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Original Article

The morphofunctional features of platelets against the background of metabolic syndrome in patients with generalized marginal periodontitis

Yu L. Vasil'ev [a, *], O.V. Kytko [a], N.S. Smetneva [b], I.V. Goloborodova [b], M.V. Nelipa [a]

[a] I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University), Department of Operative Surgery and Topographic Anatomy, Moscow, Russia
[b] A.I. Evdokimov Moscow State University of Medicine and Dentistry, Department of Faculty Therapy and Occupational Diseases, Moscow, Russia

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ABSTRACT

According to modern ideas, disorders in the system of hemostasis in cardiovascular diseases are mainly due to an increase in the functional activity of platelets, namely their increased ability to adhere and aggregate. Aim of the research was to evaluate morphofunctional platelets of patients with marginal periodontitis. Material and methods of the study involved 129 men aged 28–50 years (mean age 42.97 ± 1.31 years) suffering from a MS with generalized periodontitis of mild and moderate degree. We also formed a control group of 90 practically healthy men (mean age 42.17 ± 1.34 years). Exclusion criteria are symptomatic AH, unstable angina, myocardial infarction or stroke in history, severe chronic heart failure, severe liver and kidney disease, diabetes, complete secondary adentia. Patients were performed the dental examination with determination of community periodontal index of treatment needs (CPITN), X-ray examination, physical examination with determination of anthropometric data, as well as clinical, the definition of MS laboratory criteria, insulin resistance indices, 24-h blood pressure monitoring. Platelet morphology was studied using scanning electron [17 clauser] microscopy (PhilipsPSEM 550 h) on samples prepared by a special method. The results of the study of platelet morphology in patients with MS and control group revealed a non-uniform pattern. The number of platelets in both groups being almost equal (249,6 ± 8,3x109/l and 256,8 ± 8,7x109/l respectively). Conclusion: The presented data provide strong evidence for changes in the morphofunctional state of peripheral blood platelets in patients with generalized marginal periodontitis of mild and moderate degree against the background of metabolic syndrome.

1. Introduction

The importance of morphological disciplines for medical science and practice is very high. On the one hand, new morphological disciplines play a special role in teaching some clinical disciplines, making a huge contribution to the formation of a clear idea of the material nature of the disease [1,2]. Correlation of morphological and clinical manifestations of diseases at all stages during their development allows young doctors to learn the skills of clinical and anatomical analysis, contributing to the formation of clinical thinking.

The study about the relationship between periodontal diseases and the components of the metabolic syndrome (MS) is relevant [3] because of the increase in the number of patients diagnosed with MS and the vast spread of this disease over the past two decades. The analysis of microcirculation values proved not only the reduction of blood flow intensity but also the decreased vasoactivity of microvessels essential to maintain normal microcirculation in periodontal tissues, as it provides active modulation of tissue blood flow and its adaptation to local metabolic needs [4]. To date, the number of adult patients with this pathology is about 20% of all nosological units. Foreign statistics say [5,6] that 25–35% of the population suffer from MS in Western countries.

On the other hand, the study about the morphological features of diseases both at different levels (subcellular, cellular, tissue, organ, systemic and organismic) and at various stages of development allows us to hope for the possibility of reversibility of the

* Corresponding author.
E-mail address: dr.vasiliev@gmail.com (Y.L. Vasil'ev).

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pathological process under the condition of appropriate correction.

Finally, now, during the life of the patient, the diagnosis of the disease becomes more morphological. Modern classifications of diseases increasingly include this particular component in the diagnosis [7].

Taking the above into account, understanding the morphological features of diseases, ultimately, is of great importance for the practical activities of a doctor.

According to modern ideas, alteration in the system of hemostasis in cardiovascular diseases (CVD) are mainly due to an increase in the functional activity of platelets, their increased ability to adhesion and aggregation. This information explains the growing interest of researchers in studying the morphofunctional state of thrombocytes in various pathological states [8]. Disfunction of hemostatic homeostasis is the cause of intravascular coagulation of blood and can lead to a variety of etiologies and clinical manifestations of diseases, as well as influence the course and outcome of the disease. The patient may have a myocardial infarction (MI), unstable angina (HC), cerebral stroke, sudden death due to acute thrombosis and occlusion of the arteries [7,8].

