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Rīga Stradiņš University

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Evaluation of Signs of Skin Aging in Patients with Metabolic Syndrome after Substitution Therapy with Antioxidants

Summary of the Doctoral Thesis
for obtaining a doctoral degree (*Ph.D.*)

Sector – Clinical Medicine
Sub-sector – Internal Medicine

Riga, 2021

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Abbreviations

AD	atopic dermatitis
AGEs	advanced glycation end products
AhR	aryl-hydrocarbon receptors
AH	arterial hypertension
AN	acanthosis nigricans
AO	antioxidant
AU	attributed units
BCC	basal cell carcinoma
BG	Birbeck granules
BMI	body mass index
CVD	cardiovascular diseases
COX-1	cyclooxygenase 1
COX-2	cyclooxygenase 2
CRP	C-reactive protein
DNA	deoxyribonucleic acid
DM	diabetes mellitus
ECM	extracellular matrix degradation
ERC	endosomal recycling compartment
FOXO	transcription factor
FR	free radicals
Glu	glucose
HDL	high-density lipoprotein
Chol	cholesterol
ICD	International classification of disease
IDF	International Diabetes Federation
IGF	interstitial growth factor

IL-6	interleukin-6
IR	insulin resistance
LDL	low-density lipoprotein
LHš	Langerhans cells
LP	lipid peroxidation
LTB	leukotriene B
MACE	major cardiac event
MetS	metabolic syndrome
MMP	matrix metalloproteinase
MMP-1	metalloproteinase-1
NMF	natural moisturising factors
OS	oxidative stress
P53	tumour protein p53
PM	particulate matter
ROS	reactive oxygen species
SAI	skin aging index
SCC	squamous cell carcinoma
Se	selenium
SIRT1	sirtuin 1
SIS	skin immune system
SPF	sun protective filters
TEA	telangiectasia
TG	triglycerides
TNF-beta	tumour necrosis factor-beta
TOR	target of rapamycin
T2DM	type 2 diabetes mellitus
UV	ultraviolet
UVI	ultraviolet index

UVA	ultraviolet radiation A
UVB	ultraviolet radiation B
UVC	ultraviolet radiation C
WHO	World Health Organisation
AI	apoptotic index

Preface

Skin aging is a complex biological phenomenon consisting of two overlapping components: internal (true aging) and external. Internal aging is largely genetically determined and is an inevitable change that can only be attributed to time. It is reminiscent of aging which occurs in most internal organs, and its underlying mechanisms are likely to be associated with reduced proliferative capacity, leading to cell aging and changes in skin cell biosynthetic activity. In the case of MetS, one of the pathogenetic mechanisms is oxidative stress (OS), which maintains chronic latent inflammation in the body and also in the skin. OS, which is believed to play a central pathogenic role in the development of MetS, is a state of prooxidant/antioxidant imbalance in which the net amount of Reactive Oxygen Species (ROS) exceeds the body's antioxidant defenses. Excess ROS can cause lipid, protein oxidation, and oxidative damage to DNA. One of the internal factors in accelerating skin aging is overweight, central obesity, and metabolic syndrome (MetS), which is now defined as a separate nosology.

MetS is a syndrome, of which main symptoms are obesity, insulin resistance (IR), dyslipidemia, and hypertension or high blood pressure. About a third of the adult population in developing countries is currently classified as having MetS according to various criteria. (Moore et al., 2017). Antioxidants (AO) or substances that prevent oxidation of other molecules are nutritional factors associated with MetS (Gregorio, 2016). Intake of Se and other AOs is associated with lower C-reactive protein (CRP) in women, indicating a possible anti-inflammatory effect of dietary AO. The above is also relevant in Latvia but has not been studied. There are patients with diabetes, light skin phototype, MetS, cardiovascular diseases in Latvia, but visual changes do not provide entire scope of the aging processes in the skin.

Aim of the Thesis

The aim of the Thesis was to study the signs of skin photoaging and evaluate the effectiveness of AO therapy in patients with MetS in the Latvian population.

Objectives of the Thesis

The following objectives have been put forward to reach the aim of the Thesis:

1. Evaluate MetS symptoms and skin parameters in MetS patients and control groups.
2. Identify and describe signs of skin aging in the group of MetS patients (visually, as well as using dermatoscopic and histological examination).
3. Evaluate skin changes after organic selenium substitution.
4. Evaluate interrelationships between MetS symptoms, OS parameters, and skin changes in MetS patients and control groups.

Hypothesis of the Thesis

AO substitution improves skin aging parameters in MetS patients.

Novelty of the Thesis

1. For the first time in Latvia, signs of skin aging and OS parameters in patients with MetS in the Latvian population will be described.

2. For the first time, histological features of skin structure in MetS patients and patients without MetS in the Latvian population will be thoroughly studied, compared, and described in regard to the AO therapy.
3. For the first time, functional condition of the skin will be determined and tested using dermatological equipment, evaluating the skin's microrelief, signs of photoaging, and facial skin capillaries in patients with and without MetS.

1 Materials and Methods

The study was conducted among the residents of Latvia, who came to Pauls Stradins CUH and prof. Janis Kisis' private practice. The prospective study was performed on 196 patients of both sexes. The study examined a total of 165 MetS patients as well as 31 practically healthy patients (control group). Patients were divided into four groups:

Group 1 – patients with MetS who use selenium as a substitution;

Group 2 – patients without MetS receiving selenium substitution;

Group 3 – patients without MetS who do not receive any substitution (control group);

Group 4 – patients with MetS who do not use selenium substitution.

Table 1.1

Criteria for inclusion and exclusion of patients

Patient inclusion criteria	Patient exclusion criteria
Patients between January 1 and December 31 of 2012	Patients between January 1 and December 31 of 2012
Patients with metabolic syndrome (IDF 2006)	Patients with skin phototype III–IV
I–II Fitzpatrick skin classification phototype (8)	Patients under 40 and over 55 years of age
Patients aged 40–55 years	Patients with a history of facial aesthetic surgery, peeling, dermabrasion
No history of aesthetic surgery	Patients who sunbathe more than 20 times a year
Use a sunscreen (SPF) during sunbathing	Patients who do not use sunscreen
Do not attend solariums	Patients attending solariums
No chronic diseases (oncological diseases, autoimmune diseases, psoriasis, fungus, onychomycosis)	Presence of chronic disease (oncological diseases, autoimmune diseases, psoriasis, fungus, onychomycosis)
Do not use glucocorticoids	Patients with glucocorticoid therapy, oral contraceptive therapy

Table 1.1 continued

Patient inclusion criteria	Patient exclusion criteria
Patients do not work in places exposed to long-term UV radiation (construction, building, road repairs, athletes).	Work involving long-term UV exposure to the skin.
Patients with a moderate degree of insolation (moderate sun exposure, patients spend the whole season in central or northern Europe).	Hypersensitivity to selenium or its components.

Patients in the control group:

1. Patients without MetS
2. Age group 40–55 years
3. Practically healthy people (no chronic diseases, oncological diseases)
4. Fitzpatrick classification I–II skin phototype (Table 3)
5. No history of cosmetic surgery
6. Use sunscreen (SPF) filters during tanning
7. Do not visit solariums
8. Patients do not take glucocorticoids
9. Patients with a moderate degree of insolation (moderate sun exposure, patients spend entire season in central or northern Europe)

Table 1.2

Research variables

Dependent variables	Independent variables
SAI	Body mass index
Dyslipidemia	SOD
Arterial hypertension	-
Metabolic syndrome	-
Central-type obesity	-
Insulin resistance	-
UV overexposure	-

Table 1.2 continued

Dependent variables	Independent variables
Photoaging	-
Antioxidant deficiency	-
High risk of pre-malignant skin conditions (actinic keratoses)	-

The patients in the study used selenium (*SelenoPRECISE, PharmaNord*, Denmark) at 200 μgrams per day for 3 months; serum selenium levels were assessed prior to the antioxidant administration. *SelenoPRECISE* was received from the Danish company *PharmaNord* in the form of humanitarian aid; it contains high-quality selenium and was registered as a food supplement at the Latvian Food Center (*Opinion No. 141*).

For 56 patients, a water-based selenium-containing composition (cream) was applied topically to the facial skin.

The research was performed with the permission and examination protocol of the Ethics Committee of Rīga Stradiņš University, the consent protocol, and the participation questionnaire comply with the European Directive "Good Clinical Practice" and the Declaration of Helsinki. There is no conflict of interest.

1.1 Methods of obtaining primary data or sources of secondary data

Primary patient data were analysed during the study; clinical instrumental examinations were performed: a physical examination of the patient with determination of anthropometric parameters. Body mass index was determined according to the formula: body weight (kg)/height (m^2). Abdominal circumference was measured to determine visceral type obesity (using a meter):

women > 88 cm, men > 102 cm. Blood pressure was measured by the Korotkoff method.

The following parameters were determined: MetS serum blood component parameters (fasting glucose, LDL, HDL, total cholesterol, triglycerides), OS parameters (selenium, SOD, MDH, GPx), vitamin D and selenium levels. The following parameters were also evaluated from blood biochemical parameters: P, IL-6, and TNF-beta as markers of inflammation.

For laboratory analysis, all study participants had blood taken from the vein in the morning, and it was collected in a vacutainer using *LiHep* as an anticoagulant. Chemically pure reagents "Merck analytical grade" were used in the study.

Biochemical analyses were performed in **E. Gulbis laboratory**.

OS parameters (selenium, SOD, MDA, GPx) were determined in the RSU Biochemistry Laboratory.

The principle for determination of **malondialdehyde (MDA)** is to determine MDA alone (in hydrochloric acid) or MDA in combination with HNE (in the presence of methanesulphonic acid). Two molecules, 4-HNE and MDA, react with N-methyl-2-phenylindole at 45 °C to form a stable chromophore with an absorption peak at 586 nm (Esterbauer, 1990; Janero, 1990).

Determination of **Cu, Zn-Superoxide dismutase is performed** by automated spectrophotometry according to the manufacturer's instructions for the *RX Daytona* analyser (*Randox laboratories*, Ltd.). Heparinised blood is centrifuged at 3000 rpm for 10 minutes and the supernatant is aspirated. Erythrocytes (Er) are then washed three times with the isotonic solution, centrifuging at 3000 rpm for 10 minutes each time. The washed erythrocytes (0.2 ml) are lysed to 2.0 ml with cold redistilled water, mixed and kept for 15 minutes at a temperature of + 4 °C. The lysate is diluted with 0.01 mol/L

phosphate buffer pH 7.0 so that the percent of inhibition is between 30 % and 60 % (lysate dilution for human samples – 25) (Woolliams et al., 1983).

The fluorimetric method for **selenium** is based on the reduction of selenium to tetravalent compounds that form complexes with 2.3-diaminonaphthalene which are then read at an excitation wavelength of 369 nm and an emission wavelength of 518 nm with a maximum in the range of 518–515 nm (Alfthan, 1984).

Glutathione peroxidase is detected automatically by spectrophotometry according to the manufacturer's instructions for the *RX Daytona* analyser (*Randox laboratories*, Ltd., Crumlin, UK). Glutathione peroxidase catalyses oxidation of glutathione (GSH) in the presence of cumene hydroperoxide. Oxidised glutathione (GSSG) is further converted to a reduced form, GSH, by glutathione reductase (GR) and NADPH, while NADPH is oxidised to NADP+. Glutathione peroxidase activity corresponds to a decrease in absorbance at 340 nm caused by NADP oxidation. One unit corresponds to the amount of enzyme produced by the oxidation of 1.0 μM NADPH to NADP+ per minute at 340 nm at 37 °C.

The structure and condition of the skin were evaluated at **various stages,** starting with visual inspection using the skin aging index (SAI) and continuing with dermatological professional equipment (*Dermlite* DL3), where peculiarities of premature skin aging were identified and the skin aging index was evaluated.

Basic elements of clinical skin diagnostics

Visual examination of the skin, during which the dermatological status of the skin is assessed, its general condition (colour, turgor, peeling), as well as the basic problems (pore enlargement, microrelief roughness, wrinkles) are evaluated. It is important to note that healthy skin manifestations are smooth skin, no hypo- and/or hyperpigmentation, good tone (skin turgor), no wrinkles, scars

or other skin defects (skin has a good texture), no dryness of the skin (good degree of skin hydration), there are no clinical manifestations of skin diseases, the skin has good immunity to environmental factors and infections.

