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Hemostatic Parameters of Blood in Patients with Chronic Generalized Parodontitis Associated With a Metabolic Syndrome

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Abstract

The purpose of this study is to assess hemostatic blood counts in patients with chronic generalized periodontitis associated metabolic syndrome. Twelve practically healthy persons and 72 patients with CGP of medium degree were examined. Of these, 24 patients without concomitant diseases; 48 patients - with CHC associated with MS. The anticoagulant and fibrinolytic activity of vascular endothelium, the content of endothelin I and homocysteine in blood serum were studied. It was found that the increase in the time of HAGEMAN-dependent fibrinolysis and a decrease in fibrinolytic endothelial activity, as well as an increase in endothelin-I, which may be due to the activation of oxidative stress in endothelial cells in this pathology.

INTRODUCTION

Currently, periodontal disease is a complex problem, which acquires not only medical, but also social significance. This is due primarily to the high prevalence and intensity of damage to periodontal tissue. The most common among inflammatory periodontal diseases is the chronic generalized periodontitis (CGP), which is a kind of dystrophic inflammatory process that occurs as a result of combined effects of various exogenous and endogenous factors [1, 2, 4].

Numerous studies have established that the emergence of significant functional and morphological changes in the periodontal complex is facilitated by universal pathogenetic mechanisms that are formed in various diseases of organs and systems. The relationship between obscheomatic diseases and the state of the oral cavity is conditioned by metabolic, hemodynamic, microcirculation, immunological and neuro-regulatory changes and microbiocenosis shifts. Diseases that directly affect the periodontal condition in patients are primarily diabetes mellitus, diseases of the cardiovascular system, chronic diseases of the respiratory system and osteoporosis [3].

The rapid increase in the frequency of metabolic syndrome or insulin resistance syndrome (IRS) is currently one of the most important public health problems in developed countries.

In most cases, the initiating moment of the onset of IRS is excess body weight, which in turn leads to the development of arterial hypertension and a decrease in the sensitivity of the peripheral tissues to insulin, followed by a progressive accumulation of excess body weight. The leading pathogenetic mechanisms in the realization of components of the metabolic syndrome (MS) are the activation of inflammatory factors, endothelial dysfunction, the violation of fibrinolysis processes, the change in procoagulant activity of blood plasma, oxidative stress, pronounced immunological shifts, disorders of autonomic nervous regulation, which are realized at the level of various organs and systems. The majority of pathophysiological manifestations of a complex of metabolic disturbances, conditioned by IRS, are closely intertwined with the leading pathogenetic links in the development and progression of chronic generalized periodontitis. It is possible that inflammatory processes and metabolic disturbances affect each other when they are simultaneously present. Since there are significant gaps in our knowledge of the relationship between periodontal diseases and insulin resistance, more fundamental and invasive studies are needed [5, 6, 7, 8, 9].

The aim of the study was to evaluate hemostatic blood indices in patients with CGP associated metabolic syndrome.

MATERIALS AND METHODS

To achieve this goal, under our supervision, there were 12 practically healthy individuals and 72 patients with moderate-to-moderate CGP. Of these, 24 patients without concomitant diseases, 48 patients with CGP in combination with MS. Patients were on out-patient treatment in the clinic of the Tashkent State Stomatological Institute (TSDI). Patients with MS at the age of 40-65 years were mainly contingents suffering from metabolic disorders, in particular insulin resistance syndrome and outpatient observation. Concomitant hypertension and obesity were noted in 86.8% of patients.

To assess the state of the vascular endothelium in patients with CGP, the following was done: the definition of anticoagulant activity of the vascular endothelium; the determination of fibrinolytic activity of the vascular endothelium; determination of endothelin I content in blood serum; determination of homocysteine content in blood serum. In all cases, blood sampling was performed in the morning hours, on an empty stomach, by gravity into a plastic test tube. The blood for the study was taken from the ulnar vein twice: in an amount of 10 ml before the cuff test (3-5 minutes clamping of the vessels of the shoulder

