doi:10.25143/prom-rsu_2017-01_dts

Rīgas Stradiņa Universitāte

Jūlija Kalniņa

OZONE THERAPY IN PREVENTION AND TREATMENT OF IN CARIES IN PERMANENT TEETH

Summary of Doctoral Thesis
for obtaining the degree of a Doctor of Medicine

Speciality – Dentistry

Riga, 2017

RĪGAS STRADIŅA UNIVERSITĀTE

Jūlija Kalniņa

OZONE THERAPY IN PREVENTION AND TREATMENT OF IN CARIES IN PERMANENT TEETH

Summary of Doctoral Thesis
for obtaining the degree of Doctor of Medicine

Speciality – Dentistry

Riga, 2017

The study was carried out at Rīga Stradiņš University Department of Dental Therapy and Oral Health.

Scientific supervisor:
Dr. med., Professor **Rūta Care**,
Rīga Stradiņš University, Latvia

Official reviewers:
Dr. med., Professor **Ilga Urtāne**,
Rīga Stradiņš University, Latvia
Dr. habil. med., Professor **Pēteris Apse**,
SIA Adenta, Latvia
Dr. med. **Daiga Kviļūna**,
University Children's Hospital, Latvia

Defence of the Doctoral Thesis will take place at the public session of the Doctoral Council of Medicine on 1 March 2017 at 14.30 in Hippocrates Lecture Theatre, Dzirciema Street 16, Rīga Stradiņš University.

Doctoral Thesis is available at the library of Rīga Stradiņš University and on the website www.rsu.lv.

Secretary of the Promotional Council:
Dr. med. Professor **Anda Brinkmane**

CONTENT

SUMMARY

Caries is one of the most common oral diseases. Ozone therapy is one of the latest techniques in dentistry which is applied for caries prevention and treatment. Ozone destroys cariogenic bacteria, thus eliminating one of the main caries aetiological factors. It is believed that ozone therapy can prevent or stop progression of caries, which usually leads to drilling and filling of teeth. Caries prevention and treatment using ozone is a new method in Latvia.

Several studies have demonstrated beneficial effects of ozone on treatment of root caries both *in vitro* and *in vivo*. However, data on the role of ozone in prevention and treatment of fissure and pit caries, which is one of the most frequently occurring types of caries in children and adolescents, is still very controversial. Given the fact that caries incidence in Latvia is high, it is important to pay close attention to prevention of early enamel caries.

The aim of the study was to determine the efficiency of ozone in caries prevention in premolars and treatment of initial enamel caries treatment in permanent molars in children by assessment of changes in mineralization of dental tissues.

The main objective of the study was to assess changes in dental tissues after ozone therapy in the period of 24 months.

The study included ten-year-old children. At this age, it is important to protect teeth from occlusal surface caries, because it is difficult to provide good cleaning of dental surfaces and self-cleaning by means of chewing. During the study, the incidence of caries, oral hygiene and periodontal status of children was determined and assessed, and analysis of dietary behaviour was performed. Active dental change takes place during early adolescence which we were able to follow up during the study. In addition, there are very few studies worldwide, which include children of this age group.

Many different caries prevention methods are available world-wide, our task was to assess the efficiency of application of ozone, fluoride varnish and sealants on development of caries in newly erupded premolars. This study is unique for it is the first time in Latvia when ozone therapy was studied in pediatric dentistry.

By using laser infused fluorescence device *DIAGNOdent* which is not very popular in Latvia, but is well-known abroad, efficacy of ozone and fluoride varnish in therapy of initial occlusal enamel caries in permanent molars was determined. Our study is the only study in Latvia, in which this early caries diagnostic and monitoring instrument was applied.

Caries in premolars developed equally often in ozone, in fluoride varnish, and in sealants groups. Therefore, it is not important what preventive measures we use. We concluded that ozone is an effective way of treatment of initial enamel caries in permanent molars. This indicates the need for the earliest possible commencement of preventive treatment of initial enamel caries.

ABBREVIATIONS USED IN THE STUDY

AAPD	− American Academy of Paediatrics Dentistry
B	− buccal
BW	– BiteWing X-ray images
CO_2	− carbon dioxide
CPITN	− Community Periodontal Index of Treatment Needs
d	− dent
DB	− disto-buccal
DL	− disto-lingual
y.	− year
y.o.a.	− years of age
Ci	− initial enamel caries
DMF S	− Caries intensity index for dental surfaces in permanent occlusion
DMF T	− Caries intensity index for dental in permanent occlusion
L	− lingual
MB	− mesio-buccal
ML	− mesiolingual
n	− numero (number)
p	− filled primary teeth
p	– statistical significance (the probability of obtaining at least as extreme results given that the null hypothesis is true)
pH	− a numeric scale used to specify hydrogen ion concentration in solution
ppm	− part per million (equals to mmol/l)
X-ray	– radiological imaging
SD	− standard deviation
V	− vestibular
WHO	− World Health Organization

INTRODUCTION

Caries is the main oral health problem affecting 60–90% of school-age children (Petersen, 2003; Rickard et al., 2004; Almaz and Sonmez, 2013). Thanks to modern prevention methods, which allow treatment and stopping of caries at an early stage of its development, it is possible to achieve significant reduction in intensity of caries (Marthaler, 2004; Neuhaus et al., 2009). It is important to identify as early as possible the initial enamel lesions to prevent their progression, and it is considered to be more effective means than filling (Johansson et al., 2014).

In recent years, we have been faced with highly neglected dental decays in increasingly younger children. Latvian children under 18 years of age have high caries prevalence and intensity. In Riga in 2001, (carious, filled and extracted teeth) index in 11-year-old adolescents was 1.75, while in the group of 15-years-old children it was – 7.04 (Bērziņa and Care, 2003). Data of the Department of Children Dentistry of Rīga Stradiņš University show sharp growth of caries incidence in early age, as in 2010, 926 children underwent dental treatment under general anaesthesia in the Department of Children Dentistry, while in 2001 – 85 children. From 2000 to 2014, 15 594 children were treated under general anaesthesia (Ciganoviča and Care, 2012). This fact once again underlines the importance of timely protection of teeth from caries, to reduce the number of children in need of dental treatment.

It is known, that micro-organisms play an important role in causing and development of caries (Polydorou et al., 2012). One of caries prevention strategies is reduction of the number of caries-causing micro-organisms in plaque, thereby preventing formation and development of caries (Johansson et al., 2009). For this purpose, various antimicrobials are used, which can mechanically and / or chemically reduce development of dental biofilm and amount of bacteria (Polydorou et al., 2012).

Ozone therapy is one of the new methods in dentistry, making it possible to treat dental decay without drilling (Castillo et al., *2008*). Ozone is widely used due to its antibacterial, disinfectant and healing properties (Nogales et al., 2008). Ozone destroys cariogenic bacteria, thus eliminating one of the main caries aetiological factors (Azarpazhooh and Limeback, 2008). Interest in “biological” approach in treatment of caries rapidly increases, considering caries as an infectious disease (Tyas et al., 2000).

First permanent molars are subjected to the greatest risk of caries because they erupt as early as at six to seven years of age and often are insufficiently cleaned. Occlusal fissure and pit caries constitutes majority of carious lesions in children aged from 8 to 15 years (Baysan and Lunch, 2006). To protect premolar and molar fissures, traditionally sealants and fluoride containing preparations are used. In recent years, prevention and treatment of caries using ozone, increasingly becomes more and more popular, while in Latvia it is still a novelty. It is a non-invasive, painless and quick method. This is a very important factor because many children are still afraid of dentists. Ozone has highly strong antimicrobial properties; it disrupts bacterial cell membranes, in the result of which bacteria die (Nogales et al., 2008). Laboratory studies have shown that ozone significantly reduces the number of micro-organisms in carious dentin in a very short period of time, thus improving dental remineralisation (Nagayoshi et al., 2004). Taking into account the properties of ozone, it would be clinically important to check properties and effectiveness of ozone in carious prevention and dental treatment in children as introduction of new technologies enhances quality of prevention and treatment, shortens time required for treatment and is a benefit not only for a patient but also for a dentist.

The Aim of the Study

To determine efficiency of the use of ozone through assessment of changes in mineralization of dental tissues, in prevention of initial enamel caries of children, and in treatment of permanent teeth.

Tasks of the Study

1. To analyze the oral health status in the study group.
2. To evaluate the changes in dental tissues after ozone, fluoride varnish and sealants use after 6, 12, 18 and 24 months, and to determine the effects of ozone to mineralization process in caries prevention in premolars.
3. To evaluate the changes in dental tissues after ozone and fluoride varnishes use after 6, 12, 18 and 24 months, and to determine the effects of ozone to mineralization process at an early enamel caries treatment in molars.

Hypothesis of the Study

1. Ozone is an effective agent in prevention of early enamel caries in premolars.
2. Ozone is an effective agent in treatment of early enamel caries in molars.

Scientific novelty of the Study

For the first time in Latvia, there was conducted a study with application of a new method in treatment and prevention of early enamel caries – ozone therapy. In the scientific paper, caries development was assessed with laser infused fluorescence device *DIAGNOdent* which is a new method in Latvia. Within two years we assessed changes in dental tissues after the use of ozone and various popular prophylactics (fluoride varnish and sealants). The study results showed potentialities of ozone in prevention and treatment of caries in permanent premolars and molars.

1. MATERIAL AND METHODS

1.1. The design of the study

Cohort, longitudinal study "Application of ozone in prevention and treatment of caries in permanent teeth" was conducted at the Institute of Dentistry of Riga Stradins University in the period from September 2012 to October 2014. The study included ten-year-old children. This age of children was selected based on stage of development of molars and premolars – teeth necessary for the study.

Patients who were selected from the database of the Institute of Dentistry of RSU (n = 1648), were born from 1 January 2002 until 31 December 2002. Of them, 400 children (200 girls and 200 boys) were randomly included in the study, and letters of invitation to participate in the study were dispatched to their parents. Only 158 children (70 girls and 88 boys) attended the first appointment. The study was approved by the Ethics Committee of Rīga Stradiņš University.

The study included three parts: (See Figure 1.1.)