Examination of the skin with a dermatoscope using the skin aging index (SAI). Dermatoscopy is a highly informative non-invasive skin examination method used in visual clinical examination of the skin. Dermatoscopy allows evaluating the relief and morphology of the skin. During it, the skin turgor in the décolleté and cheek area was evaluated, the degree of skin peeling and dryness was evaluated, the amount of skin wrinkles and their properties were determined: number, width, depth, as well as relief. Pigmentation disorders assessed: lentigo-type pigmentation and/or dyschromia (light/dark spots).

Patients had their AGE levels in the skin determined using an autofluorescence device (*AGE Reader, Diagnoptics Technologies B.V.*, The Netherlands). It is a non-invasive monitoring device that uses UV light to promote autofluorescence in human skin tissues. Autofluorescence depends on the amount of AGEs. From the obtained value the degree of cardiovascular risk is expressed (Stirbann, Heinemann, 2014):

1. up to 2.5 AU (inclusive) – no CVD risk;
2. 2.6–2.8 AU – medium degree of CVD risk;
3. 2.9 AU and more – high degree of CVD risk.

Skin biopsy material (punch biopsy) was used for structural and morphological evaluation of the skin, and light microscopy was used for histological evaluation of the skin preparation. Biopsy instrument: trepan-biopsy.

During the study, the patients underwent a skin biopsy under 5 % lidocaine local anesthesia. Analyses were performed with the aim of further immunohistochemical examination; collagen, fibrin, and elastin were evaluated as markers of skin aging and in turn CD3, CD68, CD20, CD34, macrophages –

as markers of inflammation. Biopsy was performed with a *DERMO-PUNCH* instrument (Audin, 2001; Balaban et al., 2005). The patient was administered local anesthesia with a 5 % solution of lidocaine. The *DERMO-PUNCH* tool was immersed into the skin to a depth of 4 mm with rotating movements. The skin material was then cut with a scalpel. The duration of the procedure was 3–8 minutes. For histological evaluation of the skin, the biopsy material was fixed in a 10 % formalin solution and poured with paraffin. Paraffin plates of 7 μm thickness were stained with hematoxylin-eosin and picrofuccin according to the Van-Gizon method. The skin preparation was then evaluated under a light microscope at a magnification of 400 times.

Table 1.3

Research examinations

<table>
<tr><th colspan="2">Laboratory tests</th></tr>
<tr><td rowspan="3">Blood</td><td>Total cholesterol in plasma
• HDL
• LDL
• Glucose level in plasma
• Triglycerides</td></tr>
<tr><td>25(OH) D level in serum</td></tr>
<tr><td>Oxidative stress parameters
1. total AO capacity
2. MDA
2. SOD
3. Selenium level
4. 5. GPx</td></tr>
<tr><td></td><td>CRP- proinflammatory marker</td></tr>
<tr><td colspan="2">Skin instrumental diagnostics (Dermatoscopy, Aramo SG equipment)</td></tr>
<tr><td colspan="2">• Visual diagnostics of the skin
• Dermatoscopic examination of the skin
• Skin examination with professional equipment, sebumetry, hydration (Derma-Scope, AGE-reader)
• Skin biopsy (punch-biopsy), immunohistochemical, electron microscopic examination</td></tr>
</table>

Table 1.3 continued

Laboratory tests		
Immunohistochemical examination	Epidermis, dermis Collagen Fibrine Elastine CD3 CD68	• CD34 • CD 31 • Macrophages • Types of collagen • Condition of vascular capillaries, nerve fibers
Objective determination of patient skin parameters before and after treatment with AO using Derma-Scope system	1. Determination of the degree of hydration of the skin 2. Skin sebumetry 3. Determination of skin elasticity, wrinkle size, depth of wrinkles 4. Determination of pigmentation 5. Determination of skin aging index (using SAI)	
AGEreader	Determination of glycation end products using skin fluorescence	

1.2 List of methods for statistical analysis of data and grounds for choice

The obtained data were entered into a *Microsoft Excel* table, then statistically processed and analysed in *SPSS* programme with a 22.0 programme version. In accordance with the general principles, a value of p = 0.05 was considered as the statistical reliability threshold for bilateral test results. The Kolmogorov Smirnov test was used in the course of the work. Quantitative variables for normally distributed data were described by the arithmetic mean and standard deviation; for data not normally disaggregated – by median and quartiles. Categorical or quantitative variables were characterised by a percentage. The relationship of all variables was analysed using Spearman correlation.

1.3 Research project cooperation partners

1. *Pharma Nord ApS*: Sadelmagervej 30–32, DK-7100, Vejle, Denmark. In the form of humanitarian aid. Phone: (+45) 75 85 74 00; Fax: (+45) 75 85 74 74.
2. RAKUS Pathology center, Professor Regina Kleina.
3. RSU Biochemistry laboratory.
4. ERDF Project "Development of a new dermo-cosmetic product for patients with metabolic syndrome to restore skin barrier function"; agreement No. 2014/0024/2DP/2.1.1.1.0/14/APIA/VIAA/076
5. E. Gulbis laboratory, Riga, Latvia, in the form of humanitarian aid (contract No. PEM-13-2013)

2 Literature review

2.1 Skin structure

The skin is the outer tissue of the body, with an area of 1.5–2 m². The sebaceous glands produce sebum, a skin barrier that consists of fatty acids and cleanses the body of excess cholesterol (Zhou et al., 2012).

The dermis is the connective tissue of the skin. The thickness of the dermis is 0.5–5 mm. It distinguishes between a wart layer and a mesh layer. Skin biological barrier and lipid layer.

On a daily basis, the skin is irritated by exogenous and endogenous factors. Endogenous factors are dietary components, synthetic vitamins, metabolic breakdown products (Table 2.1).

Table 2.1

Factors influencing the biological barrier of the skin

Exogenic and endogenic factors	Lifestyle
Decreased humidity	Diet
Psychoemotional stress	Drugs
Dry skin	Obesity
High temperature (equatorial)	Alcohol and smoking
UV radiation	Skin maceration

The skin needs to produce about 100–150 mg of lipids a day. Lipids in complex with keratinocytes form a skin lipid barrier.

If a person has a damaged skin barrier, the loss of water (transepidermal water loss) is intensified and clinically the skin becomes dry, rough, itchy, and even cracks may form.

Keratinocytes produce natural moisturizing factor (DMF), which plays an important role in skin hydration and keratinocyte differentiation. In the case of DMF deficiency, the elasticity of the skin changes and its hydration decreases.

2.1.1 Skin barrier and filaggrin

Dry skin or xerosis is a common skin condition that manifests as skin roughness, peeling, itching and redness. Factors causing and provoking dry skin are presented in table 2.2.

Table 2.2

Skin dryness risk factors

Determinant factors	Trigger factors
Individual characteristics	Inherited predisposition
Provoking factors	Age
Diseases	Atopic dermatitis, psoriasis, hypothyroidism
Trigger	Temperature, humidity, sun UV light, conditioners, heating
Chemical substances	Soaps, bath soaps, perfumes, detergents
Physical irritants	Rub, radiation, abrasion

Filaggrin binds to keratin filaments in epithelial cells (profile). Upon hydroxylation of filaggrin, amino acids are formed, which help to retain water in the skin and are an important factor in the adequate skin barrier (Fig. 2.1). Individuals with a mutation in the filaggrin gene may develop dry skin, atopic dermatitis, ichthyosis, eczema (A Pons-Guiraud et al., 2007).

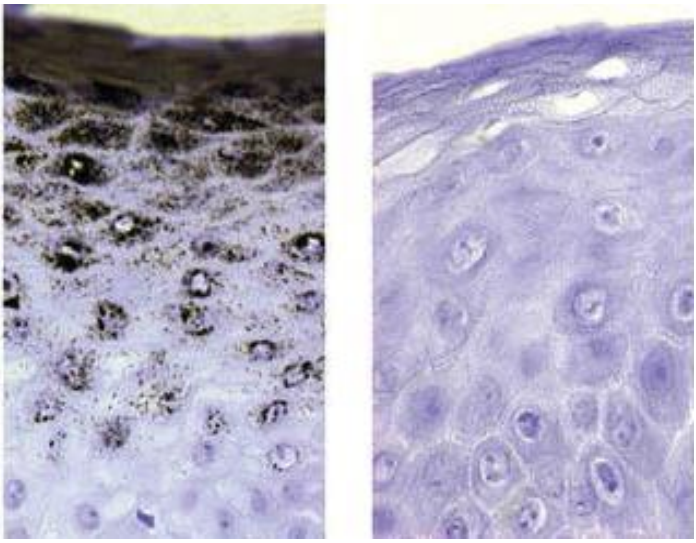

Figure. 2.1 **Filaggrin granules in a patient with normal skin and granule deficiency** *(Ring, 2016)*

Skin aging can be divided into the following main types: one, which is regulated by genetics (physiological skin aging) and the other, which is influenced by external environmental factors – the sun, smoking, excessive alcohol consumption. When telomeres reach a critical size, the cell cycle stops or the mechanism of apoptosis is activated (Toussaint, 1994).

2.1.2 Skin physiologic and pathologic aging pathways

Physiological skin aging is a natural aging process that begins at the age of 20. The most common pathological cause is solar ultraviolet radiation (Shigenaga, Hagen and Ames, 1994).

2.2 UV radiation and skin aging as a result

UV radiation is that part of the sun's electromagnetic radiation in which the wavelength is between 100 and 400 nm (one nanometer is between 10 and 9 meters). It is divided into three categories (Table 2.3).

Table 2.3

Wavelength distribution of ultraviolet radiation

Wavelength	UV radiation
315–400 nm	UVA
280–314 nm	UVB
100–280 nm	UVC

As the sun's rays pass through the atmosphere, all the UVC and 99 % of the UVB radiation is absorbed by the ozone layer, water vapor, oxygen, and carbon dioxide in the atmosphere. As a result, UVA radiation and a small part of UVB radiation reach the Earth (Berneburg et al., 2000). The elastic fibers of the dernal layer of the skin stick together and become amorphous, which clinically manifests itself as yellowish, thick skin – *actinic elastosis*. The skin is drier because it has a harder time retaining water (Stoebner et al., 2008). The synthesis of type 1 procollagen in human fibroblasts in photoblotted *skin in vivo* and in fibroblasts from photobonded skin cultured *in vitro* has been determined (Stoebner et al., 2008).

2.3 Skin immune system: its function

The cell populations that interact with each other to form the basis of the skin immune system (AI) are: keratinocytes, dendritic antigen-presenting cells, monocytes, macrophages, granulocytes, mast cells, lymphoid, endothelial cells, and T lymphocytes.

2.3.1 The concept of a three-stage response of the skin immune system

The primary AIS response begins with the response of the acquired immune system to the invaded antigen. Active dendritic, Langerhans cells bind Ag to cell surface receptors and travel to peripheral lymph nodes, where they are presented to native T lymphocytes.

The secondary AI response is mediated by a rapid and effective local acquired immune system.

The tertiary AIS response is characterized by central memory. Central T cells recirculate throughout the body through the lymph nodes and enhance immune responses to previously encountered Ag in various tissues.

2.3.2 Langerhans cells

There is evidence that various immune response cells, including Langergan cells, change during the aging process (LHš; Cumberbath, Fielding and Kimber, 1994; Mahbub, Brubaker, & Kovacs, 2011).

They play a role in the pathogenesis of various dermatoses and participate in the neoplastic transformation of the skin. The number and appearance of LHs varies depending on the type of dermatosis and also in the aging processes of the skin (Lang, Mitchell and Lapenna, 2010).

2.3.3 Birbeck granules

Birbeck granules (BG) have an unusual rod-shaped structure characteristic of Langerhans cells in the epidermis. Although their existence was first described in LHs more than 50 years ago, the origin and functions of BG are still difficult to determine.

Like other mannose-binding type C lectins found on immune cells, langerin is thought to bind to microorganisms with mannose-containing surface glycoconjugates, including mycobacteria and fungi, although only association with Candida albicans has been demonstrated to date (Takahara et al., 2004).

