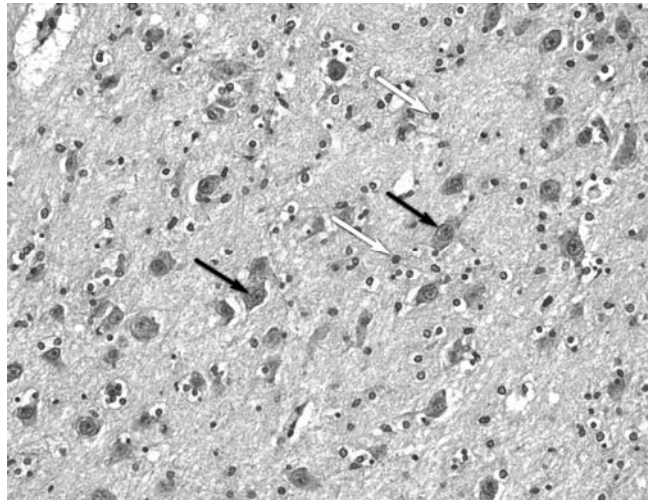

Figure 3. TUNEL-positive astrocytes (white arrows) and TUNEL-positive neurons (black arrows) in the basal ganglia region. Original magnification x250.

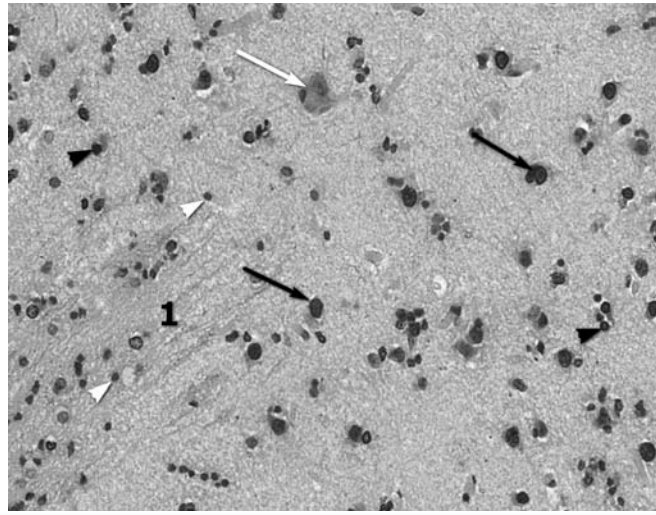

Figure 4. TUNEL-negative oligodendroglia (white arrowheads) in the white matter (1), TUNEL-positive (black arrows) and astrocytes (black arrowhead) and TUNEL-negative (white arrow) neurons in the cortex. Original magnification x400.

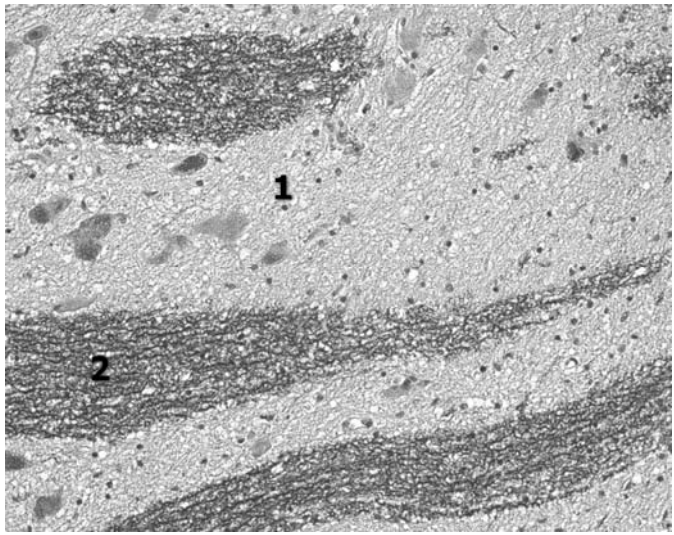

Figure 5. Negative NGFR expression in the basal ganglia (1) and strong expression in the white matter (2). Original magnification x250.

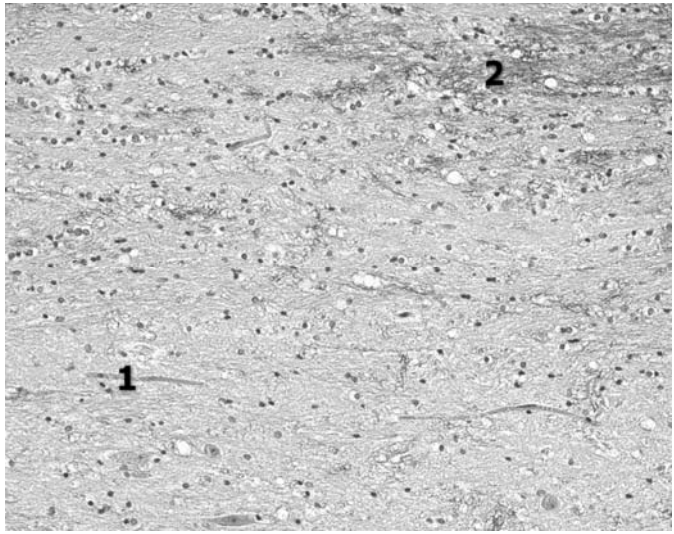

Figure 6. Low NGFR expression in the cortex (1) and moderate in the subcortex (2). Original magnification x250.

DISCUSSION

Cu/Zn superoxide dismutase, which is a key antioxidant enzyme, is present in the central and peripheral nervous systems [3, 12, 22], however, the cellular and intracellular localizations of SOD1 are not well defined. Our data suggest that SOD1 is strongly expressed in neurons, and to a lesser extent – in neuroprotective astrocytes. The highest expression of SOD1 enzymes is observed in the basal ganglia neurons, and it may be caused by the motor system vulnerability underlying chronic alcohol exposure. Moderate or low expression of antioxidant enzymes in the basal ganglia astrocytes suggests an increased activity of the nerve tissue protective system. Alcohol related changes are characterized by the increased expression of SOD1 in neurons paralleled by moderate or low, and even negative enzymatic protection in the astroglial cells of cortical regions, providing additional information on nerve tissue response to oxidative stress. This study evidenced the lowest subventricular expression of astroglial SOD1 suggesting poor reactivity within a region responsible for potential neurogenesis. Occasianally, antioxidant enzyme immunoreactivity was observed in the ependymal cells of lateral ventricles.

Cortical, subcortical and basal ganglia regions show variability in the apoptotic cell death rates [4]. TUNEL-positive staining is significantly higher in the brain tissue in the cases of chronic alcoholism compared with the control group. TUNEL-positive staining in all the cases of alcohol abuse identified the cell types which are the targeted for alcohol toxicity. Our findings suggest that the programmed cell death of both neurons and astrocytes is enhanced in the cortex and the basal ganglia regions. Our observations are in accordance with the recent studies [2, 16] and confirm a high TUNEL-positive neuronal staining in the cerebral cortex associated with the loss of brain volume. TUNEL-positive astrocytes were most frequently detected in the white matter in subcortical regions. Neurons and astroglial cells are sensitive to alcohol consumption, which promotes an apoptotic pathway of neuro-degeneration. Endothelial cell apoptosis allows considering a possibility of the blood-brain barrier disfunction [18]. Despite the fact that the alcohol-induced oligodendroglial cell death has been reported previously [1], we were unable to confirm oligodendroglial damage underlying alcohol consumption.

The number of neurons bearing low affinity p75 neurotrophin receptor is higher in the alcohol abuse cases compared to the control group. Our observations of significantly elevated expression of NGFR in the white matter of the basal ganglia region, chronically affected by alcohol, are in accord with the previous work [21]. Whilst cortical regions show the increased activity of antioxidative enzymes and the TUNEL-positive staining of neuronal cells, p75 neurotrophin receptor expression appears to be negative in the cortex and moderate in subcortical axons. A decreased p75 neurotrophin receptor expression may be suggestive of the cell damage occurring in the brain tissue [23].

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TRAINING PROGRAMME TO DEVELOP YOUNG VOLLEYBALLERS' JUMPING ABILITY

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ABSTRACT

In the current study, the jumping ability development programme created by A. V. Belyayev and L. V. Bulykina was used in training groups of young volleyballers of different sex and age during 54 days.

The sample being trained consisted of two groups. The first group, aged 16–20 years, consisted of 6 girls and 6 boys. The second group, aged 8–13 years, consisted of 4 girls and 6 boys. All of them were beginning volleyballers who had practised for 1–3 years. The control group consisted of 8 boys aged 17–18years. At the beginning of the experiment all of them underwent anthropometric measuring (6 measurements) and tests – height of standing and running vertical jump, height of block jump (standing jump with both arms stretched upwards and speed of motion was assessed by the method of zigzag run [13]. The control group underwent the same studies but did not participate in the jumping ability development programme. The results revealed that in all groups the results of jumping tests and speed tests improved. Linear correlation analysis of anthropometric measurements and tests results showed a significant correlation between body measurements and tests results. The authors recommend to apply this development programme as an aid for coaches.

***Key words*:** *young volleyballers, jumping ability development programme, anthropometry.*

In volleyball physical abilities serve as a basis for technical performance [2, 6, 11]. Particularly great importance has been attached to jumping

ability, as most elements that bring points to a team – attack, block and serve – are performed from a jump [5, 8, 9, 10].

Considering the above mentioned, coaches are attempting to create increasingly efficient jumping ability development programmes for young volleyballers.

The aim of this study was to apply the jumping ability programme created by A. V. Belyayev and L. V. Bulykina [1] specially for volleyballers in training groups of young volleyballers of different sex and age.

SUBJECTS OF THE STUDY

The subjects of the study were beginning young volleyballers who had practised volleyball for 1–3 years. The first group consisted of 12 players aged 16–20 years. They were members of the volleyball hobby group of Tallinn Secondary School No. 23, six girls and six boys. The second group consisted of ten children aged 8–13 years. They were the volleyball group of Tallinn Kalev Sports Society – four girls and six boys. In addition, there was a control group that consisted of students of Form 11 at Tallinn Secondary School No. 23 – eight boys aged 17–18 years. They did not attend volleyball training and did not regularly practise any other sport. They only attended physical education classes at school.

