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Comparative assessment of macro-, micro- and molecular rheological factors in patients with ischemic stroke. (Preliminary results)

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Abstract

Currently, the main cause of death in the developed countries of the world are diseases of the circulatory system, including vascular diseases of the brain. The development and implementation of new programs and methods for diagnosing, treating and preventing cerebrovascular diseases is the most important medical, social and economic task. Prioritization of fundamental studies in the fields of neurology and angiology has been the study of pathophysiological mechanisms of ischemic stroke development, as study of rheological properties. Our goal was to study a group of patients with ischemic brain stroke. Our group was from 10 people with an average age 72±5,4 years, the control group consisted of volunteers aged 69±2,3 years (n=12). All subjects underwent the following studies 1) To monitor acrorheological factors, we examined hematocrit and plasma viscosity. For this we used HumanCounter, Germany. 2) To monitor macrorheological factors, we studied the aggregability of erythrocytes and the deformability of erythrocyte membranes. For this we used the texture analysis method and the filtration method. It turned out that in the group of patients with ischemic stroke, all parameters were changed, but the percentage of signifi-

cant destruction was most observed in the direction of microrheological factors, more precisely, the aggregation of erythrocytes changed by more than 2 times compared to the control group. Our preliminary experiments have shown that erythrocyte aggregation is a necessary parameter for assessing blood rheology.

KEYWORDS: stroke; rheology; erythrocytes

Introduction

Currently, the main cause of death in the developed countries of the world are diseases of the circulatory system, including vascular diseases of the brain. The development and implementation of new programs and methods for diagnosing, treating and preventing cerebrovascular diseases is the most important medical, social and economic task.

Prioritization of fundamental studies in the fields of neurology and angiology has been the study of pathophysiological mechanisms of ischemic stroke development. The raw oxygen demand of the naked brain is about 20%-25% of the circulating oxygen in the roofs. A decrease in oxygen and glucose uptake into the cerebral cortex results in a condition that drags in the room for irreversible changes in the cerebral cortex. The poisonous cerebral cortex is considered to be one of the basic indicators of perfusion of cerebral tissues. This amount in the norm is 50-55 ml per 100 g of cerebrospinal fluid per minute. The nature of changes in the circulatory system of the cerebellum depends on the duration and magnitude of the reduction of the cerebral cortex, as well as the sensitivity to hypoxia of various parts of the cerebral cortex. The degree of reduction of the cerebral cortex and the sensitivity of the cerebellum to hypoxia determine the reversibility/irreversibility of pathological changes in ischemia. The zone of irreversible injury is called ischemia, the zone of reverse injury is called “penumbra”.

Microcirculation determines the adequacy of adequate blood supply and unsuitable oxygen supply.

Organs with the development of the capillary set, as well as the cerebral cortex, suffer to greater degrees in microcirculatory disorders. The microcirculation occupies the peritoneal position between the arteries and veins, the border of which is read with a diameter of 100 μm [1]. Microcirculation in fabrics depends on the composition of the capillary surface and the rheological properties of the roofs.

The roof fulfills all its important functions thanks to the current. Properties of fluidity,

determination of ability is current, called rheological. Hemorrhage – This is a science that studies the processes of flow and deformation of roofs and elements (erythrocytes, leukocytes, thrombocytes) and their interaction with subdued endothelium in the stream [1,2,3].

There are several groups of factors that affect the rheological properties of roofs:

1) Macrorheological factors, determining the properties of roofs as a whole: viscosity of roofs and plasma, hematocrit, the diameter of the vessel;

2) Microrheological, stimulant properties of cell roofs: functional composition of elemental elements – deformability and aggregation activity of erythrocytes;

3) Molecular factors: plasma lipids, fibrinogen, etc., which induce activation of erythrocyte aggregations;

The aim of our work was to find out the complex change in rheological factors, i.e. change in macrorheological factors, microrheological factors, as well as molecular factors in patients with ischemic stroke in patients from 67 to years 77.

