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Glutathione-Dependent Enzymes - Biomarkers of Inflammatory Process in Patients with Cellulitis of Maxillofacial Area

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SUMMARY

Introduction. *Inflammation-destructive process, accompanying cellulitis, leads to the disturbances of metabolic processes and an imbalance of the antioxidant defense system. Increased free radical generation and lipid peroxidation has been considered to play an important role in the pathogenesis of cellulitis. Salivary parameters (components) reflect the patients' metabolic state and have clinical-diagnostic significance.*

Aim. *To examine glutathione enzymatic redox-system, including glutathione, glutathione reductase, glutathione peroxidase and glutathione-S-transferase in plasma and leucocytes of the patients with cellulitis of maxillofacial area, treated with complex therapy.*

Material and Methods. *20 patients (18-45 years old) and 15 healthy subjects were examined. Patients were treated with comprehensive therapy, including antioxidant remedy "Aevitum" (35 mg retinol acetate and 100 mg α-tocopherol acetate) during 7 days. The activities of antioxidant glutathione-dependent enzymes and content of glutathione were determined in blood plasma and leucocytes of the patients with cellulitis using spectrophotometry.*

Results. *Inflammation process led to the imbalance of antioxidant glutathione-dependent defense system in patients with cellulitis. The results suggest that complex therapy with "Aevitum" was effective and partially restored imbalance of the antioxidant defense. Spearman's correlation analysis showed positive correlation between glutathione-dependent enzymes in blood plasma and leucocytes of the patients with cellulitis in a week time of complex therapeutic course.*

Conclusion. *Glutathione-dependent enzymes may be biomarkers of the inflammation process in patients with cellulitis; their activities reflect a degree of pathological process activity.*

Keywords: Glutathione-reductase; Glutathione-peroxidase; Glutathione-S-transferase; Cellulitis

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Introduction

Cellulitis of the maxillofacial area is infectious destructive process. Odontogenic inflammation in oral cavity is most frequently primary lesion, followed by sinusitis, otitis, as well as radiation therapy and surgical procedures[11,15]. Odontogenic infections are still a common problem in daily dental practice. Fundamental management principles are keys to successful outcome of patients with cellulitis: early diagnosis, early initiation of antimicrobial therapy, and correction of metabolic[9]. An antimicrobial therapy plays a significant role in the treatment of maxillofacial cellulitis. Course and duration of treatment

depend on the patient immune response and microbial and environmental factors[3].

Inflammatory disorders are the result of complex interactions between pathogens and the host's immune response. Important and interrelated factors that are involved in the patho-physiologic progression of cellulitis are activation of oxygen radicals and their related metabolites. Increased production of oxygen radicals may contribute to oxidative stress[16,17]. Reactive oxygen species (ROS) lead to oxidative stress and moderate to low level of function in cellular signaling pathways. Most reactive oxygen species are generated in cells by mitochondrial respiratory chain[13]. Especially important are free radicals in hypoxic signaling pathways, which have important implications for inflammation in patients with cellulitis.

Inflammation is an activating factor of peroxide oxidation of lipids (POL) in soft tissues of patients with cellulitis, metabolic disturbances of protein, lipid, carbohydrate and water-mineral metabolism[8,19]. Search of the newest and most effective drugs and methods for treatment of patients with cellulitis at the early stage of the disease has special value in modern surgical practice of maxillofacial area. For solving this problem doctors use different drugs and methods for comprehensive treatment: surgical procedures, antibiotics, perftoran, poli-vitamin therapy, etc[2,10,12]. Sometimes doctors use the complex therapy, included the traditional therapy and additional remedies with homeopathic, anti-oxidative, immuno-modulating, hormonal and other properties.

The ***purpose*** of this investigation was to examine glutathione enzymatic redox-system, including glutathione, glutathione-reductase, glutathione-peroxidase and glutathione-S-transferase in plasma and leucocytes of peripheral blood of patients with cellulitis in the maxillofacial area treated with complex therapy: common surgical procedures and anti-oxidative therapy.

Material and Methods

20 patients (18-45 years old) with cellulitis of maxillofacial area and 15 healthy subjects (control group) were examined. Complex therapy included the traditional surgical procedures and the additional traditional therapeutic course with "Aevitum" (35 mg retinol acetate, 100 mg α-tocopherol acetate) during 7 days.

