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HEMORHEOLOGIC PROFILE AND MICROCIRCULATORY HEMOSTASIS IN PATIENTS WITH CEREBROVASCULAR DISEASE IN DIABETES MELLITUS

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ABSTRACT — Diabetes mellitus is one of the most serious issues faced nowadays both by medicine and society in general, which is due to the wide spread of endocrine issues affecting nearly every country globally, the growing incidence rate, as well as the severity of complications that are hard to treat. Type 2 diabetes mellitus increases significantly the risk of developing acute cerebral blood circulation disorders, which urges further comprehensive studies focusing on the role played by the vascular-platelet relation and coagulation hemostasis in the development and progression of diabetic vascular complications. The results of our study, which involved 74 patients with acute cerebral circulation disorders against type 2 diabetes mellitus revealed alterations affecting the hemostasis system. This could be seen from activated vascular-platelet and coagulation links, decreased anticoagulant activity, and a slowdown in fibrinolysis. The severity of disorders induced by the alterations in the hemorheological profile and the microcirculatory hemostasis are associated with the duration of type 2 diabetes mellitus and the carbohydrate metabolism indicators (hyperglycemia, increased HbA1c levels and glycation end products).

KEYWORDS — diabetes mellitus; acute disturbance of cerebral circulation; blood rheological properties; hyperglycemia.

INTRODUCTION

The medical and social significance of cerebrovascular accident (CVA) is due to the respective significant rate of mortality, disability, persistent working incapacity, as well as socio-psychological maladjustment and economic losses manifested through treatment and rehabilitation costs [7, 14]. According to the Russian Statistics Agency (Rosstat) data, CVA accounts for 15.9% (2.9 cases per 1,000 inhabitants) in the overall number of deaths in the Russian Federation, giving way only to the deaths caused by cardiovascular issues. During that, the mortality rate resulting from brain vascular diseases is 4 times that observed in the USA and Canada [6]. One of the key risk factors for CVA development is type 2 diabetes mellitus (DM), whereas protein glycosylation, an increase in the plasma atherogenicity index, activation of coagulation and depression of blood fibrinolytic and anticoagulant properties, a reduction in the athrombogenic reserve, dysregulation of hemostasis and hemorheology occur under hyperglycemia. The basic mechanisms triggering cerebrovascular pathology in patients with type 2 diabetes are not only microcirculatory disorders, yet also small and large cerebral arteries atherosclerosis. It is notable that cell apoptosis, chronic vascular inflammation, activated intercellular adhesion molecules synthesis serve conditions for further progression of atherosclerotic lesions [1, 5, 9, 10].

Speaking of the pathogenesis related to later diabetic complications progress associated with microcirculation disorders and damage to the vascular bed of organs and systems, the authors point not only at structural and functional changes in the vascular wall, but at hemocoagulation and rheological disorders, too, the most sensitive being the erythrocyte and platelet link. Circulating platelets and erythrocytes are represented as populations featuring many different parameters (cell stability, cell age), while the transition from the physiologically normal status to the pathological one comes along with a change in the structure, properties of cell populations and their relationships [4, 13]. As respective literature holds it, patients with a long history of endocrinopathy, have hemorheological disorders manifesting through an increase in the plasma and whole blood viscosity, an increase in the red blood cells aggregation activity, erythrocyte membrane inability to resist destruction, low capacity of red blood cells for deformation, as well as exces-

sive development of leukocyte-erythrocyte aggregates [2, 3, 8, 11, 17].

Experts have shown that an increase in the glycated hemoglobin (HbA1c) levels, taken as a parameter for controlling carbohydrate metabolism and a marker of cardiovascular disorders, is an independent risk factor for CVA development, with females revealing a higher potential for its development compared to males [12, 16].

A significant number of works, both by national and foreign researchers, focus on early diagnosing of diabetic angiopathies, the development of algorithms for individual preclinical forecast, the choice of proper treatment tactics and prevention of vascular complications occurring through the toxic effect of hyperglycemia [15]. However, the data on the specific features pertaining to the population structure of peripheral blood platelets and erythrocytes, as well as data on the correlations of hematological parameters and the endocrinopathy duration in patients with type 2 diabetes and CVA, are of research interest, and are far from being complete and arranged in a systematic way.

