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Disaggregation Control Of Blood Vessels Over The Activity Of Platelets In Patients With Type 2 Diabetes Mellitus.

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ABSTRACT

In modern society, there is still a very high incidence of type 2 diabetes mellitus. The special danger of this disease in addition to the violation of carbohydrate metabolism is associated with frequent thromboses associated with the development of vasopathy, the nature of which has been studied insufficiently. The goal is to assess the level of disaggregation capacity of blood vessels in relation to platelets in type 2 diabetes mellitus. We examined 36 patients of the second mature age (mean age 47.4±2.1 years) with type 2 diabetes mellitus. 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. There were applied biochemical, hematological and statistical methods of investigation. High thromboses' frequency of various localizations at type 2 diabetes mellitus is closely connected with angiopathy development against their background. Weakening of plasma antioxidant protection with activation of lipids' peroxidation processes in it leading to alteration of vascular wall, is noted in conditions of type 2 diabetes mellitus. The persons with type 2 diabetes mellitus are detected to have evident weakening of disaggregating vascular impacts of vascular wall on strengthening aggregative ability of platelets. In the result of it given patients get sharply increased risk of thromboses of any localization which can lead to invalidism and lethal outcome.

Keywords: platelets, vasopathy, type 2 diabetes mellitus, vascular wall, antiaggregation.

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INTRODUCTION

In recent decades, violations of carbohydrate metabolism in the form of type 2 diabetes mellitus remain high and do not tend to decrease [1,2]. It has been repeatedly noted that the development of vascular thrombosis leading to disability and early death is very often registered in this contingent of patients [3]. Previous studies have shown that at the heart of these thromboses is very often vasopathy, the frequency of occurrence of which in these patients has recently increased [4]. It is known that vasopathy, as a rule, is manifested by the weakening of vascular control over the aggregation of blood cells and the activity of hemostasis mechanisms [5,6,7]. The manifestations of vasopathy are based on the depression of the synthesis of vascular deaggregants, the most active of which are prostacyclin and nitric oxide [8,9]. Given the high incidence of type 2 diabetes, it seemed important for science and practice to assess the level of vascular control of platelet aggregation in such patients [10]. In this connection, in this paper, the goal is to evaluate the level of disaggregation capacity of blood vessels in relation to platelets in type 2 diabetes mellitus.

MATERIALS 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±2.1 years) with type 2 diabetes mellitus [11]. 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 those surveyed agreed to participate in the study [12].

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) [13]. Antioxidant abilities of liquid part of blood were determined according to the level of its antioxidant activity [14].

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 [13]. 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 CPL according to the content of phosphorus in them.

Evidence of vascular wall's control over platelets' aggregation was detected according to its weakening in the test with temporal venous occlusion [15].

The activity of the platelet aggregation process (AP) was assessed using a visual micromethode [16] in plasma obtained after temporary venous occlusion and without it using ADP ($0.5x10^{-4}$ M), collagen (1: 2 dilution of the base suspension), thrombin 0.125 units / ml), ristomycin (0.8 mg / ml), epinephrine ($5.0×10^{-6}$ M) and with combinations of ADP and epinephrine; ADP and collagen; adrenaline and collagen in the same doses in a platelet-rich plasma standardized for platelet count to $200 × 10^9$ platelets. The magnitude of the vascular wall antiggregational activity index (IAASC) was calculated in the course of dividing the time of onset of AP in plasma taken against the background of venous occlusion for the duration of development of AP in the intact plasma. The level of disaggregation effects of blood vessels on the processes of intravascular aggregation of platelets was determined using a phase contrast microscope and considering the number of small, medium and large aggregates and the degree of involvement of platelets in plasma taken without the use of temporary venous occlusion and in plasma obtained against its background [17,18].

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 $p<0.05$.

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).

