Lipid peroxidation and antioxidant protection in patients with papulo-pustular rosacea

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ABSTRACT

Purpose: To examine lipid peroxidation, antioxidant protection and L-arginine-NO – system in patients with papulo-pustular rosacea.

Materials and methods: The study included 128 women with papulo-pustular rosacea aged 40.1±0.99 years (range: 18-68 years). The patients were divided into three groups based on the severity of the symptoms: group I –patients with mild rosacea (n=42), group II – patients with moderate rosacea (n=49), group III – patients with severe rosacea (n=37). Indicators of lipid peroxidation and antioxidant protection were defined in all patients by a spectrophotometric method.

Results: The first group of patients showed a significant decrease in superoxide dismutase (SOD) and ceruloplasmin when compared to the control group. A significant increase in diene conjugates (DC), malondialdehyde (MDA), liposoluble antioxidants (retinol, α-tocopherol) and decrease in SOD, catalase and ceruloplasmin were observed in the second group. Patients in the third group had similar dynamics with a worsening of lipid peroxidation.

Conclusions: The changes in some parameters of lipid peroxidation and antioxidant protection were revealed in patients with papulo-pustular rosacea. The nature of these changes depends on the severity of the disease. Evaluation of the antioxidant imbalance may be informative to determine the understanding of the genesis of dermatosis and to study therapeutic strategy aimed at reducing the generation of reactive oxygen species (ROS), leading to a decrease in the capacity of the antioxidant defense.

Key words: rosacea, papulo-pustular subtype, etiopathogenesis, lipid peroxidation, antioxidant protection.

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INTRODUCTION

Today rosacea is a common disease. According to different authors, frequency of dermatosis varies from 5 to 10%, and women suffer from this disease three times more often than men [1,2]. The high prevalence of the disease, the implementation of multifactorial and genetically determined mechanism of chronic, often relapsing course, as well as resistance to conventional therapies allows rosacea to refer to the most vital diseases in modern dermatology and cosmetology [3-5].

Despite the increased interest in recent research to this dermatosis, etiopathogenetic aspects of rosacea are not fully understood and are often contradictory. Several authors assign an important role in the development of rosacea to the disturbance of prooxidant-antioxidant balance, to the factors that stimulate angiogenesis and vasodilation, as well as nitric oxide which is constantly produced in the endothelium from L-arginine with the participation of NO-synthase [6-9].

Skin is the largest organ in organism, which prevents it from different environmental pollutants or toxicants. Most of them are oxidants. These substances directly or indirectly lead to production of reactive oxygen species (ROS). ROS are short-lived entities that are continuously generated at low levels during the course of normal aerobic metabolism. ROS include singlet oxygen (O_2), superoxide anion (O_2^-) H_2O_2, the hydroxyl radical (OH^-), etc. [10]. Increased production of ROS is involved in the pathogenesis of a number of skin disorders including vitiligo, lupus erythematosus, psoriasis, acne vulgaris, rosacea, different allergic reactions in the skin, etc. [11-14].

There is compelling evidence that oxidative stress drives the production of oxidation products, which can denature proteins, alter apoptosis, and influence the release of proinflammatory mediators, such as cytokines, which may be critical for the induction of some inflammatory skin diseases. This is also based on the recognition that ROS can act as second messengers in the induction of several biological responses, such as the activation of TNF, the generation of cytokines, the modulation of signaling pathways [10]. However there is own complex antioxidant system (AOS) in human organism for prevention of lipid peroxidation. These AOS includes ceruloplasmin, retinol, α-tocopherol, reduced glutathione, superoxide dismutase (SOD), catalase, etc. Antioxidants interact with ROS or their by-products to either eliminate them or to minimize their deleterious effects.

In this context, it is important to study the state of free-radical processes and antioxidant L-arginine-NO-system in patients with papulo-pustular rosacea, which will enhance the understanding of dermatosis mechanism and improve the treatment.