Aim of study:
to analyze the status of the vascular-platelet link and peripheral blood coagulation hemostasis in patients with CVA in 2 diabetes mellitus.

MATERIALS AND METHODS

The study involved 74 patients (39 women, 35 men; mean age 63.7±11.2) with CVA and type 2 diabetes. The patients were admitted to the Neurology Ward of the Volgograd Regional Clinical Hospital #1 on the first day following the development of acute neurological symptoms. All patients underwent overall physical and neurological examination as well as general clinical and biochemical tests. The diagnosis of CVA was verified subject to the results of magnetic resonance imaging (MRI) and the clinical presentation. While the patient was undergoing the MRI procedure, the nature of the stroke (ischemic or hemorrhagic), the magnitude and prevalence of focal brain changes were clarified. The atherosclerosis degree affecting the head main arteries was identified and evaluated based on Duplex scanning. All the patients were divided into three groups: Group 1 — the history of type 2 diabetes ≤ 1 month; Group 2 — the history of type 2 diabetes — about 1 year; Group 3 — the history of type 2 diabetes ≥ 2 years. The diagnosis of DM was set subject to the WHO criteria (1999–2006) and the Russian specialized medical care algorithms for patients with diabetes mellitus (2017). On the day of admission, the patients were evaluated for reactive changes (blood smear test, stained by Romanovsky-Giemsa). The morphometric parameters of peripheral blood cells were studied using a Mekos-C3 hardware and software unit (light microscopy, ×1000). When identifying the HbA1c percentage, a Quo-Lab (EKF-diagnostic) glycosylated hemoglobin analyzer was employed. The hemostasis basic parameters were identified on an automatic Sysmex CS-2100i coagulometer.

The obtained data were processed employing the one-factor analysis of variance (ANOVA) with the Microsoft Excel 2019 software packages, while identifying the differences at the achieved level $p \leq 0.05$, provided that the observed Fisher criterion ($F_{obs.}$) exceeded the Fisher critical criterion ($F_{crit.}$). The analysis of the relationship between the poikilocytosis indicators and those of glycated hemoglobin was preformed using the Pearson (rP) and Spearman (rS) correlation coefficients.

RESULTS AND DISCUSSION

Of the total number of the examined patients, 91.9% were diagnosed with ischemic stroke; 8.1% — with hemorrhagic stroke; atherosclerotic lesions of the head main arteries were detected in 89.2% of the cases, while another 35.1% of patients were found to have hemodynamically significant stenoses (≥60%). Tables 1–3 offers a view at the indicators of the peripheral blood erythrocyte and platelet levels.

A comparative analysis of hematological indices of the erythron system (Table 1) points not only at a significant increase in the RDW (Red blood Cell Distribution Index) and HCT (Hematocrit number), yet also is indicative of an increasing anisocytosis and poikilocytosis, as well as a change in the plasma vs. red blood cell mass ratio towards an increase in the latter with an increase in the endocrinopathy history. The identified trend indicates accelerated cell aging under hypoxia and an increase in the peripheral blood viscosity. The other hematological parameters failed to reveal any significant change in the erythrocyte indices.

An analysis of peripheral blood preparations using the MEKOS-C3 units reveals a statistically significant ($P \leq 0.001$, $F_{obs.} > F_{crit.}$) increase in the anisocytosis coefficient (normocytes — 54%; microcytes — 44%; macrocytes — 2%) and poikilocytosis (echinocytes — 46%; acanthocytes — 28%; eliptocytes — 3%; discocytes — 15%; reversibly altered forms — 8%) (Fig. 1A). The data obtained from the erythrocyte formula have been confirmed through a significant increase in the diameter, area of red blood cells as well as a significant increase in their shape factor, which indicates progressive degenerative changes in the erythrocyte membrane due to lengthy metabolic disorders (increased HbA1c concentration, exposure to the lipid peroxidation system). Besides, an increase

Table 1. *Data from the hematological study of the peripheral blood erythrocyte link*