METHODS

An anthropometric study was carried out with the students of the first and the second group and with the control group. Their height, weight, waist circumference, upper and lower leg circumference, and arms spread were measured according to the methodology of Martin [4]. Thereafter all the subjects underwent tests of physical ability which assessed the height of standing and running vertical jump [12] and the height of block jump (standing jump with two arms stretched upwards). In addition, the subjects' speed was assessed by zigzag run known from literature [13].

After testing the first and second group participated in Belyayev and Bulykina's jumping ability training programme. The authors recommend to conduct such a programme during the preparatory period.

Stage 1. Jumping ability training every day. During each training session jumping exercises 2, 3, 4, 7 are performed. Number of repetitions for each exercise – 20 jumps, 2–3 series. Pauses between series and exercises 1–2 minutes.

Stage 2. Jumping ability training on alternate days. Exercises 1, 5, 8 are performed. Number of repetitions for each exercise – 20 jumps, 2 series. Pauses between series and exercises 2–3 minutes.

Stage 3. Jumping ability training on alternate days. Exercises 6, 9, 10 are performed. Number of repetitions for each exercise – 25 jumps, 3–4 series. Pauses between series and exercises 2–3 minutes.

The subjects in A. Belyayev and L. Bulykina's programme were the male team of Russian super league Odintsovo Iskra. In our study Natasha Sorgina conducted the programme with young volleyballers; therefore, a few changes were made in it.

Catalogue of jumping exercises for the current study

1. Jumps upwards from half squat with maximum effort (angle between upper and lower leg 130–140°).
2. Jumps onto a gymnastic bench.
3. Step jumps
4. Scissor jumps on the spot
5. Step – squat – jump (step, drop into a deep squat, jump upwards with straight back, arms upwards)
6. Jumps upwards with straight legs forward (with a soft landing)
7. High knee run (at maximum speed, knees high)
8. Jumps over an obstacle (a gymnastic bench) with a turn from deep squat into a deep squat on the other side of the bench (with face always turned to the bench)
9. Imitation of block at the net
10. Jumping with spiking steps with touching a mark above the head

The programme lasted for 54 days for both groups. Thereafter the subjects underwent testing (anthropometric measuring and tests) as at the beginning of the study.

The students of the control group underwent an anthropometric study and test of physical abilities at both times but did not attend jumping ability training meanwhile.

Statistical analysis of data

Analysis was performed by Master of Mathematical Statistics Säde Koskel. Primary statistical analysis, linear correlation analysis, t-test to find significant differences between the means of the groups and multivariate regression analysis were applied.

RESULTS

The analysis began with finding the mean height and weight of the boys and girls of the first and the second group, and the corresponding data of the control group during the first and the second measuring.

Table 1. Mean height and weight of the subjects during the first and the second measuring

Group	Variable	First measuring (K1)	Second measuring (K2)
Group 1 (boys)	Height cm	185.550	185.663
	Weight kg	71.300	71.533
Group 1 (girls)	Height cm	167.467	167.550
	Weight kg	61.100	60.283
Group 2 (boys)	Height cm	161.917	163.000
	Weight kg	49.000	49.667
Group 2 (girls)	Height cm	161.250	161.625
	Weight kg	52.000	51.250
Group 3 (control)	Height cm	181.625	181.625
	Weight kg	73.250	73.750

The first group included 6 girls and 6 boys aged 16–20 years. The girls' mean height was 167.5 cm and weight 61.1 kg. The boys' mean height was 185.6 cm and weight 71.3 kg. The second group consisted of 6 boys and 4 girls aged 8–13 years. The girls' mean height was 161.3 cm and weight 52 kg. The boys' mean height was 162 cm and weight 49 kg. The control group consisted of eight young men aged 17–18 years with a mean height 181.6 cm and mean weight 73.25 kg.

Table 2 presents the differences between the results of the first and the second series of measurements.

Table 2. Differences between the first and second measuring

		Height	Weight	Upper leg circum-ference	Lower leg circum-ference	Waist circum-ference	Arms spread	Standing jump	Running jump	Block jump	Speed test
Group 1	**N**	6	6	6	6	6	6	6	6	6	6
Boys	**Mean**	0.083	0.233	–0.167	0.083	–0.167	0.917	7.833	7.333	6.000	–0.982
	STD	0.204	1.547	0.408	0.204	2.017	1.281	1.941	2.875	1.673	0.319
Group 1	**N**	6	6	6	6	6	6	6	6	6	6
Girls	**Mean**	0.083	–0.817	–0.167	0.083	–1.667	0.167	6.167	6.833	4.500	–1.113
	STD	0.204	2.102	0.516	0.376	2.401	0.408	1.472	3.971	1.643	0.178
Group 2	**N**	6	6	6	6	6	6	6	6	6	6
Boys	**Mean**	1.083	0.667	0.200	0.500	1.417	1.833	5.000	8.333	5.500	–0.752
	STD	0.492	1.633	0.400	1.049	1.497	1.472	1.789	2.503	3.271	1.543
Group 2	**N**	4	4	4	4	4	4	4	4	4	4
Girls	**Mean**	0.375	–0.750	0.500	0.375	0.375	1.125	5.000	3.500	3.250	–1.035
	STD	0.479	0.957	0.408	0.946	0.750	0.854	1.826	2.887	1.258	0.206
Control	**N**	8	8	8	8	8	8	8	8	8	8
group	**Mean**	0.000	0.500	0.000	0.000	0.000	0.125	0.125	0.250	0.500	0.148
	STD	0.000	1.069	0.535	0.535	0.000	0.354	0.835	1.389	1.069	0.530

The table shows that the results of jumping tests and speed tests improved in all groups. It is also interesting to note that the students were not only influenced by the jumping ability programme, but during these 54 days they also grew. The increase in height was particularly great in the group of younger boys – 1.08 cm on the average.

One of the aims was to find whether the speed abilities of volleyballers improve if only jumping ability is specially trained.

During those 54 days, we did not do any special exercises to develop the players' speed abilities. Thus, we can conclude that speed abilities and jumping ability are closely related. We found that the time of zigzag run decreased in the group of older boys by 0.98 sec on average, in the group of older girls by 1.11 sec, in the group of younger boys by 0.75 sec and in the group of younger girls by 1.03 sec. In the control group where jumping ability was not trained, the time of the zigzag run even increased by 0.15 sec.

The improvement in tests results was also proved by t-test.

Table 3. Assessment of physical abilities by t-test

	Standing jump	**Running jump**	**Block jump**	**Zigzag run**
	Test 1 – Test 2	**Test 1 – Test 2**	**Test 1 – Test 2**	**Test 1 – Test 2**
Group 1 Boys	286.67 – 294.50	294.83 – 302.17	280.33 – 286.33	23.30 –24.28
(n=6)	(p= 0.0001)	(p= 0.00077)	(p= 0.00016)	(p= 0.0003)
Group 1 Girls	248.17 – 254.33	248.50 – 255.33	244.17 – 248.67	26.51 – 27.62
(n=6)	(p= 0.0001)	(p= 0.00418)	(p= 0.0006)	(p= 0.000)
Group 2 Boys	245.50 – 240.50	250.83 – 242.50	239.50 – 234.00	27.98 – 28.73
(n=6)	(p= 0.000508)	(p= 0.000225)	(p= 0.00459336)	(p= 0.1431) Cannot be statistically proved
Group 2 Girls	237.75 – 232. 75	237.75 – 234. 25	232.75 – 229.50	31.99 – 33.025
(n=4)	(p= 0.00598)	(p= 0.04688)	(p= 0.00704)	(p= 0.001052)

The table shows that in the first and the second group the results of jumping tests improved significantly in both boys and girls; the time of zigzag run also decreased significantly. The change could not be

statistically proved only in the zigzag run test of the boys of the second group.

We also compared changes in the jumping and speed abilities in the boys of the first group and the control group.

Table 4. Check-up of essential differences between the means of the boys of the control group and group 1

	Standing jump	**Running jump**	**Block jump**	**Zigzag run**
Group 1 (boys)	7.833	7.333	6.000	–0.982
Control group (boys)	0.125	0.250	0.500	0.148
Calculated value of p	P= 0.0000	P= 0.0005	P= 0.0001	P= 0.0002

Here the results of the boys of the first group improved significantly compared with the boys of the control group who did not attend jumping ability training.

In addition, we also performed correlation analysis of anthropometric data and tests results of all the subjects participating in the study. As repeatedly shown in literature, anthropometric data form a system where all the variables are in mutual statistically significant correlation and the leading variables in this system are height and weight [3, 7]. We also got the same result when analyzing the anthropometric data of young volleyballers. The anthropometric data were in significant correlation between themselves and also in significant correlation with the results of all tests. Linear regression models proved that, for example, the height of standing vertical jump could be predicted from the number of the group, the child's sex and his/her height when measured for the second time ($R^2 = 0.9529$). An analogous result was received when predicting the height of running vertical jump where the independent variables were also the number of the group, the subject's sex and height when measured for the second time ($R^2 = 0.9562$).

In conclusion, we can say that the jumping ability programme used by us is suitable for young volleyballers, both boys and girls, aged 8–20 years. The authors recommend using the above-mentioned programme as an aid for coaches.

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DISTRIBUTION OF NEUROPEPTIDES IN NASAL AND NASOPHARYNGEAL MUCOSA IN PATIENTS WITH THE POST NASAL DRIP SYNDROME

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ABSTRACT

OBJECTIVE The post nasal drip is a very common symptom of sinusitis, allergic rhinosinusopathy, the gastroesophageal reflux disease, but there are some patients, who have a post nasal drip, sensation of a foreign body in the nasopharynx and a non-specific irritant cough with no others symptoms or signs of sinus inflammations or allergy. In the posterior rhinoscopy mucus discharge is seen. Ethiology and pathogenesis of this syndrome evolution are still unclear. The aim of the study was to identify the neuropeptide appearance and distribution in nasal and nasopharyngeal mucosa in the patients with the isolated post nasal drip syndrome and the control group, for the comparison of the data.