Methods

We studied a group of patients of 10 people with an average age 72 ±5,4 years, the control group consisted of volunteers aged 69 ±2,3 years (n=12). All subjects underwent the following studies.

1) To monitor macrorheological factors, we examined hematocrit and plasma viscosity. For this we used HumanCounter, Germany.

2) To monitor macrorheological factors, we studied the aggregability of erythrocytes and the deformability of erythrocyte membranes. For this we used the texture analysis method and the filtration method.

Erythrocytes deformability index. Evaluation of erythrocytes deformability was performed with an aid of the nucleopore membrane filter method, which is based on assessing the velocity of the erythrocytes passage through the very small pores (5 μm, which is a diameter of the smallest capillary) of the filter, at constant pressure (10 cm of water column) and temperature (37°C). Obtaining the pure erythrocytes was performed by centrifuging the blood sample at 3000 rpm, for 15 min. The resulting plasma was aspirated with a micropipette and the remaining blood cells were added with bovine serum albumin (0.2 mg per 5 ml) dissolved in the phosphate buffer. Then the blood was centrifuged a second time at 1000 rpm for 5 min. The precipitated erythrocytes, as well as a thin layer of leukocytes and thrombocytes, were separated from the phosphate

buffer. This procedure was repeated three times. Purified erythrocyte mass was diluted in the phosphate buffer, with a hematocrit of 10%. Evaluation of the deformability index implied measuring the velocity of the erythrocyte passage through the filter (mm/min) was recorded. The high-quality polycarbonate filters (with 5 μm diameter pores) were used in measuring procedures [4].

Erythrocytes aggregability index. The index of erythrocytes aggregability, which represents aggregated erythrocytes area ratio against whole area of the erythrocytes. Erythrocyte aggregation was evaluated with the recently developed "Georgian technique" providing us with direct and quantitative data., Blood samples (4ml) from the cubital veins were centrifuged and about 0.1 ml blood was diluted 1:200 in own plasma in the Thoma pipettes preliminary rinsed with 5% sodium citrate solution without addition of any other anticoagulants to the blood under study. Following standard mixing the diluted blood was placed into a glass chamber 0.1 mm high. The quantitative index of erythrocyte aggregation, which was assessed with a special program at the Texture Analysis System (TAS-plus, "Leitz, Germany), represented itself the relationship of the aggregated and unaggregated red cells [5,6].

3) To monitor molecular factor, we examined fibrinogen. For this we used standards mini analizator (Coatron 8.1, Germany).

Results

It turned out that in the group of patients with ischemic stroke, all parameters were changed, but the percentage of significant destruction was most observed in the direction of microrheological factors, more precisely, the aggregation of erythrocytes changed by more than 2 times compared to the norm. See tab. 1

Table 1. *Percentage change in rheological factors in a group of patients with ischemic stroke compared with the control group. (n in group with patients – 10 people, n in the control group – 12 people).*

Δhematocrit,%	Δplasma viscosity, %	Δ RBC aggregation, %	ΔRBC deformation,%	Δfibrinogen,%
11	20	201	25	29

Discussion

Analysis of hemorheological parameters in patients with different severity of ischemic stroke showed an increase in blood viscosity in patients. Data have been obtained indicating a direct relationship between the increase in fibrinogen levels and other parameters. Increased erythrocyte aggregation is a significant pathogenetic mechanism in stroke patients. Thus, neurological disorders in patients with ischemic strokes are associated with a pronounced impairment of the rheological properties of blood.

Structural changes in the erythrocyte membrane leading to an increase in their aggregability and a decrease in deformability, which aggravates cerebral ischemia. Violations of the functional state of erythrocytes lead to microcirculation disorders. This is an important factor in the pathogenesis of ischemic cerebrovascular accidents [1,7,8,9,10]. In recent years, a large number of experimental and clinical studies have been carried out, which have shown that a good in-depth diagnosis and adequate treatment at first is an undeniable advantage for the management of patients with cerebral stroke. Our preliminary experiments have shown that erythrocyte aggregation is a necessary parameter for assessing blood rheology.

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