Leucocytes were separated from blood using Boyum method[1]. Leucocytes were prepared during incubation with 0.1% Triton X-100 solution (final concentration) during 30 min and centrifugation at 600g 10 min. Content of glutathione and activity of enzymes have been determined with spectro-photometry (Humalyzer 2000, DE). Glutathione-reductase (GR, EC 1.6.4.2) and glutathione-peroxidase (GP, EC 1.11.1.9) activities were determined with our own methods[5]. Glutathione-S-transferase (GST, EC 2.5.1.18) activity was determined with Habig and Jacoby method[7]. Glutathione's content was determined with Sedlak, Lindsay method[14], and protein content with pyrogallol photometric test[18]. The results were calculated with the help of statistical Student's method and Microstat: Microsoft Excel 2003 program. Spearman's method of nonparametric correlation was used for examination of interrelations between the salivary parameters[6].

Results

Our results of the activity of glutathione-dependent enzymes and content of glutathione in blood plasma of the patients with cellulitis during therapeutic course are shown in figure 1. As it can be seen, glutathione-reductase activity before treatment was 84.6%, which was significantly different in comparison with the healthy individuals ($p>0.05$). On the 7th day of the treatment course of complex therapy with "Aevitum", glutathione-reductase activity decreased to 38.5% ($p<0.05$). Plasma activity of glutathione-peroxidase in patients with cellulitis before treatment was 72.4%, which was significantly different in comparison with the healthy individuals ($p<0.05$), and increased to 114.6% ($p>0.05$) after the anti-oxidant treatment. Glutathione-S-transferase activity in plasma of patients with cellulitis decreased after the anti-oxidant course from 48.36% to 41.1% ($p<0.05$). Glutathione content in blood plasma of patients with cellulitis at the initial stage of the disease was increased in comparison with healthy subjects ($p<0.05$), and decreased after the anti-oxidant treatment ($p>0.05$).

Results of investigation of the glutathione-dependent enzymes activities and content of glutathione in leucocytes of the patients with cellulitis are presented in figure 2. Activity of glutathione-reductase in leucocytes of patients with cellulitis before treatment decreased after the anti-oxidant treatment from 73.5% to 38.7% ($p<0.05$). Glutathione-peroxidase activity was increased at the first stage of cellulitis in patients' leucocytes and significantly decreased ($p<0.05$) after the anti-oxidant treatment. Similarly, the activity of glutathione-S-transferase in leucocytes of patients with cellulitis was increased before the treatment and decreased significantly after the treatment ($p>0.05$). Content of glutathione in leucocytes of the patients with cellulitis was increased before the treatment and decreased after the anti-oxidant treatment ($p<0.05$).

Results of Spearman's correlation analysis of the results are shown in table 1. Spearman's nonparametric analysis indicated the strong positive interrelation between activities of glutathione-reductase and glutathione-S-transferase in blood plasma and leucocytes of patients with cellulitis before and after treatment.

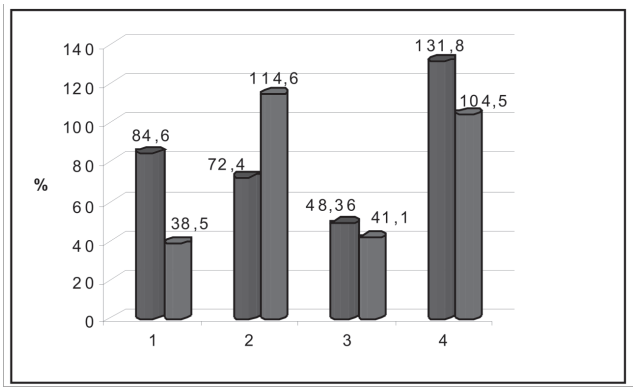

Figure 1. Activities of glutathione-dependent enzymes and content of glutathione in blood plasma of patients with cellulitis during therapeutic course 1 – GR; 2 – GP; 3 – GST; 4 – GSH. The first column - before treatment; the second column – after treatment. Healthy – 100%.