with a cuff from the sphygmotonometer) and 5 ml after the cuff test. 5 ml of blood obtained before the cuff test was centrifuged (3000 rpm) for 10 minutes to obtain serum for the study of homocysteine and endothelin-1. Serum samples were rapidly frozen and stored at minus 20 ° C in well-closed test tubes. 5 ml of blood obtained before the cuff test and 5 ml of blood obtained after the cuff test were stabilized with a 3.8% sodium citrate solution in a ratio of 9: 1. The studies were performed on a platelet-poor plasma, which was obtained by double centrifugation: first at 1000 rpm (7 minutes), then at 3000 rpm (15 minutes). Centrifugation was performed immediately after blood sampling, selection of plasma for study - immediately after centrifugation. Plasma samples were analyzed no later than 2 hours after the blood was taken. To determine the anticoagulant activity of the vascular wall endothelium, the level of antithrombin III (AT III) activity in the blood before and after the cuff test was determined. The ratio of the activity of AT III before and after the cuff test characterizes the isolation of its endothelial cells. Normally, after the cuff test, an anticoagulant is released into the blood (AT III activity increases). To determine the activity of AT III, a set of the firm “Human” was used. To determine the fibrinolytic activity of the vascular wall endothelium, the speed of Hageman-dependent fibrinolysis of blood plasma was determined before and after the cuff test (3-5 minute clamping of the vessels of the shoulder with a cuff). The ratio of the rate of Hageman-dependent fibrinolysis after and before the cuff test characterizes the isolation by the endothelial cells of the tissue activator plasminogen (t-PA) and plasminogen activator inhibitor (PAI-1). Normally, after the cuff test, t-PA is released into the blood and PAI-1 production decreases, which leads to an increase in the rate of Hageman-dependent fibrinolysis. Hageman-dependent fibrinolysis was determined using the Renam kit (Russia), which included the following reagents: Imidazole Concentrated Buffer (2 ml) - 1 vial. Calcium chloride 0.025 M solution (10ml) - 2 bottles. Acetic acid 1% solution (10ml) - 1 bottle. Kaolin 0.5% suspension (5ml) - 2 bottles. Conducting the study: The plasma was mixed with distilled water, a kaolin suspension and a solution of acetic acid. After incubation at 37 ° C, the mixture was centrifuged for 5 minutes at 1500 rpm. The supernatant was removed and the pellet resuspended in buffer. After this, a solution of calcium chloride was added. The time from formation to complete lysis of the clot was recorded. Determination of the level of endothelin I and homocysteine in the serum was performed by an enzyme immunoassay using the Human kit. Laboratory tests were carried out on the enzyme

immunoassay of the firm "ROSH". All analyzes were carried out in the Clinical and Biochemical Laboratory of the Scientific and Practical Center for Dentistry and Maxillofacial Surgery of the TSDI.

Statistical processing of the data was carried out using the Statistica 6.0 software package. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

As a result of the conducted studies, it was found that in patients with chronic generalized periodontitis, the thrombolytic resistance of the vascular wall decreases, which is manifested by inhibition of anticoagulant and fibrinolytic activity of the endothelium. Changes in the thrombolytic resistance of the vascular endothelium depend on the combination of the underlying disease, i.e. chronic generalized periodontitis with MS. With the combined form of the disease, these changes are more pronounced than with chronic generalized periodontitis. Reduction of anticoagulant activity of the vascular endothelium in patients with periodontitis is 13% manifested by inhibition of the release of antithrombin III by the vascular wall endothelium. It is known that thrombomodulin binding thrombin causes changes in the conformation of its active site, as a result of which the rate of inactivation with its antithrombin III increases. On the other hand, it has been established that a number of inflammatory cytokines, in particular interleukin 1, as well as tumor necrosis factor, cause a decrease in the anticoagulant activity of the vascular wall endothelium. The data obtained in Table 1 show that in patients with CGP combined MS there is an increase in the time of Hageman-dependent fibrinolysis and a decrease in the fibrinolytic activity of the vascular endothelium by an average of 9-10%. The inhibition of fibrinolytic vascular endothelial activity in a combined form of the disease can be associated with a decrease in the release of the tissue plasminogen activator t-PA. At the same time, there is evidence in the literature that patients with periodontitis are increasing the production of tissue-activating plasminogen t-PA, but at the same time the production of inhibitor of the tissue activator PAI-I is increasing. As a result of the conducted studies, it was revealed that in patients with CGP of moderate severity of combined MS, in contrast to CGP, an increase in the content in the blood serum of not only homocysteine but also endothelin I in 1.6 and 2.5 times, respectively. At the same time, the concentration of homocysteine in the blood serum is higher than in CGP without accompanying pathology by a factor 1.3 times. This leads to the emergence of hyperhomocysteinemia

and, consequently, toxic effects on endothelial cells. It is known that with periodontitis there are numerous metabolic disorders in the periodontal tissues. Thus, the change in phagocytosis of bacteria by polymorphonuclear leukocytes in combination with delayed apoptosis of neutrophils is accompanied by hyperproduction of reactive oxygen species, which aggravates the course of the pathological process. In the periodontal tissues, the intensity of free radical oxidation increases, the amount of SH-groups decreases, which leads to significant damage to cell membranes. Probably, hyperhomocysteinemia revealed in patients with CGP combined with MS is a consequence of metabolic disorders and, first of all, lipid peroxidation in periodontal tissues. In the blood, homocysteine can undergo an oxidation process, which releases the superoxide anion and other free radicals that damage the endothelium. The result of the damaging effect of homocysteine is the development of endothelial dysfunction, which is accompanied by a change in the production of a number of regulatory substances produced by the endothelium, in particular, a reduction in the synthesis of nitric oxide, and prostacyclin and an increase in the formation of thromboxanes. It is known that homocysteine reduces the anticoagulant activity of the vascular wall endothelium, by degrading thrombomodulin, reducing the expression of antithrombin III-heparin complexes on the surface of endothelial cells, and significantly reduces the activity of the protein C system. In addition, homocysteine causes a decrease in plasminogen activation, by stimulating the thrombin-activated inhibitor fibrinolysis - TAFI (thrombin activatable fibrinolysis inhibitor). It is important to note that homocysteine increases the expression of the plasminogen activator inhibitor gene (PAI-1), which inhibits fibrinolysis.