MATERIALS AND METHODS. We investigated the biopsies of nasal and nasopharyngeal mucosa from 11 adult patients, who had the isolated post nasal drip syndrome and from 2 control group patients without post nasal drip, by conventional light microscopy and immunohistological techniques for the protein gene product 9.5 (PGP), Neuropeptide Y (NPY), serotonin, Substance P (SP), vasoactive intestinal peptide (VIP), Calcitonin gene related peptide (CGRP) and Chromogranin A (CgA).

RESULTS. The conventional light microscopy showed a very thick basal membrane, the sclerosis of small blood vessels, the hyperplasia of mucosal glands- the neurogenic inflammation, mostly in nasopharyngeal mucosa. The abundance of PGP-containing nerve fibres was found around glands, sclerotic arterioles in almost all the cases. The main neuropeptides that were found in the mucosa of the patients were VIP, NPY, CgA, mostly in the nasopharyngeal mucosa. There were diffe-

rences in PGP-containing structures and the selective neuropeptide (NPY, CgA, and VIP) distribution in nasal and nasopharyngeal mucosa in comparison with the control group.

CONCLUSIONS. The main histological changes in the patients with the isolated post nasal drip syndrome are a thickened basal membrane, the hyperplasia of basal cells, pronounced hyperplasia of mucosal glands, the sclerosis of small arterioles. The main neuropeptides that are found in nasal and nasopharyngeal mucosa samples are PGP 9.5, vasoactive intestinal peptide, neuropeptide Y and chromogranin in the post nasal drip syndrome group. There is an imbalance of common neuropeptide-containing innervations, mainly sympathic nerves, the precursors of neuropeptides- chromogranins in the nasopharyngeal mucosa of the patients with the post nasal drip syndrome. SP and CGRP are not the most characteristic neuropeptides in the case of the post nasal drip syndrome pathogenesis.

Key words: *neuropeptide, neurogenic inflammation, nasal mucosa.*

INTRODUCTION

The post nasal drip is usually a symptom of acute or chronic rhinosinusitis, allergic rhinosinusitis, gastroesophageal reflux disease. There is a group of patients, who have no other symptoms of sinus inflammation, allergy or reflux, but still they have mucus discharge in nasoparynx, the sensation of a foreign body in the pharynx and irritant cough, the so-called post nasal drip syndrome (23).

The nasal mucosa is richly innervated by sensory, sympathetic and parasympathetic nerve fibres which secrete, when they become activated, a variety of transmitter molecules. The source of neuropeptides may be the nerve cells and the neurons associated cells, like glyocytes and fibroblasts. C and Aδ fiber stimulation causes the release of neuropeptides. The release of neuropeptides by sensory nerve endings produces vasodilatation and increased vascular permeability, the phenomena primarily described in the rodents that have been collectively termed neurogenic inflammation (4, 16, 22).

The substance P (SP), serotonin, the calcitonin gene related peptide (CGRP), the gastrine releasing peptide, the neuropeptide Y (NPY), the vasoactive intestinal peptide (VIP), chromogranin are found in nasal mucosa (4). These neuropeptides cause vasodilatation and upper airways

oedema, characterized by nasal obstruction, and bronchial constrictions, increased mucus formation, the leukocyte recruitment and differentiation and the activation of various immune cells, including lymphocytes, eosinophils, mast cells, and macrophages (23, 24).

In the case of chronic upper and lower respiratory tract illnesses, respiratory mucosa shows an increased quantity of pro-inflammatory sensory neuropeptides. Neuropeptide concentrations correlate with the patients' symptom intensity (16).

The concentration of neuropeptides in the bronchial and nasal secretions is relatively low for asymptomatic patients, but it is highly elevated in the patients with allergic respiratory diseases, including nasal polyps (4, 7). Since neuroendocrine cells in the airway mucosa are discovered, increasing interest is attached to the yet unexplored neurogenic inflammation in the case of chronic airway inflammation, especially if it is characterized by increased airway reactivity.

The aim of the study was to identify neuropeptide appearance and distribution in nasal and nasopharyngeal mucosa in the patients with the isolated post nasal drip syndrome and the patients without the post nasal drip and to compare the data.

MATERIAL AND METHODS

Inferior nasal turbinate and nasopharyngeal mucosa specimens were obtained from 11 adult patients (age 18–58) with the isolated post nasal drip syndrome and from 2 control group patients (age 28–36).

All the patients were examined by ***an otolaryngologist*** to exclude rhinosinusopathy (the normal pneumatisation of all the sinuses at the CT scan), allergy (no changes in skin prick tests, IgE range (total and specific)) and gastroesophageal reflux disease (clinically and by performing endofibrogastroscopy). All the patients have had symptoms of the post nasal drip for more than 6 months and had no reaction to common therapy: topical steroid (fluticasone), antihistamine (loratidin), anti-reflux therapy (diet and omeprasole).

The control group was formed from 2 voluntary patients, who where undergoing rhinoseptoplasty, these patients where without any symptoms of the post nasal drip.

Tissue pieces (1–2 mm²) from the middle part of the lower nasal turbinate and the middle part of the nasopharyngeal arch area were taken

under control of the endoscope, under local anaesthesia (submucosal administration of 1–2 ml of 1% lidocaine solution) or general anaesthesia (control group).

For immediate fixation previously prepared saturated picric acid solution (formaldehyde 2%, 0.2%o Picric acid, 1 M phosphate buffered (pH 7.2) was used. Tissue slices were stained with haematoxylin and eosin, and by the use of immunohistological technique (Hsu et al 1981) for the protein gene product 9.5 (PGP), substance P (SP), Neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), serotonin, calcitonin gene related peptide (CGRP) and chromogranin A (CgA) (Table 1).

The samples were examined under the Leica microscope. The results of immunohistochemistry were listed by the semi-quantitative counting method (Tobin et al., 1990; Pilmane, 1997) (Table 2).

Table 1. The data of the antibodies applied in immunohistochemistry

	Obtained from	Working dilution	Manufacturer	Code
PGP	rabbit	1:600	DAKO (Denmark)	Z5116
Serotonin	mouse	1:10	DAKO (Denmark)	M758
NPY	rabbit	1:10	DAKO (Denmark)	B48-100
VIP	rabbit	1:400	abcam (UK)	Ab22736
SP	mouse	1:1000	abcam (UK)	Ab14184
CGRP	rabbit	1:20	Quartet (Germany)	281328
CgA	rabbit	1:400	DAKO (Denmark)	A0430

Table 2. The semi-quantitative analysis of the immunohistochemically determined structures

Applied markings	Semiquantitive explantation
–	No positive structure seen in the visual field
0/+	Rare positive structures seen in the visual field
+	Few positive structures seen in the visual field
+/++	Few to a moderate number of positive structures seen in the visual field
++	A moderate number of positive structures seen in the visual field
++/+++	Moderate to numerous positive structures seen in the visual field
+++	Numerous positive structures seen in the visual field
+++/++++	The abundance of positive structures in the visual field

RESULTS

Nasal mucosa demonstrated the pronounced partially patchy thickened basal membrane. Epithelium showed basal cell hyperplasia and intraepithelial infiltration with lymphocytes, marked hyperplasia of glandulocytes was seen along the epithelial lining (Figure 1). Two of the patients showed the metaplasia of the epithelium (the stratified squamous epithelium instead of the pseudostratified ciliated epithelium). *Lamina propria* also demonstrated infiltration with lymphocytes, the hypertrophy of glands and the sclerosis of small arterioles (Figure 2). One patient showed the granulation tissue of subepithelium and marked infiltration of lymphocytes, and another patient had the lymphatic nodule of the subepithelium.

The control group patients' nasal mucosa samples where without marked inflammation – no lymphocyte infiltration was seen, sero-mucosal glands where without hyperplasia.

Numerous PGP-containing nerve fibres (+++) were observed mainly around the secretory parts of seromucosal glands and sclerotic arterioles (Figure 3). The patient with lymphatic nodules in the submucosa demonstred an occasional amount of PGP-containing nerve fibres. The patient who had granulations and the methaplasia of nasal epithelium,

showed almost negative PGP structures in all the mucosa samples (0/+) (Table 3).

The control group patients' nasal mucosa samples showed moderate (++) PGP-containing nerve fibres, mostly around blood vessels and seromucosal glands (Table 4).

Nasopharyngeal mucosa showed even more expressed changes in comparison to nasal mucosa – the very thickened basal membrane, the hyperplasia of basal cells, the pronounced hyperplasia of mucosal glands, the sclerosis of small arterioles, some patients had infiltrations of lymphocytes in the submucosa. One patient, the same with granulations in the nasal mucosa, also showed the granulation tissue in the nasopharyngeal submucosa. One of the control group patients had mucoid respiratory epithelium in the nasopharynx, another one had normal respiratory epithelium in the nasopharynx without marked inflammation or hyperplasia.

Table 3. The results of immunohistochemical findings in nasal and nasopharyngeal mucosa

	PGP	**NPY**	**VIP**	**SP**	**CGRP**	**Serotonin**	**CgA**
Glands – nasal mucosa	++/+++	0/+	++	0/+	0/+	–	+++
Glands – nasopharyngeal mucosa	++/+++	+	++	0/+	0/+	–	+++
Blood vessels – Nasal mucosa	++/+++	0/+	++	0/+	0/+	–	++
Blood vessels – Nasopharyngeal mucosa	+++	+	++	0/+	0/+	–	++/+++
Total	++/+++	+	++	0/+	0/+	–	++/+++

(–) No positive structure seen in the visual field, (0/+) rare positive structures seen in the visual field, (+) Occasionally positive structures seen in the visual field, (+/++) few to moderate number of positive structures seen in the visual field, (++) a moderate number of positive structures seen in the visual field, (++/+++) moderate to numerous positive structures seen in the visual field, (+++) numerous positive structures seen in the visual field, (+++/++++) the abundance of positive structures in the visual field.