The aim of our investigation was to study of lipid peroxidation, antioxidant protection and L-arginine-NO – system in patients with papulo-pustular rosacea.

MATERIALS AND METHODS

The study involved 128 women with papulo-pustular rosacea, seeking medical care in out-patient and in-patient department of Grodno Regional Dispensary of Skin and Venereal Diseases and at the department of Dermatovenereology of Grodno State Medical University. The control group consisted of almost 41 healthy women.

Study inclusion criteria: papulo-pustular rosacea (L71); progressive stage of the disease; female; age over 18 years; informed consent to medical intervention and compliance with doctor's instructions regarding the prescribed therapy; the absence of concomitant diseases in the acute phase, requiring constant medical treatment; negative pregnancy test.

In order to determine the clinical form of the disease, we referred to the international classification which was developed by the National Rosacea Society Expert Committee on the Classification and Staging of Rosacea [15]. This classification includes erythemato-telangiectatic rosacea, papulo-pustular rosacea, phymatous rosacea (hypertrophic) and ocular rosacea.

According to the scale of the diagnostic evaluation of rosacea, which included a qualitative assessment of the severity of the main symptoms of the disease, all the subjects were divided into three groups based on the severity: group I – patients with mild rosacea (n=42), group II – patients with moderate rosacea (n=49), group III – patients with severe rosacea (n=37).

The activity of the various stages of free radical processes was evaluated on the content of the primary (diene conjugates (DC)) and secondary (malondialdehyde (MDA)) lipid peroxidation products in the red blood cells and blood plasma using spectrophotometric method. The state of non-enzymatic and enzymatic components of AOS was studied on the content of ceruloplasmin, retinol, α-tocopherol in the blood plasma, as well as reduced glutathione, SOD and catalase activity in erythrocytes with use of spectrophotometric method [16].

Nitric oxide production was determined by the total content of nitrate/nitrite (NO^{3-}/NO^2) in the blood plasma. In order to estimate the total nitrite NaOH, blood plasma was deproteinized with zinc sulfate, followed by reduction of nitrate to nitrite by means of granules of cadmium. Measuring the level of NO^{3-}/NO^2 in blood plasma was carried out by

spectrophotometric method at 540 nm with Griess reagent [17,18].

Statistical analysis of digital data was performed using the application package Microsoft Excel and Statistica 6.0. In order to describe the results obtained, we calculated the frequency of the studied phenomena (p) with the arithmetic mean (M) and the standard error of mean (m). The figures and the text data are expressed as M±m. As the group variances were not homogeneous, analysis was performed by nonparametric Mann-Whitney U test. If the group variances were homogeneous, independent-samples t-test was used for comparing two groups. $P < 0.05$ between the two groups was accepted as significant.

RESULTS

The study involved 128 women with papulo-pustular rosacea. The average age of patients was 40.1±0.99 years (range: 18-68 years). The dominant age group was aged 31-40 years and consisted of 49 participants (38.3%). Moderate rosacea existed in all age ranges. However, age group from 31 to 40 years showed the majority of patients, suffered from severe rosacea.

The disease duration ranged from 1 month to 10 years with an average of 44.5±2.5 months. The proportion of patients with disease duration of up to one year was 14.1%, from 1 year to 5 years – 60.2%, from 5 to 10 years – 25.8%. Within these groups, results showed the largest number of patients suffered between 1-5 years. Additionally, the results also showed that the majority of patients who suffered either from 1-5 years or 5-10 years were affected with moderate rosacea (21.9% and 12.5%, respectively). In patients with disease duration up to a year, the majority of patients suffered from mild rosacea (8.6%). The maximum duration of dermatosis was in the 51-60 year-old age bracket. For patients under the age of 60, the following pattern was defined: the older the patient is, the longer the continuation of dermatosis lasts.

