Serum Levels of 8-hydroxy-deoxyguanosine under the Chemicals Influence

LUCIA DINU[1], CORINA DANIELA ENE (NICOLAE)[2], ILINCA NICOLAE[3]*, MIRCEA TAMPA[2,3], CLARA MATEI[2], SIMONA ROXANA GEORGESCU[2,3]

[1] MedLife Clinic, 1 Hans Christian Andersen Str., 040289, Bucharest, Romania

[2] University of Medicine and Pharmacy Carol Davila, 8 Eroii Sanitari Blvd., 060474, Bucharest, Romania

[3] Victor Babes Hospital of Clinical and Infectious Diseases, 281 Mihai Bravu Blvd, 030303, Bucharest, Romania

Many environmental factors such as chemicals (plastics, aditives, solvents, pesticides), smoking, ionizing or UV radiations, infections, modulate cellular processes that often result in disruption of redox homeostasis, leading to accumulation of reactive oxygen species (ROS). We conducted an experimental model with the purpose to evaluate a possible role of redox imbalance, assessed by serum levels of 8-hydroxy-deoxyguanosine (8-OhdG) and total antioxidant status (TAS), to induce pruritus, in patients exposed to chemicals from occupational and non-occupational clothing. Our results are congruent with several recent studies that allowed oxidative stress as a possible inducer factor of pruritus.

Keywords: oxidative stress, 8-hydroxy-deoxyguanosine, total antioxidant status, chemicals, urticaria

Protective occupation-specific uniforms and equipment and non-occupational clothing involve the exposure to a variety of allergens implicated in the occurence of some dermatological diseases. Of these, contact dermatitis occupy a central position followed by pruritus and contact urticaria. About 40000 of dyes are used in the textile industry. They involve over 9000 different chemical structures (1).

A large variety of materials are used for the production of textiles, some of them having animal (wool, silk), vegetal (cotton) or mineral source. Textile industry also use synthetic fibers (polyester, acrilic, nylon, polypropylene).

Along with the use of textiles, manufacture of clothing, including protective equipment, requires the use of natural or synthetic leather, latex and metals. The latex based products such as surgical gloves, elastic bandages and prints with aesthetic purpose, involve the use of other additives, such as vulcanizing agents, accelerators (thiurams) activators, blockers, anti-oxidants (in order to reduce the degradation of latex), preservatives, dyes, stabilizers. After the process of vulcanization results a stronger and more elastic latex [2].

All these compounds may be responsible for the induction of allergic skin reactions.

The most used metals are nickel, cromium and cobalt. Dyes, natural or artificial, may also induce an inflammatory process.

Using fabric finishing treatments (table 1) in order to improve the clothing properties (resistance to creasing, flame-retardant, water-repelent, antimicrobial effect) also contribute to the occurrence of the skin manifestations. Among the chemicals used, formaldehyde ustilized in the textile and leather industry, is most commonly responsible for the appearance of the allergic reactions. Formaldehyde is a colorless gas with a characteristic pungent odor, easily soluble in water, ethanol and diethyl ether, very commonly used in the textile industry to obtain a permanent press finish [1].

Organic solvents, such as aliphatic hydrocarbons, used for cleaning the equipment used in the textile and leather industry, also have their contribution in the development of the allergic skin reactions.

Clothing may contain other possible allergens not involved in the production process, such as pollen and insecticides (from storages).

The same compound can generate more than one allergic skin conditions. A variety of factors favour the occurrence of dermatological manifestations. Among them

Agents for water-repellent effect	silicon, fluorocarbon, zirconium emulsions
Antisoiling agents agents	aluminum, titanium oxides, silicon, polymers, fluorocarbon
Antistatic agents	polyethylene glycols, epoxy resins
Antimicrobial agents	quaternary ammonium compounds, tributyltin oxide
Antimildew agents	copper and zirconium compounds and pentachlorophenyl laurate
Softening agents	Silicone compounds, cationic surfactants (quaternary ammonium salts of aminoamide salts)

Table 1
EXAMPLES OF FABRIC FINISHING TREATMENTS [1]

* *email-drnicolaei@yahoo.ro*

are atopic dermatitis, hyperhidrosis and the skin friction in the intertriginous areas, which favour the penetration of allergens from clothing.

The skin has an important sensory function, being the body interface with the external environment and ensuring the tactile, thermal and pain sensitivity. It has the role in integrating into the external environment and in active defense, following the action of external stimuli.

Myelinated nerve endings in the skin, such as Ab and Ad fibers transmit stimuli of touch and other mechanical stimuli and fast conducted pain [3].

Unmyelinated C fibers are specialized in stimuli such as heat, cold, burning, itching and slow transmitted pain [4-7]. C fibers have contact and maintain the connection with keratinocytes, mast cells, Langerhans cells and inflammatory cells [8-15] stimulating skin cells to secrete some neuropeptides [16-18].

Pruritus is the most frequent symptom of dermatological diseases, expecialy of atopic dermatitis and urticaria. Prevalence of acute pruritus in general population is 8.4% [19]. Pruritus originates within the skin free nerve endings and the sensation of itching is transmitted through C fibers to the posterior horn of spinal cord and then to the cerebral cortex via the spinothalamic tract [20]. Itching generates a spinal reflex response, the scratching. A single mechanism does not explain all the causes of pruritus.

The study of mast cells raised a special interest, being the main sourse of histamine and also responsable for the release of other mediators involved in the development of itch, like interleukins, proteasis and TNF-alfa. Mast cell activation leads to the tyrosine phosphorylation and mobilisation of intracelular Ca^{2+}, followed by activation of protein kinase C, MAPKs (mitogen activated protein kinases) and nuclear factors such as NF-Kb. According to some studies, MAPK and NF-Kb play an important role in regulating the synthesis of proinflammatory molecules on cellular response, especially TNF-alpha, IL-1 beta and IL-6 [21].

While histamine is stored in mast cells, basophils and keratinocytes, H1 to H4 receptors are present on the sensory nerve fibers and inflammatory cells [22-24].