Table 4. The results of immunohistochemical findings in nasal and nasopharyngeal mucosa – the control group

	PGP	**NPY**	**VIP**	**SP**	**CGRP**	**Sero-tonin**	**CgA**
Glands – nasal mucosa	++	0/+	0/+	0/+	0/+	–	+
Glands – nasopharyngeal mucosa	++	0/+	0/+	0/+	0/+	–	+
Blood vessels – Nasal mucosa	++	0/+	0/+	0/+	0/+	–	+
Blood vessels – Nasopharyngeal mucosa	++	0/+	0/+	0/+	0/+	–	+
Total	++	0/+	0/+	0/+	0/+	–	+

(–) No positive structure seen in the visual field, (0/+) rare positive structures seen in the visual field, (+) Occasionally positive structures seen in the visual field, (+/++) few to moderate number of positive structures seen in the visual field, (++) a moderate number of positive structures seen in the visual field, (++/+++) moderate to numerous positive structures seen in the visual field, (+++) numerous positive structures seen in the visual field, (+++/++++) the abundance of positive structures in the visual field.

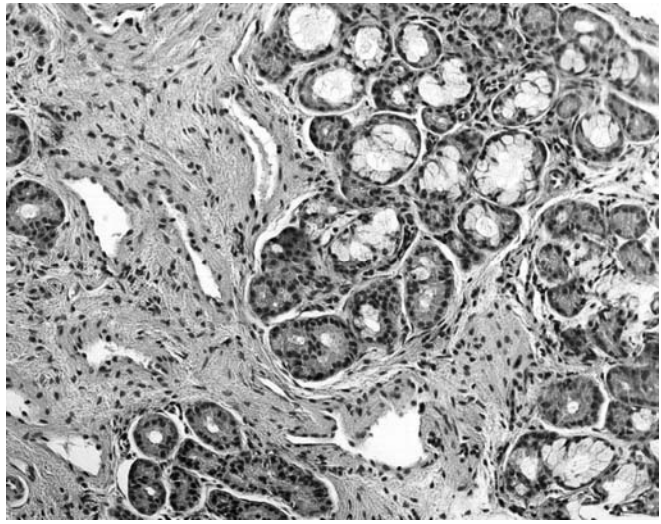

Figure 1. The marked hyperplasia of seromucosal glands, the infiltration of lymphocytes in nasal mucosa, HE x 200.

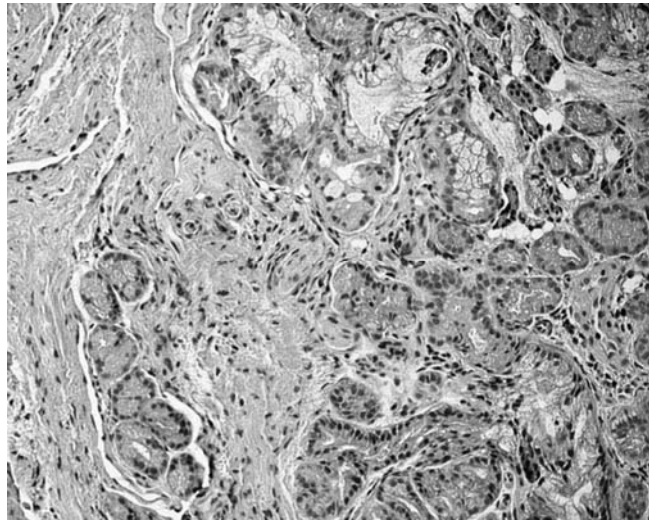

Figure 2. Sclerotic small arterioles, the hyperplasia of seromucosal glands and the infiltration of lymphocytes in nasopharyngeal mucosa. HE x 200.

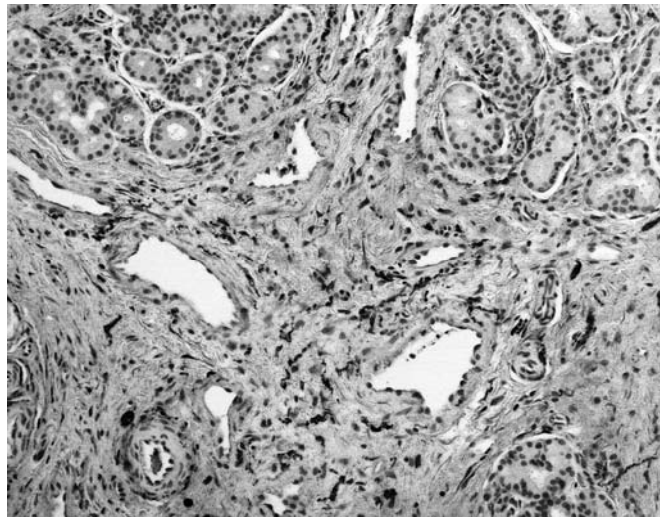

Figure 3. The abundance of PGP immunopositive structures around sclerotic arterioles and hyperplased seromucosal glands in nasopharyngeal mucosa. PGP 9.5 x 200.

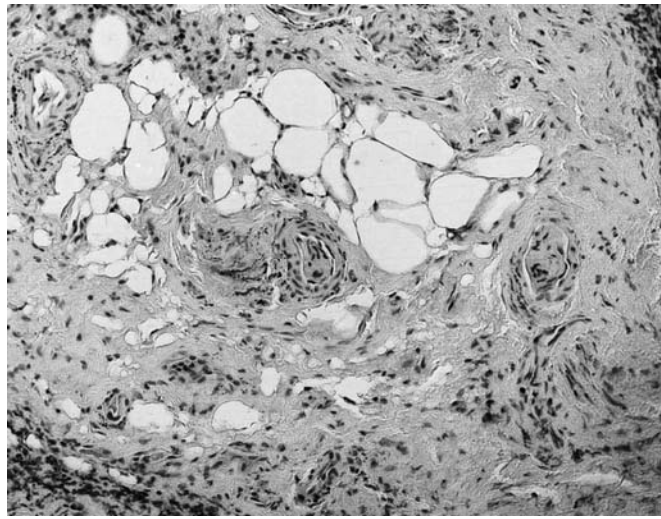

Figure 4. VIP containing nerve fibres around blood vessels in nasopharyngeal mucosa. VIP x200.

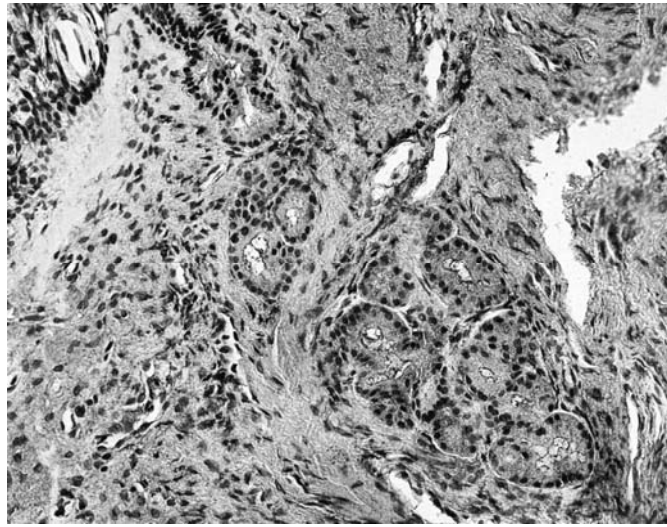

Figure 5. Numerous Chromogranin A granules containing cells next to the hyperplased seromucosal glands. CgA x250.

The abundance of PGP-containing nerve fibres (+++/++++) was found around glands, sclerotic arterioles in almost all the samples, except the patient with granulations in sub mucosa – he had only few PGP-containing fibres (+) and only next to some seromucos glands (Figure 4) (Table 3).

The control group patients demonstrated moderate PGP immuno-positive structures (++) in nasopharyngeal mucosa, mostly next to seromucosal glands and arterioles.

All the nasal and nasopharyngeal mucosa samples, also in the control group, were serotonin negative (Table 4).

Most of the nasal and nasopharyngeal mucosa samples showed few NPY positive structures (+), mostly around small sclerotic arterioles in the nasopharyngeal mucosa. Exceptions were mucosal samples from the patient with lymphatic nodules in the submucosa, in this case we saw more positive structures in comparison to other patients' mucosa samples around the arterioles in nasopharyngeal mucosa (++) (Table 3).

The control group patients' nasal and nasopharyngeal mucosa showed occasional NPY immunopositive structures (+) next to sero-mucosal glands and blood vessels (Table 4).

Nasal mucosa showed moderate to a large number of VIP-containing nerve fibres (++/+++) around submucosal glands and small sclerotic arterioles, but in the nasopharyngeal mucosa samples VIP-containing nerve fibres were even more, especially around sclerotic arterioles (++/+++) (Figure 5) (Table 3). The control group patients' nasal and nasopharyngeal mucosa samples showed few VIP immunopositive structures mostly around blood vessels, similarly in nasal and naso-pharyngeal mucosa (Table 4).

In all the nasal and nasopharyngeal mucosa samples, including the control group patients, rare (0/+) or negative Substance P containing structures were found (Table 3) (Table 4).

Occasional CGRP positive structures (0/+) in the visual field were seen in the nasal and nasopharyngeal mucosa, mainly in the naso-pharyngeal mucosa next to blood vessels and around seromucosal glands, the same findings were seen in the control group patients' mucosa samples (Table 3) (Table 4).

Almost in all of nasal and nasopharyngeal mucosa samples moderate to numerous (++/+++) CgA-containing cells were found, mainly in seromucosal glands and the basal layer of epithelium (Figure 6) (Table 3). Moderate CgA immunopositive structures were found in the

control group nasal and nasopharyngeal mucosa samples next to blood vessels and glands (Table 4).