Indicators	group I n = 22	group II, n = 25	group III, n = 27	P at $F_{crit.} > F_{cr/criterion.}$
Hematology analyzer				
RBC ($\times 10^{12}$/l)	4,73±0,12	4,57±0,20	4,73±0,16	P≥0,5
MCV (fl)	89,8±0,7	89,2±1,4	91,9±1,9	P≥0,5
RDW (%)	13,5±0,3	14,9±0,4	15,9±0,3	P≤0,001
HCT (%)	38,2±0,9	40,3±0,9	42,6±1,0	P≥0,001
HGB (g/l)	145±7	145±4	151±5	P≥0,5
MCH (pg)	31±0,7	32±0,7	31,9±0,7	P≥0,5
MCHC (g/l)	350±7	344±2	344±1	P≥0,5
MEKOS-C3 hardware and software unit				
Ovalocytosis coefficient	0,84±0,02	0,83±0,01	0,84±0,01	P≥0,5
Poikilocytosis coefficient, (%)	9±1	16±1	44±3	P≤0,001
Anisocytosis coefficient (%)	7±0,4	7,5±0,4	10±0,4	P≤0,001
The area of red blood cells,(mm^2)	143,6±0,3	146,4±0,2	150,5±0,2	P≥0,05
The average diameter of the red blood cell (microns)	7,5±0,2	7,3±0,2	6,5±0,1	P≤0,05
Red blood cell form factor	33,6±1,2	40,9±1,0	43,4±1,0	P≤0,001