Recently, a study on mice, reported that, in addition to receptors H1, H3 and H4 receptors on sensory nerve fibers are also involved in itching [25,26]. H4 receptor shows a higher affinity for histamine compared to H1 receptor and appears to be expressed more selectively, being involved in chemotaxis and release of inflammatory mediators by eosinophils, mast cells, monocytes, dendritic cells and T lymphocytes. Furthermore, experimental models of asthma and itching, using H4 receptor antagonists, obtained promising results, which lead to the possibility of new therapeutic options for chronic pruritus [27].

Histamine released from mast cells acts on keratinocytes, leading to increased production and release of nerve growth factor (NGF) [28]. NGF also induces the release of histamine from mast cells and sensitizes various neuroreceptors, including TRPV1 (transient receptor potential V1) [29]. According to studies, histamine regulates substance P release via histamine H3 prejonctional receptors, located on the peripheral sensitive nerve endings [30].

This may have an impact on substance P-dependent diseases, such as atopic dermatitis.

In the course of time, several modalities of pruritus assessment have been proposed, including visual analogue scale (VAS) and the verbal intensity score.

Visual Analogue Scale (VAS), used to assess the intensity of itching, is the oldest graphic rating scale, being used since 1960 [31].

On a horizontal line, 100 mm in length, anchored by verbal itching characterization („absent" and „very intense"), the patient draw a perpendicular line or put an X on the line to indicate the intensity of pruritus [32].

The response to pruritus may present variations according to disease.

While patients with atopic dermatitis resort to an intense scratching, leading to excoriations with or without bleeding, patients with urticaria resort rather to friction, explaining the absence of scratching lesions in their skin.

Urticaria is one of the most common dermatological disease, affecting approximately 15-20% of the population at some moment during their life [33]. The causes of urticaria are multiple, sometimes unidentifiable, despite a detailed anamnesis and paraclinical investigations (idiopathic urticaria).

Textile and footwear industry have a very important potential for generating contact urticaria .

Mechanisms underlying the contact urticaria are divided into two main types: immunologic and non-immunologic. However, there are substances that cause immediate contact reactions, whose mechanisms are not yet kown [34].

Most patients with contact urticaria are type 1 IgE – mediated, in previously sensitized individuals. An important cause is rubber latex. Immunologic contact urticaria is caused by latex itself, unlike allergic contact dermatitis to rubber, due almost exclusively to the antioxidants, vulcanizing agents and chemicals used in the manufacturing process.

Almost any normal subject can develop non-immunologic contact urticaria, because it occurs in individuals non previousely sensitized. Low molecular weight chemicals like aldehydes and weak acids and their salts can cause non-immunologic contact urticaria [35].

In chronic urticaria (hives evolving over 6 weeks) pruritus intensity is correlated with stress, but this relationship is less tight than in other pruritic conditions including psoriasis [36].

Recent researches focuse on the role played by the oxidative stress in the development or progression of certain diseases, including skin diseases.

Reactive oxygen species (ROS) are continuously formed in living cells of aerobic organisms as part of physiological and metabolic processes and other biochemical reactions. ROS have endogenous physiological functions, but due to their reactivity can lead to oxidation of protein, lipid cell membranes and cellular DNA [37].

Exogenous factors such as certain chemicals (especially carcinogenic), infections (bacterial, viral etc.), smoking, ionizing radiation or UV can produce, under certain conditions, oxygen free radicals [38].

Under normal conditions, ROS generation is a process of maintaining physiological homeostasis redox processes of cells, providing aerobic cells the ability to adapt to the extracellular changes.

Under physiological conditions, reactive oxygen and nitrogen species produced in human body are inactivated by antioxidant defense systems. Of these, intracellular antioxidant enzyme systems (superoxide dismutases, glutathione peroxidases, catalase, glutathione S-transferase, glutathione reductase, glucose-6-phosphate dehydrogenase) and non-enzymatic extracellular compounds (albumin, ceruloplasmin, ferritin, transferrin, reduced glutathione, 10 ubiquinone, uric acid, bilirubin, lipoic acid, methionine, ascorbic acid, alpha tocopherol, beta carotene, selenium, zinc) play an active role. A variety of kits have been developed and marked for the quantitative

determination of antioxidants present in biological products.

The reality seems to be that, despite the antioxidant defense mechanisms mentioned above, oxidative damage remain an inevitable result of the existence of aerobic existence.

These free radicals are highly reactive with biological macromolecules, generating lipid peroxides, inactivating proteins and causing DNA distortion. (production of 8-hydroxy- deoxyguanosine and breaking chains) [39].

A variety of markers were invoked to assess oxidative stress, but in most cases, they didn't show clinical significance. In the late 80's some studies demonstrated that the 8-hydroxy- deoxyguanosine (8-OHdG) level is increased in the serum or urine of patients with diseases associated with oxidative stress [40]. Recently, numerous studies examined the level of 8-OHdG in human organs, urine and in DNA of leukocyte, in relation to oxidative stress, diet, aging and cancer incidence. Follow these studies, it was concluded that 8-OHdG is an important biomarker of oxidative stress [41,42], of the aging process, including degenerative diseases [43,44] and of carcinogenesis due to free radicals [45,46]. In addition, 8-OHdG is also considered a biological marker of lifestyle and diet effect [47-49].

The estimation of antioxidant potential of human serum can be done quickly and safely by determining total antioxidant status.

Currently we assist to a continuous increase of the need for identifying new ways to monitor health in order to reduce diagnostic errors and to draw coordinates of new possible therapeutic options. This experimental model aims to evaluate a possible role of 8-hydroxy-deoxyguanosine and total antioxidant status in the pathogenesis of pruritus.