When assessing subjective sensations, patients with papulo-pustular rosacea reported that they suffered from: itch of the skin (61.0% of cases), a burning sensation in the area of lesions (45.3%), hot flushes (33.6%), tightening of the skin (28.9%), pain in the rash (21.1%). Only 10.9% of patients reported that there were no subjective sensations.

In all patients, the clinical picture was characterized by the presence of papules and pustules against a background of persistent erythema and telangiectasia. The incidence of erythema differed among patients. It was observed that in 123 (96.1%) patients it affected the cheeks, in 115 (89.8%) – the chin, in 99 (77.3%) – the forehead, in 37 (28.9%) – the nose, in 16 (12.5%) – perioral region, in 3 (2.3%) – eyelids, in 1 (0.8%) – neck. Against erythema background both visual and dermatoscopic treelike branching network of blood vessels were verified. Multiple papular and pustular elements were placed on the skin of the cheeks for 83 (64.8%) patients, on the skin of the chin for 39 (30.5%), on the forehead for 24 (18.8%), on the nose for 23 (18.0%), in the perioral area for 3 (2.3%). The size of the papules and pustules ranged from 1 to 4 mm in diameter.

From the 128 patients in this study, 94 (73.4%) patients used systemic medications such us antibiotics, antihistamines and desensitizing agents, metronidazole and vitamins. Such drugs as metronidazole, azelainic acid, antibiotics, agitated slurry containing alcohol, acaricidal and / or antibacterial component were topically prescribed. Additionally, 23 (18.0%) patients independently and 46 (35.9%) patients on prescription used outside agents containing steroids. The therapy gave moderate inconstant effect. These patients had repeatedly received out-patient treatment from dermatologists and cosmetologists near their places of residence or in-patient treatment from the department of Grodno Regional Dispensary of Skin and Venereal Diseases.

The next stage of this research was to study the lipid peroxidation in patients with papulo-pustular rosacea. For this purpose, an evaluation of primary (DC) and secondary (MDA) lipid peroxidation products in the erythrocytes and blood plasma were carried out (Tab. 1).

As seen in Table 1, when evaluating the primary (DC) and secondary (MDA) lipid peroxidation products in the erythrocytes and blood plasma of patients with mild papulo-pustular rosacea, significant differences from those of the control group were not found ($p>0.05$).

In erythrocytes of the patients with the moderate rosacea compared to the control group it was found a reliable growth of indicators of both primary (DC) (11.6±0.33 U/ml and 10.3±0.35 U/ml respectively, $p<0.01$) and secondary (MDA) (10.5±0.28 mmol/L and 9.6±0.25 mmol/L respectively, $p<0.05$) lipid peroxidation products. In regard to plasma, no significant differences in the content of DC and MDA were detected ($p>0.05$).

When investigating the similar indicators of the patients with severe rosacea, it was observed that the concentration of DC and MDA in erythrocytes was 1.2 times higher than that in the control group. The concentration of DC in erythrocytes was noted at 12.8±0.53 U/ml and 10.3±0.35 U/ml respectively, $p<0.001$. For MDA, the concentration was 11.5±0.41 mmol/L and 9.6±0.25 mmol/L respectively, $p<0.001$.

These results are possible due to the accumulation and subsequent activation of free radicals (Tab. 1). The blood plasma of the patients in this group showed a significant increase of MDA

concentration (1.9±0.14 mmol/L and 1.5±0.08 mmol/L respectively, p<0.05) compared to the control group.

Table 1. Indicators of the lipid peroxidation in women with papulo-pustular rosacea

The test indicator		Control group (n=41)	Group I (n=42)	Group II (n=49)	Group III (n=37)
Erythrocytes	DC (U/ml)	10.3±0.35	9.8±0.46	11.6±0.33*#	12.8±0.53*#†
	MDA (mmol/L)	9.6±0.25	9.5±0.25	10.5±0.28*#	11.5±0.41*†
Plasma	DC (U/ml)	1.2±0.12	1.3±0.15	1.3±0.14	1.5±0.18#
	MDA (mmol/L)	1.5±0.08	1.6±0.07	1.7±0.05	1.9±0.14*

* – significant differences between the control and the first group, between the control and the second group, between the control and the third group; # – significant differences between the first and second group, between the first and the third group; † – significant differences between the second and third group.

