SKIN CHANGES AND PECULIARITIES IN PATIENTS WITH METABOLIC SYNDROME

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Approximately 20-25 % of the World's adult population aged 40 - 75 years have metabolic syndrome (MS). MS is one of the most widespread risk factors for: diabetes mellitus, cardiovascular disorders and skin disorders MS, due to the oxidative stress, supports a chronic inflammatory reaction in the skin and in the other parts of the body. During oxidative stress the net amount of reactive oxygen species (ROS) exceeds the antioxidant capacity of the body causing lipid peroxidation, protein oxidation and oxidative DNA damage. The role of oxidative stress in pathogenesis of early skin changes in patients with MS is not clearly defined. The aim of our study was to compare visual skin changes between patients with MS and without it, and to reveal early histological manifestations of MS in the skin. The study was conducted at the *Clinic of Aesthetic Dermatology*, Riga, Latvia. 50 patients aged 45-55 were enrolled. The research consisted of a clinical examination, biochemical testing and *Punch* biopsies. Tissue samples were stained with haematoxylin-eosin, Masson's Trichrome and also immunohistochemically stainining with antibodies to CD34, CD117, CD20, CD8 and bcl-2. CD1a positive Langerhans cells were evaluated in 3 fields of vision. Data was analyzed with Microsoft Excel 2010 software. Gender ratio was women: men= 1.6:1. Histological changes in the skin of patients with MS were: hyperkeratosis, acanthosis, dermal fibrosis, elastosis and mild thickening of the stratum spinosum. Infiltrates around blood vessels were composed of T lymphocytes (CD3+). More significant expression and accumulation of apoptotic protein Bcl2 in skin of patients with MS in comparison to patients without MS was noted. Initial skin histological changes in MS are dermal elastosis, thickening of stratum spinosum and acanthosis. Mild T lymphocytic infiltration around capillaries possibly reflects the inflammatory component of MS. Increased accumulation of bcl2 anti-apoptotic protein in epidermis was more significantly expressed in patients with MS.

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Introduction

Approximately 20-25 % of the World's adult population aged 40 - 75 years have metabolic syndrome and they are twice as likely to die from and three times as likely to have a heart attack or a stroke in comparison to those without MS. According to the International Diabetes Federation definition (Alberti et al., 2006) a person can be characterized as having the metabolic syndrome if he has central obesity (waist circumference for women ≥ 80 cm, man ≥ 94 cm) and additionally two of any of the

following factors: raised triglycerides, reduced HDL cholesterol, raised blood pressure, raised plasma glucose. The cluster of cardiovascular disease (CVD) risk factors that defy the metabolic syndrome is now considered to be the driving force for the uprising CVD epidemic (Hanefeld et al., 2010). MS maintains a chronic inflammatory reaction throughout the body due to the presence of oxidative stress. Application of early treatment strategies may result in control of symptoms and complications of the MS. However, severe sequelae such as myocardial infarction, cerebral stroke and disability still remain very high (Ma et al., 2013).