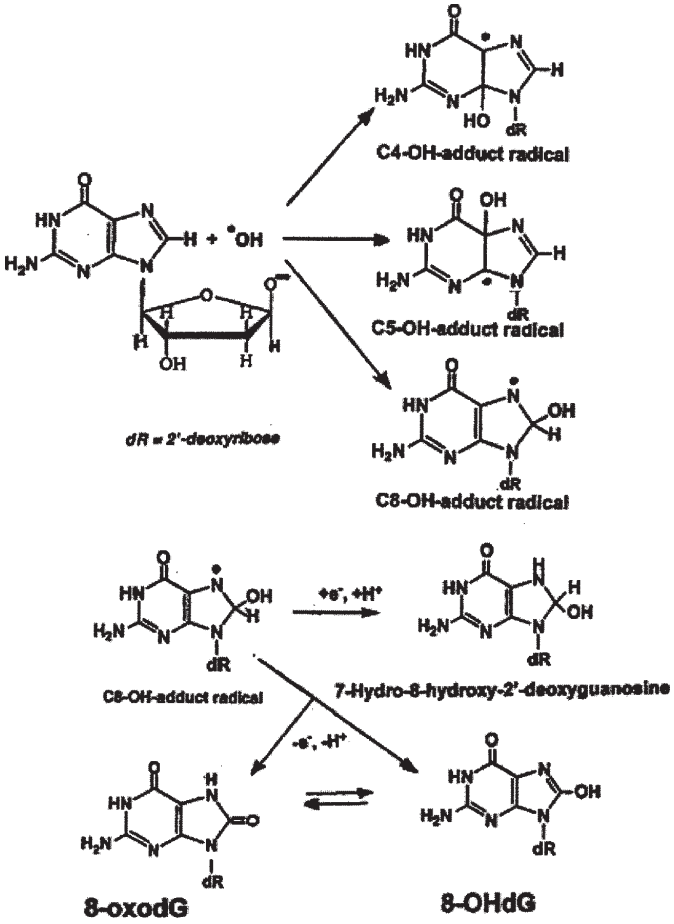

Fig. 1. Reaction of 2'-deoxyguanosine with hydroxyl radicals, radical adducts followed by reduction to 7-hydro-8-hydroxy-2'-deoxyquanosine, and by oxidation to 8-hydroxy-2'-deoxyguanosine (8-OHdG) or its tautomer 8-oxo-7-hydro-2'-deocyguanosine (8-oxodG)

Experimental part

Materials and methods

We conducted a prospective study, which included 84 subjects aged 18 and over, divided into 3 groups: 27 subjects with cutaneous pruritus, 27 subjects with chronic idiopathic urticaria associating pruritus and 30 healthy volunteers. We used as an experimental model for contact urticaria a group of 27 subjects with chronic idiopathic urticaria. The intensity of pruritus was assessed using visual analogue scale (VAS).

The study was made between march 2010 and December 2013 within the Dermatology Clinic of " Victor Babes" Hospital, Bucharest.

Inclusion criteria

Untreated subjects with cutaneous pruritus and chronic idiopathic urticaria associating pruritus, with adequate nutritional status.

Exclusion criteria

We excluded from the study subjects with urticaria vasculitis, subjects with positive intradermal skin testing to autologous serum (ASST), pruritus and urticaria subjects with known etiology, such as: physical urticaria, cholinergic urticaria, hives and pruritus caused by food allergy, medications, connective tissue, thyroid diseases, malignancies and other itching dermatological skin diseases, subjects who were receving corticosteroids and immunosuppressive therapy. We also excluded pregnant and lactating women and also subjects with other causes of pruritus than those mentioned above (psychogenic, metabolic pruritus and itching as a result of parasites on the skin).

Investigations

At study entry, all the subjects were evaluated clinical and paraclinical (complete blood count, biochemical, serological, immunological, parasitological, bacteriological and allergy tests). Hematologic tests were performed using ABX Pentra 60 automatic analyzer (France) and biochemical/serological determinations were performed using HumaStar Analyzer (Germany) and ELISA system (Austria).

To assess total antioxidant status we used spectrophotometric method. The principle of the method was based on the capacity of human serum antioxidants to inhibit the oxidation of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)). Final product of the reaction is a relatively stable blue-green compound, colorimetrable at a wave length of 600 nm. The assessment of total antioxidant status use the following enzymatic reaction (fig. 2):

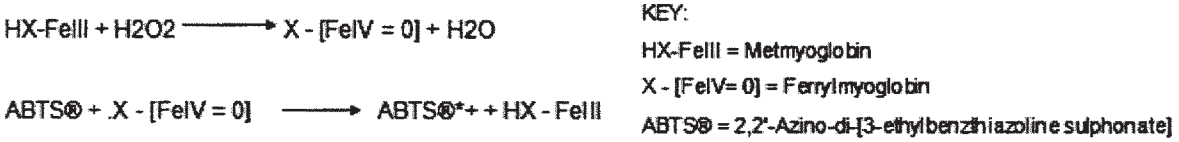

Fig. 2. Enzymatic reaction to achieve the total antioxidant status

Quantitative determination of hs 8OHdG (higly sensitive oxidative DNA adduct 8-hydroxy-2-deoxyguanosine) was made in human serum by ELISA (in vitro enzyme-linked immunosorbent assay) method. The principle of the method was based on the ability of DNA oxidation products of interacting with 3,3,5,5-tetramethylbenzidine (TMB). Method uses DNA specific monoclonal antibody which cross-react with the oxidative degradation products of DNA (8-hydroxy-guanine, 8-hydroxy-guanosine). The final product of the reaction is colorimetrable at a wave length of 450nm. Hs8OHdG determination involves the evaluation of the (fig. 3) compounds.

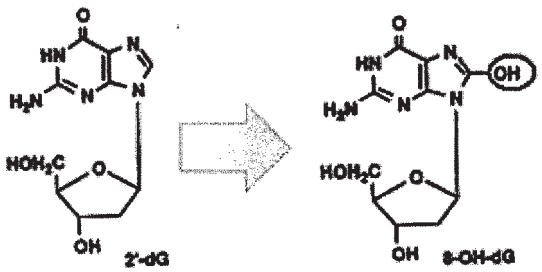

Fig. 3. Generation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) by oxygen radicals

Statistical analysis of data

The clinical and laboratory quantitative data were expressed by the mean and standard deviation. The correlation between the phenomena was expressed by the correlation coefficient r. The value threshold for the statistical significance was 0.05. The processing of data was performed using SPSS software.