2.4 Oxidative stress and its impact on skin: antioxidants and selenium

OS is a condition of the body in which there is an imbalance between AO and prooxidants because the net amount of BR exceeds the body's antioxidant capacity. Excessive BR levels in tissues lead to lipid peroxidation, protein oxidation, and direct DNA damage (Zhou et al., 2011; Grattagliano et al., 2008). ROS in the skin are formed as a result of several different processes, of which the exogenous stress caused by UV radiation is the main cause of ROS in the skin exposed to sunlight. Other important contributing factors are plasma membrane proteins, such as increasing levels of NADPH oxidases. In addition, H2O2, which is formed as a by-product of fatty acid degradation in peroxisomes, should be considered as an endogenous source of ROS. This can also be attributed to the formation of ROS during the oxidative explosion of phagocytes in inflammatory reactions, as well as to the activity of various cytosolic enzymes such as cyclooxygenases. Although all of these sources contribute to the overall oxidative burden of the cell, most of the cell ROS (approximately 90 %), which is formed in cells regardless of UV stress, is formed in mitochondria as by-products of mitochondrial respiration. Mitochondrial oxidants are a major source of oxidative damage that increases with age.

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ROS in the skin are formed as a result of several different processes, of which the exogenous stress caused by UV radiation is the main cause of ROS in the skin exposed to sunlight. Other important contributing factors are plasma membrane proteins, such as increasing levels of NADPH oxidases. In addition, H2O2, which is formed as a by-product of fatty acid degradation in peroxisomes, should be considered as an endogenous source of ROS. This can also be attributed to the formation of ROS during the oxidative explosion of phagocytes in inflammatory reactions, as well as to the activity of various cytosolic enzymes such as cyclooxygenases. Although all these sources contribute to the role of cellular total AO in patients with premature skin photoaging and cancer development is highly relevant. Data from the United Kingdom on the prevalence of melanoma (a type of skin cancer) (5,800 new cases each year and 28 fatalities out of 100 melanomas) raise serious concerns about the negative effects of UV radiation on the skin. The study mentions the important role of AO selenium in oncogenic transformation, but clearly the role of selenium was described in 2013, which confirmed that selenium is a necessary and powerful component of AO and skin melanoma prevention (Cassidy and Fain, 2013). Studies confirming the hypothesis that low selenium levels are associated with MetS (Arnaud et al., 2012; Zulet et al., 2009; Puchau et al., 2009).

Selenium is a component of the daily diet with strong antioxidant properties, which in daily products are mainly in beef, lamb, poultry, seafood (cod, herring, sardines), milk, onions, eggs, beans, whole grains. It is recommended to take approximately 55–60 micrograms per day. After the food and nutrition board (FDA). Selenium is a trace element that is very important in human metabolism. New research has shown the importance of this element in human health. (Rayman, 2000). It has both antioxidant and anti-inflammatory properties due to selenium-dependent glutathione peroxidase, which can reduce hydrogen peroxide, lipid, and phospholipid hydroperoxides and reactive oxygen

radicals. This trace element may play a protective role against cardiovascular disease, as glutathione peroxidase is able to reduce the oxidative modification of lipids and reduce platelet aggregation. Because selenium is usually found in significant amounts in immune tissue and has a major effect on the immune system, selenium deficiency is accompanied by a loss of immune competence (Brenneisen, Steinbrenner and Sies, 2005).

Low levels of selenium in the diet increase the body's susceptibility to diseases associated with OS. Several *in vitro* animal models and human studies have shown an inverse relationship between selenium intake and cancer risk (Bera et al., 2013). The beneficial effects of selenium are due not only to selenoproteins, which play a very important role in antioxidant protection and preservation of the cell-reducing environment, but also to an increase in some DNA glycosylase activities involved in repairing oxidative DNA damage and DNA repair pathways mediated by p53, BRCA1. and Gadd45. Selenium and selenoproteins are essential for keratinocyte function and skin development. Lack of selenoenzymes in the mouse epidermis causes skin and hair follicle abnormalities, premature skin aging, and even premature death (Sengupta, Lichti and Carlson, 2010).

2.5 Metabolic syndrome

2.5.1 Skin, metabolic syndrome and xenobiotics

MetS affects the skin in different ways, the most widely accepted theory of the pathophysiology of MetS is insulin resistance. MetS is characterized by visceral obesity, insulin resistance, dyslipidemia and hypertension.

AO, or substances that prevent the oxidation of other molecules, are considered nutritional factors associated with MetS (Gregorio et al., 2016). It has been found that the use of AO reduces the OS and inflammatory response that play a role in the development of MetS (Soory, 2012).

MetS affects the skin in different ways, the most widely accepted theory of the pathophysiology of MetS is insulin resistance. MetS is characterized by visceral obesity, insulin resistance, dyslipidemia and hypertension.

AO, or substances that block other molecules Muscle, fat and liver cells do not respond properly to the hormone insulin and are no longer able to easily absorb glucose from the bloodstream. Pancreatic beta cells initially try to produce more insulin to achieve euglycemia. Over time, the pancreas is unable to keep up with the growing demand for insulin and excess glucose accumulates in the bloodstream (Leroith, 2012). Any pathophysiological dysfunction that results in a loss of metabolic control in the body can lead to skin disease. Fat accumulation in MetS with progressive development of insulin resistance causes a cascade of hormonal changes, such as effects on growth hormone. Hormones synergistically follow the principle of autoregulation. As a result, androgen-dependent skin conditions such as acne or androgenic alopecia are expected to worsen.

IR appears several years before the diagnosis of diabetes. Hyperinsulinemia activates insulin growth factor-1 (IGF-1) receptors on cutaneous fibroblasts and keratinocytes, directly and indirectly stimulating their proliferation. IR-induced skin changes appear very early, so recognizing IR skin changes is a safe and easy way to detect insulin resistance.

Skin changes that develop due to IR allow rapid, reliable, simple, and safe detection of insulin resistance (González-Saldivar, 2017). An interesting aspect of skin function is being studied, which is related to its ability to remove xenobiotics from the body, get rid of BR with the help of AO and even reduce

cholesterol levels through the excretory function performed by the sweat and sebaceous glands.

Basically, ROS or reactive oxygen species are xenobiotic degradation products, metabolites. Xenobiotics include medicines, cosmetics, chemicals, artifacts, dietary components. Xenobiotics are metabolized in the body in 2 phases:

1. Cytochrome P450s via monoxygenase.
2. Hydroxylated molecules are conjugated to the hydrophilic substance glutathione, glucuronic acid or because of a methylation process.

The skin, as the largest human organ, participates in the elimination and degradation of xenobiotics, which involves the methylation of endogenous substrates, competing for free labile methyl groups. In lipid metabolism, there is an increased need for methyl groups in the body, because of which liver steatosis develops with subsequent dyslipidemia, metabolic syndrome may be an additional influencing factor.

An imbalance between ROS production and the body's overall antioxidant defense system in MetS may be a combination of excessive xenobiotic exposure (including high-concentration dietary supplementation with synthetic vitamins) and reduced detoxification / elimination of xenobiotic agents due to lifestyle and genetic factors.

The antioxidant and excretory function of the skin can be one of the main components of the body's antioxidant defense system and plays an important role in protecting against MetS.

Specifically, the skin expresses all enzymes of xenobiotic excretory systems, such as cytochrome P450 enzymes, flavin-dependent monoxygenase, monoamine oxidase, alcohol dehydrogenase, and aldehyde dehydrogenase. In addition, the skin is "equipped" with drug metabolism systems and antioxidant enzymes. Sweat has been found to contain large amounts of toxic substances.

In addition, sebum, which is a protective barrier of the skin and consists of fatty acids, cleanses the body of excess cholesterol (Zhou S. S et al., 2012). The potential role of sebum production in lipid and cholesterol homeostasis, its effects on MetS, and its association with several skin diseases (*acanthosis nigricans*, *acne*, burns) have been extensively discussed (Zhou S. S et al., 2012).

Metabolic syndrome (MetS) is characterized by visceral obesity, insulin resistance, dyslipidemia, and hypertension. MetS is a major cause of the prevalence of modern type 2 diabetes and cardiovascular disease (PANS, Esposito, 2012).

AO, or substances that prevent the oxidation of other molecules, are considered nutritional factors associated with MetS (Gregorio et al., 2016). The use of AO has been found to reduce the OS and inflammatory response that play a role in the development of MetS (Soory, 2012).

2.5.2 Skin diseases most associated with metabolic syndrome

Some inflammatory skin diseases such as vitiligo, scleroderma, recurrent aphthous stomatitis, Behcet's disease, rosacea, lipoid necrobiosis, ring granuloma, fibroepithelial polyps, holoderma and eruptive xanthomas may be associated with MetS (Seremet and Gurel, 2018). According to *Diris et al., 2003*, the most common non-inflammatory skin diseases observed as metabolic disorders are cutaneous xerosis, diabetic dermopathy, facial erythrosis, xanthromy, pseudoscleroderma, *acanthosis nigricans*.

2.5.3 *Acanthosis nigricans*

Acanthosis nigricans is a skin dermatosis characterized by thickened skin, hyperpigmentation of the neck and neck area, as well as in wet and sweaty areas (inguinal region, armpits).

The pathogenesis of *acanthosis nigricans* is based on insulin receptor dysfunction or autoantibodies to insulin receptors. In MetS, increased growth hormone expression is observed, leading to increased proliferation of keratinocytes and fibroblasts (PANS, Kluczynik, 2012).

MetS negatively affects the skin, impairing its basic functions (Akase et al., 2012). Cells with molecular aging properties accumulate in the skin, the amount of these cells can reach up to 15 % of the total number of cells.

2.6 Skin aging index **Skin changes due to free radicals**

One of the most productive theories of recent years is the free radical aging theory, proposed almost simultaneously by Harman (1956; Harman, 1994) and Emanuel (1958; Tabas et al., 2007). These changes include partially swollen mitochondria with unusual and rare crystals, heterogeneity or absence of crystal size and shape, and almost complete absence of branched mitochondria (Brantova et al., 2006). The characteristic morphological features of mitochondria in aged cells can also be induced in mitochondria from otherwise normal cultured primary skin fibroblasts by inhibiting energy metabolism with anti-respiratory drugs (Guillery et al., 2008).

It is known that premature skin aging due to free radical damage can also be induced by ultraviolet (UV) radiation (Gentlelift, 2019). The presence of UV-induced mtDNA mutations and the resulting vicious circle with further increases in mtDNA mutations create a situation reminiscent of a defective driving force in which insufficient energy production causes chronic oxidative stress.

Experimental and clinical studies have shown that impaired T cell reactivity, cytokine production, and other immunological reactions are associated with changes in skin appearance, including the development of various diseases and premature skin aging (Zegarska, 2010).

3 Results

A total of 196 patients were surveyed. Computer programmes *MS Excel* and *SPSS 22* were used for data analysis. Literature was also used for data analysis (Teibe and Berķis, 2001; Teibe, 2007). The Kolmogorov–Smirnov test with Lilliefors correction was calculated for quantitative data in order to decide on the use of parametric or non-parametric statistics. Most of the variables did not correspond to the normal distribution ($p \leq 0.05$), so non-parametric statistics were used. The following statistical methods were used:

- descriptive statistics (frequencies for qualitative data; medians and quartiles for quantitative data) for all patients together and for each of the four study groups;
- the McNemar test was used for qualitative data to determine the efficacy of Se therapy; for quantitative data, the Wilcoxon test was used;
- Spearman rank correlation was used to determine the relationship between all variables;
- linear regression analysis was used to predict the skin aging index depending on the factors influencing it.