DISCUSSION

This study shows the infiltrations of lymphocytes, thick basal membrane and sclerotic small arterioles and the hyperplasia of seromucosal glands in the nasal and nasopharyngeal mucosa of the patients with the post nasal drip syndrome in contradistinction to the control group patients, whose nasal and nasopharyngeal mucosa samples showed no marked signs of chronic inflammation. Similar results describe also Stephanie and Kunal (2008) by the histological examination of nasal mucosa in the case of allergic rhinosinusitis and hyperreflectoric rhinitis (24).

In the nasopharyngeal mucosa PGP-containing neural fibres were more observed than in the nasal mucosa (in the control group as well as in the post nasal drip patients group), probably due to the density of innervations, which is more pronounced in the mucosa of nasopharynx compared with the mucosa of lower nasal turbinate (3, 7, 24). PGP imunopositive structures were found less in the control group patients' mucosa samples; probably this finding can be associated with the increased production of neuropeptides in the case of post nasal drip. Fisher & al (2005) describe similar histological findings and the abundance of PGP-containing nerve fibres in nasal mucosa in the case of persistent perennial allergic rhinitis (9), so we can not exclude some analogy with allergic inflammation in the case of the post nasal drip syndrome pathogenesis.

VIP-containing nerve fibres are usually located around arterioles, glands, and muscle fibres and the most important effects of VIP are vasodilatation, bronchodilatation and the activation of glandular secretion (9). Our study showed moderate to a large number of VIP-containing nerve fibres around seromucosal glands and small sclerotic arterioles, especially in the nasopharyngeal mucosa samples, it suggests that VIP could implicate in the pathogenesis of mucus discharge in the nasopharynx and probably also stimulate the sclerotisation. Especially according to the fact, that the control group patients' nasal and nasopharyngeal mucosa samples contained a smaller (occasionaly to few) number of VIP imunopositive structures and there were no sclerotic arterioles (11, 12, 20).

In our study just rare NPY positive structures were found in nasal and nasopharyngeal mucosa. The control group patients' nasal and nasopharyngeal mucosa samples contained rare NPY immunopositive structures. Heppt & al (2004) describes similar results in the patients with environmentally triggered hyperreflectory rhinitis (10). Neuropeptide Y is co-localized with norepinephrine in a population of sympathetic neurons in the walls of human nasal mucosal arterioles (4). Sympathetic stimulation induces vasoconstriction and increased nasal airway potency. There is also some evidence that sympathetic activity can induce airway glandular secretion through the stimulation of serous cells through β receptors (4, 19). There are studies that reported about the NPY effect on vasoconstriction, it is effective at reducing the symptoms of nasal obstruction and the weight of mucus secretions after the nasal allergen challenge (24). In this case, our findings explain increased mucus formation in the patients' nasopharyngeal mucosa.

In our study we investigated the substance P appearance in nasal and nasopharyngeal mucosa. The results showed rare or negative (0/+) SP containing structures, also in the control group patients' mucosa. Sensory nerve neuropeptides include the tachykinins (i.e., substance P and neurokinin A), the calcitonin gene-related peptide (4, 23). The effects of these neuropeptides include glandular activation, leukocyte recruitment, differentiation and the activation of various immune cells, including lymphocytes, eosinophils, mast cells and macrophages (15, 24, 25). According to these known effects, it is unusual that the substance P was not found in nasal and nasopharyngeal mucosa samples, and the acquired results may suggest that the substance P is not so deeply involved in the pathogenesis of the post nasal drip syndrome.

Our results showed the absence of serotonin in all the nasal and nasopharyngeal mucosal samples and also in the control group patients. Serotonin effects are smooth muscle contraction, vasodilatation, and increased vascular permeability. Serotonin as well as histamine, leukotrienes, prostaglandins and tryptase are released and account for the immediate airway allergy symptoms and it is often found in the case of hyperreflectoric rhinitis as well (9). That fact can suggest that serotonin as well as the substance P, has no significant influence on the post nasal drip pathogenesis, and it makes a significant difference from the described study results until now in the case of allergic and hyperreflectory rhinitis (7, 8, 9,10, 16).

In the studies described in literature, CGRP is usually found in allergic and hyperreflectoric rhinitis (16, 20). Calcitonin gene-related peptide is one of the neuropeptides that is released in the human nasal mucosa after trigeminal nerve stimulation. The main effects of this neuropeptide are vasodilatation, mucus secretion, plasma extravasations; CGRP exerted also a significant dose-dependent stimulation on ciliary's beat frequency (4). According to these known effects the negative CGRP-containing nerve fibre in the present study is inexplicable. That seemly suggests the difference between the allergic and the neurogenic inflammation.

Chromogranin A belongs to the granin family of uniquely acidic secretory proteins co-stored and co-secreted with other hormones and peptides in the elements of the diffuse neuroendocrine system. In nasal mucosa chromogranin mediates as a marker of neuropeptides, neuro-endocrine cells. CgA peptides take part in the regulation of calcium and glucose metabolism, cardiovascular functions, gastrointestinal motility and nociception, tissue repair, inflammatory responses and as host defence peptides in the first phase of microbial invasions (4, 26, 27). Our results showed moderate to numerous CgA immunopositive cells in nasal and nasopharyngeal mucosa in the case of the isolated post nasal drip syndrome. The fact must be mentioned that the control group patients… nasal and nasopharyngeal mucosa samples contained less CgA immunopositive cells in comparison with the main group patients.

CONCLUSIONS

The main histological changes in the patients with the isolated post nasal drip syndrome are the thickened basal membrane, the hyperplasia of basal cells, the pronounced hyperplasia of mucosal glands, the sclerosis of small arterioles. The main neuropeptides that are found in nasal and nasopharyngeal mucosa samples are PGP 9.5, the vasoactive intestinal peptide, the neuropeptide Y and chromogranin in the post nasal drip syndrome group. There is an imbalance of common neuropeptide-containing innervations, mainly sympathic nerves, the precursors of neuropeptides – chromogranins in the nasopharyngeal mucosa of the patients with the post nasal drip syndrome. SP and CGRP are not the most characteristic neuropeptides in the case of the post nasal drip syndrome pathogenesis.

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BODY PROPORTION CHANGES OF NURSING HOME OLIGOPHRENICS IN WESTERN HUNGARY (1991–2011)

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ABSTRACT

In 1991, somatometric investigation of oligophrenics living in specialized nursing homes in Western Hungary was carried out by the authors. Focusing on the most important body measurements, we repeated the study 20 years later in order to observe the changes in the body measurements of the mentally retarded groomed in the nursing home. The proportionality (z-score) was analyzed by comparing to the corresponding values of the UHP. The favourable changes in the body proportion are due to the following environmental factors: 1. general inference of the social changes after 1989, 2. healthier nutrition through employing dietetic professionals in the nursing home, 3. physical training programmes through employing curative gymnastics professionals in the institute, 4. adequate medication; replacing older drugs, frequently causing obesity as a side effect, with the new generation ones.

***Key words*:** oligophrenics patients, z-score, UHP, Hungary

INTRODUCTION

In 1991, somatometric investigation of 46 male and 58 female oligophrenics living in specialized nursing homes in Western Hungary (Europe) was carried out by the authors. By ethnics, all the subjects were Hungarian, belonging to European races. Most of them were diagnosed as imbeciles (mentally moderately retarded). As oligophrenia has a heterogeneous background [1], the results of the somatometric

measurements may reflect the different appearance. The results have been summarized in our paper "Study of physique in psychiatric and oligophrenic patients" [10]. As to the monitoring of body proportions [6], a high z-value of the suprailiac skinfold was found to be characteristic in both sexes. Abdominal fat apposition and abdominal-type obesity is a well-known severe risk factor of several internal diseases [4].

Furthermore, the knowledge of body disproportionalities proved to be useful in the practical management of caregiving.

Assuming that somatometric characteristics are influenced by both genetic and environmental factors (abundant nutrition and insufficient physical activity, respectively), based on the results of our study, a recommendation has been addressed to the leader of the nursing home. Focusing on the most important body measurements, we repeated the study 20 years later in order to observe the changes in the body measurements of the mentally retarded groomed in the nursing home.

MATERIAL AND METHODS

The majority of the 30 male and 25 female subjects involved in the 2011 study were mentally moderately retarded. The age range was, just like in the earlier study, 35–45 years.

All extents have been measured according to the Martin's technique [5], taking the recommendations of the IBP/HA into consideration [9].

The proportionality (z-score) was analyzed by comparing to the corresponding values of the Unisex Human Phantom [6], just like 20 years earlier. The Unisex Human Phantom (UHP) is a suitable benchmark for studying body proportions of individuals or groups of Homo sapiens. Its use is criticised by some researchers [7], all the same it is a convenient tool for monitoring the changes of body proportions [11], for example, in sports anthropometry [3, 8] and clinical anthropometry, respectively.

RESULTS

The body measurements to be compared are presented in Table 1.

Table 1. Body measurements investigated

Body measurements investigated	Male 1991		Male 2011		Female 1991		Female 2011	
	x	SD	X	SD	x	SD	x	SD
Weight (kg)	67.72	15.0	73.55	16.2	60.64	13.8	57.77	12.6
Height (cm)	166.49	9.3	170.32	10.1	151.96	6.1	152.35	10.4
Subscapular skinfold (mm)	15.39	9.7	18.03	8.4	24.76	10.9	15.55	8.7
Suprailiac skinfold (mm)	25.00	11.3	17.39	8.6	32.24	12.6	17.12	9.5
Medial calf skinfold (mm)	12.74	8.2	9.04	5.2	29.60	9.6	16.00	8.7
Bicondylar width of humerus (mm)	68.13	6.3	69.23	5.3	62.38	7.7	59.42	10.5
Bicondylar width of femur (mm)	94.52	24.4	95.30	6.5	93.19	9.6	86.48	9.5

In males, both weight and height proved to have increased. These changes correspond to the secular trend observed in the adult population [2]. As to the skinfold values, subscapular skinfold values have increased, while suprailiac skinfold values have notably decreased and the values measured at the medial side of the calf have decreased as well. Thus, the femoral and humeral bicondylar width measurements did not increase significantly, albeit it seems to be logical that supporting a greater body mass would require a more robust bony frame. The BMI value [13] in the former study proved to indicate normal weight (24.6 kg/m^2), while the 25.4 kg/m^2 value of the recent study clearly represents overweight.