The study was approved by the Hospital Committee of Ethics. All subjects consented for the use of their biological samples in research and for teaching.

Variable	Pruritus (n=27)	Urticaria associating pruritus (n=27)	Control (n=30)
Age (years)	36.2±9.4	34.5±8.2	35.6±6.2
Women/men	17/10	15/12	18/12
Smokers/non-smokers	7/20	5/22	3/27
Alcohol consumers/non-alcohol consumers	5/22	2/25	4/26
ASST	Negative	Negative	Negative
Intensity of pruritus (VAS)	6.5±3.6(1)	6.3±3.8(1)	0.3±0.3
Systolic pressure (mmHg)	117.7±3.1	114.3±2.2	116.3±2.9
Diastolic pressure (mmHg)	68.2±0.9	63.2±1.0	64.3±0.8
Hemoglobin (g/dl)	14.1±0.9	13.8±1.1	14.2±0.7
Glucose (mg/dl)	89.4±18.1	83.7±14.1	86.2±14.6
Creatinine (mg/dl)	0.81±0.11	0.79±0.09	0.77±0.06
Uric acid (mg/dl)	3.8±0.5	3.7±0.6	3.6±0.4
AST (U/L)	17.1±3.8	16.4±4.5	15.2±2.2
ALT (U/L)	15.3±4.5	15.7±3.1	15.0±2.7
GGT (U/L)	19.3±3.3	21.2±4.2	17.2±2.6
Bilirubin (mg/dl)	0.21±0.03	0.25±0.05	0.22±0.02
CRP (mg/dl)	0.41±0.28(1)	0.46±0.20(1)	0.17±0.17
Triglycerides (mg/dl)	88.5±9.2	94.3±0.6	79.2±11.3
Cholesterol (mg/dl)	163.9±17.8	159.8±21.4	155.3±16.1
HDL-cholesterol (mg/dl)	42.7±6.2	40.3±5.5	42.1±4.2
LDL-cholesterol (mg/dl)	104.5±8.7	101.4±7.1	96.2±5.3
8-OHdG (ng/ml)	5.2±2.1(1)	8.61±3.1(1,2)	3.46±0.6
TAS (mmol/l)	0.95±0.2(1)	0.87±0.1(1,2)	1.42±0.2

Table 2
CLINICAL AND BIOLOGICAL CHARACTERISTICS OF THE STUDY PARTICIPANTS

1 = p <0.05 statistically significant variation between pruritus versus control and urticaria associating pruritus versus control

2 = p <0.05 statistically significant variation between urticaria associating pruritus versus pruritus

n = number of subjects, AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = gamma-glutamyltransferase, CRP = C-reactive protein, 8-OHdG = 8-hydroxy-deoxyguanosine, TAS = total antioxidant status

Results and disscutions

The investigation of serum levels of 8-hydroxy-deoxyguanosine and total antioxidant status in subjects with pruriginous skin diseases and control subjects, required the selection of representative study groups (table 2). The analyzed groups (pruritus, urticaria associated with pruritus and control group) are similar in terms of age, gender, dietary habits, response to intradermal skin testing to autologous serum (ASST), blood pressure, hemoglobin and serum glucose level, liver and kidney function tests and lipid profile. We obtained statistically significant differences between the groups with itching skin diseases and control group, regarding severity of pruritus, serum levels of 8-hydroxy-deoxyguanosine and total antioxidant status. Compared to control group (0.4 ± 04), VAS score was increased in subjects with pruritus (6.5 ± 3.6, $p < 0.05$) and in subjects with urticaria associating pruritus (6.3 ± 3.8, $p < 0.05$). Compared to control group (3.4 ± 0.6 ng/mL) we obtained elevated serum concentrations of 8-hydroxy-deoxyguanosine in subjects with pruritus (5.2 ± 2.1 ng/mL, $p <0.05$) and in urticaria associating pruritus subjects (8.6 ± 3.1 ng/ml, $p <0.05$) (fig. 4). Compared to control group (1.42 ± 0.27 mmol/L) we obtained significantly lower serum concentrations for total antioxidant status in subjects with pruritus (0.95 ± 0.26 mmol/L, $p < 0.05$) and urticaria associating pruritus subjects (0.87 ± 0.13 mmol/L, $p < 0.05$) (fig. 5)

The analysis of statistical relationships between serum levels of 8-hydroxy-deoxyguanosine and total antioxidant status showed a weak negative correlation without statistical significance in control group ($r = -0.14$, $p>0.05$) and a strong negative correlation with statistical significance in subjects with pruritus ($r = -0.72$, $p< 0.05$) and in subjects with urticaria associating pruritus ($r = -0.88$, $p < 0.05$) (table. 3).

A special attention was given to the relationships between the variations of serum 8-hydroxy-deoxyguanosine levels and VAS score (fig. 6) and between the variations of serum levels of total antioxidant status and VAS score (fig. 7) in subjects with pruriginous skin disorders .

At low levels of VAS score (0.0-3.0) we did not obtained any relationship between the serum levels of 8-hydroxy-deoxyguanosine and VAS score nor in subjects with pruritus ($p> 0.05$), nor in subjects with urticaria associating pruritus ($p > 0.05$) (table 4). We did not obtain a strong correlation between VAS score and the elevated serum levels of 8-hydroxy- deoxyguanosine in subjects included in the study. So, we obtained moderate positive correlations ($r = 0.37$, $p < 0.05$ in subjects with pruritus and $r = 0.43$, $p < 0.05$ in subjects with subjects associating pruritus) between VAS score ranging from 3.1 to 6.0 and the serum levels of 8-hydroxy-deoxyguanosine and strong positive correlations ($r = 0.49$, $p < 0.05$ in subjects with pruritus and $r = 0.61$, $p < 0.05$ in subjects with urticaria associating pruritus) between VAS score ranging from 6.1 to 10.0 and the serum levels of 8-hydroxy-deoxyguanosine (table 4) .