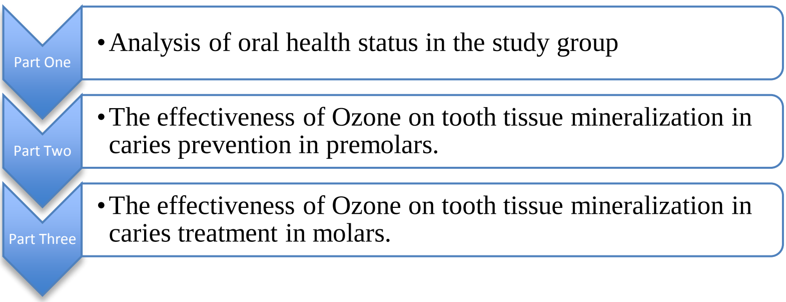

Figure 1.1. **Schematic representation of the study**

158 children participated (70 girls and 88 boys) in **the first part of the study** who responded to an invitation to participate in the study and to attend

the first appointment. 158 parents with their children during this visit filled out a questionnaire about the children's oral care and dietary habits. Repeated examinations were performed every 6 months within the period of 2 years. The study concluded with 120 children remained therein. The total of compliance of patients was – 75.9%. Delays of appointment schedules were the most common reasons for withdrawal from the study.

Of all 158 children who arrived for the first appointment, 122 children were selected for the **second part of the study**. Inclusion criteria: at least 2 intact, erupted premolars. Exclusion criteria: history of bronchial asthma.

Children were divided into 4 groups: 3 therapy groups (applying different means of prevention) – fluoride varnishes, sealants, ozone and 1 control group. The groups of fluoride varnishes and sealants were used as an active control group. For distribution of patients in groups see Figure 1.2.

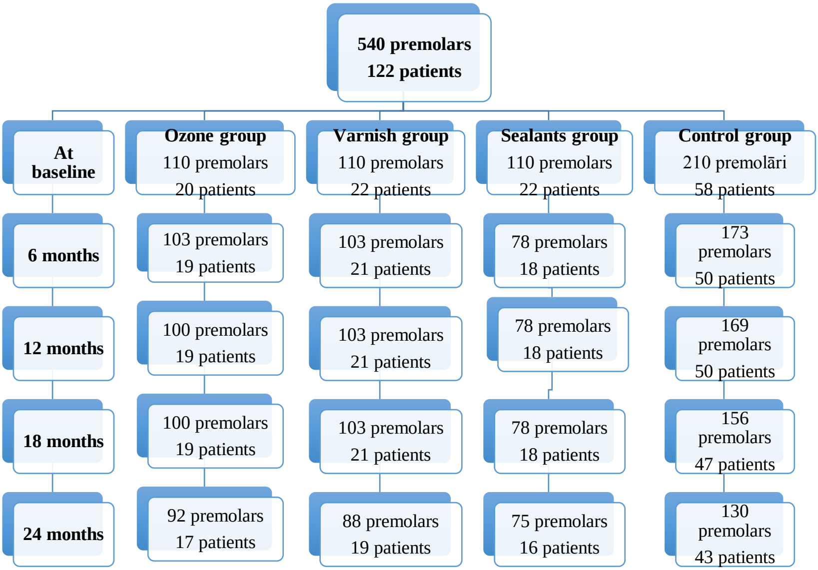

Figure 1.2. **Distribution of patients of the second part of the study**

During the study, for various reasons, participation in the study was suspended by 15% of children in the ozone group, 13.7% – in the varnish group, and 27.3% – in the sealants group, while in the control group – 25.9% of children. In total, 27 children not arrived for a dental appointment. Delays in appointment schedules were the most common reasons for withdrawal from participation in the study.

Of all 158 children who arrived for the first appointment, 49 children were selected **for the third part of the study**. Inclusion criteria: at least 2 fully erupted molars with early enamel caries diagnosed by laser infused fluorescence device (*DIAGNOdent, KaVo*) in occlusal surfaces – *DIAGNOdent* value – 11 to 20. Exclusion criteria: history of bronchial asthma, glucose-6-phosphate dehydrogenase deficiency, hyperthyroidism, severe anaemia, thrombocytopenia.

Children were divided into 3 groups: the ozone group (new method of therapy), the varnish group (conventional method of treatment, as an active control group), and the control group (see Figure 1.3.). In total, 88 teeth were studied. Patients' compliance in this part of the study was 93.3% in the control group and 94% in the varnish and ozone groups. 6% (n=3) of children suspended their participation in the study. Delays of appointment schedules were the most common reasons for withdrawal from the study.

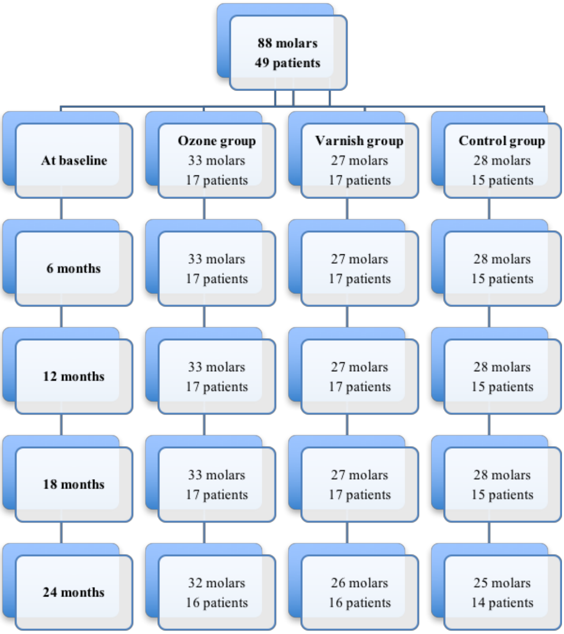

Figure 1.3. **Distribution of patients of the part three of the study**

1.2. Assessment of oral health

DMF, CPITN and Greene-Vermillion index was determined in the children of the study. Data were recorded in patient records (WHO Oral Health Assessment Form, 1987). X-ray examination (BW×2) was carried out for the teeth.

1.2.1. Assessment of dental status

Caries intensity was determined for teeth and surfaces by DMF index. This characterizes the intensity of caries in an individual or a group of people.

The DMF expresses the amount of carious, filled and extracted teeth or surfaces.

Dental status was investigated using radiographic examination (BW×2). Twice a year measurements were made using laser infused fluorescence device *DIAGNOdent* on occlusal surfaces of molars in order to determine changes of mineralization in dental tissues. Measurements were recorded in a table.

Initial enamel caries was defined as primary or clearly visible changes in dental enamel. In registration of data the following codes were used:

Table 1.1.

Codes for recording of initial enamel caries

Scores	Criteria
0 	No shadow or stained area
1 	Lesion stays the same width when transilluminated / Thin grey shadow into enamel when transilluminated
2 	Wide grey shadow into enamel when transilluminated

1.2.2. Assessment of plaque and dental calculus

For assessment plaque and dental calculus, a simplified Greene - Vermillion oral hygiene index (Greene and Vermillion, 1964) was used, which is determined for six teeth (dd 16, 11, 26, 31 buccal and 46, 36 lingual). It is marked by codes – for plaque and for dental calculus (see Table 1.2.).

Table 1.2.

Applicable codes for assessment of plaque and dental calculus

Criteria for classifying debris	
Scores	**Criteria**
0	No debris or stain present
1	Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered
2	Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.
3	Soft debris covering more than two thirds of the exposed tooth surface.
Criteria for classifying calculus	
Scores	**Criteria**
0	No calculus present
1	Supragingival calculus covering not more than third of the exposed tooth surface.
2	Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.
3	Supragingival calculus covering more than two third of the exposed tooth surface or a continuos heavy band of subgingival calculus around the cervical portion of the tooth or both.

1.2.3. Assessment of periodontal status

Periodontal status was assessed by CPITN (Community Periodontal Index of Treatment Needs) index, which determines periodontal status and treatment needs. For children up to the age of 19 – only six teeth are being examined: d17/16; d11; d26/27; d36/37; d31; d46/47. The following codes were used for registration of periodontal status (see Table 1.3.).

Table 1.3.

Periodontal status recording criteria

Scores	Criteria
2	calculus felt during probing but all the black area of the visible
1	bleeding observed, directly or by using mouth mirror, after sensing
0	healthy
9	Tooth is not possible to investigate (difficult access or patient cooperates)
X	If there are not at least two teeth remaining and not indicated for extraction in a sextant, the appropriate box should be cancelled

1.3. Clinical examination

Clinical examination was carried out in a dental chair, using standard lighting and dental instruments: mirror, blunt probe and graduated button probe. Examination of patients was conducted by the author of the study.

All children of the study, every 6 months for 2 years underwent determination of caries intensity both in permanent and milk teeth and surfaces by DMF index.

X-ray examination (BW×2) was carried out once a year. Plaque and dental tartar, as well as CPITN index in children was determined every six months for two years.

Twice a year measurements were made using laser infused fluorescence device *DIAGNOdent* on occlusal surfaces of molars in order to determine changes of mineralization in dental tissues. Before each measurement, calibration was carried out for each tooth individually. The highest value was recorded in each tooth.

The initial caries was determined every 6 months for two years.

1.4. Preventive treatment of premolars with ozone, fluoride varnish, and a sealant

In the beginning of the study and every 6 months for 2 years, 122 children of three premolar caries prevention groups of the study (ozone, fluoride varnish, sealants groups), as well as children of the control group underwent polish of teeth with fluoride-free polishing paste and:

- children in the **ozone group** underwent 6-second ozone application on intact premolars. After ozonation, teeth were covered with a fluoride solution. After the application, patients were not allowed to eat foods and rinse mouth for 4 hours. They were instructed in oral hygiene, which included mandatory brushing twice a day with fluoride containing toothpaste (at least 1000 ppm).
- In the **fluoride varnish** group, fluoride containing varnish (*Fluocal solute, Septodont, France*) was applied on intact premolars of children. After the application, patients were advised to avoid rinsing their mouths, eating solid foods for 4 hours and cleaning their teeth until the next day morning. Patients were instructed in oral hygiene, which included mandatory brushing twice a day with a fluoride containing toothpaste;
- in the **group of sealants**, intact premolar fissures were treated with 37% orthophosphoric acid, which was rinsed away after 15 seconds of etching. Tooth drying was followed by application of a sealant (*Clinpro 3M ESPE Dental Products,* St. Paul, USA). The highest points were tested with articulation wrapper which in case of need were removed by a micro-motor and a polishing-drill. Patients were instructed in oral hygiene, which included mandatory brushing twice a day with a fluoride containing toothpaste;

- in the **control group** the intact premolars remained untreated.

Every 6 months' patients had dental appointments in order to determine efficiency of chosen prevention methods (development of caries in treated surfaces). If caries had not developed, an application of fluoride varnish and re-application of ozone was carried out. If necessary, if any of the sealants was expelled or partially expelled, it was applied repeatedly.