Histological data / findings:

Table 3.1

Comparison of patients with and without MetS

Histological properties	With MetS (n = 27)	Without MetS (n = 23)
Epidermal thickness	0.71 mm	0.56 mm
Stratum corneum thickness	0.18 mm	0.16 mm
Stratum spinosum (number of rows)	9	7.5
Stratum granulare (number of rows)	2.25	1.75

Table 3.1 continued

Histological properties	With MetS (n = 27)	Without MetS (n = 23)
Basal membrane thickness	n = 14	n = 11
Average volume of adipocytes	0.33 mm	0.3 mm
Dermal elastosis	n = 9	n = 3
Perivascular infiltration	n = 26	n = 13
Narrowing of the dermal capillaries	n = 26	n = 13

In patients in the MetS group, the following histological findings were observed, which were compared with those in the non-MetS group: acanthosis or thickening in the stratum spinosum layer, which varied; flattened dermal-epidermal junction; enlarged adipocytes in the hypodermic layer; thick collagen fibers. In patients in the MetS group, the total number of CD34-positive endothelial cells in more than 4.5 capillaries was about 1 mm^2, but in patients without MetS 3.1 capillaries were about 1 mm^2, respectively. In patients with MetS, the basement membrane was thickened. CD117-positive mast cells were found in the dermis and between adipocytes (8 CD117-positive cells per 1 mm^2). Elastosis in the superficial dermis was observed in patients in the MetS group.

Immunoreactivity bcl-2 protein was accumulated in basal cells with a mean volume of 39.1 per 100 basal cells, but in the group without MetSt – 6.4, respectively. The average thickness of the epidermis was 0.71 mm with MetS and 0.46 mm without MetS. *Stratum spinosum* thickness was 0.48 mm in the MetS group and 0.25 mm without MetS. The *stratum corneum* was 0.18 mm with MetS and 0.16 mm without MetS. Cumulation of dendritic cells around the inflammatory cell foci could be observed in MetS patients with signs of acanthosis and hyperkeratosis. Different levels of accumulation for Birbeck's granules were found in the MetS group, as well as some CD 1a+ cell

migration in the papillary dermis. Increased adipocyte volume up to 0.13 mm and fibrosis in the deep capillary structure was observed in MetS patients.

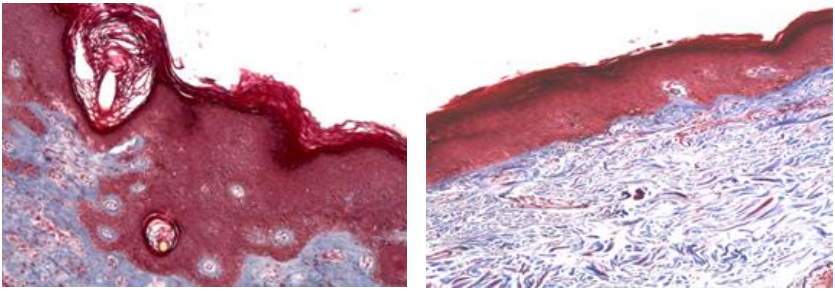

Figure 3.1 **Early skin changes in patients with MetS: hyperkeratosis (left side), dermal elastosis (right side)**, (100× Masson's trichrome)

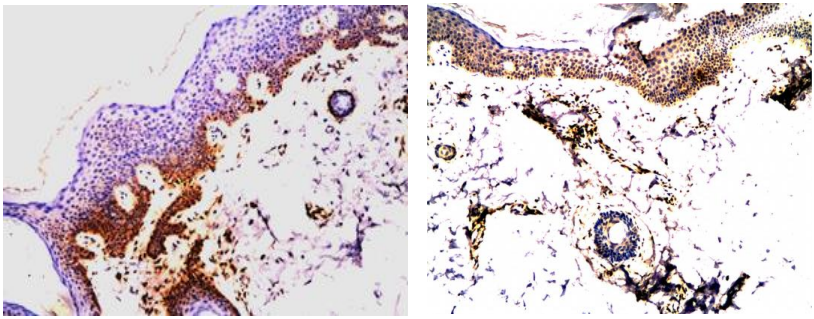

Figure 3.2 **Expression of apoptotic protein (BCL-2) in patients with MetS (left). Perivascular infiltration around the vessel in the MetS group (right)**, (EnVision method, DakoCytomation, 100×)

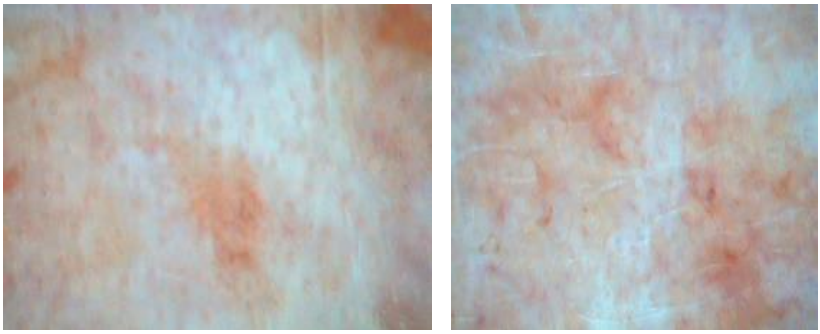

Figure 3.3 **Optical dermatoscopy: dyspigmentation visualisation; Skin in patients without MetS (left side) and skin in the presence of MetS (right side)** (Skin analyses, Aramhuvis Co, 60×)

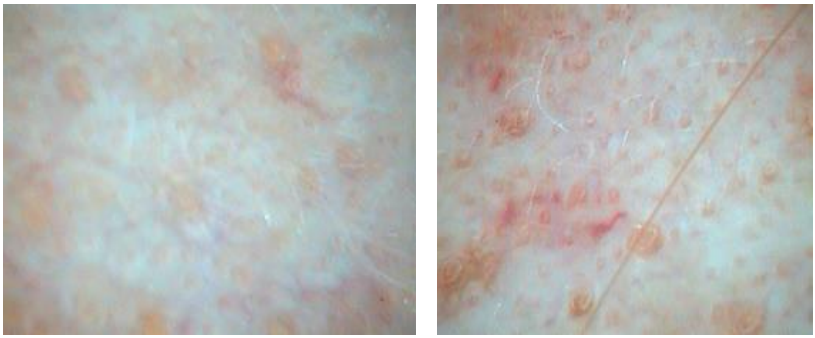

Figure 3.4 **Dermatoscopy finding for telangiectasias: skin without MetS (left) and skin with MetS (right)**

Regarding the cutaneous optical dermatoscopy, the dyspigmentation of the skin, predominantly hyperpigmentation, and pigment accumulation were more pronounced in patients with MetS (Figure 3.3), and telangiectasias and superficial capillaries were more visualised in MetS patients as well (Figure 3.4).

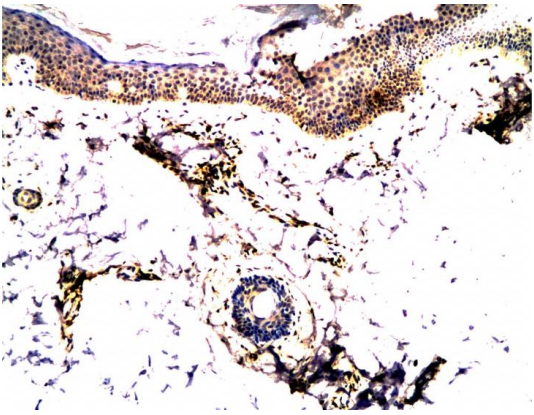

Figure 3.5 **Mast cell accumulation in patients in the MetS group**
(En Vision method. DakoCytomation 100×)

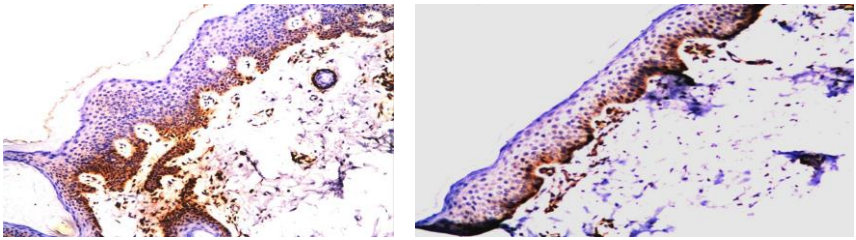

Figure 3.6. **Apoptosis protein accumulation in bcl-2 patients in MetS group (left) and non-MetS group (right) (comparison)**

3.1 Descriptive statistics for quantitative data

Frequencies were calculated for qualitative data and plotted graphically. For example, distribution by age is shown in (Figure 3.7). It shows that all patients had a median (bar height) of 52 years, a first quartile (lower cut-off point) of 44 years, and a third quartile (upper cut-off point) of 57 years. In contrast, the group without MetS who underwent selenium substitution had 46, 38, and 54 years, respectively. The MetS group with Se substitution had 54, 47, and 58 years, respectively. The group without MetS and without selenium

substitution had 45, 34, and 54 years, respectively. The MetS group with no Se substitution had 55, 47, and 58 years, respectively.

A total of 85 patients without MetS, 111 with metabolic syndrome, 168 women and 28 men were studied. In the group without MetS and Se preparation administration, there were 31 women and 4 men (n = 35). There were 47 and 15 (n = 62) in the MetS group with Se administration, respectively. There were 45 and 5 (n = 50) in the group without MetS and without Se preparations, respectively. In the group with MetS and no Se preparations: 45 and 4 (n = 49), respectively. The studied groups were comparable and homogeneous by age, sex and UV index.

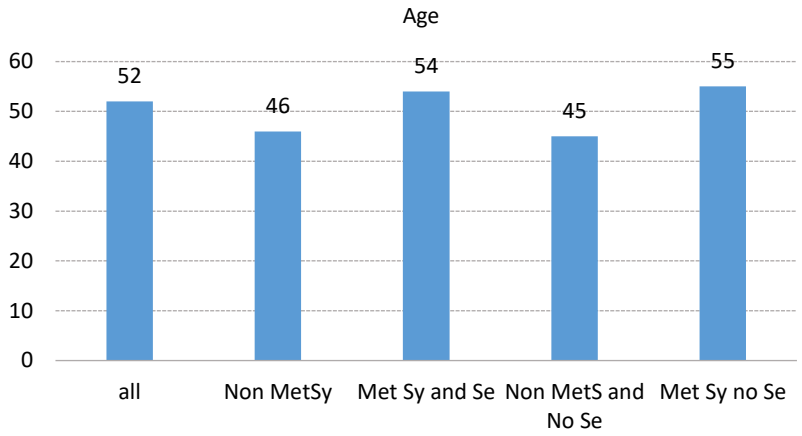

Figure. 3.7 **Distribution of patients in groups by age**

3.2 Dependent sample tests

The aim of the study was to investigate whether the skin condition and laboratory parameters of patients improve after the use of Se preparations, mainly in patients with MetS. Dependent sample tests were used to check for

improvement – the McNemar test for qualitative data and the Wilcoxon test for quantitative data.

3.2.1 McNemar test results

The McNemar test was performed in groups: one, which underwent a biopsy, had no MetS, and underwent Se substitution (n = 7) and one which had MetS and underwent Se substitution (n = 25). The number of patients without MetS who received Se preparations was small (n = 6) and it was not possible to judge the population by this number. Therefore, the group that was more important in the author's study – the one with MetS that was given Se preparations (n = 23) – was analysed. The frequency and McNemar test's p values are shown in the graphs (Figures 3.8–3.11).

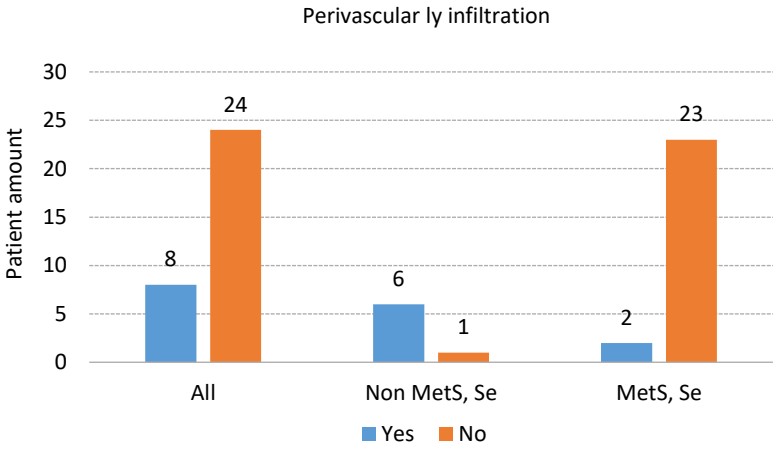

Figure 3.8 **Results of descriptive statistics and McNemar test for perivascular lymphocyte infiltration**

In 8 patients who initially had perivascular lymphocyte infiltration (Figure 3.8), this was no longer found due to Se substitution. These differences are statistically significant. Let us have a look at patients (n = 13) who underwent biopsy and received Se tablets.