Thus, the results of the study are consistent with the data of domestic and foreign authors and confirm the diagnostic and pathogenetic significance of homocysteine concentration in the blood in chronic generalized periodontitis, especially when combined with MS.

In healthy people, the level of endothelin I in the blood is low, which, combined with a short half-life, limits its hemodynamic effects. At a number of pathological conditions endothelin I, being a powerful vasoconstrictor. The vasoconstrictor effect is realized by binding endothelin I to the ETA receptors, which, with the participation of the Gq protein activates phospholipase C, producing diacylglycerol and inositol-3-phosphate, which activate the C kinase and cause calcium ions to enter the smooth muscle cells of the vessels. It is

the differences in the physiological and pathological roles of endothelin I that determine its diagnostic significance as a marker of damage and dysfunction of endothelial cells. The main stimulator of endothelin I production with endothelium of the vascular wall are active forms of oxygen, inflammatory cytokines, such as IL-1, IL-6 and TNF. It has been experimentally proved that the inflammatory process in the periodontium promotes the development of oxidative stress in the vascular wall. Therefore, the potential mechanism of the observed increase in endothelin I concentration in periodontitis by a factor of 1.4 and in a combined form of the disease by 3.5 times can be an inducer of oxidative stress in endothelial cells. It is likely that an increase in serum endothelin I concentration in patients with chronic generalized periodontitis combined with MS should be considered as a response to systemic manifestations of the inflammatory process. The uneven increase in the concentration of markers of endothelial dysfunction in CGP and its combination with MS is probably due to complex biochemical mechanisms of the development of dysregulation of endothelin I production against the background of hyperhomocysteinemia.

Table 1

Indicators of the system of hemostasis and homocysteine in the blood of patients with CHC combined with MS.

№	Indicators	Healthy persons with intact periodontium N =12	Patients with chronic generalized periodontitis N= 24	Patients with CHC combined with MS N=48
1	Antithrombin-III before the cuff test in %	98,27 + 8,11	86,11 + 6,54	76,24 + 5,44*
2	Antithrombin-III after cuff test in%	118,9 + 9,74	109,8 + 8,76	87,6 + 5,84
3	Index of anticoagulant activity of endothelium	1,27 + 0,09	1,16 + 0,08	1,15 + 0,06

4	XII-dependent fibrinolysis before the cuff test in sec.	601,6 + 14,8	556,9 + 10,4	698,4 + 12,7*
5	XII-dependent fibrinolysis after a cuff test in sec	367,8 + 10,9	401,8 + 12,3	507,1 + 13,5*
6	Index of fibrinolytic activity of endothelium	1,61 + 0,08	1,45 + 0,07	1,31 + 0,06
7	Concentration of homocysteine µMol / l	7,95 + 0,71	10,12 + 0,81	16,17 + 0,61*
8	Concentration of endothelin-1 of blood plasma µMol/l	1,57 + 0,14	2,23 + 0,17	5,52 + 0,41*
9	Soluble fibrin monomer complex mg/dL	2,42 + 0, 15	5,07 + 0,37	8,14 + 0, 72*

Note: * - the reliability of differences when comparing the control group P <0.05

Thus, the results of our own studies and literature data allow us to conclude that in patients with CGP associated with MS an increase in the content of markers of endothelial dysfunction occurs.

CONCLUSIONS

1. In patients with CGP combined MS, the time of Hageman-dependent fibrinolysis increases and the fibrinolytic activity of the vascular endothelium decreases by an average of 9-10%.

2. In patients with CGP of moderate severity of combined MS, in contrast to CGP, an increase in the serum content of not only homocysteine but also endothelin I is 1.6 and 2.5 times, respectively. At the same time, the concentration of homocysteine in the blood serum is higher than in CGP without accompanying pathology by a factor of 1.3 times.

3. Potential mechanism of the detected increase in endothelin I concentration in case of periodontitis by a factor of 1.4 times and with a combined form of the disease by 3.5 times can be an inducer of oxidative stress in endothelial cells in this pathology.

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