Human skin is the largest organ and besides its obvious aesthetic functions it is also responsible for thermoregulation, sensation, evaporation, absorption, water resistance and protective and immunological functions that are violated by the presence of MS (Zhou et al., 2012). MS, due to the oxidative stress, supports a chronic inflammatory reaction in the skin and in the other parts of the body (Roberts et al., 2009). Oxidative stress is a condition of oxidant/antioxidant imbalance, in which the net amount of reactive oxygen species (ROS) exceeds the antioxidant capacity of the body. Excessive ROS can react with cellular macromolecules and cause lipid peroxidation, protein oxidation and oxidative DNA damage (Grattagliano et al., 2008). The skin is a major target for a toxic assault by a broad spectrum of physical (i.e. UV radiation) and chemical (xenobiotic) agents that are capable of altering its structure and function (Cao, 2009; Gauglitz, 2009). Many environmental pollutants are either oxidants or substances that directly or indirectly catalyze the production of ROS. ROS are short-lived entities that are continuously generated at low levels during the course of normal aerobic metabolism (Bickers, & Athar, 2006). According to Gosenca et al. (2011) and Grattagliano et al. (2008) ROS are believed to activate signalling pathways that induce proliferation and are responsible for survival of cells, and (Bickers, & Athar, 2006) can also alter apoptotic pathways that may be involved in the pathogenesis of a number of skin disorders, including photosensitivity diseases and some types of cutaneous malignancy. ROS act largely by driving several important molecular pathways that play important roles in diverse pathologic processes including ischemia–reperfusion injury, atherosclerosis, and inflammatory responses (Bickers, & Athar, 2006). Neutrophils contain catalase and glutathione peroxidase (GP) for protection against free radical (ROS) damage. These enzymes catalyze decomposition of hydroperoxides without formation of toxic by-products, thus H_2O_2 is eliminated by catalase via reduction to H_2O (Keller et al., 2006). Antioxidants attenuate the damaging effects of ROS and can impair and/or reverse many of the events that contribute to epidermal toxicity and disease (Higgins et al., 2008).

Zhou et al. (2012) writes "The skin is a major component of the body's antioxidant defense system, primarily through its xenobiotic/ drug biotransformation system, reactive oxygen species - scavenging system and an excretory system mediated via sweat and sebaceous glands. In the circumstances of chronic excess energy intake and inhibition of sebum secretion, excess lipids are only stored as adipose tissue, whereas excess cholesterol can accumulate in the arterial wall."

Inhibition of sebum secretion increases the levels of circulating lipids and cholesterol, and consequently the risk of dyslipidemia and MS (Zech et al., 1983; Bershad et al., 1985; Rodondi et al., 2002). Originally, skin diseases were associated with isolated components of the metabolic syndrome, e.g. obesity and diabetes was associated with acne in polycystic ovary syndrome (Rodondi et al., 2002; Trouba et al., 2002). *Acne* is a common skin condition closely related to increased sebum production (Janiczek-Dolphin, 2010). Increased sebum secretion may be a compensatory reaction to remove excessive lipids and cholesterol from the body. In fact, numerous studies have found that long- term medication- induced inhibition of sebum secretion can lead to significant increases in the levels of lipids and cholesterol in the circulation, and consequently to an increased risk of MS (Petersen et al.,

2000; Roberts et al., 2009; Zhou et al., 2012. *Acanthosis nigricans*, a hyperplastic skin lesion is associated with insulin resistance, obesity, MS and type 2 diabetes mellitus (Higgins et al., 2008; Ice et al., 2009). Recently, several additional skin diseases, e.g. psoriasis, were also associated with MS (Svacina et al., 2008), where perhaps common pathogenic mechanisms are present (Trouba, 2002; Svacina, 2008). Therefore, evaluation of early cutaneous manifestations of the metabolic syndrome is relevant due to the severe systemic sequelae associated with progression of the metabolic syndrome.

Aim of the study

Evaluate the relationship between signs of skin aging and MS syndrome, and compare dermatological peculiarities in patients with MS to those who do not have MS.

Materials and methods

Altogether 50 patients with a I-II skin phototype (Heather, 2012) were included in a study conducted at the *"Clinic of Aesthetic Dermatology"* in Riga, Latvia. Permission to conduct the study was provided by the Riga Stradins University committee No. E-9 (2). Generally, 27 patients were clinically and biochemically diagnosed with MS based on the IDF (2006) criteria (Alberti et al., 2006):

- Essential major criteria:
    - Central obesity with a waist circumference of 80 cm (women) and 94 cm (men),
- Additional 2 minor criteria out of the following:
    - Raised triglycerides >1.7 mmol/l,
    - Reduced HDL-cholesterol <1.3 mmol/l (women) and <1.0 mmol/l (men),
    - Raised blood pressure ≥130 mmHg (systolic) or/and ≥85 mmHg (diastolic),
    - Raised fasting plasma glucose ≥5.6 mmol/l.