In 6 patients who initially had perivascular Ly infiltration this was no longer present after Se treatment. These differences are statistically significant.

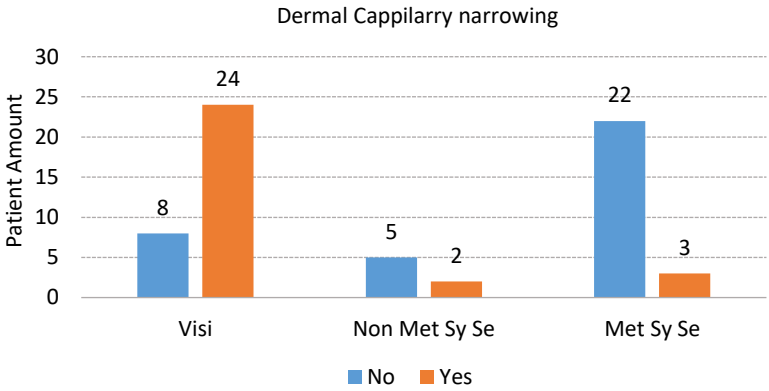

Figure 3.9 **Descriptive statistical and McNemar test results for narrowed dermal capillaries**

In 8 patients who initially had narrowed dermal capillaries (Figure 3.9), this was no longer found due to Se substitution. These differences are statistically significant. In 2 patients the initially narrowed dermal capillaries were absent after Se treatment. These differences are not statistically significant.

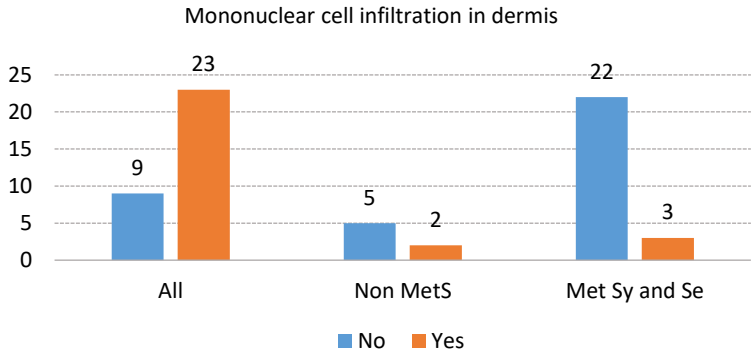

Figure. 3.10 **Results of descriptive statistics and McNemar test for dermal infiltration of mononuclear cells**

In 3 patients who initially had dermal mononuclear cell infiltration (Figure 3.10), this was no longer present after Se treatment. These differences are not statistically significant.

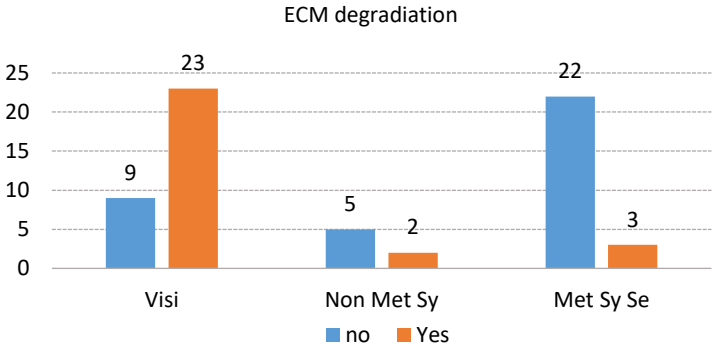

Figure. 3.11 **Results of descriptive statistics and McNemar test for ECM degradiation**

In 8 patients who initially had ECM degradiation (Figure 3.11), this was no longer found after Se treatment. These differences are statistically significant.

Thus, after Se treatment, there was a decreased amount of:

- perivascular lymphocyte infiltration;
- narrowed dermal capillaries;
- ECM degradiation.

In 6 patients who initially had ECM degradiation (Figure 3.11), this was no longer present after Se treatment. These differences are statistically significant.

Thus, after Se treatment, there was a decreased amount of:

- perivascular lymphocyte infiltration;
- narrowed dermal capillaries;
- ECM degradiation.

3.2.2 Wilcoxon test results

The Wilcoxon test was performed in groups: those without MetS and Se administration (n = 35) and those with MetS and Se administration (n = 62). Column p gives the values of the Wilcoxon test, for statistically significant differences, the p values are given in bold.

Table 3.2

Wilcoxon test results

Variable	n	Before treatment			After treatment			p
		Q_1	Me	Q_3	Q_1	Me	Q_3	
No MetS, Se								
Skin aging index	35	4.0	5.0	7.0	4.6	5.2	5.9	**< 0.001**
Total cholesterol I, mmol/	35	4.5	5.2	5.9	4.6	5.2	5.9	**0.009**

Table 3.2 continued

Variable	n	Before treatment			After treatment			p
		Q_1	Me	Q_3	Q_1	Me	Q_3	
No MetS, Se								
LDL, mmol/l	35	2.5	2.9	3.3	1.5	1.8	2.2	< **0.001**
HDL, mmol/l	35	1.4	1.6	1.9	4.5	4.9	5.5	< **0.001**
Glucose, mmol/l	35	4.9	5.0	5.6	4.7	4.9	5.6	0.080
Vitamin D, ng/mL	35	18.1	30.6	34.3	18.1	30.6	33.5	0.125
CRP, mg/L	35	0.1	0.3	1.3	0.1	0.3	1.3	0.180
MDA, mM	35	2.3	2.5	2.7	2.2	2.3	2.5	**0.018**
Selenium mg/L	35	57.0	61.0	65.0	88.0	97.0	98.0	< **0.001**
SOD, U/g Hb	35	1637.0	1701.0	1824.0	1769.0	1922.0	2021.0	**0.001**
Degree of elastosis	7	0.0	1.0	1.0	0.0	1.0	1.0	1.000
Langerhans cells	7	2.0	9.0	16.0	2.0	10.0	15.0	1.000
MetS, Se								
Skin aging index	62	4.0	6.0	6.3	5.4	6.0	6.9	**0.022**
Total cholesterol I, mmol/	62	5.4	6.1	6.9	5.4	6.0	6.9	**0.004**
LDL, mmol/l	62	3.1	3.6	4.3	1.5	1.7	2.6	< **0.001**
HDL, mmol/l	62	1.4	1.6	1.9	4.5	5.0	5.5	< **0.001**
Glucose, mmol/l	62	4.9	5.3	5.8	4.9	5.2	5.7	0.237
Vitamin D, ng/mL	62	15.2	25.8	30.6	16.6	27.0	30.7	**0.013**
CRP, mg/L	62	0.4	1.4	3.1	0.3	1.4	2.9	0.109
MDA, mM	62	3.2	3.7	4.1	2.9	3.3	3.7	0.062
Selenium mg/L	62	73.8	80.5	90.3	111.0	117.0	124.5	< **0.001**
SOD, U/g Hb	62	1371.3	1439.5	1508.0	1452.0	1577.5	1698.0	< **0.001**
Degree of elastosis	25	2.0	2.0	2.0	1.0	2.0	2.0	**0.014**
Langerhans cells	25	22.0	27.0	33.5	22.0	27.0	33.5	**0.046**

For those who had MetS and were given Se, after the treatment:

- the average skin aging index decreased – the medians were the same, but the arithmetic means were 5.6 and 5.4, respectively;
- the medians for total cholesterol decreased;
- the median for LDL decreased;
- the median for HDL increased;
- the median for in vitamin D level increased;
- the medians of Se and SOD levels increased;
- the average degree of skin elastosis decreased – the medians were the same, but the arithmetic means were 1.9 and 1.6, respectively.

3.3 Skin aging index

3.3.1 Method

The new method would make it possible to timely identify among practically healthy patients those who have already developed FR-induced damage and to take preventive and therapeutic measures while this prevention is still effective. The method is informative, reliable, non-invasive, and easily reproducible. This method can be used on its own, as well as in combination with already known scales for assessing skin aging.

3.3.2 Inspection

The duration of the patient's visual inspection procedure is 7–9 minutes. During the visual inspection, the dermatological condition of the skin, its general condition (colour, turgor, desquamation) are determined, as well as the main problems (roughness, enlarged pores, microrelief changes, wrinkles, etc.) are identified.

3.3.3 Prediction of skin aging index depending on factors influencing it

Multiple linear regression was used to predict the skin aging index depending on the factors influencing it. The model is based on the following assumptions.

For a young, low-blood, slim person: if the Se is high, the MDA is low, the SOD is low, the skin is expected to be in very good condition (SAI = 0).

For an old, hypertensive, obese person with low Se, high MDA, high SOD: very poor skin condition is expected (SAI = 11).

The minimum and maximum values of the variables are used in the model (Table 3.3).

Table 3.3

Minimum and maximum values of the variables included in the model

	Minimum	Maximum
Age	21	78
Systolic blood pressure	80	180
BMI	18.5	42
Se	24	131
MDA	1.51	5.75
SOD	1145	2240
SAI	0	11

The expression of the model is as follows (see table 3.3) and the determination coefficient R^2 is close to the maximum value 1.

$$y = (0.099 \times (x_1 - 21) + 0.024 \times (x_2 - 80) + 0.129 \times (x_3 - 18.5) - 0.031 \times$$
$$\times (131 - x_4) + 1.219 \times (x_5 - 1145) + 0.001 \times (x_6 - 1145)) \times (11 \div 14),$$
$$R^2 = 0.996$$

x_1 – age, in years

x_2 – systolic blood pressure

x_3 – BMI

x_4 – Se

x_5 – MDA

x_6 – SOD

y – SAI

4 Discussion

The above-mentioned research results have made it possible to solve the research tasks. SAI is obtained, which is a diagnostic technique for evaluating the interaction between OS and skin aging, as well as for visualising the possible aging of internal blood vessels and the risk of CVD. **Skin xerosis and dyspigmentation observed in the case of MetS indicate OS and chronic latent inflammation of the skin, which prognostically indicates an accelerated skin aging process not only visually but also morphologically.**

Skin elasticity and narrowing of the dermal capillaries, as well as the immune response to lymphocytic infiltration in the MetS group, which are both interrelated with OS parameters, have been observed through a deeper examination of skin structure and extraction of material using skin punch biopsy. This confirms the idea that systemic damage in the case of MetS is not only the effect of metabolic processes but also the functional and physiological condition of the skin as such. It is known that imbalance between FR production and the body's overall AO defense system in the case of MetS may be a combination of excessive xenobiotic exposure as well as reduced detoxification / elimination of xenobiotic agents due to lifestyle and genetic factors. More specifically, all enzymes in xenobiotic excretory systems are found in the skin, such as cytochrome P450 enzymes, flavin-dependent monooxygenase, monoamine oxidase, alcohol dehydrogenase, and aldehyde dehydrogenase (Shigenaga et al., 1993).

In the course of this study, the following skin changes were frequently observed in MetS patients but less frequently in the control group: dehydration, sometimes even xerosis, reduction of turgor and elasticity, uneven skin relief, gravitational wrinkles, which is one of the parameters measured in SAI, accordingly in MetS patients the SAI (Me = 6) is higher compared to the control group (Me = 4), ($p < 0.005$).

In MetS patients, the study participants, epidermal damage, dyspigmentation (including lentigo) and dyskeratosis, skin peeling, dry skin, keratosis, including actinic keratosis was observed. As a result of metabolic disorders and the development of MetS, the microvascular network in the skin was also altered, vascular lumens were narrowed (before Se substitution – 92 %, after – 40 % ($p < 0.001$)). Histological findings include skin hyperkeratosis and epidermal thickening, as well as dermal elastosis with extracellular matrix degradation, perivascular infiltration with dermal capillary narrowing. The authors of the study suggest that these changes indicate the cumulative accumulation of MetS aging processes and MetS skin symptoms.