1.5. Treatment of occlusal caries of permanent molars with ozone and fluoride varnish

At baseline of the study and after every 6 months for 2 years, for treatment of caries in permanent molars in both therapy groups (ozone and fluoride varnishes), as well as in the control group, teeth were polished with fluoride-free polishing paste and:

- **In the ozone group,** after polishing, a molar was dried and a measurement with laser infused fluorescence device (*DIAGNOdent, KaVo*, *Germany*) was made for registration of depth of dental caries (demineralization) in occlusal surface of a tooth. Before each measurement, calibration was performed for each tooth individually. The highest reading was recorded for each tooth. Then teeth were treated with ozone (*Prozone, W & H, Austria*) for 6 seconds. After ozonation, teeth were coated with fluorides containing solution. After that the patient were asked to avoid eating and rinsing of his/her mouths for 4 hours. Patients were instructed in oral hygiene, which included mandatory brushing twice a day with a fluoride containing toothpaste;
- **In the fluoride varnish group**, like in the ozone group, a measurement was made with laser infused fluorescence device (*DIAGNOdent, KaVo, Germany*). The highest level of each tooth was

recorded. Then fluoride varnish (*Fluocal solute, Septodont, France*) was applied on molars. After the application, patients were advised to avoid rinsing their mouths, eating solid foods for 4 hours and cleaning their teeth until the next day morning. Patients were instructed in oral hygiene, which included mandatory brushing twice a day with a fluoride containing toothpaste;

- **In the control group,** molars were left untreated.

1.6. Questionnaire on oral care and children's dietary habits

At baseline of the study, a survey of parents and children was conducted during dental examination in order to obtain data on children’s oral hygiene and eating habits. Modified validated questionnaires from WHO international collaborative study carried out in Latvia (International Collaborative Study, 1993) were used.

1.7. Processing of statistical data of the research

The database of the study was created in *Microsoft Office Excel* program. Statistical data analysis was performed using IBM SPSS version 20. In the analysis of data of the study subjects, descriptive and analytical statistical methods were used. DMF index for teeth and surfaces, as well as for Greene–Vermillion index, mean and standard deviation values were determined. Frequency tables were used for description of periodontal health status. Both indicators were compared between the study groups and within the same group at different time points (6, 12, 18 and 24 months).

Depending on correlation of data distribution and normal distribution, the analysis included parametric (*t-test, Pearson's x2* or *Fisher's exact* test) and non-parametric (*Wilcoxon* or *Mann–Whitney* test) methods of statistical analysis. $P < 0.05$ was considered as the threshold of statistical significance.

The analysis of the survey results included frequency tables, calculation of percentage distribution of various factors in various groups of the study.

1.8. Ethical considerations of the study

The study was approved by the Ethics Committee of Rīga Stradiņš University. A permission of the Department of Dental therapy and oral health of Rīga Stradiņš University was received for conducting of the study. All actions were carried out in accordance with the Declaration of Helsinki (The World Medical Association Declaration of Helsinki). The study was conducted in accordance with law of the Republic of Latvia and requirements for protection of personal data.

2. RESULTS

2.1. Analysis of oral health status in the study group

The first part of the study included 158 ten-year-old children (70 girls, 88 boys) who responded to a call to participate in the study and came to the first appointment.

2.1.1. Caries intensity

At baseline, the mean index was 1.88 (SD=2.01), the mean DME S – 2.99 (SD=3.59). After 24 months, increased 1.9 times ($p<0.001$), reaching 3.71 (SD=2.78). The mean also increased ($p<0.001$), reaching 6.06 (SD=5.45) after 24 months, which is 2 times greater than at baseline. Changes of caries intensity throughout the study are shown in Figure 2.1.

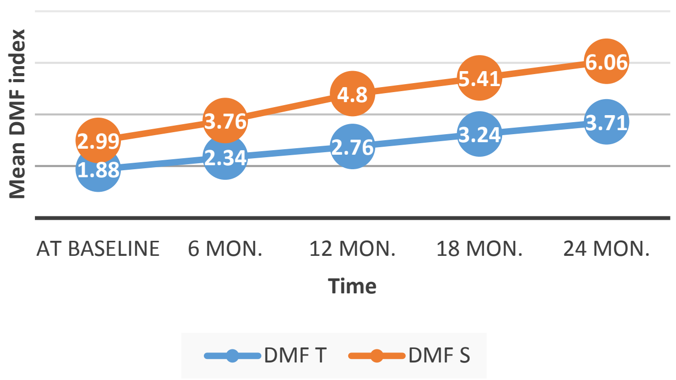

Figure 2.1. **Changes of DMF T and DMF S index throughout the study**

At baseline, the mean number of carious teeth was 0.70 (SD=1.26), filled teeth – 1.16 (SD=1.5), extracted teeth – 0.03 (SD=0.16). After 24 months, the highest increase in the number of filled teeth – 2.4 times, reaching 2.73 (SD=2.19) ($p<0.001$). The number of carious teeth also increased – up to 0.94 (SD=1.54) and the number of extracted teeth – up to 0.05 (SD=0.22), however this increase was not statistically significant.

Similar changes were also observed in the structure of index. At end of the study, compared to the baseline, the highest increase in the mean number of filled surfaces – up to 2.4 times, reaching 4.73 (SD=4.3) of filled surfaces ($p<0.001$). Like with, the mean increase of the number of carious and extracted surfaces was not statistically significant during the study.

At baseline, 129 children still had milk teeth. At baseline, the mean do in teeth was 3.54 (SD=2.17), while in surfaces – 7.36 (SD=5.35). The mean do in teeth after 24 months decreased by almost a half ($p<0.0005$) to 1.93 (SD=1.28).

At baseline, the mean number of teeth affected by initial enamel caries (Ci) was 1.74 (SD=1.45) (see Figure 3.2.). After 6 months, the number did not change, but after 12 months, it statistically significantly increased ($p=0.02$). After 18 months Ci decreased, however these changes were not statistically significant. Comparing Ci values at baseline and after 24 months, statistically significant ($p=0.005$) increase of 1.42 times, reaching 2.42 (SD=1.95) was observed.

The mean number of surfaces affected by Ci, at baseline was 2.12 (SD=1.91) (see Figure 2.2.). After 6 months, the number of dental surfaces with Ci statistically significantly remained unchanged when compared with baseline values. After 12 months, their number increased statistically significantly ($p=0.0003$). Also, after 18 ($p=0.013$) and 24 months ($p<0.0001$), the mean number of surfaces with Ci increased 1.6 times, reaching 3.38 (SD=2.59).

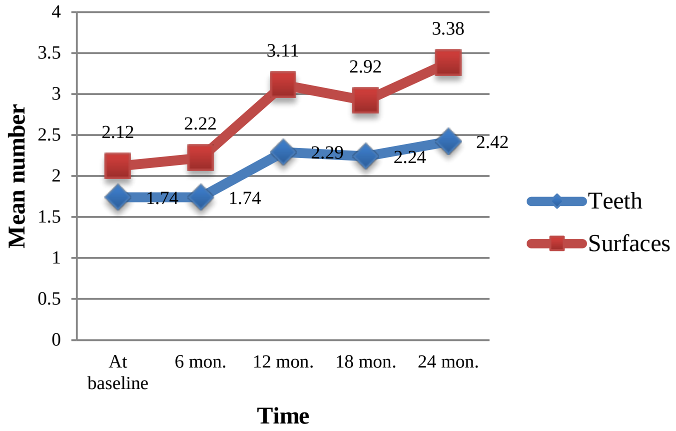

Figure 2.2. **Changes in number of teeth affected by initial enamel caries and surfaces throughout the study**

At baseline, status of first molars was examined separately, because these permanent teeth erupt as the first ones. Of all the examined 632 molars, approximately one third (29.6%) were healthy (see Figure 2.3.). The mean number of healthy and filled first molars showed no statistically significant difference (p=0.472). 14.1% of first molars were carious. The number of Ci affected teeth reached 29.4%.

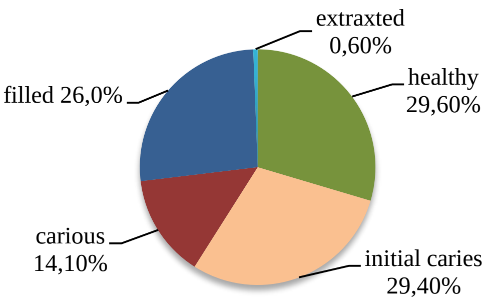

Figure 2.3. **Status of first molars**

2.1.2. Oral hygiene

The mean Greene – Vermillion index was 2.21 (SD=0.99). In all subsequent time points compared to baseline, the mean Green – Vermillion index statistically significantly decreased 1.4 times ($p<0.05$), after 24 months, this index reaching 1.55 (SD=0.64).

2.1.3. Periodontal status

Changes of periodontal status during the study see in Figure 2.4. At baseline, the mean number of sextants with healthy periodontium was 1.36 (SD=1.99). During the study, it increased, however these changes were not statistically significant. The mean number of sextants with bleeding gums until the 12th months increased statistically significantly ($p<0.002$), however starting with the 18th month the number decreased. Changes in the number of bleeding sextants were not statistically significant compared to baseline. The mean number of sextants with dental calculus was 1.31 (SD=2.11) at baseline. During the study, the number decreased two times ($p<0.001$) after 24 months.

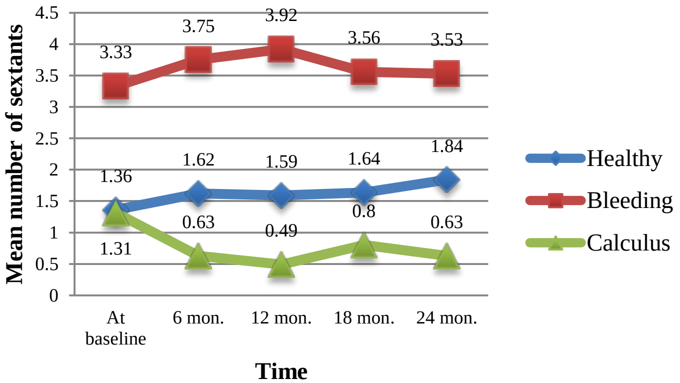

Figure 2.4. **Changes of periodontal status during the study**

2.1.4. Analysis of children dietary and oral care habits

When analysing data on frequency of eating, it was found that most often children eat three or more times a day (44.9% and 54.4%), and only 0.63% eat twice a day. Most children (91%; n=143) tend to eat between meals on an average 2.3 times a day. Mostly children eat two kinds of products between meals. Only a small part of the study subjects eats up to 4 different types of products. Most frequently children eat fruit and sweets (candies, chocolate, ice cream, etc.); (See Figure 2.5.).