At low levels of VAS score (0.0-3.0), this was not associated with serum levels of antioxidant status in subjects with pruriginous skin diseases. We obtained weak negative correlations between VAS score ranging from 3.1 to 6,0 and the serum level of total antioxidant status in pruritus subjects ($r = -0.19$, $p = 0.05$) and in subjects with urticaria associating pruritus ($r = -0.26$, $p < 0.05$) . We have also obtained strong negative correlation between VAS score ranging from 6.1 to 10.0 and the level of total antioxidant status in subjects with pruritus ($r = -0.85$, $p < 0.05$) and subjects with urticaria associating pruritus ($r = -0.79$, $p < 0.05$) (table 5).

The estimation of the oxidative stress is very useful to monitor the population health status. The disruption of the balance between prooxidant and antioxidant factors and chronic inflammation may increase the susceptibility of the organism to harmful environmental factors [35,50,51].

Table 3
CORRELATIONS BETWEEN THE SERUM LEVELS OF 8OHdG (ng/mL) AND TAS (mmol/L) IN THE STUDIED GROUPS

The study group	r	p
Control (n=30)	-0.14	0.11
Pruritus (n=27)	-0.72	0.00
Urticaria associating pruritus (n=27)	-0.88	0.00

n=number of subjects; r=correlation index; p=statistical significance

Table 4
VARIATIONS OF THE 8-OHDG SERUM LEVELS (ng/mL) ACCORDING TO VAS SCORE IN SUBJECTS WITH PRURIGINOUS SKIN DISEASES

The study group	Variable	VAS		
		0.0-3.0	3.1-6.0	6.1-10.0
Pruritus	n	4	11	12
	8-OHdG	3.8±1.7	5.2±2.2	5.7±1.4
	r	0.07	0.37	0.49
	p	0.69	0.05	0.00
Urticaria associating pruritus	n	3	14	10
	8-OHdG	6.9±1.3	8.2±2.1	9.1±2.6
	r	0.15	0.43	0.61
	p	0.54	0.04	0.00

n= number of subjects; 8-OHdG=8-hidroxi-deoxiguanosine;r=correlation index; p=statistical significance

Table 5
VARIATIONS OF SERUM TAS LEVELS (mmol/L) ACCORDING TO VAS SCORE IN PATIENTS WITH PRURIGINOUS SKIN DISEASES

The study group	Variable	VAS		
		0.0-3.0	3.1-6.0	6.1-10.0
Pruritus	n	4	11	12
	TAS	1.30±0.10	1.02±0.11	0.84±0.16
	r	0.06	-0.19	-0.85
	p	0.35	0.05	0.00
Urticaria associating pruritus	n	3	14	10
	TAS	1.01±0.04	0.89±0.12	0.79±0.11
	r	-0.11	-0.26	-0.79
	p	0.88	0.04	0.00

n= number of subjects; r=correlation index; p=statistical significance;TAS: total antioxidant status

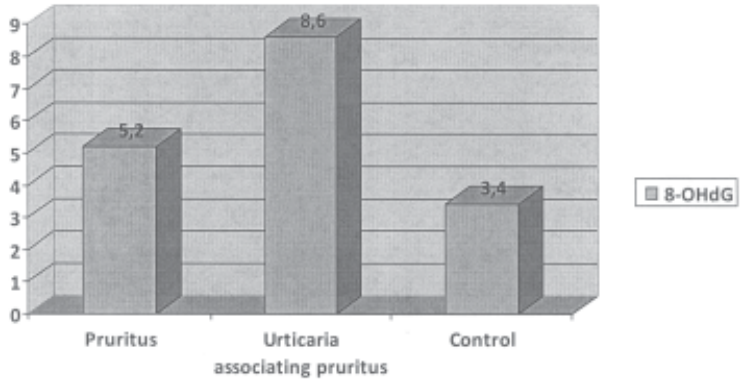

Fig. 4. Graphical representation of 8-hydroxy-deoxyguanosine serum levels (8-OHdG-ng/mL) in subjects with pruritus, urticaria associating pruritus and in the control group ($p < 0.05$)

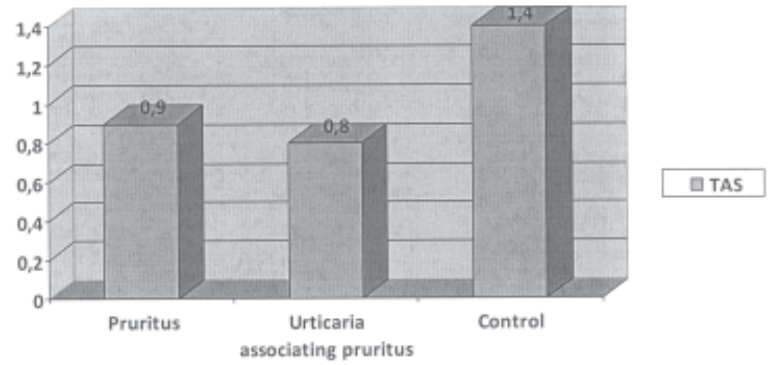

Fig. 5. Graphical representation of serum levels of the total antioxidant status (TAS-mmol/L) in subjects with pruritus, urticaria associating pruritus and in the control group. ($p < 0.05$)

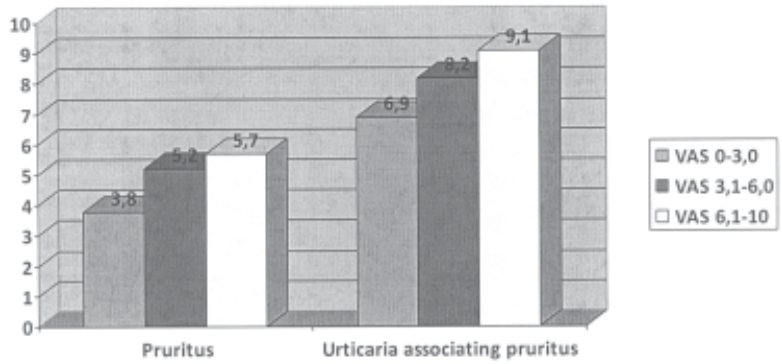

Fig. 6. Graphical representation of the serum levels of 8-OHdG (ng/ml) according to VAS score in subjects with pruritus and those with urticaria associating pruritus ($p < 0.05$)