In the course of the study several parameters were evaluated in all patients:

- Clinical examination:
    - measurement of the arterial blood pressure,
    - measurement of the waist circumference,
- Instrumental examination of the skin surface with "ARAMO SG" – Skin Analyzer (Aramhuvis Co) with 3 different lenses (1x; 10x; 60x) for determining the moisture and sebum level, evenness of the surface, pore size, spot diagnostics, wrinkle evaluation.
- Biochemical testing: total cholesterol level (TH), fasting plasma glucose, high density lipoproteins (HDL), low density lipoproteins (LDL) levels.
- Full- thickness circular 4 mm *Punch* biopsies were obtained from the dorsal surface of the palm. Specimens were stained with haematoxylin-eosin and with Masson's trichrome. Immunohistochemical antibody staining was conducted with DakoCytomation EnVision system (Denmark) for the following antigens: CD 34, CD 117, CD20, CD8 and bcl-2. Subsequently, capillaries and CD117, CD3, CD20, CD8 positive cells and fibres were quantified per 1 mm^2. Axiostar Plus microscope with a ruler was used to measure adipocyte

dimensions under 40x and 100x magnification. Bcl-2 expression was evaluated per 100 cells. CD1a positive Langerhans cells were evaluated in 3 fields of vision. The average quantity of CD1a positive Langerhans cells was calculated under a 400x magnification.

Data were analyzed using Microsoft Excel 2010.

Results

In the group of patients with MS mean waist circumference for both genders was 95 cm. Average body mass index - 25.5 kg/m². Women: men ratio was 1. 6:1. Results of histological examination of skin in both groups of patients are shown in Table 1.

Table 1: Comparison of histological early skin changes in patients with MS and without

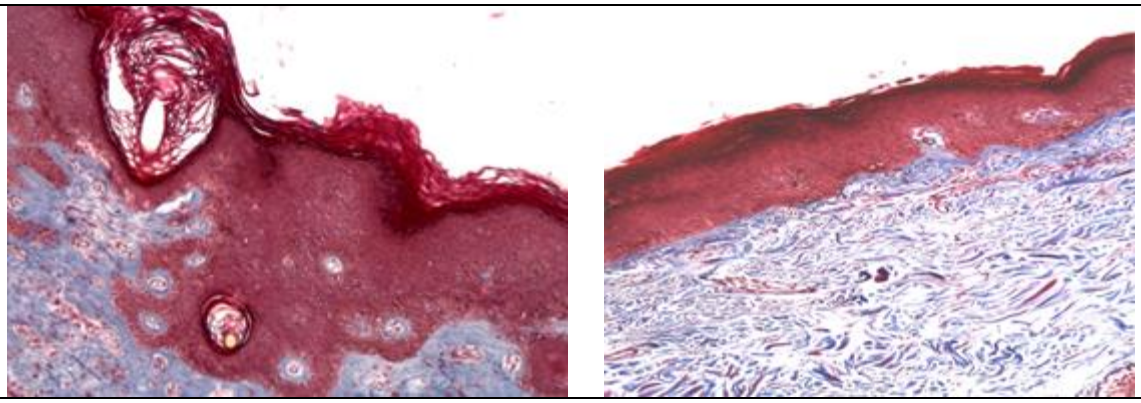
Histological pattern	Patients with MS (N=27)	Patients without MS (N=23)
Thickness of epidermis	0.71 mm	0.56 mm
Thickness of *stratum corneum*	0.18 mm	0.16 mm
Stratum spinosum (number of rows)	9	7.5
Stratum granulare (number of rows)	2.25	1.75
Thickening of basal membrane	N=14	N=11
Average size of adipocytes	0.33 mm	0.3 mm
Dermal *elastosis*	N=9	N=3
Perivascular lymphocytic infiltration	N=26	N=13
Narrowing of dermal capillaries	N=26	N=13