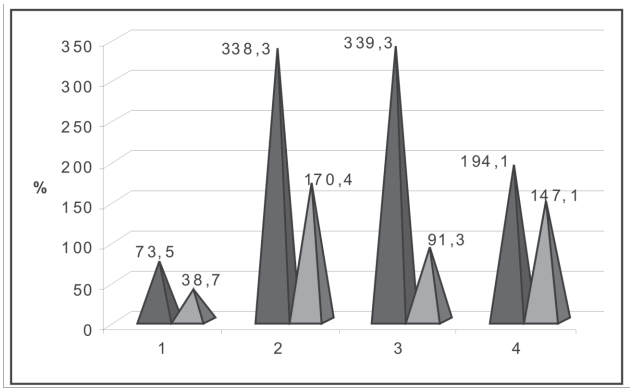

Figure 2. Activities of glutathione-dependent enzymes and content of glutathione in leucocytes of the patients with cellulitis during therapeutic course 1 – GR; 2 – GP; 3 – GST; 4 – GSH. The first column - before treatment; the second column – after treatment. Healthy – 100%.

Table 1. Interrelation between glutathione-dependent enzymes and glutathione in blood plasma and leucocytes of patients with cellulitis

Enzymes	Healthy		Patients with cellulitis			
			Before AOT		After AOT	
	r	p	r	p	r	p
			Blood plasma			
GR-GP	+0.830	<0.005	-0.277	>0.05	+0.697	<0.025
GR-GST	+0.714	<0.025	+0.503	<0.05	+0.818	<0.005
GR-GSH	+0.603	<0.05	+0.091	>0.05	+0.867	<0.001
			Leucocytes			
GR-GP	+0.685	<0.025	-0.313	>0.05	+0.927	<0.001
GR-GST	+0.728	<0.025	+0.446	<0.05	+0.816	<0.005
GR-GSH	+0.721	<0.025	+0.280	>0.05	+0.830	<0.005

Analysis between activities of glutathione-reductase and glutathione-peroxidase in plasma and leucocytes of patients before treatment didn't show the interrelation ($p>0.05$). In a week, complex therapy including the anti-oxidant treatment restored their functional interrelation ($p<0.001$). Relative results of correlation analysis existed between glutathione-reductase activity and content of its coenzyme glutathione. Only after therapeutic course with the anti-oxidant treatment, their interrelation was restored, both in plasma ($p<0.001$) and leucocytes ($p<0.005$).

Discussion

Inflammation-destructive process, accompanying cellulitis, leads to metabolic imbalance and disturbance of enzymatic glutathione redox-system. The main biological role of glutathione-reductase is based on the reduction of oxidized glutathione (GSSG) to its reduced form (GSH) with utilization of $NADPH^+$. Hydrophilic antioxidant glutathione is the main component of redox-buffer of the intracellular medium. Glutathione-associated metabolism is a major mechanism for cellular protection against agents which generate oxidative stress and peroxide oxidation of lipids (POL). Recent genetic and biochemical evidence has demonstrated that glutathione and glutathione-dependent enzymes play a central role in the cellular defence against toxic agents. Glutathione peroxidase protects our cells against lipid peroxides and hydro-peroxides. Another enzyme, glutathione-S-transferase, catalyzes the conjugation of GSH with different toxic and mutagenic compounds, which are generated during lipid peroxidation processes.

Inflammation process leads to metabolic imbalance of antioxidant enzymes and intoxication, which reflects the increase of glutathione content and activities of glutathione-peroxidase and glutathione-S-transferase in plasma, and especially in leucocytes of patients with cellulitis before treatment. Complex therapy, including the anti-oxidant treatment decreased activities of glutathione-dependent enzymes and content of glutathione, already in a week. Leucocytes participate in the process of phagocytosis, which takes place in blood of patients with cellulitis. Our results of the state of enzymatic redox-system of glutathione reflect the activity of inflammation process in tissues of patients with cellulitis.

In our early study, we treated patients with cellulitis of maxillofacial area with complex therapy, including the anti-oxidant treatment with "Aevitum", and investigated the dynamics of activities of the glutathione-reductase, glutathione-peroxidase and content of glutathione during the treatment in tissues around cellulitis, skin and muscles[4]. In a week of the complex therapy, including the anti-oxidant treatment, the imbalance of the glutathione-reductase/glutathione-peroxidase system in the skin and muscles has partially been corrected. This fact was confirmed by the dynamics of activities of glutathione-dependent enzymes, the more effective improvement of the patient's health status, and treatment course duration.

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