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

Table. Registered indicators in the surveyed

Registrated parameters	Patients, n=36, M±m	Control, n=26, M±m
acylhydroperoxides plasma, D_{233}/1ml	3.10±0.10	1.42±0.09 p<0.01
TBA-compounds, umol/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 platelets		
cholesterol of platelets, umol/10^9 platelets	1.02±0.002	0,67±0,005 p<0,01
common phospholipids of platelets, umol/10^9 platelets	0.34±0.014	0,49±0,004 p<0,01
acylhydroperoxides of platelets, D_{233}/10^9 platelets	3.32±0.08	2,20±0,04 p<0,01
malonic dialdehyde of platelets, nmol/10^9 platelets	1.40±0.15	0,68±0,02 p<0,01
catalase of platelets, ME/10^9 platelets	5400.0±20.31	9790,0±20,10 p<0,01
superoxidismutase of platelets, ME/10^9 platelets	1250.0±8.62	1650,0±3,00 p<0,01
aggregation of platelets in intact plasma		
aggregation with ADP, s	25.7±0.15	41,0±0,12 p<0,01
aggregation with collagen, s	22.0±0.16	33,2±0,10 p<0,01
aggregation with thrombin, s	38.6±0.18	55,3±0,05 p<0,01
aggregation with ristomycin, s	29.1±0.12	45,2±0,06 p<0,01
aggregation with epinephrine, s	74.2±0.14	93,0±0,07 p<0,01
aggregation with ADP and epinephrine, s	21.0±0.13	34,5±0,04 p<0,01
aggregation with ADP and collagen, s	16.3±0.14	26,6±0,05 p<0,01
aggregation with epinephrine and collagen, s	14.1±0.18	29,2±0,12 p<0,01
The number of platelets in the aggregates, %	13.2±0.23	6,5±0,07 p<0,01
Number of little aggregates (in 100 free thrombocytes)	17.0±0.24	3,1±0,03 p<0,01
Number of medium and large aggregates (in 100 free thrombocytes)	1.51±0.05	0,14±0,03 p<0,01
cardiovascular control of platelet aggregation		

IAAVW with ADP	1.24±0.10	1,53±0,16 p<0,01
IAAVW with collagen	1.19±0.18	1,48±0,16 p<0,01
IAAVW with thrombin	1.21±0.25	1,44±0,13 p<0,01
IAAVW with ristomycin	1.27±0.18	1,56±0,11 p<0,01
IAAVW with epinephrine	1.33±0.10	1,62±0,13 p<0,01
IAAVW with ADP and epinephrine	1.27±0.17	1,49±0,12 p<0,01
IAAVW with ADP and collagen	1.25±0.19	1,51±0,10 p<0,01
IAAVW with epinephrine and collagen	1.19±0.20	1,53±0,11 p<0,01
The number of platelets in the aggregates after temporary venous occlusion, %	9.3±0.13	4,5±0,15 p<0,01
Number of little aggregates (in 100 free thrombocytes) after temporary venous occlusion	6.3±0.12	2,1±0,15 p<0,01
Number of medium and large aggregates (in 100 free thrombocytes) after temporary venous occlusion	0.21±0.010	0,02±0,005 p<0,01

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

In patients with type 2 diabetes mellitus, the onset of AT with all inducers and their combinations was accelerated (Table). The earliest time the AT developed with collagen, a little later with ADP, even later with ristomycin, thrombin and adrenaline. The onset of AT with the tested combinations of inductors was greatly accelerated. The importance of blood-free patients with platelet aggregates and the degree of platelet involvement in diabetes mellitus type 2 exceeded control figures.

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

Patients with type 2 diabetes showed a decrease in IAAWW with all tested inducers (for adrenaline 1.33±0.10, for ADP 1.24±0.10, for ristomycin 1.27±0.18, for collagen and thrombin 1.19 ±0.18 and 1.21±0.25, respectively) and with all their combinations (for ADP and adrenaline 1.27±0.17, for ADP and collagen – 1.25±0.19, for adrenaline and collagen – 1.19±0.20). At the same time, in a plasma obtained against a background of temporary venous occlusion, the number of platelet aggregates in the blood of patients and the excessive involvement of platelets in them decreased little.

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 – platelets [19,20]. At type 2 diabetes mellitus the depression of plasma antioxidant activity is formed which provides the increase of LPO activity in it [21,22]. The increase of freely radical processes in liquid part of blood inevitably promotes the damage of platelets' membranes. The development of these manifestations in combination with found in these patients' platelets lipid imbalance leads to their hyperaggregability. The level of disaggregating impacts from the side of vascular wall [23,24] lowers simultaneously with it in respect of platelets.

The decrease in IAAVW with inductors and their combinations is caused by the growth of AP and depression of the disaggregation capacity of the vessels [25,26]. An important reason for this is the activation of LPO in plasma [27,28]. The early onset of AP in response to ristomycin in patients is associated with increased vWF generation in the vessels [29,30]. Previously, the development of AP in response to two inducers and an excessive number of platelet aggregates in the blood of patients before and after temporary

venous occlusion should be considered a manifestation of increasing depression of the disaggregation capacity of the vessels [31, 32].

CONCLUSION

An important manifestation of vasopathy is the depression of the disaggregation properties of blood vessels in relation to platelets. Quite often this is observed in patients with metabolic pathology, including those with type 2 diabetes. Recently, it is often accompanied by various vascular thrombosis. The study revealed that in patients with type 2 diabetes mellitus there is a strong depression of the disaggregation properties of blood vessels in relation to platelets. These disorders inevitably lead to the activation of hemostasis mechanisms in patients with type 2 diabetes and promote the development of vascular complications in them [33,34,35].

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