Source: Authors

In the group that consisted of patients with metabolic syndrome the following histological findings in comparison to the group without MS were documented: a*canthosis* or thickening of the *stratum spinosum* was variable, the dermal-epidermal junction (DEJ) was flattened, adipocytes in hypodermis were increased in size, collagen bundles were thickened. In patients with MS CD 34 positive endothelial cells composed up to 4.5 capillaries per 1mm^2, in patients without MS 3.1 capillaries per 1 mm^2, respectively. The epidermal basal membrane was thickened in MS cases. CD117 positive mast cells were evenly distributed in the dermis and between adipocytes (8 CD117 positive cells per 1 mm^2 on average). Patients with MS had *elastosis* (Figure 1) in superficial dermis. Immunoreactivity to bcl-2 protein had a patchy pattern in basal cells with a mean quantity of 39.1 per 100 basal cells in MS and 6.4 in persons without it (Figure 2). Average thickness of epidermis was 0.71 mm (patients with MS) and 0.46 mm (without MS) Thickness of *stratum spinosum* was 0.48 mm (patients with MS) and 0.25 mm (without MS). Thickness of *stratum corneum* was 0.18 mm (patients with MS) and 0.16 mm (without MS). Significant accumulation of DCs around a cluster of inflammatory cells, *acanthosis* and

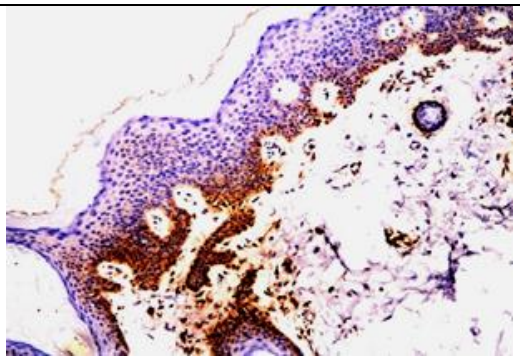
hyperkeratosis was observed in patients with MS. Variable content of *Birbeck* granules in patients with MS was evaluated, as well as, in some cases migration of CD 1a+ cells into pappilary dermis. Enlargement of adipocytes up to 0.13 mm and fibrosis of deep vessels has been revealed.

Figure 1: Early skin lesions in metabolic syndrome patients: *hyperkeratosis* (left), expressed dermal *elastosis* (right), (100 x, Masson's trichrome)



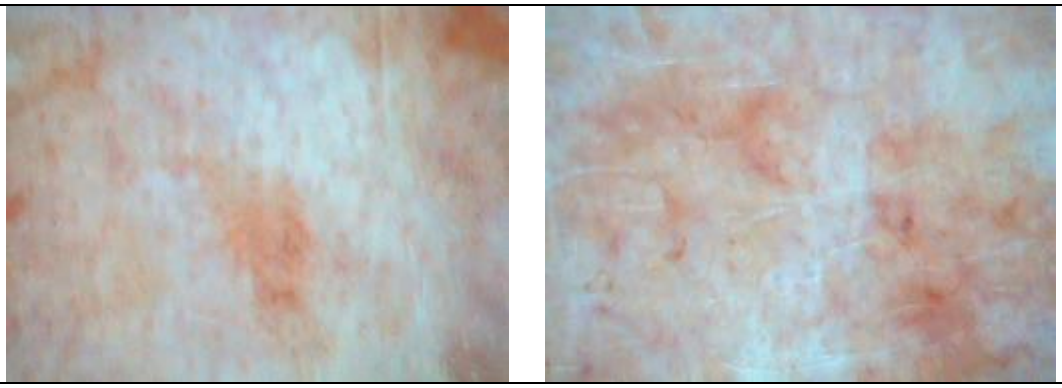
Source: Authors

Figure 2: Overexpression of apoptotic protein (bcl-2) in person with metabolic syndrome (En Vision method, DakoCytomation, 100 x)