In females we found decreased weight, while increasing of height did not prove to be significant. As to the skinfold values, subscapular and suprailiac skinfold values, and those measured at the medial side of the calf have notably decreased. The bicondylar width of the thigh bone and humerus was found to have decreased. The BMI value [13] of females in the former study proved to indicate overweight (26.3 kg/m^2), while the 24.0 kg/m^2 value of the recent study represents normal weight.

The proportionality (z-score) values [6] (Table 2. Figures 1–2.) of males show increased weight and moderate increasing in subscapular skinfold values, while suprailiac skinfold values proved to decrease notably. The medial calf skinfold values and bicondylar values decreased as well. The proportionality changes in females indicate minimally increased weight, notably decreased skinfold values as well as decreased bicondylar values.

Table 2. Proportionality of the oligophrenic patients (z-score)

Body measurements investigated	Male z-score		Female z-score	
	1991	2011	1991	2011
Weight	0.28	1.05	2.39	1.91
Height	–0.02	–0.25	0.01	0.07
Subscapular skinfold	–0.39	0.07	1.95	–0.02
Suprailiac skinfold	2.27	0.4	4.63	0.83
Medial calf skinfold	–0.50	–1.48	3.48	0.4
Bicondylar width of humerus	1.49	0.77	1.65	0.49
Bicondylar width of femur	0.32	–0.18	1.91	0.35

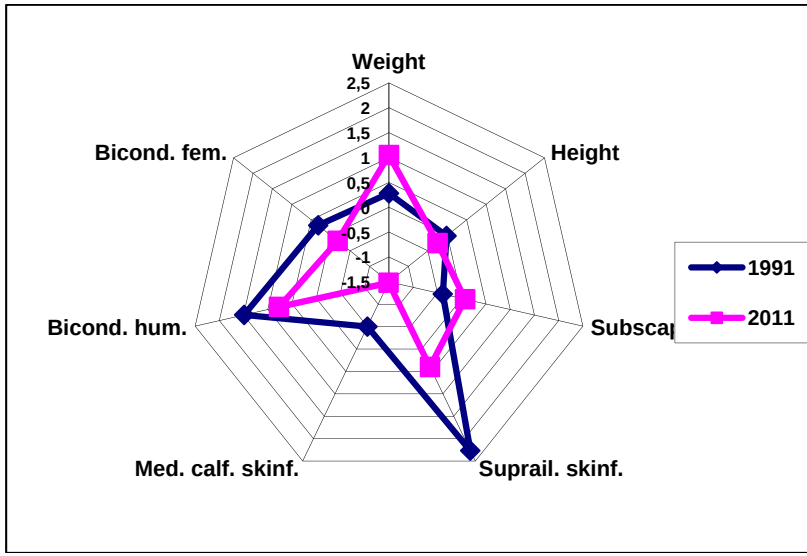

Figure 1. Proportional profile of the male oligophrenic patients (z-score).

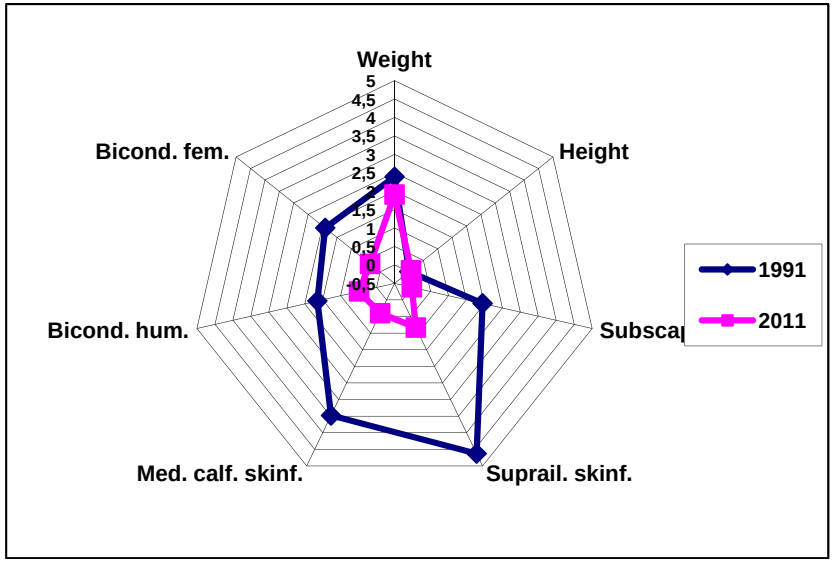

Figure 2. Proportional profile of the female oligophrenic patients (z-score).

DISCUSSION

The decreased proportionality of skinfold values in both sexes is to be considered as an important change. This change is most pronounced in the suprailiacal region. Thus, the value of an important risk factor has decreased. Another important observation is the decreased proportionality of the body mass, even in males, despite their numerically higher values. The bicondylar value is an indicator of bone maturity; its decreasing is to be considered as an unfavourable tendency. However, decreasing values correspond to the changes observed in the healthy population [12].

The favourable changes in the body proportion (i. e. decreased skinfold measurements and body mass) are due to the following environmental factors:

– general inference of the social changes after 1989 (the downfall of the communist regime),
– healthier nutrition through employing dietetic professionals in the nursing home,

– physical training programmes through employing curative gymnastics professionals in the institute,
– adequate medication; replacing older drugs, frequently causing obesity as a side effect, with the new generation ones.

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RELATION BETWEEN SERUM ENZYMES AND LIVER HISTOPATHOLOGY IN MINK WITH HEPATITIS

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ABSTRACT

The dystrophy of the mink liver is widely distributed in mink farms of Latvia. However, clinical diagnostics of the liver dystrophy is quite difficult. The hypothesis of this study was to clarify the indicators of ALT (alanine aminotransferase) and ALP (alkaline phosphatase) as the criteria in the early diagnostics of the mink liver injury. The goal of this study was to determine the potential correlation between the expressed functional indicators and the histopathology of the liver. Blood samples were obtained from ten minks, seropositive against the Aleutian disease. Liver injury was assessed histologically. Through application of the biotin-streptavidine immunohistochemical method, the presence of the hepatocyte growth factor, as well as the basal membrane components was established in the mink liver tissue, but liver apoptosis was determined by TUNEL. For the assessment of cytokines, a semi-quantitative counting method was used. The results showed the apoptosis of the hepatocytes in the whole area of the liver parenchyma. The correlation between the apoptosis and the expression of inflammation cells in the mink liver was non-existent. Constitutive release of cytokines (Hsp 70 and HGF) was detected in the liver hepatocytes centrilobular or around the periportal area. We established that have the mean correlation existed between the moderate steatosis and the weak HGF expression. Hsp 70 expression in hepatocytes was higher when moderate and numerous apoptotic cells were seen. The ALT level of the experimental mink in 40% cases was incresed above the standart. Interconnection between the degree of gravity of steatosis and the increasing of ALP and ALT levels was not found. We concluded that the simultaneous presence of the infiltration of inflammatory cells and the

moderate expression of HGF detect the still maintained regeneration ability of liver despite the persisting inflammation. The increased activity of liver enzymes (ALT and ALP) does not directly depend on the damaged mink liver disorders and can be suggested only as a common indicators for the liver disfunctioning of mink with the Aleutian disease.

***Key words*:** *mink, liver, blood, histopathology, the Aleutian disease.*

INTRODUCTION

The liver is the largest metabolism centre and organ in the body. The disfunctions of liver can cause various reasons, one of them for mink is the Aleutian disease. The pathogenic agent (parvovirus) not only multiplies in macrophags, but suppresses CD8+ cells, stimulates plasmocitosis, and initiates the process of glomerulonepfritis and hepatitis [9; 22].

Hepatocytes have several hundred functions and are a frequent target in infectious, toxic, metabolic and immunologic injury to the liver when injured, hepatocytes may produce and release oxygen free radicals, lipid peroxide products, proteases, cytokines and growth factors that injure adjacent cells [27]. It has been shown in numerous studies that cytokines are involved in the inflammatory syndrome and it may counterpart with the potential to limit an inflammatory response. The heat shock protein (Hsp) and the hepatocyte growth factor (HGF) are types of indicators of the cellular condition [8]. Liver function tests are a group of the biochemical measurements that are used to identify patients who are suffering from the liver or the biliary tract disease [18].

Although the liver has a great capacity for the regeneration of the hepatocyte mass [1], in the mink farms of Latvia the liver steatosis is widely distributed. Clinical diagnostics of the liver dystrophy is quite difficult. Reports of the relationship between the serum chemistry data and the liver morphology for mink are limited, therefore to establish the potential marker in serum as an early index for the morphopathology of liver were important. It made a choice of well timed methods of treatment. The hypothesis of this study was to clarify ALT (alanine aminotransferase) and ALP (alkaline phosphatase) as the criteria in the early diagnostics of the mink liver injury. The goal of this study was to

determine the potential correlation between the expressed liver functional indicators and the histopathology of the liver.

MATERIAL AND METHODS

*Animals and samples.*To detect the histopathological changes in liver, 10 dark brown minks of seven months of age without clinical signs of any disease were randomly selected. All the mink were positive to the virus of AD according to the reaction of imunoelectroosmophoresis.

For obtaining of blood samples the mink were positioned on their back and heart puncture of animals was carry out [11]. Approximately 4 ml of blood was placed in the sterile vacutainer without anticoagulant. The blood samples for serum chemistry ALT (alanine aminotransferase) and ALP (alkaline phosphatase)) determinations in time of 2 h were sent to the National Diagnostic Center of Latvia and analyzed on the same day.