It is known that the action of various inducers, which stimulate the process of thrombus formation, is accompanied by an increase in the functional activity of thrombocytes. This process is due to the morphological changes in the platelets that determine their activation [8]. In the process of adhesion, the form of platelets from the disk form turns into a spindle-spherical shape, their ultrastructure changes, the outer membrane becomes more elastic, which facilitates the contact of platelets with each other and other structures [10,11].

Thus, the study of the hemostasis system in patients with coronary heart disease (CHD) revealed that the platelets in these patients are larger and have increased aggregation activity. Morphological composition and functional activity of thrombocytes in patients with arterial hypertension (AH) also were studied. At the same time, it was revealed that in this category of patients the morphological composition among platelets is represented by a large number of active forms possessing high adhesive and aggregation activity [McGill D.A., 1994].

Despite the existence of literature data, scientists continue to investigate the morphology and functional activity of platelets in patients with CHD and metabolic syndrome (MS), as well as determining the significance of these disorders in changing the morphofunctional state of thrombocytes. Of particular interest is the study of the morphological features among platelets in MS patients. It is known that MS is detected mainly in middle-aged people, for a long time it proceeds without distinct clinical symptoms (therefore patients with MS fall into the field of vision of the doctor in the last stage). Similar changes in the indices were observed in spheroehinocytes, which are platelets of spherical shape with large pseudopodia. However, it is the precursor of such serious diseases as CHD, AH, type 2 diabetes mellitus, as well as severe cardiovascular complications. That is why current study and identification of signs of MS contributes to the development of effective regimens for the treatment and prevention of cardiovascular disasters [1,3,12].

The formation of endothelial dysfunction activates the processes of lipid peroxidation and is accompanied by universal microcirculatory disturbances. Accumulation of under-oxidized products leads to free radical damage to most tissues, including, apparently, to more aggressive manifestations of activation of free radical oxidation and antioxidant damage in periodontal tissues. It is known that an important link in the pathogenesis of periodontal diseases of inflammatory nature is the disturbance within microcirculation in periodontal vessels. Disturbances also occur due to changes in the system of hemostasis and rheological properties of the blood that correlate with the severity of periodontitis.

According to many authors, the presence of MS creates factors for the formation of inflammatory-destructive lesions of periodontal disease [9,11,16].

Aim of the research was to evaluate morphofunctional platelets of patients with marginal periodontitis.

2. Methods

The study involved 129 men aged 28–50 years (mean age 42.97 ± 1.31 years) suffering from a MS with generalized periodontitis of mild and moderate degree. The diagnosis of MS is established following the recommendations of The Russian Scientific Society of Cardiology [17]. We also formed a control group of 90 practically healthy men (mean age 42.17 ± 1.34 years). The selection of patients was based on the inclusion/exclusion criteria, without the inclusion and exclusion.

An essential criterion for patients is abdominal obesity (waist circumference (WC) > 80 cm). There are additional criteria: arterial hypertension (AD≥140/90 mm Hg..); elevated triglyceride levels ≥ 1.7 mmol/l; elevated cholesterol (CS) level of low-density lipoprotein> 3.0 mmol/l; reduced level of high-density lipoprotein cholesterol ≤1,2 mmol/l; blood glucose level of fasting blood ≥6.1 mmol/l; body mass index (BMI) > 30 kg/m2. Also taken into account the index of oral hygiene (unsatisfactory or poor) and the depth of dentogingival pockets from 3.5 mm to 5 mm with bone resorption not exceeding 1/2 the length of the root of the tooth.

Exclusion criteria are symptomatic AH, unstable angina, myocardial infarction or stroke in history, severe chronic heart failure, severe liver and kidney disease, diabetes, complete secondary adentia. Patients were performed the dental examination with determination of community periodontal index of treatment needs (CPITN), X-ray examination, physical examination with determination of anthropometric data, as well as clinical, the definition of MS laboratory criteria, insulin resistance indices (insulin, C-peptide, indexes HOMA-IR, Caro), 24-h blood pressure monitoring. Platelet morphology was studied using scanning electron [17 clauser] microscopy (PhilipsPSEM 550 h) on samples prepared by a special method (AS Shitikova, 1991), aimed at preserving the morphology of platelets circulating in the blood stream. According to this method, a 3 mm prick is applied to the end phalanx of the finger, after which the tip of the finger is immersed in a beaker with 5 ml of isotonic sodium chloride solution and the bleeding time in transmitted light is taken into account. If the solution is very intensely stained with blood, then for further observation the finger moves to another such glass.