Increased activation of accumulation of free radicals in patients resulted in a significant increase in lipid peroxidation for all three groups. Furthermore, the more severe the symptoms, the more dramatic the results become. By studying the results in patients of the second and first groups, it was found that the concentrations of DC (11.6±0.33 U/ml and 9.8±0.46 U/ml respectively, p<0.01) and MDA (10.5±0.28 mmol/L and 9.5±0.25 mmol/L respectively, p<0.05) in erythrocytes of the patients in the second group were significantly higher than that of in the first group. When comparing the data obtained in patients from the second and the third group data showed a significant increase in concentration of both DC (p<0.05), and MDA (p<0.05) in the red cell mass of the patients of the third group compared with the patients of the second group. Concentrations of DC in blood plasma (p<0.001) and erythrocytes (p<0.001) were significantly higher in patients from the third group in relation to those in the first group.

When evaluating the content of nitric oxide (Fig. 1) was found an increase in patients with different severity of papulo-pustular rosacea. Thus, the first group showed (11.3±0.6 mkmol/L), the second group showed (13.0±0.6 mkmol/L) and the third group showed (14.9±0.71 mkmol/L) a significant increase of nitric oxide compared to the control group which only showed (9.4±0.39 mkmol/L) (Fig. 1). Evaluation of the content of the nitric oxide found in patients showed that as the severity of the disease increased, so did the concentration of nitric oxide.

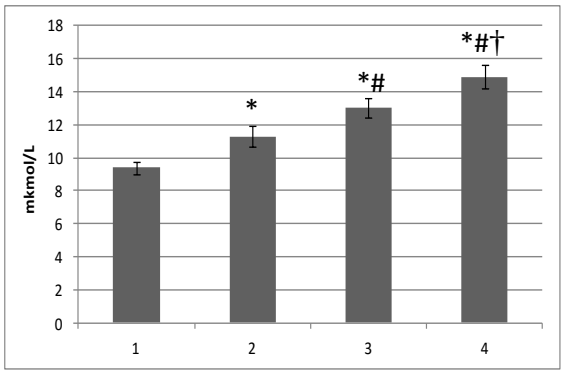

Figure 1. Level of nitric oxide (mkmol/L) in patients with papulo-pustular rosacea in the control group – 1, in the first group – 2, in the second group – 3, in the third group – 4; * – significant differences between the control and the first group, between the control and the second group, between the control and the third group; # – significant differences between the first and second group, between the first and the third group; † – significant differences between the second and third group.

To evaluate indicators of AOS condition, ceruloplasmin, retinol, α-tocopherol in the blood plasma, as well as reduced glutathione, catalase and SOD in red blood cell mass were tested (Tab. 2).

Performance analysis of the enzymatic chain of AOS showed a significant decrease in the activity of the main intracellular antioxidant – SOD in erythrocytes of patients of the first (43.5±2.01%, p<0.01), of the second (40.7±1.94%, p<0.001) and of the third (36.71±2.05%, p<0.001) groups compared with the control group (50.3±1.38%). In addition, there was a significant decrease in the activity of the enzyme studied in patients with mild papulo-pustular rosacea compared with severe (p<0.05) (Tab. 2).

The activity of catalase in red blood cells of the first group of patients showed a tendency of its reduction (29.9±0.42 mmol H_2O_2/min/g Hb and 30.5±0.53 mmol H_2O_2/min/g Hb respectively, p>0.05) compared with the control group. The activity of this enzymatic antioxidant was significantly lower in patients with moderate rosacea (28.6±0.43 mmol H_2O_2/min/g Hb, p<0.01), than in the control group. The most pronounced changes verified in erythrocytes of patients with severe rosacea (27.5±0.54 mmol H_2O_2/min/g Hb, p<0.001) compared with the control group. This data clearly showed a significant decline of catalase in the second and third groups compared to the first group (Tab. 2).