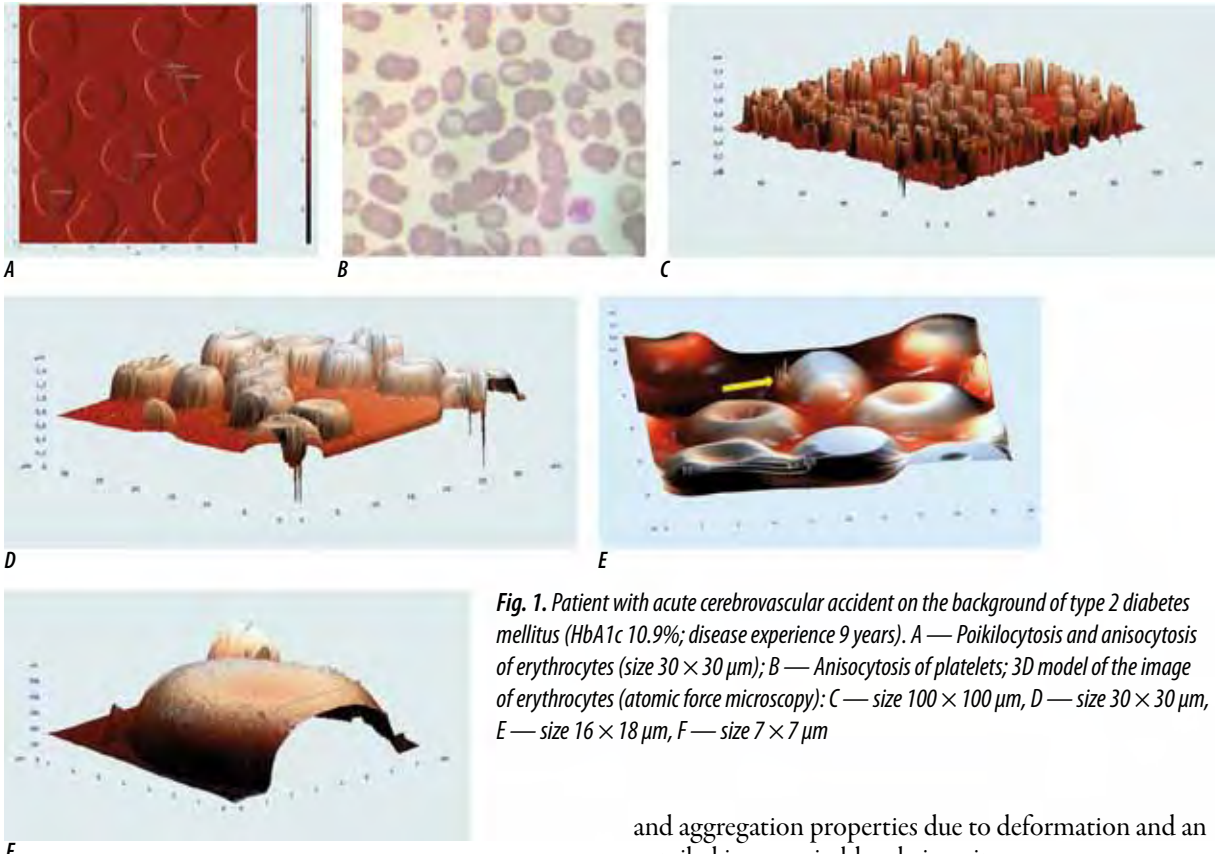

Fig. 1. *Patient with acute cerebrovascular accident on the background of type 2 diabetes mellitus (HbA1c 10.9%; disease experience 9 years). A — Poikilocytosis and anisocytosis of erythrocytes (size 30 × 30 μm); B — Anisocytosis of platelets; 3D model of the image of erythrocytes (atomic force microscopy): C — size 100 × 100 μm, D — size 30 × 30 μm, E — size 16 × 18 μm, F — size 7 × 7 μm*

in the number of aggregated forms of red blood cells and erythrocyte-platelet conglomerates was revealed (Fig. 1B), which means an increase in their adhesive and aggregation properties due to deformation and an entailed increase in blood viscosity.

A comparative assessment of platelet indices reveals a statistically significant increase in PCT (thrombocrit), PDW, MPV (average platelet volume), which points at an increasing thrombocytosis, a growth

in the degree of platelet anisocytosis, an increased number of their young and giant forms. This means an enhanced activation of the hemostasis system against an increase in the endocrinopathy history complicated by CVA.

A test of peripheral blood smears also showed high platelet anisocytosis (macrothrombocytes — 25%, normal platelets — 75%) (Fig. 2A, 2B) with their giant forms appearing in the decompensation stage in patients with different experience of endocrinopathy against CVA. The appearance of a large number of macrothrombocytes in patients with angiopathies and type 2 diabetes as early as starting from the 1st month of the disease progress means a high stress on the megakaryocytic germ of the red bone marrow, which results in immature hypo-granulated platelets separating from the megakaryocyte. The body's response involves compensating for the hypogranulation of platelets by their size, thus leading to the development of macrothrombocytes.

their aggregation delay stage decreases. An increase in platelet aggregates during ristocetin induction may be associated with an increased release of Willibrand factor from platelet α-granules, this leading to increased platelet agglutination.

In order to identify the correlations between an increase in the level of HbA1c and poikilo-, anisocytosis of red blood cells, an analysis of the respective relationship was carried out based on the Pearson coefficient (rP) and the Spearman coefficient (rS) (Table 3).

Following the Cheddock scale, a *strong* correlation was observed between the red blood cell poikilocytosis coefficient and the HbA1c level in Group III of patients, whereas in Group II the observed correlation could be described as *notable* (Table 3). High (strong) correlation was also to be observed in patients belonging to Groups II and III between the anisocytosis coefficient and the HbA1c level. The obtained outcomes indicate that patients with type 2 DM history

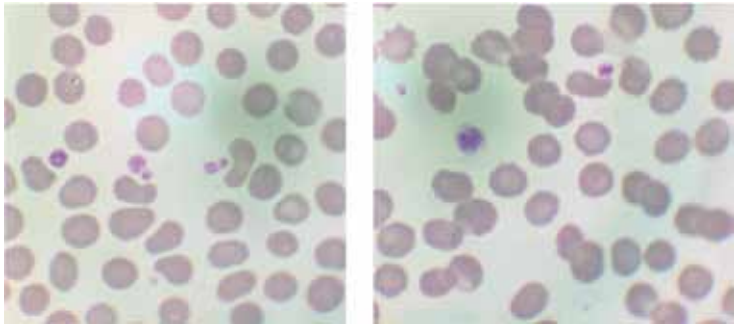

Fig.2. *Platelet anisocytosis in a patient with CVA and type 2 diabetes (HbA1c 7.9%)*