The amount of lost blood is determined by the increase in the volume of fluid in the cups (bleeding time norm - up to 4 min, the volume of lost blood - from 0.01 to 0.4 ml). This technique practically excludes the activation of platelets at the pre-analytic stage. The number of cells of each form was expressed as a percentage of the total number of cells in the sample. The results were processed using the Student and Wilcoxon criteria.

The research was carried out by dental clinics “Center for Biotic Dentistry” and the State Clinical Hospital named after E.O. Mukhina.

3. Results

The study of platelet morphology in patients with MS and control group revealed a non-uniform pattern. The number of platelets in both groups being almost equal (249,6 ± 8,3x109/l and 256,8 ± 8,7x109/l respectively).

The results of the study of the morphology of platelets in MS patients and control subjects were shown in Table 1.

Table 1
The composition of the main morphological forms of platelets in the blood plasma of patients with MS and control group, (M ± m).

Item	Patients with MS, n = 129	Control group, n = 90
Total number of platelets (10^9/l) (%)	249,6 ± 8,3	256,8 ± 8,7
Discocytes (%)	51,08 ± 0,20*	84,56 ± 0,06
Disco Echinocytes (%)	28,80 ± 0,24*	9,98 ± 0,12
Spherocytes (%)	14,49 ± 0,01*	2,79 ± 0,04
Spheroechinocyte (%)	4,06 ± 0,03*	1,95 ± 0,01
Bipolar forms (%)	1,57 ± 0,01*	0,72 ± 0,05
Sum of active forms (%)	48,92 ± 0,18*	15,44 ± 0,08
The number of platelets in the aggregates	13,30 ± 0,12*	6,21 ± 0,02
The number of small aggregates containing 2–3 thrombocytes per 100 freely lying platelets (%)	17,59 ± 0,23*	3,3 ± 0,04
The number of medium and large aggregates containing four platelets and more per 100 freely lying platelets (%)	5,10 ± 0,04*	0,09 ± 0,005

* - statistical significance in results compared to control group (p < 0,05).

4. Discussion

In patients with MS compared to the control group was detected a decrease in the number of basic physiological forms - discocytes and an increase in various activated forms of platelets.

In the control group, the number of discocytes exceeded their content in patients with MS more than 1.5 times.

The decrease in the substance of the basic physiological form of platelets in the blood plasma of MS patients is significant from the viewpoint of the functional activity of thrombocytes. It is known that the discoid form of the platelet mediates its intact state, due to the special internal organization of platelet microstructures and metabolic processes that ensure low functional activity. It is also known that platelet-shaped platelets carry a negative charge on their surface (as well as the endothelium of the vessel wall) and have a small contact surface area, which prevents platelets from performing adhesive-aggregation functions. The loss of the disk form leads to the activation of platelets and the growth of their functional activity. Together with the activation, there is an internal rearrangement of platelets ("internal contraction") with a change in their configuration and the formation of a multitude of pseudopods [11,13].

According to our research, the active forms of platelets in patients with MS and healthy individuals are primarily represented by disco echinocytes. These are thrombocytes with pointed processes - pseudopodia, which increase the area of the contact surface and provide contact between activated platelets during thrombosis. In patients with MS, the number of disco echinocytes was almost 3 times higher than the content of disco echinocytes in the group of healthy individuals, and more than twice times higher the upper limit of the norm for this indicator.

Also, patients with MS have a higher level of spherocytes - platelets of a disorderly coarse spherical configuration, which also contributes to an increase in the area of the contact surface. It is also known that the acquisition of a spherical form by thrombocytes is accompanied by a change in the physicochemical properties of their membranes, which becomes more elastic and adhesive, which facilitates the contact of platelets with each other and with other structures [10,11,13,14].

The number of spheroechinocytes (Fig. 1) in patients with MS was 4.06 ± 0.03%, which is almost 1.5 times higher than the permissible norm and more than twice as high as in the control group.

Thus, patients with MS had 48.92 ± 0.18% of active forms of platelets, which was almost half of the total number of platelets (in percent) and was almost equal to the number of discocytes - the basic intact morphological form of a platelet.

In the control group, the sum of the active forms of platelets was 15.44 ± 0.08%, while the number of intact forms was 84.56 ± 0.06%.

