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## Severity Of Aggregation Neutrophils In Patients With Type 2 Diabetes Mellitus.

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### ABSTRACT

High pathological burden of the population of different countries of the world is in many respects connected with an increase in prevalence in persons of working age of type 2 diabetes. A very high risk of this disease is associated with a high incidence of various thromboses with it. The leading cause of this complication is the hyperaggregation of blood cells. The goal is to evaluate the aggregation activity of neutrophils in patients with type 2 diabetes mellitus. We examined 36 patients of the second adulthood (mean age  $47.4 \pm 2.1$  years) with type 2 diabetes mellitus. The control group consisted of 26 clinically healthy people of the same age. All the examined persons gave written informed consent to participate in the study. Biochemical, hematological and statistical methods of investigation were used. It was found that with type 2 diabetes mellitus, antioxidant protection of the plasma is weakened with activation of lipid peroxidation processes in it. These changes are largely the cause of increased aggregative capacity of neutrophils. These changes are exacerbated by the weakening of the disaggregating properties of these cells. As a result, patients receive a sharply increased risk of thrombosis of any location, which can lead to disability and death.

**Keywords:** neutrophils, pathology, type 2 diabetes mellitus, thrombophilia, aggregation.

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## INTRODUCTION

The high incidence of type 2 diabetes mellitus [1,2] among the population of many countries of the world is one of the reasons for the high prevalence of vascular complications threatening disability and early death [3]. It is believed that the basis for the development of thrombosis in type 2 diabetes mellitus is a marked increase in the aggregation of all blood cells [4,5]. As a result, the activation of hemostasis, leading to the development of thrombophilia, inevitably occurs [6,7,8]. An important triggering factor of this process is a decrease in the sensitivity of blood cells of patients to the action of prostacyclin and nitric oxide [9,10]. Considering the high prevalence of type 2 diabetes mellitus and great significance for the processes of microcirculation of neutrophil aggregation, it was considered important to elucidate the characteristics of neutrophilic leukocyte aggregation in this patient population [11].

The aim of the study is to evaluate the aggregation activity of neutrophils in patients with type 2 diabetes mellitus.

## MATERIAL AND METHODS

The research was approved by the Ethics Committee of Russian State Social University (record №5 from 12.05.2014).

We examined 36 patients of the second mature age (mean age  $47.4 \pm 2.1$  years) with type 2 diabetes mellitus [12]. The control group was composed of 26 clinically healthy people of the same age. All the examined persons gave written informed consent on participation in the research. All participants in the study gave their written consent to participate in it [13].

Intensity of lipids' peroxidation (LPO) processes in plasma was estimated according to the content of thiobarbituric acid (TBA)-active products by a kit "Agat-Med" and acylhydroperoxides (AHP) [14]. Antioxidant abilities of liquid part of blood were determined according to the level of its antioxidant activity [15].

LPO activity in studied regular blood elements was determined according to the quantity of malon dialdehyde (MDA) in reduction reaction of thiobarbituric acid in washed and resuspended cells and the content of AHP in them [14]. In studied washed and resuspended regular blood elements we estimated the levels of cholesterol by enzymatic colorimetric method with the help of a kit "Vital Diagnostikum" and total phospholipids according to the content of phosphorus in them.

Aggregation of neutrophils was recorded on a photoelectrocolorimeter [16]. Inductors were used lectin wheat germ at a dose of  $32 \mu\text{g/ml}$ , concanavalin A -  $32 \mu\text{g/ml}$  and phytohemagglutinin -  $32 \mu\text{g/ml}$ .

The results were processed by Student's criterion (t). Statistical processing of received information was made with the help of a program package "Statistics for Windows v. 6.0", "Microsoft Excel". Differences in data were considered reliable in case of  $p < 0.05$ .

## RESEARCH RESULTS AND DISCUSSION

The patients were noted to have evident plasma LPO activation – the content of AHP in it surpassed the control value in 2.2 times, TBA-active products – in 1.5 times, being accompanied by suppression of antioxidant plasma activity in 1.4 times (Table).