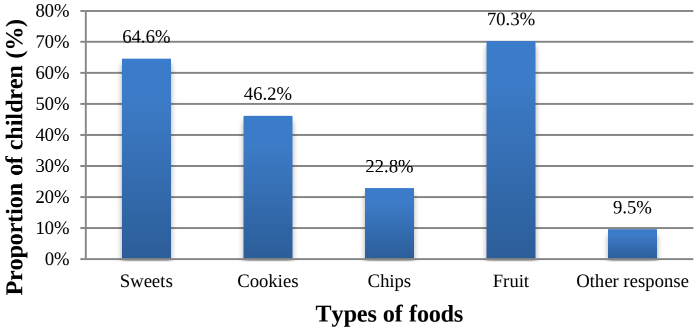

Figure 2.5. **Foods that are most widely eaten between meals**

Between meals, most children tend to choose two or three kinds of drinks, the least – four different kinds of drinks. Of drinks, the most children drink water, tea with sugar and a variety of juice (see Figure 2.6.).

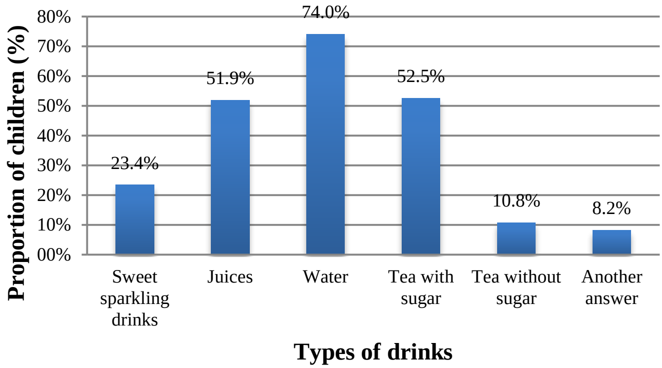

Figure 2.6. **Drinks that are most widely chosen used between meals**

The analysis of tooth brushing habits showed that 87.4% of children brush their teeth regularly. More than a half brushes their teeth twice a day. 32.9% brush their teeth once a day (in the morning or in the evening) (see Figure 2.7). 21% (n=33) of children brush their teeth under parental supervision.

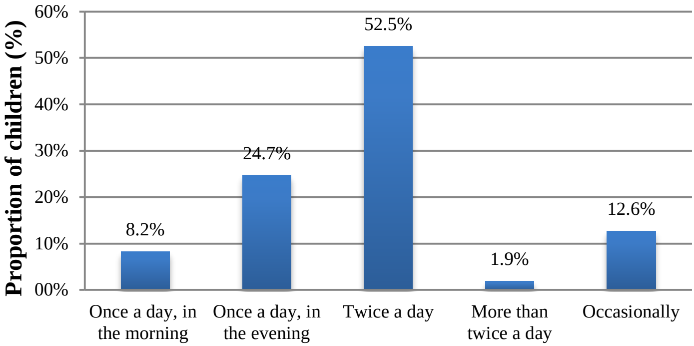

Figure 2.7. **Tooth brushing habits**

68% (n=108) of children brush their teeth with a fluoride containing toothpaste. Only 3% (n=4) of children use fluoride-free toothpaste, 29% (n=46) of parents did not know, whether toothpaste chosen by them for their children contains fluorides. Fluoride tablets use 16% (n = 25) of children.

Tooth cleaning aids such as dental floss, mouthwash, tongue cleaner, special toothbrushes use 58% (n=91) of children. Most often, only one type of aid (85.9%) is used. The most popular aids are mouthwashes and dental flosses (see Figure 2.8.).

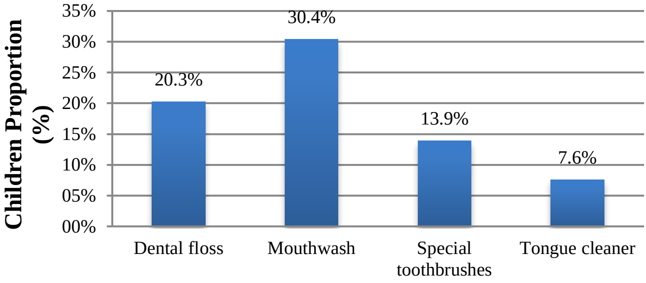

Figure 2.8. **The use of oral care aids**

60% (n=94) of parents on an average 3–4 times a year change toothbrushes of their children. 23% (n=37) – do it 1–2 times a year, and 17% (n=27) – only when the toothbrush has worn out.

80% (n=126) of children make dental appointments on regular basis. Most children (92%, n=114) do it once or twice a year. A dental hygienist is regularly visited by only 58% (n=92) of children, 29% (n=46) do it occasionally, while 13% (n=20) of children have never visited a dental hygienist. Children who attend dental hygienists on regular basis – 70% (n=64) do it only once a year.

2.2. The effectiveness of Ozone on tooth tissue mineralization in caries prevention in premolars

2.2.1. Impact on development of caries

The second part of the study included 122 children, analysing in total 457 premolars. Caries development in the study groups was assessed after 6, 12, 18 and 24 months. During 24-months period, caries developed in the sealants group in 4 teeth, in the varnish group – in 2 teeth, in the ozone group – in 3 teeth and in the control group – in 14 teeth, in total – in 23 teeth (see Table 2.1.). Caries development incidence in premolars in the study groups showed no statistically significant difference after 24 months. Most of carious teeth were observed after 18 months, the least – after 12 and 24 months.

Table 2.1.

Caries development in premolars in the study groups after 6, 12, 18 and 24 months

Time lapse	Ozone group		Sealants group		Varnish group		Control group	
	Number of carious teeth n (%)	Number of teeth (n)	Number of carious teeth n (%)	Number of teeth (n)	Number of carious teeth n (%)	Number of teeth (n)	Number of carious teeth n (%)	Number of teeth (n)
6 months	3(2.9)	103	–	78	–	103	4 (2.3)	173
12 months	–	100	–	78	–	103	2 (1.2)	169
18 months	–	100	3 (3.8)	78	2 (1.9)	103	7 (4.5)	156
24 months	–	92	1 (1.3)	75	–	88	1 (0.8)	130

Combining preventive treatment group (ozone, varnishes and sealants) in one and comparing caries development with the control group, where no preventive measures were taken, statistically significant changes in the development of caries were observed (p=0.026).

Caries development, depending on tooth surface (occlusal or another – mesial, distal, lingual, buccal) is showed in Table 2.2.

Table 2.2.

Differences of caries localization in the study groups (n = number of carious teeth)

Tooth surface	Ozone group n	Varnish group n	Sealants group n	Control group n	Total
Occlusal	3	1	0	9	13
Other	0	1	4	5	10
Total	3	2	4	14	23

Caries development both in occlusal and other surface was observed equally often. In occlusal surface caries was not observed in sealants group, but most often it was found in the control group. However, in another surface, caries was most found in the sealants group and the control group. The difference in development of caries in occlusal surface of the study groups was statistically significant (p=0.037), while in other surfaces these differences were not observed.

2.2.2. Changes of oral health during the study

Analysing oral health status of the study groups, the mean value of and index increased over the study in all groups. Changes of the mean index over the study see in Table 2.3.

Table 2.3.

Changes of the mean DMF T index over the study

	Ozone group		**Sealants group**		**Varnish group**		**Control group**	
	Mean value	**SD**	**Mean value**	**SD**	**Mean value**	**SD**	**Mean value**	**SD**
At baseline	2.20	1.79	2.82	3.06	0.95	1.76	2.16	1.85
6 mon.	3.00	2.11	2.33	1.81	1.29	1.90	2.52	1.71
12 mon.	3.37	2.11	3.33	3.22	1.81	2.44	2.92	1.78
18 mon.	3.89	2.18	3.89	3.71	2.33	2.56	3.60	2.35
24 mon.	4.29	2.17	4.63	4.30	3.00	2.88	4.00	2.64

At baseline, the mean index between the study groups did not differ statistically significantly, except for varnish and sealants groups (p=0.023). After 6 months, difference between the varnish and the ozone groups was observed (p=0.024). However, after 12, 18 and 24 months, no statistically significant differences between the study groups were observed in regard to the mean index.

Changes of the mean index during the study see in Table 2.4.

Table 2.4.

Changes of the mean DMF S index during the study

	Ozone group		**Sealants group**		**Varnish group**		**Control group**	
	Mean value	**SD**	**Mean value**	**SD**	**Mean value**	**SD**	**Mean value**	**SD**
At baseline	4.01	3.81	4.68	5.41	1.36	2.36	3.57	3.56
6 mon.	5.37	4.13	4.11	4.36	2.00	3.08	4.22	3.31
12 mon.	6.32	4.28	6.17	6.60	2.71	3.73	5.20	4.06
18 mon.	7.37	4.49	7.39	7.73	3.38	4.21	5.70	4.72
24 mon.	7.88	4.64	8.38	8.62	4.37	4.63	6.35	5.25

At baseline, the mean index between the study groups did not differ statistically significantly, except in the varnish and the sealants groups (p=0.029). After 6 months, it showed statistically significant difference between the varnish group and the ozone group – in the varnish group, the mean index was lower than in the ozone group (p=0.024). After 12, 18 and 24 months, no significant differences between the study groups were observed.

The mean number of teeth with initial enamel caries in the study groups at baseline and after 12, 18, 24 months did not show statistically significant difference (see Table 2.5.), excluding in the sealants and the ozone group, where in the sealants group this reading after 6 months was two times lower than in the ozone group (p=0.038).

Table 2.5.

Changes in the number of teeth affected by initial enamel caries during the study

	Ozone group		Sealants group		Varnish group		Control group	
	Mean number of teeth	**SD**	**Mean number of teeth**	**SD**	**Mean number of teeth**	**SD**	**Mean number of teeth**	**SD**
At baseline	2.35	1.46	1.36	1.00	1.82	1.37	1.69	1.64
6 mon.	2.47	1.54	1.17	1.34	2.09	1.26	1.68	1.48
12 mon.	3.21	1.87	1.61	1.69	2.57	1.80	2.42	2.20
18 mon.	2.79	2.53	1.44	1.89	2.62	1.94	2.36	2.43
24 mon.	3.12	2.09	1.94	1.91	2.74	2.18	2.19	1.83

Changes of the number of Ci affected surfaces during the study see in Table 2.6. The mean number of surfaces with Ci, in the study groups at baseline and after 12, 18, 24 months were no statistically significantly different, except for the sealants and the ozone group, where after 6 months this reading in the sealants group was 2 times lower than in the ozone group (p=0.014), as well as differences were also observed between the control and the ozone groups (p=0.031) – in the control group it was lower than in the ozone group.

Table 2.6.