No significant differences in the concentration of reduced glutathione in patients with different forms of papulo-pustular rosacea were not found (Tab. 2).

An analysis of the functioning of non-enzymatic components of AOS showed a significant decrease in the level of ceruloplasmin in the blood plasma of patients of the first (109.3±5.59 mg/L, $p<0.05$), of the second (92.3±5.71 mg/L, $p<0.001$) and of the third (75.9±5.44 mg/L, $p<0.001$) groups compared with the control (124.1±4.81 mg/L). In addition, there was a significant decrease in the concentration of the studied antioxidant with an increase of the severity of rosacea (Tab. 2).

Table 2 Indicators of antioxidant system in patients with papulo-pustular rosacea

The test indicator		Control group (n=41)	Group I (n=42)	Group II (n=49)	Group III (n=37)
Erythrocytes	SOD (%)	50.3±1.38	43.5±2.01*	40.7±1.94*	36.7±2.05*#
	Catalase (mmol H_2O_2/min/g Hb)	30.5±0.53	29.9±0.42	28.6±0.43*#	27.5±0.54*#
	Reduced glutathione (mkmol/g Hb)	24.7±1.05	24.6±1.26	25.7±1.31	28.6±1.72
Plasma	Ceruloplasmin (mg/L)	124.1±4.81	109.3±5.59*	92.3±5.71*#	75.9±5.44*#†
	Retinol (mkmol /L)	1.03±0.05	1.09±0.05	1.3±0.07*#	1.1±0.07
	α-tocopherol (mkmol /L)	15.6±0.71	17.6±0.83	19.7±1.10*	17.0±1.71

* – significant differences between the control and the first group, between the control and the second group, between the control and the third group; # – significant differences between the first and second group, between the first and the third group; † – significant differences between the second and third group.

In assessing the content of liposoluble antioxidants, we found a significant increase in the level of retinol in the blood plasma of patients with moderate rosacea (1.3±0.07 mkmol/L and 1.03±0.05 mkmol/L respectively, $p<0.01$) compared with the control group. Indicators of vitamin A in patients of the first and third groups were not significantly different from the control group, but tended to increase ($p>0.05$).

Furthermore, when comparing the value of this indicator between the groups of examined patients it showed a significant increase in the second (1.3±0.07 mkmol/L and 1.1±0.07 mkmol/L respectively, $p<0.05$) compared to the third group was found (Tab. 2).

The concentration of α-tocopherol in the blood plasma of patients of the second group was significantly higher (19.7±1.10 mkmol/L and 15.6±0.71 mkmol/L respectively, $p<0.01$) compared to the control group. In patients of the first (17.6±0.83 mkmol/L and 15.6±0.71 mkmol/L respectively, $p>0.05$) and the third (17.0±1.71 mkmol/L and 15.6±0.71 mkmol/L respectively, $p>0.05$) groups the above mentioned figure tended to increase, but did not differ significantly from that of the control group.

Comparison of α-tocopherol between groups revealed no significant difference (Tab. 2).

DISCUSSION

Low concentrations of free radicals and lipid peroxidation products of different stages are produced as a result of normal oxidative metabolism occurring in physiological conditions at a very low level [19].

Disorders of the antioxidant-prooxidant balance are caused by excessive activation of lipid peroxidation. When the antioxidant-prooxidant balance is disrupted, free radicals and toxic products of reactions of lipid peroxidation alter the original structure and the normal functional activity of cellular and subcellular membranes [20,21]. This further promotes the release of proteolytic enzymes in the cell cytoplasm and then into the bloodstream and alter metabolism of cellular systems.

Furthermore, it stimulates the formation of inflammatory mediators – prostaglandins, leuko-trienes, lymphokines [22].