The observed patients were noted to have increased CS content in neutrophils membranes which was accompanied by the decrease of total phospholipids in them and LPO activation on behalf of weakening of their antioxidant protection (Table).

In the observed patients, the process of neutrophil aggregation with applied inducers occurred earlier than in the control (with lectin by 48.1%, with concanavalin A by 33.8%, with phytohemagglutinin by 31.7%) (Table).

All the patients were noted to have the decrease of vessels' disaggregative impacts on neutrophils (Table).

**Table. Registered indicators in the surveyed**

Registered parameters	Patients, n=36, M±m	Control, n=26, M±m
acylhydroperoxides plasma, D <sub>233</sub> /1ml	3.10±0.10	1.42±0.09 p<0.01
TBA-compounds, µmol/l	5.34±0.14	3.56±0.07 p<0,01
antioxidant activity plasma, %	23.8±0.16	32.9±0.12 p<0.01
biochemical parameters of neutrophils		
cholesterol of neutrophils, µmol/10 <sup>9</sup> neutrophils	0.83±0.008	0.62±0.004 p<0.01
common phospholipids of neutrophils, µmol/10 <sup>9</sup> neutrophils	0.35±0.014	0.51±0.003 p<0.01
acylhydroperoxides of neutrophils, D <sub>233</sub> /10 <sup>9</sup> neutrophils	3.52±0.02	2.36±0.05 p<0.01
malonic dialdehyde of neutrophils, nmol/10 <sup>9</sup> neutrophils	1.46±0.08	0.73±0.03 p<0.01
catalase of neutrophils, ME/10 <sup>9</sup> neutrophils	6100.0±18.23	9950.0±19.77 p<0.01
superoxidismutase of neutrophils, ME/10 <sup>9</sup> neutrophils	1220.0±5.21	1780.0±4.21 p<0.01
aggregation of neutrophils		
Aggregation with lectin, %	23.1±0.14	15.6±0.07 p<0.01
Aggregation with concanavalin A, %	19.8±0.12	14.8±0.04 p<0.01
Aggregation with phytohemagglutinin, %	40.3±0.17	30.6±0.09 p<0.01

Note: p - reliability of differences in the indices of a group of patients and a control group.

Important significance in the development of rheological disturbances and thrombophilia in persons with type 2 diabetes mellitus belongs to aggregation increase of regular blood elements and especially – neutrophils [17,18]. At type 2 diabetes mellitus the depression of plasma antioxidant activity is formed which provides the increase of LPO activity in it [19]. The increase of freely radical processes in liquid part of blood inevitably promotes the damage of neutrophils' membranes [20]. The development of these manifestations in combination with found in these patients' neutrophils lipid imbalance leads to their hyperaggregability. At the same time, the level of ability to disaggregate in platelets [21,22,23].

The intensification of neutrophil aggregation in type 2 diabetes mellitus is largely due to the weakening of their sensitivity to the physiological disaggregants produced in the vessel wall. This is exacerbated by an increase in the density of receptors on leukocytes capable of interacting with lectins used in the work as aggregation inducers [24,25]. The amplification of lectin- and concanavalin A-induced aggregation of neutrophils in plasma taken against a background of temporary venous occlusion in patients with type 2 diabetes mellitus is associated with an increase in the number of adhesion receptors on neutrophils, including many sites with N-acetyl-D-glucosamine, N-acetyl-neuraminic acid and mannose [26, 27]. Growth of neutrophil aggregation with phytohemagglutinin is caused by an increase in the composition of their receptors

sites with bD-galactose [28,29] with a pronounced decrease in receptor density to prostacyclin and NO [30,31,32].

### CONCLUSION

The frequent occurrence among the population of many countries of type 2 diabetes requires further study of this pathology. Its great danger of this disease is associated with a high incidence of thrombosis in its presence. In the conducted study, it was established that in these patients lipid peroxidation in plasma was sharply increased. This is associated with an increase in these patients of excessive aggregation of neutrophils. Simultaneous depression of mechanisms of disaggregation and active mechanisms of neutrophil aggregation strongly worsens microcirculation, thereby weakening tissue trophism and increases the risk of thrombosis in individuals with type 2 diabetes [33,34,35].

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