Changes in the number of initial enamel caries-affected surfaces during the study

	Ozone group		Sealants group		Varnish group		Control group	
	Mean number of surfaces	**SD**	**Mean number of surfaces**	**SD**	**Mean number of surfaces**	**SD**	**Mean number of surfaces**	**SD**
At baseline	3.00	1.95	1.73	1.49	2.14	1.83	1.93	1.92
6 mon.	3.42	1.80	1.61	1.69	2.38	1.50	2.06	1.88
12 mon.	4.58	2.63	2.76	2.44	2.90	2.07	3.08	2.55
18 mon.	3.37	2.69	2.06	2.96	3.24	2.51	3.11	3.19
24 mon.	4.24	2.61	3.00	2.94	3.42	2.78	3.26	2.57

Analysing the mean value of oral hygiene *Greene – Vermillion* index, significant differences between the study groups were observed only after 24 months – between the varnish and the ozone groups (p=0.004) – it was lower in the varnish group than in the ozone group (1.04 compared to 1.72). Statistically significant differences were also observed between the control group and the varnish group (p>0.001) – in the control group, the index was higher than in the varnish group (1.72 compared to 1.04) (see Table 2.7.).

Table 2.7.

Changes of *Greene – Vermillion* index during the study

	Ozone group		**Sealants group**		**Varnish group**		**Control group**	
	Mean value	**SD**	**Mean value**	**SD**	**Mean value**	**SD**	**Mean value**	**SD**
At baseline	1.96	0.99	2.13	0.79	2.28	0.98	2.18	1.02
6 mon.	1.89	0.64	1.87	0.60	1.83	0.54	1.92	0.66
12 mon.	1.99	0.68	1.93	0.64	1.80	0.56	1.89	0.69
18 mon.	1.93	0.61	1.69	0.64	1.60	0.69	1.90	0.74
24 mon.	1.72	0.60	1.39	0.45	1.04	0.48	1.72	0.65

Evaluating the mean number of sextants with a healthy, bleeding periodontium or dental tartar, no significant differences between the groups were found during the study.

2.3. The effectiveness of Ozone on tooth tissue mineralization in caries treatment in permanent molars

2.3.1. Impact on the development of caries and *DIAGNOdent* readings

This part of the study was participated in by 49 children and included analysis of 88 permanent molars. Regardless to the fact that initial caries intensity indicators – DMF T and DMF S indices in the varnish group were the lowest, while in the control group – the highest, differences between the groups were no statistically significant (see Figure 2.9.). Similarly, Greene – Vermillion index in all groups at baseline did not differ (in the varnish group – 2.3 (SD=1.04), in the ozone group – 1.94 (SD=0.82) and in the control group – 1.66 (SD=0.53)).

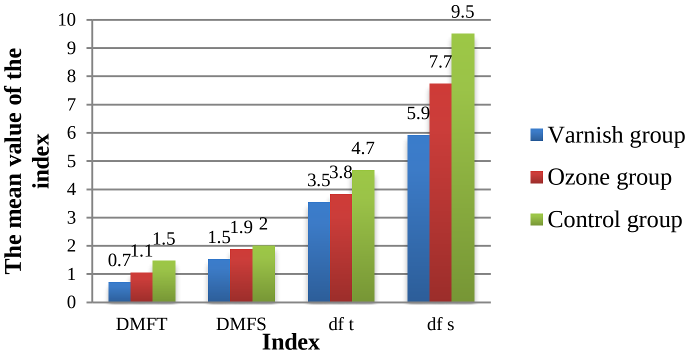

Figure 2.9. **Caries intensity in different groups at baseline**

Determining the depth of caries, at baseline and after 6 and 12 months, the mean readings of *DIAGNOdent* in the varnish, the ozone and the control groups did not differ (see Figure 2.10.). After 18 months the mean *DIAGNOdent* reading in the ozone group was 1.4 times lower than in the control group (p=0.01). This difference remained even after 24 months (p=0.03). Comparing the varnish and the ozone group, no statistically significant differences were observed in any time point of the observation.

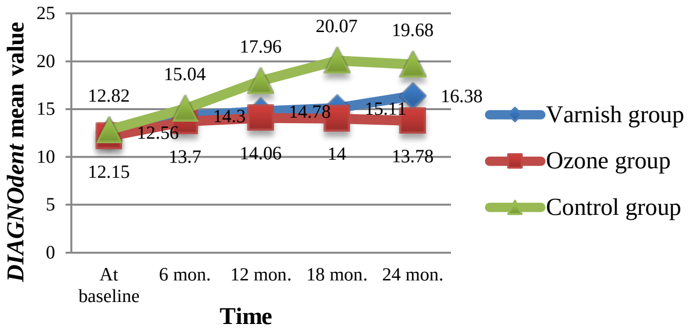

Figure 2.10. **The mean *DIAGNOdent* readings in the study groups over time**

During the study, the following impact of treatment on values of caries depth (*DIAGNOdent*) was observed in each group of the study:

- In the ozone group, the *DIAGNOdent* readings after 6 and 12 months increased, then, after 18, 24 months, it decreased however these changes were no statistically significant,
- In the varnish group, *DIAGNOdent* readings increased with every 6 months. In 6, 12, and 18 months, this increase was not significant, however after 24 months the difference was statistically significant ($p=0.02$) compared with baseline readings,
- In the control group, *DIAGNOdent* reading increased after 6 months ($p=0.006$) and 12 months ($p<0.0001$) and also after 18 and 24 months DIAGNOdent reading continued to increase ($p<0.0001$).

2.3.2. Oral health changes during the study

Changes of DMFT and DMFS indices and Greene – Vermillion during the study are reflected in Figures 2.11. and 2.12., 2.13. Differences of the mean and index between the study groups during the study were no statistically significant (see Figures 2.11., 2.12.).

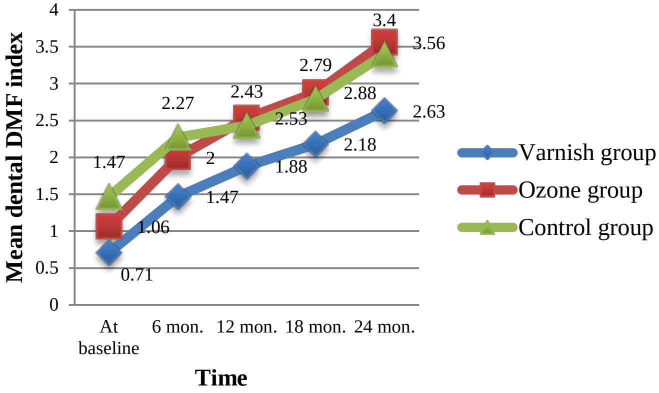

Figure 2.11. **Changes of the mean DMF T index in the groups during the study**

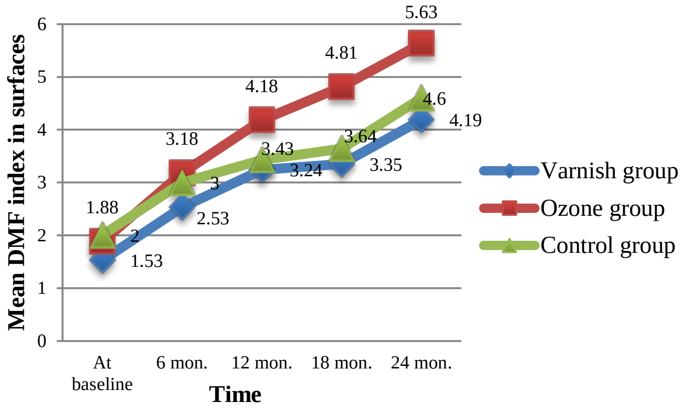

Figure 2.12. **Changes of the mean DMF S index in groups during the study**

Also readings of Greene – Vermillion index did not statistically significantly differ between the groups during the study (see Figure 2.13.)

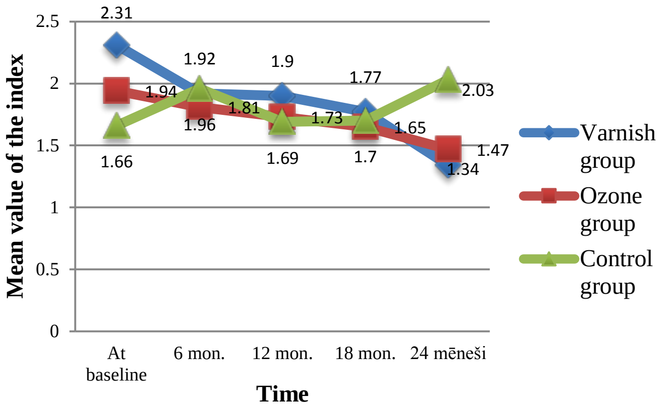

Figure 2.13. **Changes of the mean *Green – Vermillion* index in groups during the study**

The mean number of sextants with healthy, bleeding periodontium or dental tartar and its changes during the study were not statistically significant in the fluoride varnish, the ozone and the control groups.

3. DISCUSION

3.1. Analysis of oral health status in the study group

Epidemiological studies previously carried out in Latvia show moderate and high caries prevalence and intensity. Caries intensity in children of this study can be considered as moderate – reaching 3.71 by 12 years of age. Data available in the literature show that the mean index in 12–13 years old children in Latvia in 1998 was 5.75, in Riga – 3.95 (Care and Urtāne, 1999). Five years later, the mean index in 11-year-old children reached 3.9, and – 6.8, and in 13-year-old children, was 6.1 and – 11.8 (Bērziņa and Care, 2003). According to the data of Gudkina, in 2010, in 12-year-old children was 4.45 (Gudkina and Brinkmane, 2010). For comparison – in the world (data collected from 189 countries), in 2011, the mean index in 12-years-old children was 1.67. In economically highly developed countries, such as Sweden, this index was 0.8, in Germany – 0.7. In Poland the mean index already was significantly higher – 3.2, in our neighbouring countries Estonia, in 1998, it was – 2.7, while in Lithuania, in 2005 – 3.7. (http://www.mah.se/CAPP/Country-Oral-Health-Profiles/EURO/). This suggests that the intensity of caries in our study is very similar to the one in the neighbouring Lithuania.

Although all patients, regardless of the research group, 2 times a year were provided with full oral hygiene and patients were motivated to follow good oral health habits, eat healthy and so on, however, increase of caries intensity was observed during the study – DMF index both in teeth and surfaces increased. This leads to a lot of questions – what could be the reason for DMF index changes, what could be caries contributing factors, and how important is professional oral care and oral care at home? More detailed analysis of components of structure of index show that the largest increase in the study reached the proportion of filled teeth which seems logical, because the children did receive the necessary dental care – including dental filling. Proportion of carious teeth and surfaces statistically significantly did not change over time, which indicates a high caries activity. This, in turn, makes us think about possibly residual caries risk factors in children of our study – frequent snacking between meals, increased use of carbohydrates-containing products, low quality or inadequate plaque removal. The children were provided with professional dental hygiene. Therefore, additional determination of caries risk should be recommended in the following studies.