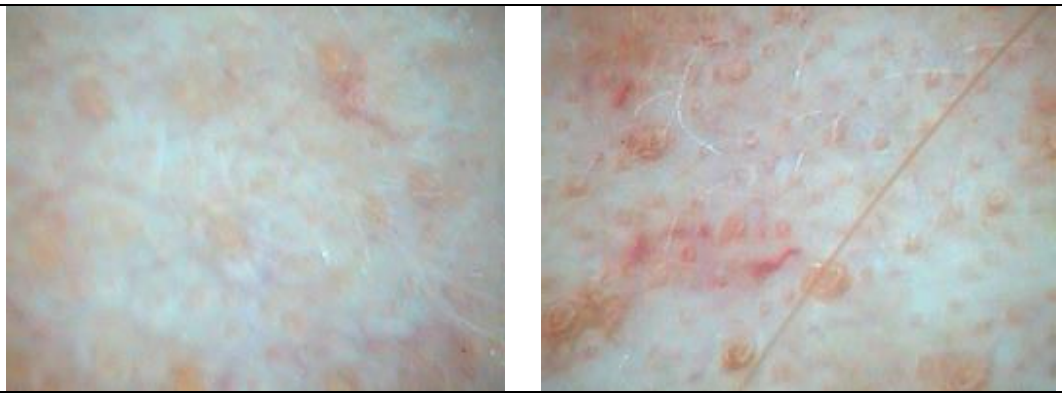
Source: Authors

Figure 3: Dermascopic features of skin dyspigmentation; skin without MS (left), skin with MS (right) (Skin Analyzer, Aramhuvis Co, 60x)



Source: Authors

Figure 4: Dermascopic findings of telangiectasia; Skin without MS (left), skin with MS (right)



Source: Authors

Regarding skin dermoscopy, patients with MS had a more significant visible dyspigmentation, such as hyperpigmentation and accumulation of a pigment (Figure 3), a more significant telangiectasia was observed in patients with MS (Figure 4).

Discussion

The most typical pathological visual features in patients with MS were dyspigmentations and teleangiectasia. According to Wang et al (2001) this is a marker of the involvement of blood vessels and melanocytes. Microscopic examinations of *Punch* biopsies in MS individuals revealed both epidermal and dermal changes. Hyperkeratosis and *acanthosis* were characteristic to MS. Endothelial cell marker CD 34 allowed to visualize narrowed capillaries with the thickening of their wall (Pusztaszeri et al., 2006). There was a perivascular accumulation of T lymphocytes that is a manifestation of oxidative stress in patients with MS (Filip et al., 2012).

Chronic inflammation due to oxidative stress in patients with MS may impact skin changes and its biological functions. The skin's antioxidative and excretory functions may be one of the major components of the body's antioxidant defence system and play an important role against the development of MS sequelae (Gosenca, 2011; Zhou et al., 2012). We speculate that the increased accumulation of bcl-2 protein in the epidermis is due to the plausible constriction of dermal capillaries that can lead to hypoxia (Zhang et al., 2010) and could influence the proliferation and exfoliation of keratinocytes. Bcl-2 is a well known substance that suppresses apoptosis (Adams et al, 2007), thus causing accumulation of senescent and poorly functioning cells and slowing the regeneration processes of the skin (Cao et al., 2009).

Dermal *elastosis* is more pronounced in skin affected by MS this is possibly due to the persistent hypoxia caused by vascular injury (Sellheyer, 2003). Oxidative stress has a pivotal role in the pathophysiology of vascular injury, especially of endothelial dysfunction (Kraemer de Aguiar et al., 2008).

Although the basic functions of skin have been well documented, the role of skin in systemic metabolic disorders is far from clear. Therefore, further studies are required for deep understanding of early manifestation of MS in skin (Zhou et al., 2012), as well as, its impact on biological and aesthetic function of the human skin.

Conclusion

Pivotal visual differences in metabolic syndrome affected skin are expressed dyspigmentations and telangiectasia. Initial histological changes of skin in patients with MS are: dermal *elastosis*, thickening of *stratum spinosum* and basal membrane, *acanthosis*, as well as, mild T lymphocytic infiltration around capillaries. Bcl-2 anti-apoptotic protein is accumulated in the epidermis more significantly in participants with metabolic syndrome.

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