A statistically significant decrease in activated partial thromboplastin time (APTT) and an increase in the fibrinogen level in hyperfibrinogenemia in the groups being compared indicates hyperproduction of fibrinogen, as well as its accelerated conversion into fibrin, thus confirming increased activation of platelet α-granules, this leading to disturbance in the coagulation hemostasis parameters (Table 2). An analysis of induced platelet aggregation with various inducers revealed no significant change in the platelet aggregation degree, yet there was a significant increase in the platelet aggregates size to be observed with each of the inducers (ADP, collagen and ristocetin) (Table 2). An increase in the size of aggregates with ADP of 1 mmol/l and with collagen points at an increased readiness of platelet activation systems, due to which — when platelet aggregation with ADP is stimulated — absorption between aggregating elements grows; when collagen is stimulated, though, it results in an increase of the thrombocytes reactivity, whereas exceeding 1 year in the decompensation stage feature an increase in the aging process of the red blood cells functional pool with an increase in the HbA1c level, which leads to increased aggregation of red blood cells; increased blood viscosity and the development of micro-and macroangiopathies. It is to be noted that patients with CVA and a type 2 diabetes history exceeding one year, at an HbA1c level of up to 8%, have more significant anisocytosis of red blood cells, while patients with an HbA1c content of above 10% have poikilocytosis prevailing over anisocytosis. We believe that this condition is triggered by the launch of compensatory mechanisms aimed at eliminating hypoxia and maintaining homeostasis.

CONCLUSIONS

1. In patients with type 2 diabetes (where the history of the issue in questions exceeds one year) and CVA, changes in the peripheral blood rheological properties (red blood cells increased aggregation;

Table 2. *The hemostasis system and platelet aggregation indicators*

Indicators	group I n = 22	group II, n = 25	group III, n = 27	P at $F_{crit.} > F_{cr/criterion.}$
Indicators of the hemostasis system				
Activated Partial Thromboplastin Time (APTT), (sec)	36,3±1,2	35,8±0,5	34,2±0,3	P≥0,001
International Normalized Ratio (INR)	1,28±0,14	1,17±0,09	1,15±0,08	P≤0,5
Prothrombin time ratio, P/C ratio (%)	97±1	98±1	99±1	P≤0,05
Pro Time, PT (sec)	12,9±0,5	12,9±0,3	13,0±0,3	P≥0,5
Fibrinogen (gramm / liter)	4,20±0,2	4,52±0,18	5,77±0,16	P≤0,001
Platelet aggregation				
ADF 1mmol/l (%)	78±2	82±1	80±5	P≤0,5
Micron	8±0,01	8,4±0,4	11,9±0,9	P≤0,001
Collagen (%)	87±2	78±4	83±4	P≤0,5
Micron	8,1±0,4	8,9±0,4	11,1±0,6	P≤0,001
Ristomycin (ristocetin) (%)	89±3	93±2	98±3	P≤0,1
Micron	7,6±0,2	8,4±0,2	9,5±0,5	P≥0,001

Table 3. *The correlation coefficients of poikilo-, anisocytosis and glycated hemoglobin indicators*

Pearson coefficient		
	Dependence of HbA1c concentration on:	
	poikilocytosis	anisocytosis
Type 2 DM ≤ 1 month	<0,1	<0,1
Type 2 diabetes about 1 year	>0,6	>0,7
Type 2 DM ≥2 years	>0,9	>0,7
Spearman's Coefficient		
	Dependence of HbA1c concentration on:	
	poikilocytosis	anisocytosis
Type 2 DM ≤ 1 month	<0,1	<0,1
Type 2 DM about 1 year	>0,5	>0,7
Type 2 DM ≥2 years	>0,9	>0,7

red blood cells increased transformation into irreversible and reversible forms; plasma and whole blood increased viscosity; leukocyte-erythrocyte aggregates excess; increased adhesion of red blood cells to vascular walls endothelial cells) the accumulation of carbohydrate metabolism products shapes the basis of pathogenetic mechanisms that promote the development of later diabetic angiopathies.

2. In patients with type 2 diabetes, CVA mainly develops against significant atherosclerotic lesions of the head main arteries, including with hemodynamically significant stenoses.
3. CVA manifests a significant parallelism between the hemostatic system activation (changes in platelet aggregation, fibrinogen level, fibrinolytic activity) and the carbohydrate metabolism indices (plasma glucose, HbA1c, glycation end products).
4. An objective investigation of the HbA1c level, seen as a key diagnostic criterion for the CVA course in patients with carbohydrate metabolism disorders, will allow setting a timely diagnosis of endocrinopathy, as well as offering a forecast for the outcome.
5. A proper individualized pathogenetic treatment administered through the acute period of stroke will help improve the potential outcome and the life forecast for patients suffering from the said combined pathology.

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