In our study, there were accounted teeth with initial enamel caries. At the age of twelve years, an average of 2.4 teeth were of a potential risk of progression of initial enamel caries and may require treatment with application of conservative methods – drilling and filling. Similar results were obtained by Abanto and co-authors, who observed that those children who are at high risk of caries, early enamel lesions often tend to progress, rather than to stop. In children with low caries risk – early enamel lesions tend to regress. (Abanto et al., 2015).

In our study, a special attention was paid to first molars of permanent teeth. At baseline, 14% of molars were carious, and even higher percentage comprised the filled teeth – 26%. As indicated in the literature, children at the age of 6 to 9 years, have at least 22 to 36% of the first permanent molars that are carious. (Cho et al., 2001). Goyal et al. emphasizes that the most often caries affects first permanent molars (80% of all carious teeth) and the most common locations are in occlusal tooth surfaces (Goyal et al., 2007). As concluded in some epidemiological study, in first 3 to 4 years after eruption of teeth, 50% of the first permanent molars become carious (Duruturk et al., 2011). To avoid these situations, it is necessary to introduce early intervention strategies.

During the study, a decrease of oral hygiene indicator Greene-Vermillion index was observed in patients, which is assessable as medium.

Changes of periodontal health status were also observed. The mean number of sextants with healthy periodontium increased, however these changes were not statistically significant. Dental calculus incidence significantly decreased – about two times, which could be explained by the fact that patients, on a regular basis – twice a year – underwent professional dental hygiene, when, if necessary, their dental calculus was removed. The mean number of sextants with bleeding gums remained unchanged. In regard to risk factors, plaque index was determined during appointment to which a child had to be prepared – they had to brush their teeth, thus eliminating the amount of plaque and, possibly, influencing final results. Without knowing an appointment time, it would be possible to address this index at more precise level.

Although the children of the study were provided with a professional dental hygiene twice a year, oral hygiene habits at home are of tremendous importance. The children of the study were at the beginning of early puberty age when parental influence reduces. The older grow children, the more frequently they have an opportunity to snack outside the home. Lithuanian colleagues in their study stress out that adolescence is the age when person’s understanding about teeth cleaning, physical activities, smoking and healthy diet take down their roots. It is known how difficult is to change bad habits in adulthood, when they are already deeply rooted in everyday life (Brukiene et al., 2010). Family is one of the most important social supporters in education of children (Gift, 1993). In early childhood both parents and other family members set models for healthy lifestyle. Later, at adolescence, they become a support group at times when children lack strength and motivation, for example, to maintain good oral hygiene. (Inglehart and Tedesco, 1995; Östberg et al., 2002). Also, it should be taken into account that a child depends on desires and financial capabilities of parents (e.g., in purchases of toothpastes and toothbrushes) (Gift, 1993).

Analysing results of the parental survey, it appears that children often (on average 2.3 times a day) tend to snack on various sweets, fruit, most often they drink water, however they also choose tea with sugar and various juices, thus exposing their teeth to persistent fluctuations of *pH*. In the study conducted in Latvia, it was concluded that 6-year-old children daily add to their tea on average 1.47 teaspoons of sugar (in teaspoon is 5g sugar), while 12-years-old children add 1.86 teaspoons of sugar, thus daily consuming on average 2.71 and 4.36 tablespoons of sugar (Gudkina and Brinkmane, 2010). Data available in literature show that caries prevalence in many EU countries have decreased, despite the fact that consumption of sugar per person per year has remained roughly the same (≈ 34 kg per person per year) (Touger-Decker, 2003), frequency of consumption of sugar-containing snacks also does not change (Reich, 2001). Reduction of caries prevalence is linked mainly to regular use of fluorides (toothpaste) and improved oral hygiene. If fluorides are taken in sufficient quantities, diet plays a lesser role in caries prevention (Van Loveren, 2000).

Teenage children would require parental supervision and support. However, the survey data show that dental cleaning under parental supervision is carried out only in 21% of 10-year-old children. The survey data show that 32.9% of children cleaned their teeth only once a day. Results of WHO's international collaborative study conducted in Latvia in 1993 show that 43% cleaned their teeth once a day (Care and Urtāne, 1999), similar data were stated also by Polk et al. According to them, 44% of children clean their teeth once a day or even less often. Tooth cleaning has several advantageous – it provides both mechanical plaque removal from teeth, as well as a supply of teeth with fluorides from a toothpaste and water (Polk et al., 2014).

Data obtained during the study show that a fluoride containing toothpaste was used for teeth cleaning by 68% of children (n=108), 29% of parents (n=46) did not know whether toothpastes chosen by them for their children contain fluoride. Results of WHO's international collaborative study carried out in Latvia in 1993 show that fluoridated toothpaste was used by 45% of children. Literature data show that fluoridated toothpastes tend to reduce caries development on average by 24% compared with fluoride-free toothpastes (Walsh, 2010). Tooth cleaning aids, such as dental floss, mouthwash, tongue cleaner, and special toothbrushes were used by 58% of children. Of those, mouthwash and dental floss were used the most widely.

Of concern is the fact that by the age of 10, only 13% of children of the study group had never visited a hygienist. Guidelines of dentists' associations of different countries recommend hygienist's appointments already from 3 years of age, at least once a year, depending on group of caries risk. Adolescents are especially advised to undergo professional removal of soft plaque and dental tartar taking into account each child's risk of caries and individual needs (Dean et al., 2011).

Summarizing findings of the study concerning the oral health of 10–12 year old children, it was concluded that caries intensity in permanent teeth was low at baseline; however, after 24 months caries intensity increased and was assessed as medium. Oral hygiene and periodontal health of 10-year-old children was moderate at baseline, but after 24 months, oral hygiene index Greene – Vermillion decreased and periodontal health status improved.

3.2. The effectiveness of Ozone on tooth tissue mineralization in caries prevention in premolars

Caries prevention is widely addressed in our study. What exactly is prevention? The term "prevention" in a dictionary is explained as - measures for prevention of outbreak the spread of infectious diseases, strengthening of human and environmental health. The term treatment – professional and individual disease prevention, diagnostic, treatment, rehabilitation and care of patients, carried out by a medical practitioner (http://www.vm.gov.lv/lv/nozare

/terminu_vardnica/). As it is known, as soon as a tooth has erupted, it is constantly exposed to mild acids leading to changes of mineralisation of permanent tooth tissues. It is very difficult to draw the line when it comes to prevention and treatment of caries. Therefore, in regard to dental caries, the definition “preventive therapy” is increasingly wider used.

Caries development is closely related to a variety of factors, including microbial reduction. Over time, various methods for disinfection of dental tissues thus preventing development or progression of caries have been proposed. Application of ozone in dentistry is a recent novelty (Almaz and Sönmez, 2013). It is used in almost all dental procedures, because it is devoid of toxicity and side effects. Ozone is the most powerful oxidant in the world, causing death of bacterial cells (Bocci et al., 2009) and enabling diffuse of calcium and phosphate ions in dental tissues, leading to remineralisation of dental tissues. Ozone destroys micro-organisms in lesions by oxidizing pyrotartaric acid and CO_2, thus increasing *pH* levels which cause remineralisation of demineralised tissues by salivary minerals or remineralizing means (Dähnhardt et al., 2006).

The importance of fluoride in caries prevention is long talked about (Petersen and Lennon, 2014). Hiiri and co-authors report that fluoride varnishes statistically significantly reduce caries development both in milk and permanent bite (Hiiri et al., 2006). Sealants also are regarded as good protectants against dental caries (Poulsen et al., 2009).

Both fluoride varnish and sealants are effective in prevention of caries. However, their application is relatively expensive. In regard to the use of ozone a clinic must take into consideration relatively high costs of an ozone generator (approximately EUR 5000), as well as needs for regular maintenance thereof. For an insight, according to price-list of RSU Institute of Dentistry ozonation of each tooth costs EUR 5, application of sealants are EUR 13 per tooth, application of fluoride varnishes per tooth – EUR 3. Children up to 18 years of age are entitled for the National Health Service-financed fluoride varnish application in entire oral cavity. Ozonation and sealants application is not financed by the NHS.

Duration of a manipulation is a very important factor for adaptation of any of caries prevention methods. Application of sealants is a relatively time-consuming process compared to application of a fluoride varnish, which is often considered to be the most effective in prevention of pit and fissure caries (Bravo et al., 1997). In contrast, application of ozone takes only a few seconds and greatly saves a dentist's time.

In the second part of our study, using various caries prevention methods (ozone, fluoride varnish and sealants) in the period of 24 months, caries developed equally in the ozone, the varnish and sealants groups (respectively, in 3; 2; 4 premolars), while in the control group, caries developed in 14 premolars. However, among the study groups, after 24 months, no statistically significant differences in effectiveness of prevention methods were observed. Combining preventive therapy groups (ozone, varnishes and sealants) in one and comparing caries development in this joint group with the control group, where no preventive measures were taken, statistically significant changes in development of caries were observed. This suggests that the use of one of these caries prevention measures can result in reduction of development of caries risk. Similarly, Bravo et al in their study found that the use of fluoride varnish and glass ionomer sealant resulted in finding of no significant differences between the two caries prevention methods (Bravo et al., 1997). This same author in another 5-year study concluded that resin based glass sealant is more effective than fluoride varnish (Bravo et al., 2005).

Caries development both in occlusal and other surfaces was observed equally often. In occlusal surface caries was not observed in the sealants group, confirming the effectiveness of sealants in prevention of occlusal surface caries. Sealants are usually applied on occlusal surfaces of teeth, thus creating a reliable barrier that protects dental fissures and pits from cariogenic bacteria and environmental impact. Many studies have shown the effectiveness of sealants in reduction of caries incidence from 87% to 60% (Gordon et al., 2012).

In our study, ozone was applied occlusally, but regardless to this, caries developed in occlusal dental surfaces rather than in another. The literature shows controversial data on antimicrobial effect and efficiency in destruction of microorganisms in occlusal dental surfaces. There are reliable data on preventive properties of ozone in therapeutic dentistry prior to etching, filling and application of sealants (Azarpazhooh and Limeback, 2008). Discussing the findings of our study, it is noteworthy to know what measures for prevention of caries were used for children of the study at home. Because it is shown that maintenance of good oral care habits at home and regular visits to a hygienist, provide low incidence of caries and periodontal diseases (Axelsson et al., 2004).

Taking into account the results obtained, in the future it would be desirable to explore opportunities of combination of preventive methods used in our study, thereby possibly improving the desired results. Axelsson et al in their study showed a statistically significant benefit in reducing caries in children and adolescents through combination of different caries prevention methods (Axelsson et al., 2004). Johansson confirms that combination of ozone and fluoride varnish is recommended means for caries prevention (Johansson et al., 2014).