As previously mentioned, the use of synthetic fibers in the textile industry, the manufacture of latex products (surgical gloves, elastic bandages, prints with aesthetic purpose), the use of additives in the clothing and foowear industries (dyes, organic solvents, preservatives, stabilizers, metals), are an important cause for the appearance of skin allergic conditions (contact dermatitis, contact urticaria, pruritus) [35]. In this study, we accorded a special attention to the ability of some external stimuli to induce itching in individuals exposed to a variety of synthetic materials. Although this study included a small number of subjects, we mention that is the first research that starts from the hypothesis that the redox imbalance, assessed by serum levels of 8OHdG and TAS, could be involved in the pathogenesis of pruritus.

The study of the prooxidant status in individuals with pruriginous skin diseases and control, reported elevated serum levels of 8-OHdG in patients with pruritus, respectively urticaria associating pruritus subjects, versus control group ($p < 0.05$). We found statistically significant differences between subjects with pruritus and subjects with urticaria associating pruritus ($p < 0.05$) (fig. 2). These results draw attention to the fact that a number of external stimuli could be a cause for the appearance of the oxidative stress and a possible trigger for some dermatological diseases associated with pruritus. For sustaining these observations we mention also the positive relationship obtained between serum levels of 8-OhdG and the intensity of pruritus assessed by VAS score (fig. 4).

The analysis of the serum total antioxidant status in subjects with pruriginous skin diseases and control group, showed significant variations between the studied groups. We obtained lower levels of total antioxidant status in subjects with pruritus, respectively urticaria associating pruritus subjects, versus control group ($p<0,05$). Statistically significant differences were obtained between patients with urticaria associating pruritus and those with pruritus ($p < 0.05$). (fig. 3). Subjects included in the study did not use dietary supplements and have not been previously diagnosed with impaired absorption or excretion. These results draw attention to the risk of the onset of pruritus in subjects with low total antioxidant status and also support the possible involvement of antioxidant status of human serum in the pathogenesis of pruritus.

Obtaining negative correlations between the serum level of total antioxidant status and the intensity of pruritus, reconfirms the observation made previously (fig. 5).

Other experimental results presented in this study, which support the possible association between the redox imbalance and the appearance of itching, are the strong negative correlations obtained between serum levels of 8-OHdG and the antioxidant status only in subjects with pruritus (table 2), the strong association between the elevated VAS score and the elevated serum levels of 8-OHdG (table 3) on the one hand and, on the other hand, between elevated VAS score and the low levels of the total antioxidat status (table 4).

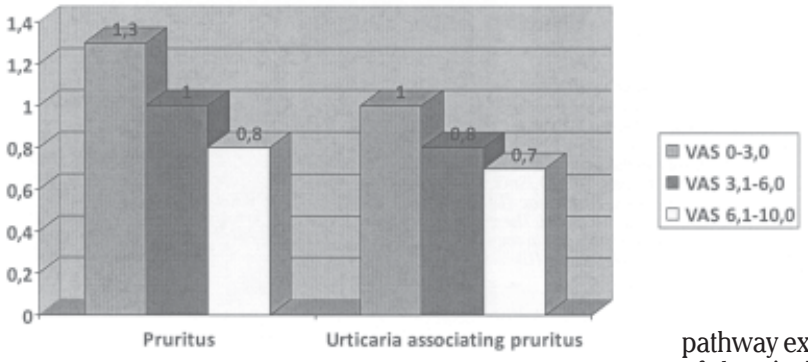

Fig. 7. Graphical representation of serum TAS levels (mmol/L) according to VAS score in subjects with pruritus and those with urticaria associating pruritus ($p < 0.05$)

To analyze this association between the oxidative stress and the appearance of pruritus, we should mention that the total antioxidant status includes a series of compounds with antioxidant activity in human serum, such as: albumin, uric acid, ascorbic acid, alpha tocopherol, bilirubin, transferrin, ferritin, ceruloplasmin, haptoglobin, serum antioxidant enzymes. From the point of view of a quantitative approach, albumin is the most abundant extracellular antioxidant compound, being the main reservoir of sulfhydryl groups (thiol). Uric acid acts as either oxidant or antioxidant, modulating the activity of the endothelial NADPH oxidase. Ascorbate serum levels, similar to serum levels of urate, are modified by the dietary intake or nutritional supplements. The antioxidant effect of alpha tocopherol is controversial because of the disputable epidemiological observations regarding the relationship between the vitamin E and the prevention or progression of certain diseases related to oxidative stress.

The inflammation tests and iron stores values were comparable in the studied groups and did not affect the total antioxidant status. The activity of the serum antioxidant enzymes (superoxide dismutase, glutathione peroxidase) are poorly represented in the extracellular compartment. Thus, determining sulfhydryl groups may be an important criterion to analyze the association between pruritus and reduced antioxidant capacity of human serum. To support this hypothesis, we mention that glutathione is the most abundant intracellular thiol. Although extracellular glutathione levels are relatively low, it is possible that the turnover of glutathione to be altered in certain pathological conditions. Under normal conditions, the cellular level of glutathione is regulated by three major mechanism: one that regulates the synthesis, one that controls the distribution between intracellular and extracellular compartments and another one which performs the glutathione degradation in extracellular space. Extracellular levels of glutathione are significantly reduced in patients with pruriginous skin diseases (unpublished results) in response to oxidative stress, to the inflammatory cytokine stimulation and the presence of transition metals.