The value of this study reflects the finding that the observed inflammatory response in the skin, comparable to chronic latent inflammation, is associated with OS (followed by perivascular and dermal infiltration, narrowed blood vessels) and may be associated with elevated serum SOD (No MetS Me = 1512 (U/g Hb), MetS Me = 1545 (U/g Hb)). Elevated SOD in MetS is thought to indirectly show antioxidant capacity and activity in the patient's skin. OS also reduces the protective functions of the skin, which may be clinically seen as skin xerosis, dyspigmentation and neoplasms (seborrheic keratoses), as additional manifestations of MetS skin (see results).

This study also shows the activity of Langerhans cells, even at minimal UV sub-erythematous doses. Elevated MDA levels (No MetS Me = 2.82, MetS Me = 2.99) may indicate extracellular matrix damage and metalloproteinase activity that can be used as a secondary MetS prognostic tool. Various skin symptoms associated with neoplasms, such as seborrheic keratoses, actinic keratoses, *acanthosis nigricans* elements – papillomas, as well may indicate an additional risk for MetS prognosis.

Since MetS affects almost all vital skin functions, MetS-impacted skin may develop skin failure syndrome, with the weakening of its physiological functions, inadequate immune responses, and reduced protection against UV, as well as the development of intolerant and sensitive skin. OS and its biomarkers are elevated in MetS: the clinical value of elevated MDA (not > 3.15 mkM/L) and SOD (normal range from 1307–1601 U/g Hb) can serve as early biomarkers for further MetS tactics and treatment plan development. Recognition provides information on the potential risk of developing CVD and DM type 2.

Changes in MDA and SOD levels may be associated with various skin changes and even degradation of the extracellular matrix in the case of MetS. The intensity and activity of MDA and SOD indicate the unfavourable course of MetS, which is shown by this study, the use of MetS can be recognised in practice. Despite the lack of central obesity (one of the most important risk factors for CVD), patients with the above-mentioned changes should be included in the severe risk group for CVD risk prevention.

Elevated serum SOD levels (Me = 1545 U/g Hb) in comparison with the control group (Me = 1512 U/g Hb) ($p < 0.005$) together with skin keratinisation and stratum corneum thickening indirectly indicate FR-induced skin damage as well as affect the skin immune system (Akdeniz, 2018). It has been shown that damaged collagen fibers, with subsequent fiber fragmentation, decrease in amount and diameter. By reducing O2- levels, it protects other AOs from its deactivating effects, especially catalase and glutathione peroxidase (GPx). Superoxide dismutases convert superoxide to hydrogen peroxide, which in turn can be converted to water by catalase or GPx. SOD is considered to be one of the most important AOs, while increased SOD activity against the background of oxidative imbalance and lack of other antioxidant components (mainly GPx) is associated with undesirable side effects in body tissues, including inflammatory reaction with subsequent cytokine release (Lazzarino

et al., 1994). Therefore, Se substitution has a positive effect on OS reduction in MetS patients of this study and is considered justified. When using Se, the developed SAI for MetS patients was significantly reduced: before it was 6.0 but decreased to 5.5 ($p < 0.003$). Examination of biopsy samples also showed the effectiveness of Se: the degree of elastosis decreased (from 2.0 to 1.0 ($p < 0.002$)). Changes in telangiectasias, dermal capillary stenosis, extracellular matrix degradation, dermal mononuclear cell infiltration, and perivascular infiltration were observed.

Selenium is an antioxidant needed by the body, it has antioxidant properties and it protects the skin from UV radiation and free radical damage. It should be noted that the "therapeutic window" of the admissible dose range for selenium is relatively narrow: the difference between the lowest dose at which there are no deficiency symptoms and the highest non-toxic level is between 0.1 and 1.0 μg/kg body weight. This complicates the study of selenium as a trace element and its effects on the skin. The study developed an innovative selenium-containing product, a cream for patients with MetS. The cream was developed by the ERDF project No. 2014/0024/2DP/2.1.1.1.0/14/APIA/ VIAA/076, 2 patents were registered (LV and international: Cream for the restoration of the epidermal lipid barrier of facial skin, neck, and décolleté area in patients with metabolic syndrome).

Skin lesions in MetS patients can be interpreted in the following ways:

1. It re-emphasises the interconnectedness of organ systems and the extent to which the skin is involved in metabolic disorders.
2. The results of this study demonstrate the value and potential of skin symptoms for early diagnosis and better treatment of patients at risk for MetS or even type 2 DM and CVD.

One of the results of this study is the development of the Skin Aging Index (SAI). The idea of a simple skin assessment protocol that could potentially provide information on the patient's metabolic status, MetS, and/or DM development is attractive and would undoubtedly improve patient care (van Waateringe, 2017). MetS is associated with CVD risk, so skin changes may have been considered when deciding on the need for CVD prevention. It is believed that it would be important to use a technique to determine the risk of developing skin damage caused by FR, and that is SAI. SAI was developed by summarising and analysing the signs of aging in the skin, dermatoscopic changes and OS parameters, as well as the authors' intuition. The above skin changes (dyspigmentation, seborrheic keratosis, hyperpigmentation, skin xerosis) conclusively coincide with the acceleration of SOD elevation activity and elevated MDA. Therefore, a coincidence of MDA levels and skin changes is expected and has been demonstrated in this study, where MDA levels are higher in the MetS group (No MetS Me = 2.82, MetS Me = 2.99). The SAI, which has an integrated relationship between skin changes and FR damage, is higher in MetS patients compared to the control group.

The new method for detecting neoplastic lesions of the skin caused by FR makes it possible to timely identify among practically healthy patients those who have clinical skin lesions caused by FR damage. The method is informative, reliable, and reproducible. The method allows for the detection of skin aging and neoplastic lesions.

The present study suggests that in MetS patients, the skin is one of the major manifestations of OS due to FR that are constantly produced in the skin in response to environmental and endogenous pro-oxidants. Physical activity and psychological stress can also cause OS on the skin. In addition to the observation of higher levels of SAI in individuals with MetS, it was also found that a higher number of individual components of MetS coincided with

even higher rates of SAI. A previous study had already shown that higher levels of MetS components were associated with higher serum AGE levels. Kleins et al. showed that individuals with one component of MetS had a 2.5 % risk of developing CVD over the next 5 years, while individuals with four or more components of MetS had a nearly 15 % risk. The five-year risk of developing type 2 DM increased from 1.1 % (one MetS component) to 17.9 % (≥ four MetS components). An 11-year observational study showed an almost linear relationship between the number of individual components of MetS and the risk of coronary heart disease in people without a history of CVD or type 2 diabetes. Therefore, it was believed that the increase in SAI may reflect an even greater risk of type 2 DM and CVD, which was described in the patent. This allowed for good statistical power and ability to perform stratified analysis. In addition, this is the largest study of MetS (n = 196) showing an association between SAI and several cardiometabolic risk factors.

The components of MetS that were significantly higher in MetS patients compared to the control group were dyslipidemia (increased predominant LDL and triglycerides): LDL levels (in MetS patients was 3.7 mmol/L and 3.0 mmol/L in the control group ($p < 0.001$)); triglyceride levels (in MetS patients were 1.9 mmol/L and 1.0 mmol/L in the control group ($p < 0.001$)); skin glycation products (AGEs), high blood pressure, high BMI and abdominal obesity. After adjusting several factors, it was observed that low LDL cholesterol levels were significantly associated with a higher level of SAI as well as elevated triglyceride levels. Therefore, it is not difficult to suspect common pathophysiological pathways. The results of this study are consistent with Nagase et al., who studied the skin aging process in animal models with MetS. They found evidence of both OS and elevated markers of inflammation, as well as increased expression of mineralocorticoid receptors in the skin. They suggested considering a mechanism of aging similar to that occurring in the internal organs of MetS

patients. More specifically, OS and inflammation are associated with MetS; therefore, FR which damage DNA and mitochondrial function and cause hormonal dysregulation (including insulin resistance) are associated with the aging process. Skin collagen glycation is thought to be associated both with MetS parameters and aging.

The findings of this study are also reflected in patents on CVD risk assessment:

1. LV15058 B. A method for determining high cardiovascular risk in the case of central obesity in patients in the Baltic Sea region; owner Rīga Stradiņš University. Patent publ. date 20.04.2016. Application No. P-15-49 (LV), date of application 27.05.2015., date of publication of the application 20.10.2015.
2. PCT application WO 2016/190723 (A1). Determination method of the high cardiovascular risk indicators in the case of central obesity in patients from the Baltic sea region; owner Rīga Stradiņš University. Date of publication of the application 01.12.2016.

Screening of high-SAI patients for risk factors for CVD may reveal both high-risk patients and under-treated patients. CVD risk factors were more common in patients with high SAI – SAI was higher in MetS patients (Me = 6) compared to controls (Me = 4), ($p < 0.005$). MetS is thought to be a chronic systemic inflammatory disease that causes insulin resistance by lowering insulin receptor expression. Th1 pathway cytokines (interferon-γ, interleukin (IL)-2, IL-12, and tumour necrosis factor (TNF)-α) predominate in foci of atherosclerosis. In addition, a decrease in insulin receptor expression in endothelial cells reduces the amount of nitric oxide (NO), a vasodilator. This results in constriction of the blood vessels, leading to increased stiffness of the arteries. OS and DNA / RNA damage are also increased in patients with MetS, very similar to those in psoriasis. Thus, evidence suggests (Audin, 2001) that chronic MetS

inflammation affects skin homeostasis in combination with genetic and other factors. Therefore, physicians should be aware of the increased risk for CVD in patients with high SAI and adjust their treatment accordingly.

The presence of the above-mentioned skin diseases in people who meet the MetS criteria is not uncommon. Within the framework of this study, this observation can be interpreted as follows:

1. It reiterates the interconnectedness of organ systems and the extent to which the skin is involved in metabolic disorders;
2. The growing interest and results of related research suggest early detection and better treatment of people at risk of developing CVD or type 2 DM.

Current findings on **elevated SAI** (SAI in MetS patients (Me = 6) is higher compared to controls (Me = 4), ($p < 0.005$)) in individuals with MetS who show a positive association between the number of individual MetS components and a higher level of ADI scores. The fact that a higher number of SAI is associated with an increased prevalence of individual components of MetS, provides additional evidence that elevated SAI may contribute to pathophysiology of several cardiometabolic risk factors. Prospective studies are needed to show whether SAI measurements can be used as an additional non-invasive screening tool to identify high-risk individuals with both CVD and type 2 CD. MetS can be managed to reduce its manifestations and prevent type 2 diabetes and coronary heart disease, taking into consideration SOD, MDA, SAI.

It is recommended that selenium be administered topically and orally to reduce skin changes caused by skin aging and MetS. Oral selenium is recommended during the winter season when food is deficient in antioxidants, but a selenium-containing cream is recommended for topical use in summer when there is a high ultraviolet effect.

Conclusions

1. The following significant differences were found in 196 patients with MetS: waist circumference, LDL levels, triglyceride levels and arterial blood pressure. The total amount of Lentigo-type pigmentation in MetS patients was higher than in control patients. Signs of acanthosis nigricans were more common in MetS patients, skin microrelief was uneven in MetS patients. **This indicates cumulative accumulation of MetS aging processes** and early development of MetS skin symptoms; therefore, skin symptomatology could be used as an additional criterion for early identification and monitoring of MetS patients.
2. In patients with MetS, in contrast to the control group, the following signs of **skin aging** were observed: dyspigmentation, seborrheic keratosis and telangiectasia, actinic keratosis and *acanthosis nigricans* (hyper-pigmentation, skin thickening, papillomatosis). **Dermoscopic** signs included dilated vascular capillaries, enhanced pigment. The histological finding was skin hyperkeratosis and epidermal thickening, as well as dermal elastosis with the degradation of the extracellular matrix. Perivascular infiltration with dermal capillary stenosis was observed. By summing and analysing the signs of skin aging, dermoscopic changes and OS parameters, the **Skin aging index (SAI)** was developed, which is higher in MetS patients ($p= 0,005$)
3. When using selenium, the developed SAI in MetS patients decreased: from 6.0 to 5.5 ($p < 0.003$). Pathological examination of biopsy samples also showed the effectiveness of selenium: the degree of elastosis decreased. Decreases severity of telangiectasias. Perivascular infiltration and mononuclear dermal cell infiltration as well as, extracellular matrix degradation and narrowing of capillaries decreases in MetS patients.