Analysing oral health status in groups of the second part of the study groups, the mean DMFT and DMFS indices between the study groups did not differ statistically significantly at baseline, except in groups of varnishes and sealants – in the group of sealants, and was higher than in the varnish group. However, after 24 months, no statistically significant differences in the mean and index were observed between the study groups. The mean value of DMFT and DMFS indices increased during the study in all groups. As previously mentioned, this could be caused by several reasons. It should be noted that caries risk depends on each individual. Individual peculiarities such as low or high salival pH levels, genetic predisposition, previous caries experience, use of medications, various immune system-affecting systemic diseases, and personal oral care habits affects the risk of development of caries (Touger-Decker and van Loveren, 2003).

The mean number of teeth and surfaces with Ci in the study groups at baseline and after 24 months showed no statistically significant difference.

Analysing the mean value of oral hygiene Greene – Vermillion index at baseline, no significant differences between the study groups were found – so it therefore be assumed that levels of oral hygiene at baseline between the groups are similar. At the end of the study, namely, after 24 months, the mean Greene – Vermillion index decreased and oral hygiene improved in all study groups, excluding the ozone group.

Evaluating the mean number of sextants with healthy, bleeding periodontium or dental tartar, no significant differences between the groups were found at baseline and during the study. It could therefore be considered that periodontal status in all study groups was similar.

Assessing the results, we concluded that ozone, a fluoride varnish and sealants are equally effective means for caries prevention in premolars.

3.3. The effectiveness of Ozone on tooth tissue mineralization in caries treatment in permanent molars

For the study purposes there were selected occlusal surfaces of teeth, as they are prone to dental caries due to various reasons. First, the newly erupted tooth enamel is immature. It contains a relatively high organic component, which is permeable, thereby exposed to caries attacks. Second, the morphology of fissures and pits provides favourable environment for retention of plaque and bacterial proliferation. Enamel is thinner in dental fissures and pits, therefore demineralisation process can be faster. Molars have relatively long eruption period (1.5–2.5 years), premolars – a few months). This lengthy eruption can interfere with adequate dental hygiene, because bristles of a toothbrush cannot easily access occlusal surfaces which are outside distal occlusion and are difficult to reach (Casamassima et al., 2013). At the age from 7 to 14 years, when children's bite changes, eruption of premolars and molars take place. These dental pits and fissures immediately after eruption are exposed to risk of caries; however, improving oral hygiene, along with the use of ozone therapy and special oral care products, it is possible to ensure the greatest protection against caries.

At baseline, differences of DMFT and DMFS index between the study groups were no statistically significant. Similarly, oral hygiene Greene – Vermillion index in all groups did not differ at baseline. Analysing oral health status in patients of this part of the study, no statistically significant differences were found in the mean value of DMFT and DMFS index between the study groups. Also, values of the mean Green – Vermillion index between the groups did not differ statistically significantly during the study, although in the control group, the mean value of Greene – Vermillion increased 1.2 times after 24 months. Changes of CPITN index components between the study groups were no statistically significant.

During the ozone therapy it is important to observe remineralization process of demineralized areas. At baseline of our study, the mean value of *DIAGNOdent* did not differ between the varnish, the ozone and the control group. After 6 and 12 months, *DIAGNOdent* value also did not differ in the study groups. After 18 months, the mean values of *DIAGNOdent* was 1.4 times lower in the ozone group than in the control group (p=0.01). This difference remained even after 24 months (p=0.03). Comparing the varnish and the ozone group, no statistically significant differences were observed in any time point of the observation. This suggests that ozone, like fluoride varnish, is an effective measure in the therapy of initial enamel caries in permanent molars.

As a risk factor that could affect the results of the study should be considered the fact that the of children of the study, if necessary, were offered free of charge dental care at the Pediatric Department of the Institute of Dentistry of RSU, however, a part of the children chose to treat their teeth at their dentist, closer to their place of residence. Unfortunately, most patients who underwent dental treatment elsewhere, had filling performed on those teeth, in which early enamel caries was diagnosed by us, thereby affecting the final study results. The second risk factor was the exactness of *DIAGNOdent* device. As already described in the literature review, the sensitivity of the device is 0.75 and its specificity is 0.96 (Shi et al., 2001). The *DIAGNOdent* device is much more sensitive than other traditional diagnostic methods. It can often give false positive results in diagnostics of early caries. Bader et al has mentioned that the use of *DIAGNOdent* device in clinical trials can cause some complications. For example, the presence of plaque or pigment can be interpreted as progression of a carious lesion, and it is very difficult to make repeated measurements in the same point of dental fissure to be able to compare results in different time intervals (Bader et al., 2004).

It is very important to detect and treat caries at its early stage. In the light of the study findings, it can be concluded that ozone not only prevents dental tissues from the use of invasive methods in removal of carious tissues, but also initiates stopping of caries and remineralisation of dental tissues. Ozone therapy is an ideal way to reduce the fear of dental treatment. Often, dental treatment may be delayed because the patient is very young, fearful and unwilling to cooperate, which can lead to neglecting of the disease and the need for radical therapy. It can be widely used in young children.

In our study, similarly to Holmes study, reduction of caries and remineralisation using ozone was observed (Holmes, 2003). It can be concluded that ozone therapy can be considered an effective means in treatment of initial enamel caries in permanent molars.

4. CONCLUSIONS

1. Intensity of caries in permanent teeth of ten-year-old children was low (according to WHO) at baseline of the study, namely, = 1.88, however, after two years it increased and could be considered as moderate (according to WHO) with = 3.71. Oral hygiene index Greene – Vermillion and periodontal health at baseline was moderate (according to WHO), after two years it Greene – Vermillion index decreased to 1.55 ($p<0.05$), however it was still considered as moderate (according to WHO). Periodontal health improved.
2. Ozone, fluoride varnish and sealants are equal means for caries prevention in premolars, as incidence of caries development in the ozone, the fluoride varnish and the sealants groups showed no statistically significant differences after 24 months.
3. Ozone therapy can be considered as effective means for treatment of initial enamel caries in permanent molars as the mean value of *DIAGNOdent*, after 24 months was 1.4 times lower in molars of the ozone group than in the control group ($p=0.03$).

5. PRACTICAL RECOMMENDATIONS

Ozone can be recommended means for caries prevention and treatment of initial enamel caries. Early treatment of initial caries, rather than long-term monitoring should be promoted in order to prevent caries progression. For this purpose, we would recommend the following:

- To organize courses and practical trainings for practitioners on ozone therapy.
- To introduce the latest caries prevention and treatment methods in training of dentistry students.
- To introduce the latest caries prevention and treatment methods in training of hygienists.

We recommend promotion of potentialities of early caries diagnostics with *DIAGNOdent* device as follows:

- To arrange courses and practical trainings for medical practitioners on early caries diagnostics.
- To introduce the latest caries diagnostics methods in training of dentistry students.
- To introduce the latest caries diagnostics methods in training of hygienists.

6. SCIENTIFIC PUBLICATIONS AND REPORTS ON THE STUDY

Articles in internationally peer-reviewed scientific journals

1. Kalniņa J., Care R. Prevention of Occlusal Caries using a Ozone, Sealant and Fluoride Varnish in Children. Stomatologija. Baltic Dental and Maxillofacial Journal. 2016; 18(1): 26–31.

Articles in peer-reviewed scientific journals in Latvia

1. Kalniņa J., Care R., Rendeniece I. Ozona terapija kariesa ārstēšanā bērniem. RSU Zinātniskie raksti, 2012; 2: 151–156.
2. Kalniņa J., Care R., Brinkmane A. Mutes veselības stāvoklis Latvijas 10 gadus veciem bērniem. RSU Zinātniskie raksti 2013; 397–403.
3. Kalniņa J., Care R. Ozona lietošana okluzālā kariesa ārstēšanā: 18 mēnešu rezultāti. RSU Zinātniskie raksti 2014; 334–339.
4. Rendeniece I., Brinkmane A., Care R., Kalniņa J. Grūtnieču mutes dobuma stāvokļa veselības novērtējums, zināšanas par bērna zobu kopšanas un jaunākām ārstēšanas iespējām. RSU Zinātniskie raksti 2012; 2: 145–150.

Reports in international scientific conferences

- Presentation at the 4th Scientific conference of Baltic dentists with the poster report “Ozone therapy for the treatment of dental caries in children”, 9 April 2012, Tartu, Estonia
- Presentation at FDI 101st Annual World Dental Congress with the poster report “Oral health status 9–11 years old Latvian children”, 30 August 2013, Istanbul, Turkey.
- Presentation at the 12th Congress of European Academy of Paediatric Dentistry with the poster report “The influence of ozone, sealants and fluoride varnish on occlusal caries development in 12 months’ period”, 7 June 2014, Sopot, Poland.
- Presentation at the seminar of the 9th European Academy of Paediatric Academy (EAPD) with the poster report “Ozone treatment of pit and fissure caries: 24 month results”, 8 May 2015, Brussels, Belgium.
- Presentation at the 25th Congress of the International Association of Paediatric Dentistry with a poster report “The influence of ozone, sealants and fluoride varnish on occlusal caries development in 24 months period”, 2 July 2015. Glasgow, United Kingdom.
- Presentation in international courses “The cavity-free future – it is possible?” with a lecture “Fluorides and caries”, 26 April 2016, Tartu, Estonia.

Reports in scientific conferences in Latvia

- Presentation at the 6th Baltic preventive dentistry conference with an oral report "Savlaicīga mutes saslimšanu diagnostika un profilakse", 11 May 2013, Riga, Latvia.
- Presentation in the Conference of Association of Dental Hygienists "Darba aizsardzība. Ozonterapija" with lecture "Ozona pielietošana zobārstniecībā", 5 April 2014, Riga, Latvia.
- Presentation at Riga Stradins University 13th Scientific conference with a poster report "Ozona terapija okluzālā kariesa ārstēšanā: 12 mēnešu rezultāti", 10 April 2014, Riga, Latvia.
- Presentation at the 7th Baltic preventive dentistry conference with a poster report "Izglītošanas un motivācijas ietekme uz mutes dobuma veselību", 17 May 2014, Riga, Latvia.
- Presentation atthe conference organized by Dentistry Institute of RSU "Zobārstniecības izglītība, zinātne un prakse neatkarīgajā Latvijā (1994–2014)" with an oral report "Ozonēšana bērnu zobārstniecībā", 27 June 2014, Riga, Latvia.