Identifying the cellular and molecular mechanisms that explain the appearance of pruritus is a major clinical challenge [52-54]. Our results are congruent with several recent studies that allowed oxidative stress as a possible inducer factor of itching. By intradermal injection of hydrogen peroxide or tertiary-butylhydroperoxide in mice, the researchers observed the pruritus induction by activating TRPA1 (transient receptor potential ankyrin subtype 1). This signaling pathway involves beta and gamma G proteins and is independent of histamine. By administration of some antioxidants (N - acetyl - L - cysteine) or Trolox (a water soluble vitamin E analogue) the researchers obtained the attenuation of itching [52]. The onset of pruritus by TRPA1 activation of the signaling pathway explain the association of pruritus with a number of chronic diseases mediated by oxidative stress. A number of modulators that explain the etiopathogenesis of pruritus were identified: capsacin (transient receptor potential TRPV1 - vanilloid1), bradykinin (B1 , B2), oxidants (TRPA1), histamine (H1, H4), endogenous serine proteases (PAR1 , PAR2 , PAR3), serotonin (5HT2, 5HT3), neuropeptides (NK1, NK2) [53].

Conclusions

Our findings suggest that oxidative stress may be a new mechanism for pruritus, although further investigations are needed to sustain this observation. Combating the oxidative stress may lead to the development of new effective therapies for pruritus.

Acknowledgement: All authors have equally contributed to this work - Conceived and designed the experiments; performed the experiments; analyzed the data; wrote the paper; Conflicts of interests: none declared.

References

1. GREENBERG M. I. Textile Manufacturing Industry. In: GREENBERG M. I., HAMILTON J. R., PHILLIPS S.D., McCLUSKEY G., J. Occupational, Industrial, and Environmental Toxicology. 2nd Edition. Philadelphia: Mosby, 2003:574-581.
2. PRASANNA A. Latex Allergy – Review Article. The Indian Anaesthetists' Forum – (www.theiaforum.org) Online ISSN 0973-0311 2005 (3) supl.
3. JULIUS D, BASBAUM AI. Molecular mechanisms of nociception. Nature. 2001;**413**:203–210.
4. CHUNG MK, LEE H, CATERINA MJ. Warm temperatures activate TRPV4 in mouse 308 keratinocytes. J Biol Chem. 2003;**278**:32037–32046.
5. CHUNG MK, LEE H, MIZUNO A, et al. TRPV3 and TRPV4 mediate warmth-evoked currents in primary mouse keratinocytes. J Biol Chem. 2004;**279**:21569–1575.
6. IKOMA A, STEINHOFF M, STÄNDER S, et al Neurobiology of pruritus. Nat Rev Neurosci. 2006;**7**:535–547.
7. STÄNDER S, SCHMELZ M. Chronic itch and pain – similarities and differences. Eur J Pain. 2006;**10**:473–478.
8. BIGLIARDI PL, STAMMER H, JOST G, RUFLI T, BÜCHNER S, BIGLIARDI-QI M. Treatment of pruritus with topically applied opiate receptor antagonist. J Am Acad Dermatol. 2007; **56**:979–988.
9. CHATEAU Y, MISERY L Connections between nerve endings and epidermal cells: are they synapses? Exp Dermatol. 2004;**13**:2–4.
10. GAUDILLERE A, MISERY L, SOUCHIER C, et al. Intimate associations between PGP9.5-positive nerve fibres and Langerhans cells. Br J Dermatol. 1996;**135**:343–344.
11. HARA M, TOYODA M, YAAR M, et al. Innervation of melanocytes in human skin. J Exp Med. 1996;**184**:1385–1395.
12. HILLIGES M, WANG L, JOHANSSON O. Ultrastructural evidence for nerve fibers within all vital layers of the human epidermis. J Invest Dermatol. 1995;**104**:134–137.
13. HOSOI J, MURPHY GF, EGAN CL, et al. Regulation of Langerhans cell function by nerves containing calcitoningene-related peptide. Nature. 1993;**363**:159–163.