4. **The findings suggest an effective AO action of selenium**: selenium, being a powerful antioxidant, will prevent skin damage caused by free radicals (FR), reduce the OS in the skin and protect skin from premature aging.
5. OS features in patients with MetS are characterized by elevated MDA and increased SOD activities. The role of MDA and SOD as early biomarkers in the monitoring, prevention, and treatment of MetS patients was demonstrated. The SAI, which contains integrated interrelationships between skin changes and free radical damage, was higher for the MetS group (Me = 6) compared to the control group (Me = 4) ($p < 0.005$). Considering the peculiarities of OS in MetS patients, namely, MDA, SOD, and free radical-induced signs of skin aging (xerosis, lentigo, *acanthosis nigricans*), the importance of the role of MDA and SOD in patient monitoring and treatment, and prevention of MetS was determined.

Practical recommendations

1. Oral organic substitution of seleno-L-methionines improves the clinical signs of the skin, including reducing hyperpigmentation, redness, and microrelief. It restores skin turgor and reduces chronic latent inflammation of the skin. It also prevents the formation of seborrheic keratoses and actinic keratoses as a result of skin damage.
2. Clinical manifestations of MetS and skin, together with oxidative stress (SOD, MDA) parameters and SAI, can be a good indicator of timely preventive measures to reduce the risk of CVD.
3. The invented SAI can provide a clear assessment in dermatological practice by assessing the severity of the signs of skin aging, as well as SAI together with the presence of MetS can be seen as an additional value for CVD risk visualization.
4. To achieve a therapeutic effect to reduce photodamage, it is recommended to use Seleno-L-methionine orally together with topical applications to the skin in the form of a cream. Oral selenium is recommended during the winter season when AO deficiency in food is possible, but selenium-containing cream is recommended for topical use in summer when the ultraviolet effect is high.

Publications on the research topic

1. LV15082 B. Cream for the restoration of the epidermal lipid barrier of facial skin, neck, and décolleté area in patients with metabolic syndrome; owner Rīga Stradiņš University. Patent publ. date 20.04.2016. Application No. P-15-90 (LV), date of application 24.08.2015., Date of publication of the application 20.12.2015.
2. PCT application WO 2017/034384 (A1). Epidermal lipid barrier restoring cream for facial skin, neck, and decollete area for metabolic syndrome patients; owner Rīga Stradiņš University. Date of publication of the application 02.03.2017.
3. LV15058 B. A method for determining high cardiovascular risk in the case of central obesity in patients in the Baltic Sea region; owner Rīga Stradiņš University. Patent publ. date 20.04.2016. Application No. P-15-49 (LV), date of application 27.05.2015., date of publication of the application 20.10.2015.
4. PCT application WO 2016/190723 (A1). Determination method of the high cardiovascular risk indicators in the case of central obesity in patients from the Baltic sea region.; owner Rīga Stradiņš University. Date of publication of the application 01.12.2016.
5. Voicehovska, J., Vlaskovska, M., Janovska, J., Babikovs, S., Voicehovskis, V., Skesters, A., Silova, A., Karpovs, J., Zubova, A., Kisis, J., Daberte, I., Sprudza, D., Kleina, R. 2018. Oxidative stress markers diagnostic value in metabolic syndrome dermal manifestations: a prospective clinical trial. *C R Acad Bulg Sci*; 71(2): 271-9. DOI 107546/crabs.2018.02.15.
6. Janovska, J., Voicehovska, J., Kleina, R., Kisis, J., Karls, R., Zubova, O., Babikovs, S. 2014. Diversity of CD1a positive cells in case of 25-hydroxyvitamin D deficiency in patients with metabolic syndrome.*UDC Classification*: 616-01, DOI: http:// dx.doi.org/10.12955/cbup.v2.475, pages 292–297.
7. Janovska, J., Voicehovska, J., Kisis, J., Kleina, R., Karls, R. 2013. A pilot study of skin aging peculiarities in patients with metabolic syndrome. *Journal of Investigative Dermatology*, 133, Suppl., 1 May.

Bibliography

1. Achanta, G., Huang, P. 2004. Role of p53 in sensing oxidative DNA damage in response to reactive oxygen species-generating agents. *Cancer Research*. 64(17), 6233–6239.

2. Adomas Urbanavicius. Free Radical Damages In Proteins. http:// www.cryst.bbk.ac.uk?

3. Afanas'ev I. B. 2010. Signaling by reactive oxygen and nitrogen species in skin diseases. *Current Drug Metabolism*. 11(5), 409–414.

4. Akase, T. et al. 2012. Aging-like skin changes induced by ultraviolet irradiation in an animal model of metabolic syndrome. *Biol Res Nurs*. 14, 180–187. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/acel.12017#references-section, [viewed 25.06.2019.].

5. Akdeniz, N., Uzuncakmak, T. K., Karadag, A. S. 2018. Cutaneous manifestations of obesity and metabolic syndrome. *Clin Dermatol*. 36, 81–88.

6. Alfthan, G. 1984. A micro method for the determination of selenium in tissues and biological fluids by single–test-tube fluorimetry. *Analytical Chimica Acta*. 165, 187−194.

7. Alissa, E. M., Bahijri, S. M., Ferns, G. A. 2003. The controversy surrounding selenium and cardiovascular disease: a review of the evidence. *Med Sci Monit*. 9(1), 9–18.

8. Ames, B. N, Shigenaga, M. K., Hagen, T. M. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA*. 90, 7915–7922.

9. Angelova-Fischer, I. et al. 2014. Skin barrier integrity and natural moisturizing factor levels after cumulative dermal exposure to alkaline agents in atopic dermatitis. *Acta Dermato-Venereologica*, Volume 94, Number 6, 640–644. Available from: doi: https://doi.org/10.2340/00015555-1815.

10. Angelova-Fischer, I. et al. 2014. A randomized, investigator blinded efficacy assessment study of stand-alone emollient use in mild to moderately severe atopic dermatitis flares. *Journal of The European Academy of Dermatology and Venereology*, Volume 28, Issue s3. Available from: https:// doi.org/10.1111/jdv.12479.

11. Arthur, J. R., McKenzie, R. C., Beckett, G. J. 2003. Selenium in the immune system. *The Journal of Nutrition*. 133(5), 1457–1459.

12. Audin, A. 2001. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. *Clinical Biochemistry*. 34, 65–70.

13. Balmain, A., Yuspa, S. 2014. Milestones in skin carcinogenesis: the biology of multistage carcinogenesis. *J Invest Dermatol.* 134(e1): E2-7. doi: 10.1038/skinbio.

14. Back, P., Braeckman, B. P., Matthijssens F. 2012. ROS in aging Caenorhabditis elegans: damage or signaling? *Oxid Med Cell Longev*. 608478.

15. Balaban, R. S., Nemoto, S., Finkel, T. 2005. Mitochondria, oxidants, and aging. *Cell*. 120, 483–495.

16. Banerjee, R. R. 2004. Regulation of fasted blood glucose by resistin. *Science*. 303, 1195–1198.

17. Bera, S., Rosa, V. D., Rachidi, W., Diamond, A. M. 2013. Does a role for selenium in DNA damage repair explain apparent controversies in its use in chemoprevention? *Mutagenesis*. 28(2), 127–134.

18. Bevan, R. J. et al. 2003. Validation of a novel ELISA for measurement of MDA-LDL in human plasma. *Free Radical Biology and Medicine*. 35, 517–527.

19. Bjelland, S., Seeberg, E. 2003. Mutagenicity, toxicity, and repair of DNA base damage induced by oxidation. Mutation Research. *Fundamental and Molecular Mechanisms of Mutagenesis*. 531(1-2), 37–80.

20. Boldirev, A. A. 2000. Discrimination between Apoptosis and Necrosis of Neurons under Oxidative Stress. *Biochemistry (Moscow)*. 65(7), 981–990.

21. Brantova, O., Tesarova, M, Hansikova, H. 2006. Ultrastructural changes of mitochondria in the cultivated skin fibroblasts of patients with point mutations in mitochondrial DNA. *Ultrastruct Pathol*. 30, 239–245.

22. Braverman, M., Fonfrko, E. 1982. Studies in cutaneous aging: II. The microvasculature. *J Invest Dermatol*. 73, 59–66.

23. Brenneisen, P, Steinbrenner, H, Sies, H. 2005. Selenium, oxidative stress, and health aspects. *Mol Aspects Med*. 26(4-5), 256-267.

24. Brys, K., Vanfleteren, J. R., Braeckman, B. P. 2007. Testing the rate-of-living/oxidative damage theory of aging in the nematode model Caenorhabditis elegans. *Exp Gerontol*. 42, 845–851.

25. Dandona, P., Chaudhuri, A., Ghanim, H., Mohanty, P. Proinflammatory effects of glucose and anti-inflammatory effects of insulin: relevance to cardiovascular disease. *Am J Cardiol*. 99(4A). Available from: https://www.ncbi.nlm.nih.gov/pubmed/17307055, [viewed 25.06.2019.].

26. Danos, N., Holt, N. C., Sawicki, G. S., & Azizi, E. 2016. Modeling age-related changes in muscle-tendon dynamics during cyclical contractions in the rat gastrocnemius. *Journal of applied physiology*. 121(4), 1004–1012.

27. Davidovic, M. et al. 2010. Old age as a privilege of the "selfish ones". *Aging and disease*. 1(2), 139–146.

28. De Leo, V. A., Dawes, L., Jackson, R. 1981. The density of Langerhans cells (LC) in normal versus chronic actinically damaged skin (CADS) of humans. *J Invest Dermatol*. 76, 330–334.

29. Douki, T., Reynaud-Angelin, A., Cadet, J., Sage, E. 2003. Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation. *Biochemistry*. 42(30), 9221– 9226.

30. Droge, W. 2002. Free radicals in the physiological control of cell function. *Physiol Rev*. 82, 47–95.

31. Ergun, T. 2018. Hidradenitis suppurativa and metabolic syndrome. *Clin Dermatol*. 36, 41–47.

32. Experientia., C. E. 1972. Increased incidence of lymphomas in thymectomized mice. *Evidence for an immunological theory of aging*. 28(4), 459.

33. Fain, H. D. 2013. Selenium for prevention of cutaneous melanoma. *Nutrients*. 5(3), 725–749.

34. Farage, M. A. 2007. Structural characteristics of the aging skin: a review. *Cutan Ocular Toxicol*. 26, 343–357.

35. Farooqi, I. S. 2001. Partial leptin deficiency and human adiposity. *Nature*. 414, 34–35.

36. Feldman, D., Bryce, G. F., Shapiro, S. S. 1990. Mitochondrial inclusions in keratinocytes of hairless mouse skin exposed to UVB radiation. *J Cutan Pathol*. 17, 96–100.

37. Finn, J. C., Cox, S. E., Earl, M. L. 2003. Social implications of hyperfunctional facial lines. *Dermatol Surg*. 29, 450–455.

38. Fisher, G. J., Datta, S. C., Talwar H. S. 1996. Molecular basis of sun-induced premature skin aging and retinoid antagonism. *Nature.* 6563(379), 335–339.

39. Fisher, G. J., Kang, S., Varani, J. 2002. Mechanisms of photoaging and chronological skin aging. *Arch Dermatol*. 138, 1462–1470.

40. Freitas, B. E, de Castro, L. L. 2015. Antioxidant capacity total in non-melanoma skin cancer and its relationship with antioxidant nutrients. *Nutr Hosp*. 31(4), 1682–1688.

41. Friedman, J. M. 2000. Obesity in the new millennium. *Nature*. 404, 632-634.

42. *General AntiOxidant Action by Ben Best*. http://www.benbest.com/nutrceut/ AntiOxidants.html.

43. Gentlelift.com. http://www.gentlelift.com/facial_aging.htm.

44. Gilchrest, B. A. 1984. Skin and Aging Processes. Boca Raton: CRC Press.

45. Godin, D. 1988. Antioxidant enzyme alterations in experimental and clinical diabetes. *Mol Cell Biochem*. 84, 223–233.

46. González-Saldivar, G. et al. 2017. Skin Manifestations of Insulin Resistance: From a Biochemical Stance to a Clinical Diagnosis and Management. *Dermatology and Therapy*. 7(1), 37–51.