It is known [15] that the activation of platelets, resulting from the restructuring of their internal structural organization and metabolism in response to the stimulating effect, is accompanied by a change in their morphological and functional characteristics. This process, in turn, promotes cell-cell interactions and promotes platelet aggregation, leading to thrombosis.

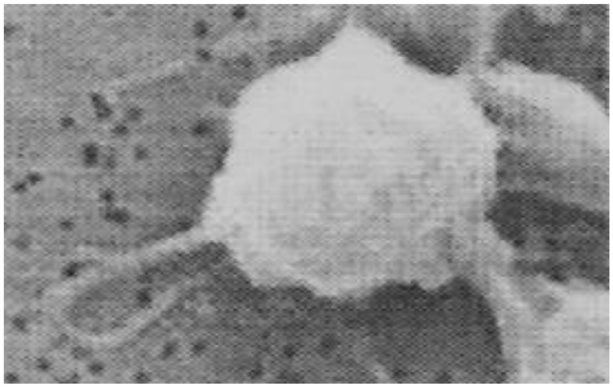

Fig. 1. Spheroechinocytes in patients with MS (scanning electron microscope).

During the study of the morphology of platelets, we also drew attention to the ratio of small, medium and large platelet aggregates in the group of patients with MS and control group.

We note significant increasing in the number of small aggregates in the blood of patients with MS (Fig. 2) - 17.59 ± 0.23 per 100

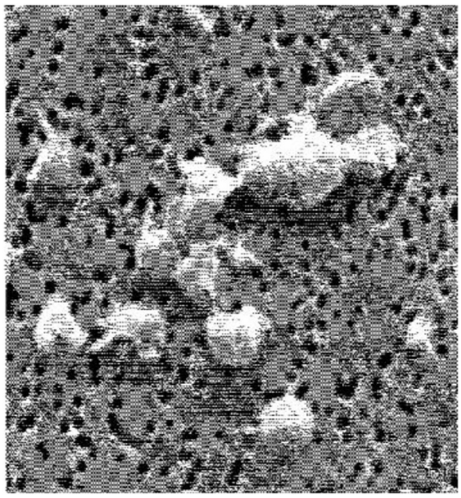

Fig. 2. Active forms of platelets form a platelet aggregate (scanning electron microscope, PhilipsPSEM 550x).

freely lying platelets (the upper limit of the norm is 3.9 per 100 freely lying platelets). In the control group, this indicator was 3.3 ± 0.04 per 100 freely lying platelets.

It is known that small aggregates of platelets can become a matrix necessary for the formation of large aggregates, the formation of which can lead to serious cardiovascular complications [19].

There are also differences in the number of medium and large platelet aggregates in the MS group and the control group. In the MS group, this index was 5.1 ± 0.04 per 100 freely lying platelets. Considering that within the permissible limit the maximum number of medium and large aggregates should not exceed 0.11 per 100 freely lying platelets, the result shows a high functional activity of platelets in patients with MS.

In the control group, the number of medium and large platelet aggregates was 0.09 ± 0.005 per 100 freely lying platelets, which is a normal level for this indicator.

It is known that during free movement in the bloodstream, aggregates can damage endotheliocytes. Subendothelial structures, including collagen fibers, are exposed; this is an additional stimulus leading to activation of primary hemostasis, which increases the risk of thrombotic complications [1,5,13,18].

5. Conclusion

The presented data provide strong evidence for changes in the morphofunctional state of peripheral blood platelets in patients with generalized marginal periodontitis of mild and moderate degree against the background of metabolic syndrome. It seems clear that in the conditions of metabolic disorders, there are pronounced disturbances of platelet hemostasis, which manifest themselves in a change in the morphology and functional activity of cells.

In turn, the parafunction of hemostasis during the metabolic syndrome probably worsens the rheological properties at all levels, including the periodontal tissues.

The results are significant for the understanding of the disease, the mechanisms of its progression and development of complications, the most successful development of methods of diagnosis of such disorders. Besides, these data allow forming a more effective interdisciplinary interaction of hematologists, endocrinologists, and dentists. Such studies help to form ideas about the need to develop schemes antithrombotic therapeutic intervention for the correction of existing violations and to prevent the development of CVD and MTR in such patients, as well as to evaluate the effectiveness of such therapy.

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