After parenteral euthanasia of mink with 1 ml 1% solution of ditilini [11] their liver samples were fixed in 12% formalin. All the experimental minks were obtained within the period of pelting and slaughtering and in accordance with the guidelines of animal protection.

Immunohistochemistry. Multiple 6 μm-thick sections of the paraffin-embedded mink liver were examined for immunohistochemistry [7]. Prior to immunostaining, sections were deparaffinized and rehydrated. Sections were processed in the microwave for 20min in 4% citrate buffer (pH 10), quenched for 10 min with 3% H_2O_2 for blocking endogenous peroxidase activity, rinsed in phosphate-buffered saline (pH 7,4), pretreated with a nonimmune goat serum for 10 min for blocking of nonspecific antibody binding and then incubated for 2 h with the primary antibodies.

The primary antibodies utilized in immunohistochemistry were rabbit polyclonal antibodies specific for the hepatocyte growth factor (HGF, dilution 1:300, R&D System, DE) heat shock protein 70 (Hsp 70, dilution 1:100, abcam, UK). Immunoreaction was visualized by the avidin-biotin (LSAB) immunoperoxidase method using the LSAB kit (DakoCytomation, DK), and the DAB (diaminobenzidine) solution (Dako, DK) was used as chromogen, but hematoxylin – as the counterstain.

TUNEL reaction was used for the detection of apoptosis [20]. *In situ Cell Death Detection*, POD (Roche Diagnostics) and the DAB substrate (Vector) were used. Deparafinised sections (xylol 2 x 4 min, 99% ethanol 2 x 2 min, 95% ethanol 2 x 2 min and 70% ethanol 2 x 2 min) were rinsed with water (7–10 min) and transferred to PBS (pH 7.5) for 10 min. Subsequently slides were placed into 50 ml PBS solution with 500 µl 30% hydrogen peroxide for 30 min on shaker to block the endogenous peroxidases. Afterwards tissue samples were washed with PBS (3 x 5 min), placed into 0.2 M boric acid (pH 7.0) and into the microwave (700W) for 10 min for the fixation of antigen, cooled to room temperature and rinsed with PBS. After that, slides were kept in the refrigerator in 0.1% BSA (bovine serum albumin) solution with PBS for 10 min and then incubated in the TUNEL mix (Tdt – mix of terminal deoxynucleotide transferase and DIG-labeled deoxynucleotide) for 1h at +37ºC. Then the slides were rinsed with PBS 1:10, and incubated for 30 min at +37ºC with POD (sheep anti-digoxygenin antibogy coupled with horseradish peroxidase Fab fragment). Then the slides were washed with PBS, covered with DAB (diaminobenzidine chromogen) for 7 min, and rinsed with running water for 5 min. Finally, haematoxylin and eosin staining was performed on each sample. Sections were covered with a polystyrene-based medium and coverslipped.

Statistical analysis. For the quantitative analysis we used a counting of inflammation cells in three fields of vision, while the semi-quantitative analysis was used to estimate the proportions of immunopositive cells in liver [21]. The designations were as follows: 1 – few positive cells in the view field; 2 – moderate and 3 – numerous positive cells in the view field. For the statistical analysis (p-value) of the growth factor data the Wilcoxon rank tests were used.

RESULTS

Inflammation cells and cytokines in the mink liver. In the mink liver inflammation infiltrate, being rich in lymphocytes, was extending from the portal tracts towards the side parenchyma. In all the histological samples of the liver, macrophages constituted 18%, neutrophil leukocytes – 27%, but lymphocytes – 55% of the total inflammation cell count. A strongly marked tissue infiltration with macrophages, neutrophil leukocytes and lymphocytes was found around the central

vein of lobuli, but the Kupffer cells infiltration – mainly around portal tract ducts.

In 10 minks, the liver histology and the TUNEL assay showed lesions and liver adaptive changes (Table 1). Fatty dystrophy was observed in all the mink liver samples, all the animals had mostly macrovesicular steatosis.

The results showed the apoptosis of the hepatocytes in the whole area of the liver parenchyma and the calculated statistical coherence shows that the influence of the inflammation cells on apoptosis is not significant ($p>0.05$). In the cases of moderate [3] or numerous [2] apoptotic hepatocytes were seen, there was no considerable difference in the expression of inflammation cells, whereas when just a few [1] apoptotic cells were present, their number was considerably lower ($p<0.05$).

The constitutive release of cytokines (Hsp 70 and HGF) was detected in the liver hepatocytes centrilobular or around the periportal area. The expression of HGF and Hsp 70 was found in the zones, where lots of the inflammation cells were observed. The study revealed that numerous [3] positive apoptotic hepatocytes have the relationship ($r=0.36$) with a weak HGF expression and have the mean correlation ($r=0.59$) between the moderate [2] steatosis and the weak HGF expression. We established that the Hsp 70 expression in hepatocytes was higher when moderate and numerous apoptotic cells were seen.

Serum enzymes. The results of biochemistry analysis are shown in Table 1. The ALT level of experimental mink in 40% cases was incresed above the standard, however, the level does not increase more than twice. Fatty distrophy was found in all the mink liver samples, however, the interconnection between the degree of gravity of steatosis and the ALT level was not found. The results showed that the increased ALT level correlated with the moderate apoptosis, but normal ALT level – with week and great apoptotic hepatocytes. Results also showed, that increased ALT level were when HGF expression was moderate [2], but normal ALT level were with weak and intense HGF expression (Figure1).

Table 1. Cytokines in the liver and serum parameters of mink with hepatitis

No.	Infiltration of inflammatory cells	Fatty dystrophy	Apoptosis of hepatocytes	HGF	Hsp 70	ALT (U/L)	ALP (U/L)
1.	3	1	3	1	1	220.6	265.9
2.	2	2	2	3	0	89.9	139.6
3.	3	1	2	3	1	194.6	165.2
4.	3	2	2	3	0	87.4	119.4
5.	2	1	1	1	1	90.4	178.6
6.	2	1	3	1	1	116.2	194.1
7.	2	2	1	2	1	157.6	279.2
8.	2	1	2	2	0	182.6	188.5
9.	2	2	3	2	2	200.8	289.7
10.	2	1	3	1	1	117.8	197.1
Standard					Hunter, Lemieux, 1996	up to 158.0	37–67
					Weiss et al., 1994	71.6–80	

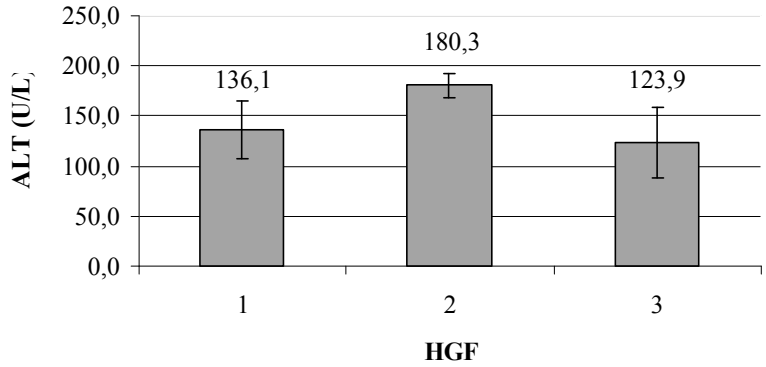

Figure 1. Relation between serum ALT level and HGF in mink liver.

Blood serum ALP level (Table 1) was increased of all mink serum (119.4 – 289.7 U/l), however in general interconnection between degree of gravity of steatosis and increasing of ALP level were not founded.

DISCUSSION

The cellular, biochemical and molecular mechanism, that cause injury of hepatocytes play a role in all liver diseases [28]. In liver samples we basically observed that the lobule architecture has been maintained, but inflammation infiltrate which were rich with lymphocytes indicate to the pathological process. As usually only few lymphocytes can be found around the triad [12] then authors [17] characterize such a picture as a chronic and active hepatitis.

Upon comparing distribution of inflammation cells, lymphocytes showed the larges distribution. In liver parenchyma neutrophil leukocytes were about 28% less than lymphocytes, that can to take for significan index of functional condition of liver. Authors [12] confirm that infiltration of neutrophil leukocytes is observed in case of hepatitis and this is a proof of a perenchymal infection.

Apoptosis is genetically determined self annihilation mechanism, which is influenced by different condition [23]. In the study we observed apoptosis of hepatocytes in liver of mink in all parts of parenchyma although physiologicallly the apoptotic process is basically going in central vein area, which means that hepatocytes are developing in acinus 1 (periportal) section of liver and slowly migrates to area 3 (centrolobular) where they are degenerating [12; 23].

Apoptosis may also develop without inflammation reaction [6]. As the results obtained did not show correlation between apoptosis of hepatocytes with infiltration of inflammation cells, we consider that there is some other reason, which causes apoptosis. Researchers [12; 26] assert that such a process is characteristic for viral infections as in the result of a combined effect of caspases activity and damage of cell mitochondrion, when the ferment endonucleases and a internucleosomal fragmentation of DNS is involved. This small number of apoptotic hepatocytes can possibly be explained by the presence of Aleutian disease virus persisting in mink organism, who can prevent the apoptotic process by inhibiting nuclear protein p53 and caspases, which damage cell DNA [23].

Hepatocyte growth factor (HGF) in normal hepatocytes persists in a non active form [4; 10; 19], but in case of liver damage it is transformed in to active form [16]. Finding of positive coherence between moderate steatosis and HGF expression indicate to ability of mink liver regeneration. It can explain with fact, that HGF is an adipokine which stimulates cell division, motility, angiogenesis, and normal morphogenesis in injured tissue [29].