The result of this process is a development of inflammatory changes in the affected skin, which clinically manifest as the main signs of dermatosis – erythema, telangiectasia, papules, pustules, edema and infiltration. In addition, disturbance of lipid peroxidation activity promotes changes of proliferative activity of lymphoid cells and (starting)

immunopathological mechanisms of inflammatory reactions [22].

Lipid peroxidation and oxidative stress have an important role in many inflammatory skin diseases. Rosacea is a disorder of unknown pathophysiology. Multiple aetiological factors have been proposed. Sunlight and heat are important factors in the pathogenesis of disease [23]. Recent studies have shown the role of oxidative stress and antioxidative system disorders in rosacea: exposure to UV light resulted in a significant increase in antioxidant enzyme activities in skin [24-26].

In our study an increase in the content of nitric oxide was revealed. The maximum increase of them being observed in cases of severe pathology. It is known that nitric oxide molecule acts as an effector, which determines the amount of blood flow in the microcirculation [19]. At the same time, this molecule when combined with superoxide anion forms peroxynitrite, which has a marked cytotoxic effect and is capable of oxidizing lipids and proteins of cell membranes. This results in damaging effects of the membranes [9].

The concentration of nitrogen monoxide in the systemic circulation may reflect not only the level of activity of L-arginine-NO-system, but the tension of other mechanisms. These are encountered when there is excess of nitric oxide, particularly an increase in lipid peroxidation and decrease in antioxidant defense. Nitric oxide can be characterized as one of the mediators of inflammation in rosacea [27].

The body has its own antioxidant system that eliminates an excess of reactive radicals, but in many pathological conditions, it is inconsistent, which results in damage to the cell membranes. This damage causes dysfunction of cells and tissues, which entails the development of oxidative stress. In patients with this disorder, the development of oxidative stress takes place, which is implemented through the NO-dependent mechanisms. The action of nitric oxide is spread through its reactive forms and is carried out with the participation of over-expression of inducible form of NO-synthase, through which a large amount of NO is produced. Thus, not only oxidation, but also nitrosative stress caused by excessive formation of NO in these regions of the body are being developed [28]. Undoubtedly, the increased activity of free radical oxidation of lipids is an important factor in the development of pathogenesis that must be considered in the therapy of this pathology.

The activation of free radical processes that causes the depletion of antioxidant defense mechanisms is an important pathogenic factor in this disease. Obviously, carrying out the treatment of patients with rosacea should include a means that strengthen the antioxidant resource and decrease the manifestations of oxidative stress. Evaluation of the mechanisms of free radical pathology will allow for justification of the therapeutic strategy aimed at reducing the generation of active oxygen forms.

CONCLUSIONS

Thus, according to the results of a comparative analysis of clinical and laboratory data of patients with papulo-pustular rosacea we can deduct the following conclusions:

1. In patients with papulo-pustular rosacea prooxidant-antioxidant disbalance was found. It was manifested in the activation of lipid peroxidation and in the change of some parameters of antioxidant protection, the nature of which depends on the severity of the disease.
2. Patients with different severity of papulo-pustular rosacea showed increase in primary and secondary lipid peroxidation products (DC and MDA). The concentration level of main components of antioxidant system was decreased. Concentration of reduced glutathione showed no change.
3. The revealed disorders of prooxidant-antioxidant balance are accompanied by an increasing concentration of nitric oxide. This reflects that L-arginine-NO system involved in the observed changes. Obviously, the NO-dependent nature of the oxidative stress is an important part of the pathogenesis of papulo-pustular rosacea for these patients.
4. Considering that the level of liposoluble antioxidants (retinol and α-tocopherol) in the blood plasma for the patients with papulo-pustular rosacea is higher than that for those patients in the control group, we raised the question whether it is compulsory to include these antioxidants in the therapy of this disease.

Conflicts of interest

The authors declare no conflicts of interest.

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