47. Grattagliano, I. 2008. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. *J Nutr Biochem*. 19, 491–504.

48. Gregorio, B. M. et al. 2016. The potential role of antioxidants in metabolic syndrome. *Curr. Pharm. Des*. 22, 859–869. Available from: doi: 10.2174/1381612822666151209152352 [viewed 25.06.2019.].

49. Gudkov, A. V. 2002. Converting p53 from a killer into a healer. *Nature Medicine*. 11(8), 1196–1198.

50. Guillery, O., Malka, F., Frachon, P. 2008. Modulation of mitochondrial morphology by bioenergetics defects in primary human fibroblasts. *Neuromuscul Disord*. 18, 319– 330.

51. Gupta, M. A., Gilchrest, B. A. 2005. Psychosocial aspects of aging skin. *Dermatologic Clinics*. 23(4), 643–648.

52. Halliwell, B., John, M. C. Gutteridge. 2015. *Free radicals in Biology and Medicine*. 5th ed. Oxford University Press, 944.

53. Hansford, R. G. 1983. Bioenergetics in aging. *Biochim Biophys Acta*. 726, 41–80.

54. Harman, D. 1956. Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. 11, 298–300.

55. Harman, D. 1994. The free-radical theory of aging. Increasing the functional life span. *Ann N Y Acad Sci*. 717(30), 1–15.

56. Helmbold, P. 2017. Basophilic (Actinic) Degeneration of the Dermis: An Easy Histological Scoring Approach in Dermal Photoaging. Farage, M., Miller, K., Maibach, H. Textbook of Aging Skin. Springer, Berlin, Heidelberg. Higgins, S. P. Freemark, M., Prose, N. S. 2008. Acanthosis Nigricans: A practical approach to evaluation and management. *Dermatology Online Journal*. Available from: https://escholarship.org/uc/item/7mf6g290.

57. Jendrach, M., Pohl, S., Voth, M. 2005. Morpho-dynamic changes of mitochondria during ageing of human endothelial cells. *Mech Ageing Dev*. 126, 813–821.

58. Jobeili, L., Rousselle, P., Béal, D. 2017. Selenium preserves keratinocyte stemness and delays senescence by maintaining epidermal adhesion. *Aging (Albany NY)*. 9(11), 2302–2315. Available from: doi: 10.18632/aging.101322 [viewed 25.06.2019.].

59. Kadono, S., Manaka, I., Kawashima, M. 2001. The role of the epidermal endothelin cascade in the hyperpigmentation mechanism of lentigo senilis. *J Invest Dermatol.*116, 571–577.

60. Kahn, B. B., Flier, J. S. 2000. Obesity and insulin resistance. *J. Clin. Invest*. 106, 473– 481.

61. Kapahi, P., Boulton, M. E., Kirkwood, T. B. 1999. A positive correlation between mammalian life span and cellular resistance to stress. *Free Radic Biol Med*. 26(5–6), 495–500.

62. Kligman, A. M. 1979. Perspectives and problems in cutaneous gerontology. *J Invest Dermatol*. 73, 39–46.

63. Kunlin, J. 2010. Aging and Disease. 2(1), 72–74. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2995895/ [viewed 25.06.2019.].

64. Kvam, E., Tyrrell R. M. 1997. Induction of oxidative DNA base damage in human skin cells by UV and near-visible radiation. Carcinogenesis. 18(12), 2379–2384.

65. Lavker, R. M., Kligman, A. 1988. Chronic heliodermatitis: a morphologic evaluation of chronic actinic dermal damage with an emphasis on the role of mast cells. *J Invest Dermatol*. 90, 325–330.

66. Lazzarino, G. et al. 1994. Superoxide radical as both initiator and terminator of lipid peroxidation: the importance of oxidative balance, in Frontiers of Reactive Oxygen Species in Biology and Medicine. *Asada, K., Yoshikawa, T. eds. Elsevier Sciences B.V*. 523–524.

67. Matsuzawa, Y., Funahashi, T., Kihara, S., Shimomura, I. 2004. Adiponectin and metabolic syndrome. Arterioscler. *Thromb. Vasc. Biol.* 24, 29–33.

68. McCord, J. M. 2000. The Evolution of Free Radicals and Oxidative Stress. *Am J Med* 108, 652–659.

69. McKenzie, R. C. 2000. Selenium, ultraviolet radiation, and the skin. *Clinical and Experimental Dermatology* 25(8), 631–636.

70. Meagher, E. A., FitzGerald G. A. 2000. Indices of lipid peroxidation in vivo: strengths and limitations. *Free Radical Biology and Medicine*. 28, 1745–1750.

71. Moore, J. X., Chaudhary, N., Akinyemiju, T. 2017. Metabolic Syndrome Prevalence by Race/Ethnicity and Sex in the United States, National Health and Nutrition Examination Survey, 1988–2012. *Preventing Chronic Disease* 14. Available from: https://www.cdc.gov/pcd/issues/2017/16_0287.htm [viewed 25.06.2019.].

72. Moslen, M. T. 1994. Reactive oxygen species in normal physiology, cell injury, and phagocytosis. *Free Radicals in Diagnostic, Edited by D. Armstrong, Plenum Press, New York.*

73. Mouret, S. et al. 2006. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. Proceedings of the National Academy of Sciences of the United States of America. 103(37), 13765–13770.

74. Mulero, M. et al. 2006. Oxidative stress-related markers and Langerhans cells in a hairless rat model exposed to UV radiation. *J Toxicol Environ Health A.* 69(14), 1371–1385.

75. Napolitano, M., Megna, M., Monfrecola, G. 2015. Insulin Resistance and Skin Diseases. *The Scientific World Journal*. 2015, Article ID 479354, 1–11. Available from: doi: 10.1155/2015/479354 [viewed 25.06.2019.].

76. Nelson, K. K, Melendez, J. A. 2004. Mitochondrial redox control of matrix metalloproteinases. *Free Radic Biol Med*. 37, 768–784.

77. Nestle, F. O., Nickoloff, B. J. 2007. Deepening our understanding of immune sentinels in the skin. *J Clin Invest.* 17, 2382–2385.

78. Pennacchi, P. C. et al. 2015. Glycated reconstructed human skin as a platform to study the pathogenesis of skin aging. *Tissue Eng Part A*. 21(17–18), 2417–2425. Available from: doi: 10.1089/ten.tea.2015.0009 [viewed 25.06.2019.].

79. Pittelkow, M. R., Coffey, R. J., Moses, H. J. 1988. Keratinocytes produce and are regulated by transforming growth factors. *Ann N Y Acad Sci*. 548, 211–224.

80. Puchau, B. et al. 2010. Nail antioxidant trace elements are inversely associated with inflammatory markers in healthy young adults. *Biol Trace Elem Res*. 133, 304–312.

81. Quan, T., He, T., Kang, S. 2004. Solar ultraviolet irradiation reduces collagen in photoaged human skin by blocking transforming growth factor-beta type II receptor. Smad signaling. *Am J Pathol*. 165, 741–751.

82. Quan, T., He, T., Voorhees, J. J. 2001. Ultraviolet irradiation blocks cellular responses to transforming growth factor-beta by down-regulating its type-II receptor and inducing Smad7. *J Biol Chem*. 276, 26349–26356.

83. Quan, T., He, T., Kang, S. 2002. Ultraviolet irradiation alters transforming growth factor-beta/smad pathway in human skin in vivo. *J Invest Dermatol*. 119, 499–506.

84. Rayman, M. P. 2000. The importance of selenium to human health. *Lancet*. 356(15), 233–241.

85. Reaven, G. M. 2011. The metabolic syndrome: time to get off the merry-go-round? *J Intern Med*. 269(2), 127–136. Available from: doi:10.1111/j.1365-2796.2010.02325.x [viewed 25.06.2019.].

86. Reiser, K. 1991. Nonenzymatic glycation of collagen in aging and diabetes. *Proc.Soc.Exp.Biol.Med*. 37, 17–29.

87. Ridley, A. J. et al. 2009. Cellular and sub-cellular responses to UVA in relation to carcinogenesis. *International Journal of Radiation Biology*. 85(3), 177–195.

88. Ring, J. 2016. Atopic dermatitis. Eczema. Springer International Publishing, 224. Available from: https://doi.org/10.1007/978-3-319-22243-1.

89. Seremet, S., Gurel, M. S. 2018. Miscellaneous skin disease and metabolic syndrome. *Clin Dermatol*, 36 (2018), 93–99.

90. Shi, Y., Massague, J. 2003. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell*. 113, 685–700.

91. Shigenaga, M. K., Hagen, T. M., Ames, B. N. 1994. Oxidative damage and mitochondrial decay in aging. *Proceedings of the National Academy of Sciences of the United States of America*. 91(23), 10771–10778.

92. Shimokawa, I. & Lucas, S. T. 2010. Dietary restriction and aging in rodents: a current view on its molecular mechanisms. *Aging Dis*, 1(2), 89–107.

93. Soory, M. 2012. Nutritional antioxidants and their applications in cardiometabolic diseases. *Infect. Disord. Drug Targets.* 12, 388–401. Available from: doi: 10.2174/187152612804142233 [viewed 25.06.2019.].

94. Teibe, U. 2007. Bioloģiskā statistika (Eng. Biological statistics). Rīga: Latvijas Universitāte, 156.

95. Teibe, U., Berķis, U. 2001. Varbūtību teorijas un matemātiskās statistikas elementi medicīnas studentiem (Eng. Elements of probability theory and mathematical statistics for medical students). Rīga: Rīgas Stradiņa Universitāte, 88.

96. Uysal, K. T. et al. 1997. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature*. 389, 610–614.

97. Van Heemst, D. 2010. Insulin, IGF-1, and longevity. *Aging Dis*. 1(2), 147–157.

98. Van Waateringe, R. P. et al. 2017. Skin autofluorescence, a non-invasive biomarker for advanced glycation end products, is associated with the metabolic syndrome and its individual components. *Diabetol Metab Syndr*. 9, 42. Available from: doi: 10.1186/s13098-017-0241-1 [viewed 25.06.2019.].

99. Wang, A. S, Dreesen, O. 2018. Biomarkers of Cellular Senescence and Skin Aging. *Front Genet*. 247(9), 23. Available from: doi:10.3389/fgene.2018.00247 [viewed 25.06.2019.].

100. Wei, Y. H. et al. 2001. Oxidative stress in human aging and mitochondrial disease-consequences of defective mitochondrial respiration and impaired antioxidant enzyme system. *Chin J Physiol*. 44, 1–11.

101. Wiseman, H., Halliwell, B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. The Biochemical Journal. 313(1), 17–29.

102. Wlaschek, M. et al. 2001. Solar UV irradiation and dermal photoaging. *J Photochem Photobiol B*. 63(1-3), 41–51.

103. Williams, J. A. et al. 1983. Variation in the activities of glutathione-peroxidase and superoxide-dismutase and in the concentration of copper in the blood in various breed crosses of sheep. *Research in veterinary science*. 34, 253−256.

104. Xu, Y., Shao, Y., Voorhees, J. J. 2006. Oxidative inhibition of receptor-type protein-tyrosine phosphatase kappa by ultraviolet irradiation activates the epidermal growth factor receptor in human keratinocytes. *J Biol Chem*. 281, 27389–27397.

105. Zegarska, B. 2010. Histopathological lesions in different types of skin aging. *Acta Dermatoven APA*. 19.

106. Zeng, Y., Shen, K. 2010. Resilience significantly contributes to exceptional longevity. *Current gerontology and geriatrics research*. 525693.

107. Zhou, S. S. et al. 2012. The skin function: a factor of the anti-metabolic syndrome. *Diabetol Metab Syndr*. 4(1), 15. Available from: doi: 10.1186/1758-5996-4-15 [viewed 25.06.2019.].

108. Zhou, S. S., Zhou, Y. M. 2011. Dietary methyl-consuming compounds and metabolic syndrome. *Hypertens Res*. 34, 1239–1245.

109. Zuber, T. J. 1994. Skin biopsy techniques: when and how to perform a punch biopsy. *Consultant*. 34, 1467–1470.

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