14. SINGH LK, PANG X, ALEXACOS N, et al. Acute immobilization stress triggers skin mast cell degranulation via corticotropin releasing hormone, neurotensin, and substance P:
A link to neurogenic skin disorders. Brain Behav Immun. 1999;**13**:225–239.
15. WIESNER-MENZEL L, SCHULZ B, VAKILZADEH F, et al. Electron microscopal evidence for a direct contact between nerve fibres and mast cells. Acta Derm Venereol. 1981;**61**:465–469.
16. YAPING E, GOLDEN SC, SHALITA AR, et al. Neuropeptide (calcitonin gene-related peptide) induction of nitric oxide in human keratinocytes in vitro. J Invest Dermatol. 2006;**126**: 1994–2001.
17. QUINLAN KL, SONG IS, NAIK SM, et al. VCAM-1 expression on human dermal microvascular endothelial cells is directly and specifically up-regulated by substance P. J Immunol. 1999;**162**:1656–1661.
18. WEIDNER C, KLEDE M, RUKWIED R, et al. Acute effects of substance P and calcitonin gene-related peptide in human skin - a microdialysis study. J Invest Dermatol. 2000;**115**:
1015–1020.
19. DALGARD F, SVENSSON , HOLM J , et al. Self-reported skin morbidity in Oslo: associations with socio-demographic factors among adults in a cross sectional study. Br J Dermatol. 2004;**151**:452–457.
20. PARKER F. Structure and function of skin. In: Goldman L, Bennett JC, eds. Cecil Textbook of medicine. 21st ed. Philadelphia: Saunders, 2000:2266.
21. PEREIRA U., MISERY L. Experimental Models of Itch. In: Misery L, Stander S. Pruritus. London: Springer, 2010:51-56.
22. CHURCH MK, EL-LATI S, CAULFIELD JP. Neuropeptide-induced secretion from human skin mast cells. Int Arch Allergy Appl Immunol. 1991;**94**:310–318.
23. HILL SJ. Distribution, properties, and functional characteristics of three classes of histamine receptor. Pharmacol Rev. 1990;**42**:45–83.
24. TOGIAS A. H1-receptors: localization and role in airway physiology and in immune functions. J Allergy Clin Immunol. 2003;**112**(suppl):S60–S68.
25. BELL JK, MCQUEEN DS, REES JL. Involvement of histamine H4 and H1 receptors in scratching induced by histamine receptor agonists in Balb C mice. Br J Pharmacol. 2004;**142**:374–380.
26. SUGIMOTO Y, IBA Y, NAKAMURA Y, et al. Pruritus-associated response mediated by cutaneous histamine H3 receptors. Clin Exp Allergy. 2004;**34**:456–459.
27. HUANG JF, THURMOND RL. The new biology of histamine receptors. Curr Allergy Asthma Rep. 2008;**8:**21–27.
28. KANDA N, WATANABE S Histamine enhances the production of nerve growth factor in human keratinocytes. J Invest Dermatol. 2003;**121**:570–577.
29. ZHANG X, HUANG J, MCNAUGHTON PA. NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J. 2005;**24**:4211–4223.
30. OHKUBO T, SHIBATA M, INOUE M, et al. Regulation of substance P release mediated via prejunctional histamine H3 receptors. Eur J Pharmacol. 1995;**273**:83–88.
31. AITKEN RCB. A growing edge of measurement of feelings. Proc R Soc Med. 1969;**62**:989–993.
32. JENSEN MP, KAROLY P, BRAVER S. The measurement of clinical pain intensity: a comparison of six methods. Pain. 1986;**27**: 117–126.
33. CALISKANER Z, OZTURK S, TURAN M, KARAAYVAZ M. Skin test positivity to aeroallergens in the patients with chronic urticaria without allergic respiratory disease. J Invest Allergol Clin Immunol 2004;**14**:50-4.
34. NICOLAE I, DINU L, ENE (NICOLAE) CD, MATEI C, TAMPA M, GEORGESCU SR.. Mat. Plast., **50**, no.4, 2013, p.290
35. LEOW YH. Contact urticaria. In: Ket NS, Leok GC. The Principles and Practice of Contact and Occupational Dermatology in the AsiaPacific Region. Singapore: World Scientific Publishing, 2001: 33.
36. YOSIPOVITCH G, GOON A, WEE J, et al. The prevalence and clinical characteristics of pruritus among patients with extensive psoriasis. Br J Dermatol. 2000;**143**:969–973.
37. Kehrer JP. Free radicals as mediators of tissue injury and disease. Critical Reviews in Toxicology 1993;**23**:21–48.
38. DROGE W. Free radicals in the physiological control of cell function. Physiology ¨
Reviews 2002;**82**:47–95.
39. OKAMOTO T, TETSUKA T. Role of thioredoxin in the redox regulation of gene expresion in inflamatory diseases.In: Winyard PG, Blake DR, Evans CH. Free Radicals and Inflammation. Berlin: Birkhauser,2000:119.
40. LUNEC J, HOLLOWAY KA, COOKE MS, FAUX S, GRIFFITHS HR, EVANS MD. Urinary 8-oxo-2'-deoxyguanosine: redox regulation of DNA repair in vivo? Free Radic Biol Med 2002; **33**: 875-885
41. KASAI, H., Analysis of a form of oxidative DNA damage, 8-hydroxy-2deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutation Research 1997;**387**:147–163.
42. SHIGENAGA MK, GIMENO CJ, AMES BN. Urinary 8-hydroxy-2-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. Proceedings National Academy of Sciences, USA 1989;**86**:9697–9701.
43. FRAGA CG, SHIGENAGA MK, PARK JW, DEGAN P, AMES BN. Oxidative damage to DNA during aging: 8-hydroxy-2-deoxyguanosine in rat organ DNA and urine. Proceedings of National Academy of Sciences, USA 1990;**87**:4533–4537.
44. SAYRE LM, SMITH MA, PERRY G. Chemistry and biochemistry of oxidative stress
in neurodegenerative disease. Current Medicinal Chemistry 2001;**8**:721–738.
45. HALLIWELL B. Can oxidative DNA damage be used as a biomarker of cancer risk in
humans? Problems, resolutions, and preliminary results from nutritional supplementation studies. Free Radical Research 1998;**29**:469–486.
46. MARNETT LJ. Oxyradicals and DNA damage. Carcinogenesis 2000;**21**:361–370.
47. KASAI H, IWAMOTO-TANAKA N, MIYAMOTO T, KAWANAMI K, KAWANAMI S, KIDO R, IKEDA M. Life style and urinary 8-hydroxydeoxygua-nosine, a marker of oxidative DNA damage: effects of exercise, working conditions, meat intake, body mass index, and smoking. Japanese Journal of Cancer Research 2001;**92**:9–15.
48. HALLIWELL B. Effect of diet on cancer development: is oxidative DNA damage a biomarker? Free Radical Biology and Medicine 2002;**32**:986–974.
49. VALAVANIDIS A, VLACHOGIANNI T, FIOTAKIS C. 8-hydroxy-2-deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. Journal of Environmental Science and Health Part C 2009; **27**:120–139.
50. NICOLAE I, ENE (NICOLAE) CD, SCHIPOR S , TAMPA M, MATEI CLARA, GEORGESCU SR. Rev Chim.(Bucharest), **64**, no.10, 2013, p.1201.
51. ENE (NICOLAE) CD, NICOLAE I, TAMPA M, MATEI C, GEORGESCU SR. Rev. Chim. (Bucharest), **64**, no. 6, 2013, p.654.
52. TONG LIU, RU-RONG JI. Oxidative stress induces itch via activation of TRPA1 in mice. Neurosci Bull. 2012; **28(2)**: 145–154.
53. La Vinka PC**,** Dong X**.** Molecular signaling and targets from itch: lessons for cough. Congh 2013;**9**-8.
54. CASSANO N, LATTANZI V, PROFETA G, VENA GA.Orticaria e prurito acquagenico. Ann it Derm Allergol. Clin 2007;**61**:41-49.

Manuscript received: 5.06.2014