The release of cytokine Hsp 70 in liver zones, where lots of the inflammation cells were observed, indicated about Hsp as a type of indicator of the cellular condition [8]. Hsp 70 may constitutes an intracellular counterpart to proinflammatory cytokines with the potential to limita n exaggerated inflammatory response [5]. Our establishing, that Hsp 70 expression in hepatocytes was higher when moderate and numerous apoptotic cells were seen is similar to other authors [13] finding, that Hsp 70 is highly protective against oxidative stress and apoptosis. The mechanism by which Hsp 70 inhibits apoptosis has been well studied. There is abundant evidence that Hsp 70 inhibits events upstream of caspase-3 and binds to the apoptotic-protease-activating factor-1 and prevents the recruitment of procaspase-9 to the apoptosome [2; 15; 24].

Serum enzymes. An initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood. Among the most sensitive and widely used liver proteins are ALT and ALP. In our study interconnection between degree of gravity of steatosis and ALT level were not founded and it agree with results of other scientists [29]. Therefore, we speculate that the different ALT level are caused by an association with cellular membranes of apoptotic cells, it explains why apoptotic hepatocytes release ALT upon apoptosis induction [25]. It enable to conclude that ALT level increased in cases of acute hepatocyte injury [18]. Also HGF activity were increased, because in case of liver damage it is transformed in to active form [16].

ALP is liver protein that associated with structures of liver cells membrane. It is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids [14]. In all mink blood samples ALP level was increased. Mean increasing of protein can establish in cases of acute hepatitis [18], when bile ducts are blocked [3], but threefold ALP level testify about liver steatosis [31].

CONCLUSION

Simultaneous presence of infiltration of inflammatory cells and moderate expression of HGF detect the still maintained regeneration ability of liver despite the persisting inflammation. The increased activity of liver enzymes (ALT and ALP) do not directly depend on damaged mink liver disorders and can be suggested only as a common indicators for liver disfunctioning of mink with Aleutian disease.

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RELATIONSHIP BETWEEN SOLDIERS' BODY HEIGHT-WEIGHT CATEGORY AND CHANGES IN THEIR SPINAL COLUMN KYPHOTIC CURVATURE DURING A LONG-TERM MILITARY MISSION

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ABSTRACT

Prolonged physical load can unfavourably influence the human vertebral column. Thirty-six well-trained male soldiers from the Estonian ESTCOY-8 infantry company were examined before and after a 6-month military mission to assess the effect of long-term physical load on soldiers' spinal column kyphotic curvature in relation to their body build (height-weight category). Body height and weight of the men under study were measured before and after the 6-month-long military mission. BMI was calculated as the body weight (kg) divided by the square of the standing body height (m). Body height-weight category was assessed according to Kaarma et al. 2008. Spine kyphotic curvature in the sagittal plane was recorded using pantography. The results of the study showed that significant kyphotic curvature appeared in half of the well-trained soldiers. Changes in kyphotic curvature were related to the person's body build (height-weight category). Subjects with a larger body seemed to have greater stability of kyphotic curvature.

INTRODUCTION

Prolonged difficult physical challenges during military service expect physical fitness and reliability of whole body systems of the army personnel. During prolonged physical load impairment of different body systems (including the immune system, cardiovascular system) is well known [2, 9]. It is also known that long-lasting high physical load can

lead to biomechanic malfunction of the vertebral column [5, 6]. Besides body weight tolerance, conservation and maintenance of the central nervous system and contribution to trunk motion, the thoracic part of the spine or the kyphotic curve supports and protects the heart and the lungs. Increasing curvature of the spine can lead to problems in normal functioning of the body (for example to overstretch of the posterior muscles, problems in breathing etc.) [9]. We were interested in examining well-trained soldiers before and after a high-load military mission to assess the effect of long-term physical load on soldiers spinal column kyphotic curvature in relation to their body build (height-weight category).

MATERIAL AND METHODS

Thirty-six well-trained male soldiers from the Estonian ESTCOY-8 infantry company were examined before and after a 6-month military mission in Afghanistan. This study was a small part of a larger examination of the Estonian ESTCOY-8 infantry company soldiers conducted by the Department of Cardiology, University of Tartu, supervised by Prof. Jaan Eha, that has been partly reported by Salum et al. in 2011 [7]. The study was approved by the Institutional Ethics Committee and an informed consent was given by each participant. Body height and body weight were measured according to Martin and Saller (1956) [4] in an ordinary way. BMI was calculated as the body weight (kg) divided by the square of the standing body height (m). Body height-weight category was assessed according to Kaarma et al. 2008 [3]. We used the Estonian height and weight norms for adult Estonian men and a height-weight classification [3] to assess the height-weight category of each participant in the study.

Spine kyphotic curvature in the sagittal plane was recorded using pantography [11, 12].

Statistical analysis was performed using the SPSS software package version 17.0 (SPSS, Inc., Chicago, Illinois). Paired Samples Test was used to compare a person's variables before and after military mission. The level of $p<0.05$ was selected to indicate statistical significance.

RESULTS

Mean height and weight and BMI of the studied men before and after the 6-month-long military mission are shown in Table 1.

Table 1. Body height, body weight and BMI of the studied soldiers before and after the 6-month long military mission

Variable	Mean		SD		Median		Significance of the change by Paired Samples Test
	Before mission	After mission	Before mission	After mission	Before mission	After mission	
Body height (cm)	180.71	180.07	7.00	7.05	179.03	178.75	P<0.0001
Body weight (kg)	78.49	78.35	10.96	10.24	76.75	78.00	NS (p=0.830)
BMI (kg/m^2).	24.03	24.13	3.02	2.81	23.35	23.90	NS (p=649)

Table 2. Distribution of soldiers by body height-weight categories

Body height-weight category	n	%
1 – small	9	25.0
2 – medium	8	22.2
3 – large	4	11.1
4 – leptomorphic	11	30.6
5 – pycnomorphic	4	11.1
Total	36	100

One third of the studied soldiers belonged to the leptomorphic category, which means that they were tall and relatively light in comparison with Estonian men of the same age. Most of the soldiers (58.3%) belonged to height-weight concordance categories (small, medium, large). A smaller part (41.7%) of the investigated men represented categories of discordant height and weight (leptomorphic and pycnomorphic categories). Large and pycnomorphic participants were in the minority among the well-trained soldiers under study. More than half (n=20, 55.6%) of the participants were small or leptomorphic. Half (50%) of participants had important kyphotic curve changes (more than 4º) during the mission and both kyphotic changes – flattening of the kyphotic

curvature and increasing of kyphotic curvature – were distributed relatively equally (22% and 28% respectively).

Figure 1 shows that increasing of kyphotic curvature during the military mission was more characteristic of leptomorphic men. Flattening of the kyphosis was characteristic of soldiers of the small category. We could not detect any large or pycnomorphic persons with increasing kyphotic curvature and any medium category men with flattening of the kyphotic curvature.

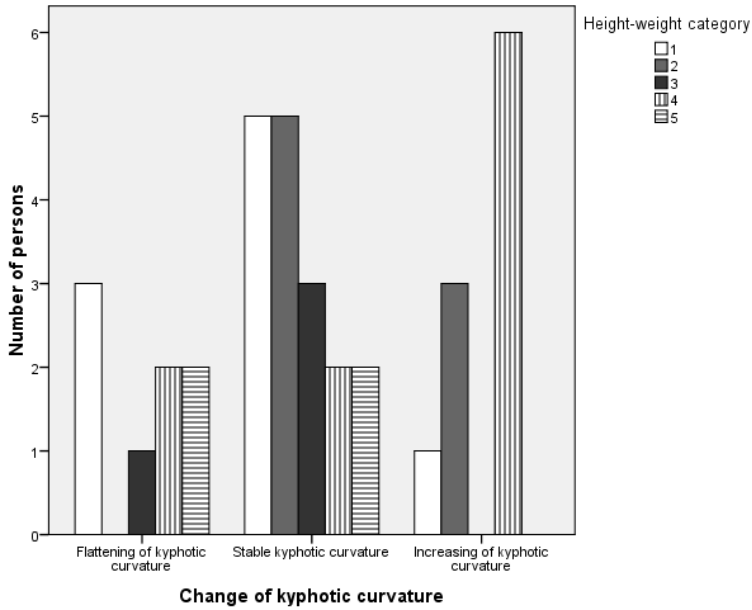

Figure 1. Relations between the height-weight category and changes of kyphotic curvature during the long-term military mission. (Height-weight categories: 1 – small, 2 – medium, 3 – large, 4 –leptomorphic; 5 – pycnomorphic).

DISCUSSION

The normal shape of the vertebral column helps the body to bear the compressive loads. The level of physical load must not exceed the boundaries of an even well-trained organism's adaptational capacity.

The level of permissible load is individual. Overload of the spine can cause deformities of the vertebral column or alteration of spinal curvatures, e.g. a flat back. Monitoring of long-term physical load effect on the musculoskeletal system could prevent the development of latter serious pathologies that could otherwise be unnoticed and untreated due to insufficient clinical symptoms [1, 6, 8].

We examined soldiers to find the effect of long-term physical load on their spinal column kyphotic curvature situation. Changes of kyphotic curvature were observed in half of the young well-trained soldiers. The five-class height-weight classification has been suggested by different studies [3, 10] as a usable basis for analysing whether the differences in distinct persons are related to their body build as a whole or not when different characteristics of the persons are studied. Our study confirmed the applicability of the height-weight classification in getting a systematic overview of relations between body build relations and changes in kyphotic curvature during a military mission. Although the number of studied soldiers was relatively small, methodical following of the changes in kyphotic curvature by body height-weight categories enabled us to notice the relationship between the body build and kyphotic curvature changes. We saw that, as Estonian young adults in general [3], the studied soldiers were also characterized by lepto-morphic body build that could be predisposed to increase of kyphotic curvature. Vice versa, well-trained soldiers with a pycnomorphic body build seems to have higher adaptational capacity to bear a high load for a long time. The large body also seems to be favourable in relation to stability of kyphotic curvature. The reason could be that the high load makes up a smaller proportion of the bigger body size. The overall changes in body height also showed that the long-term mission affects the soldiers spinal column statistically significantly. In our opinion the body build should be taken into account in military service to prevent possible later unfavourable changes in men's health, especially if the person is repeatedly involved in such long-term military missions.

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