Thesis in international scientific conferences

- Kalniņa J., Care R. Ozone therapy for the treatment of dental caries in children. Stomatologija, Baltic Dental and Maxillofacial Journal, 2012; 14(8): 30.
- Kalnina J., Care R., Brinkmane A., Gudkina J. The influence of ozone, sealants and fluoride varnish on occlusal caries development in 12 months' period. 12th Congress of European Academy of Paediatric Dentistry. 5–8 June 2014, Sopot, Poland. p. 64.
- Kalnina J., Care R. Ozone treatment of pit and fissure caries: 24 month results. 9th European Academy of Paediatric Dentistry seminar and workshop. 8 May 2015, Brussels, Belgium. p. 20
- Kalniņa J., Care R. The influence of ozone, sealants and fluoride varnish on occlusal caries development in 24 months period. Int Journal of Paediatric Dentistry, 2015; 25(1):92.

Thesis in scientific conferences in Latvia

- Kalniņa J., Care R., Brinkmane A. Mutes dobuma higiēnas stāvoklis Latvijas 9–11 gadus veciem bērniem. 6th Baltic preventive dentistry conference. 11 May 2013, Riga, Latvia.
- Kalniņa J., Care R. Ozona terapija okluzālā kariesa ārstēšanā: 12 mēnešu rezultāti. Riga Stradins University 13th Scientific conference. 10 April 2014, Riga, Latvia. P. 72.

7. BIBLIOGRAPHY

1. Abanto J., Celiberti P., Braga M.M., Vidigal E.A., Cordeschi T., Haddad A.E., Bönecker M. Effectiveness of a preventive program based on caries risk assessment and recall intervals on the incidence and regression of initial caries lesions in children. International Journal of Paediatric Dentistry. 2015; *25*(4): 291–299.
2. Almaz M.E., Sönmez I.S. Ozone therapy in the management and prevention of caries. Journal of the Formosan Medical Association. 2013; 1–9 http://dx.doi.org/10.1016/j.jfma.2013.06.020
3. Axelsson P., Nyström B., Lindhe J. The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults. Results after 30 years of maintenance. J Clin Periodontol. 2004; 31(9): 749–757.
4. Azarpazhooh A., Limeback H. The application of ozone in dentistry: a systematic review of literature. J Dentist. 2008; 36: 104–116.
5. Bader J.D., Shugars D.A. A systematic review of the performance of a laser fluorescence device for detecting caries. Journal of the American Dental Association. 2004; 135(10): 1413–1426.
6. Baysan A., Lynch E. The use of ozone in dentistry and medicine. Part 2. Ozone and root caries. Prim Dent Care, 2006; 13: 37–41.
7. Berzina S., Care R. Dental health in 11 and 13 year old children in Latvia. Stomatologija. Baltic Dental and Maxillofacial Journal 2003; 5(2): 62–64.
8. Bocci V.A., Borrelli E., Travagli V., Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. Med Res Rev. 2009; 29(4): 646–682.
9. Bravo M., Garcia-Anllo I., Baca P., Llodra J.C. A 48-month survival analysis comparing sealant (Delton) with fluoride varnish (Duraphat) in 6 to 8-year-old children. Community Dent Oral Epidemiol. 1997; 25: 247–50.
10. Bravo M., Montero J., Bravo J.J., Baca P., Llodra J.C. Sealant and fluoride varnish in caries: a randomized trial. J Dent Res. 2005; 84: 1138–1143.
11. Brukiene V., Aleksejuniene J. Theory-based oral health education in adolescents. Stomatologija. 2010; 12(1): 3–9.
12. Care R., Urtāne I. Kariesa epidemioloģija Latvijā 1990-1998. RSU Zinātniskie raksti medicīnā un farmācijā. 1999; 197–201.
13. Casamassimo P.S., Fields H.W., Dennis J., McTigue J., Nowak A. Pediatric Dentistry: Infancy through Adolescence. Elsevier. 2013; 413–415.
14. Castillo A., Galindo-Moreno P., Avila G., Valderrama M., Liebana J., Baca P. In vitro reduction of mutans streptococci by means of ozone gas application. Quintessence Int 2008; 39: 827–831.
15. Cho B.K., Kwon H.K., Kim K.S., Kim Y.N., et al. A two-year longitudinal study of dental caries in permanent first molars of Korean elementary school children. Journal of Public Health Dentistry. 2001; 61(2): 120–122.
16. Ciganoviča A., Care R. Bērnu zobu labošana vispārējā narkozē. Zobārstniecības raksti. 2012.
17. Dähnhardt J.E., Jaeggi T., Lussi A: Treating open carious lesions in anxious children with ozone. A prospective controlled clinical study. Am J Dent. 2006; 19 (5): 267–270.
18. Dean J.A., Hughes C.V., Avery D.R., McDonald R.E. Mechanical and chemotherapeutic home oral hygiene. "McDonald and Avery's dentistry for the

child and adolescent, 9th ed.” Maryland Heights (MO), Mosby Elsevier. 2011; p.205–222.
19. Duruturk L., Çiftçi A., Baharoğlu S., Öztuna D. Clinical Evaluation of DIAGNOdent in Detection of Occlusal Caries in Newly Erupted Noncavitated First Permanent Molars in Caries-Active Children. Operative Dentistry. 2011; 36(4): 348–355.
20. Fejerskov O., Kidd E. Dental Caries: The Disease and Its Clinical Management 2nd Edition. Blackwell Munksgaard. 2008; p. 20–48.
21. Gent J.F., Triche E.W., Holford T.R., Belanger K., Bracken M.B., Beckett W.S., Leaderer B.P. Association of low-level ozone and fine particles with respiratory symptoms in children with asthma. JAMA. 2003; 290(14): 1859–1867.
22. Gift H.C. Social factors in oral health promotion. In: Schou L., Blinkhorn A.S., editors. Oral health promotion. Oxford University Press; 1993. p. 65–102
23. Goyal A., Gauba K., Chawla H.S., Kaur M., Kapur A. Epidemiology of dental caries in Chandigarh school children and trends over the last 25 years. J Indian Soc Pedod Prev Dent. 2007; 25: 115–118.
24. Gordon C.I., Chadwick B.L., Hutchings S., Playle R., Pickles T., Lisles C., Kirkby N., Morgan Z.M., Hunter L., et. al. Protocol for “Seal or Varnish?” trial: a randomised controlled trial to measure the relative cost and effectiveness of pit and fissure sealants and fluoride varnish in preventing dental decay. BMC Oral Health. 2012; 12: 51.
25. Greene J.C., Vermillion J.R. The simplified oral hygiene index. J Am Dent Assoc. 1964; 68: 7–13.
26. Gudkina J., Brinkmane A. The impact of salivary mutans streptococci and sugar consumption on caries experience in 6-year olds and 12-year olds in Riga. Stomatologija 2010; 12(2): 56–59.
27. Hiiri A., Ahovuo-Saloranta A., Nordblad A., Mäkelä M. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in children and adolescents. Cochrane Database Syst Rev. 2006; 18(4): CD003067.
28. Holmes J. Clinical reversal of root caries using ozone, double-blind, randomised, controlled 18-month trial. Gerodontology. 2003; 20: 106–114.
29. http://www.mah.se/CAPP/Country-Oral-Health-Profiles/EURO/ Oral Health Database, Malmö University.
30. Johansson E., Claesson R., van Dijken J.W. Antibacterial effect of ozone on cariogenic bacterial species. J Dent. 2009; 37: 449–453.
31. http://www.vm.gov.lv/lv/nozare/terminu_vardnica/
32. Johansson E., Claesson R., van Dijken J.W. Antibacterial effect of ozone on cariogenic bacterial species. J Dent. 2009; 37: 449–453.
33. Johansson E., van Dijken J.W., Karlsson L., Andersson-Wenckert I. Treatment effect of ozone and fluoride varnish application on occlusal caries in primary molars: a 12-month study. Clin Oral Investig. 2014;18(7):1785–1792.
34. Marthaler T.M. Changes in dental caries 1953–2003. Caries research. 2004; 38(3): 173–181.
35. Nagayoshi M., Fukuizumi T., Kitamura C., Yano J., Terashita M., Nishihara T. Efficacy of ozone on survival and permeability of oral microorganisms. Oral Microbiology & Immunology. 2004a;19: 240–246.
36. Neuhaus K.W., Longbottom C., Ellwood R., Lussi A. Novel lesion detection aids. Monogr Oral Sci. 2009; 21: 52–62.
37. Nogales C.G., Ferrari P.H., Kantorovich E.O., Legw-Marques J. Ozone therapy in medicine and dentistry. J Contemp Dental Pract. 2008; 9: 1–9.

38. Ostberg A.L., Jarkman K., Lindblad U., Halling A. Adolescents' perceptions of oral health and influencing factors: a qualitative study. Acta Odontol Scand. 2002; 60:167–173.
39. Petersen P.E. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century–the approach of the WHO Global Oral Health Programme. Community Dentistry and oral epidemiology. 2003; 31:3–24.
40. Petersen P.E., Lennon M.A. Effective use of fluorides for the prevention of dental caries in the 21st century: the WHO approach. Community Dentistry and Oral Epidemiology. 2014; 32: 319–321.
41. Polydorou O., Halili A., Wittmer A., Pelz K., Hahn P. The antibacterial effect of gas ozone after 2 months of in vitro evaluation. Clin Oral Invest. 2012; 16: 545–550.
42. Polk D.E., Geng M., Levy S., Koerber A., Flay B.R. Frequency of daily tooth brushing: predictors of change in 9- to 11-year old US children. Community dental health. 2014; 31(3): 136–140.
43. Poulsen S. Fluoride containing gels, mouthrinses and varnishes. Eur Arch Paediatr Dent. 2009; 10(3): 157–161.
44. Reich E. Trends in caries and periodontal health epidemiology in Europe. International dental journal. 2001; 51(6): 392–398.
45. Rickard G.D., Richardson R., Johnson T., McColl D., Hooper L. Ozone therapy for the treatment of dental caries. Cochrane Database of Systematic Reviews. 2004; 3: CD004153.
46. Shi X.Q., Tranaeus S., Angmar-Mansson B. Validation of DIAGNOdent for quantification of smooth-surface caries: an in vitro study. Acta Odontologica Scandinavica. 2001; 59(2): 74–78.
47. Tyas M.J., Anusavice K.J., Frencken J.E., Mount G.J. Minimal intervention dentistry - a review. FDI Commission Project 1-97. Int Dent J. 2000; 50: 1–12.
48. Touger-Decker R., van Loveren C. Sugars and dental caries. Am J Clin Nutr. 2003; 78(4): 881–892.
49. Van Loveren C. Diet and dental caries: cariogenicity may depend more on oral hygiene using fluorides than on diet or type of carbohydrates. Eur J Pediatr Dent. 2000; 1: 55–62.
50. Walsh T., Worthington H.V., Glenny A.M., Appelbe P., Marinho V.C.C., Shi X. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents (Review). Cochrane Database Syst Rev. 2010; 20(1): CD007868.