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Microrheological Properties of Erythrocytes in Persons of the 2nd Mature Age with Osteochondrosis of the 2nd Degree

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EGA and INM managed the analyses of the study. Author INM managed the literature searches. All authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

The presence of osteochondrosis in a body negatively influences many blood indices. At the given state we consider to be very interesting some peculiarities of erythrocytes' rheological characteristics which determine the processes of microcirculation and metabolism in tissues. Detection of their state at developing osteochondrosis can help in a fuller understanding of its progression mechanisms and search of variants of the given process' inhibition. The aim is to estimate erythrocytes' microrheological properties in people of the second mature age with osteochondrosis of the 2nd degree. Within our research, we took 37 healthy persons of both sexes of the second mature age and also 43 people of both sexes of the same age with osteochondrosis of the 2nd degree. We applied biochemical, hematological and statistical methods of investigation. The

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quantity of acylhydroperoxides and thiobarbituric acid-products in plasma of the examined persons with osteochondrosis surpassed the control values by 38.4% and 37.4%, respectively. It was accompanied by imbalance of metabolites of arachidonic acid: the level of thromboxane B₂ in their plasma rose by 30.6%, and the level of 6-keto-prostaglandin $F_{1\alpha}$ lowered by 15.9%. At the same time, the quantity of nitric oxide metabolites in their plasma lowered by 23.6%. The quantity of acylhydroperoxides in their erythrocytes rose by 30.0% and malon dialdehyde - by 36.7%. The people with osteochondrosis were noted to have content lowering of erythrocytes-discocytes in blood by 12.7%. At the same time, the quantity of reversibly and irreversibly changed erythrocytes' forms in them increased by 35.7% and in 2.5 times, respectively. Erythrocytes' aggregative properties in people with osteochondrosis turned out to be strengthened. It was pointed by the increase of erythrocytes' summary involvement into aggregates by 32.9% in them and number growth of these aggregates - by 33.3%. People of the second mature age with osteochondrosis of the 2nd degree are characterized by strengthening of lipid peroxidation processes in plasma and erythrocytes. It is accompanied by worsening of erythrocytes' microrheological properties. Given changes can negatively influence the processes of microcirculation and worsen the trophism of tissues, including spinal column, what promotes progression of osteochondrosis. So, it is necessary to search the variants of inhibition of osteochondrosis progression and lowering of its manifestations' sharpness. At the same time, it's very important to take into account the capacities of the tested medicinal approaches. Their impact on erythrocytes' microrheological properties can be rather great.

Keywords: The second mature age; osteochondrosis; erythrocytes; microrheological properties; aggregation; membrane's surface properties.

1. INTRODUCTION

Development of dystrophic lesions of a spinal column is one of rather wide-spread chronic diseases in mammals and human beings [1]. Significant genetic component [2] is acknowledged in their genesis. It provides high frequency of osteochondrosis occurrence in all the human populations [3,4] and in different species of animals [5]. Evident clinical manifestations of osteochondrosis in a human being begins to be observed in the period of his active labor activity (at the age of 25-55 years).

They can often cause episodes of temporal disability leading sometimes to invalidism [6]. High prevalence of osteochondrosis, its susceptibility to chronicity, frequent resistance to conducted medicinal impacts dictate the necessity of detailed studying of all the changes in a body against its background [7].

It is noted that development of osteochondrosis nearly always leads to worsening of the common functional state of a body [8]. It is connected with the fact that development of osteochondrosis influences negatively to the processes of most internals' functioning [9]. As previous research showed, the presence of some trouble in a spinal column (even in the form of scoliosis) was already accompanied by microrheological dysfunctions of regular blood elements, including erythrocytes. It promoted development of hypoxia in tissues [10]. Forming in these conditions little chronic oxygen deficiency could disturb anabolic processes in a body weakening its vitality [11,12]. It created the basis for pathology development in the internals [13,14] and promoted the onset of vessels'persistent spasm [15,16]. It was traced that conditions for arterial pressure rise could be often formed against the background of degenerative changes in a spinal column. These conditions led to gradual development of arterial hypertension [17]. So, the presence of osteochondrosis burdened the course of already existing cardiovascular pathology. It also promoted the formation of resistance to the conducted hypotensive [18]. Frequent occurrence of osteochondrosis and its extremely negative impact on a body provide sharp need in continuation of research of erythrocytes' rheological characteristics at development of the given state. Clarification of peculiarities of microrheology disturbances of these regular blood elements at developing osteochondrosis can help in search of available and effective approaches to slowing-down of its progression and to prophylaxis of vascular complications' development against its background. That's why we put the following aim in our research: to estimate erythrocytes' microrheological properties in persons of the second mature age with osteochondrosis of the 2nd degree.

2. MATERIALS AND METHODS

The research was conducted on persons living in Central Russia (Moscow City and Moscow region). Into our research we took 37 healthy people of both sexes (18 men and 19 women) of the second mature age (mean age 43.5±2.5 years) who composed the control group. We also examined 43 people of both sexes (21men and 22 women) of the same age (mean age 44.7±1.9 years) with osteochondrosis of the 2nd degree who composed the group of observation. The diagnosis of osteochondrosis was confirmed clinically and rontgenologically. Existing in some persons from the group of observation concomitant chronic diseases (chronic bronchitis, chronic tonsillitis, chronic cholecystitis) were in the state of lasting persistent remission. All the persons from the group of observation and from the control group were once observed and examined. This research is approved by the local Ethics Committee of the Russian State Social University on May, 14th, 2015 (Record №5).

All the examined persons gave written informed consent on participation in conducted research.

In our research we determined the activity of the processes of lipids' peroxidation (LPO) in blood plasma which was registered according to the content of thiobarbituric acid (TBA)-active products in it with the help of a set produced by the firm "Agat-Med" (Russia) and to the level of acylhydroperoxides (AHP) [19]. We also registered antioxidant activity of blood [20].

In blood plasma of those examined we determined the content of thromboxane A_2 metabolite – thromboxane B_2 and prostacyclin metabolite – 6-keto-prostaglandin $F_{1\alpha}$ by enzymoimmunoassay with the help of sets produced by the firm "Enzo Life science" (USA). We also determined the summary content of nitric oxide metabolites [21] in plasma.

Erythrocytes were washed and resuspended. Then we estimated quantitatively the levels of cholesterol in them by enzymatic colorimetric method with the help of a set produced by the firm "Vital Diagnostikum" (Russia), and common phospholipids (CPL) – according to the quantity of phosphorus in them [22].

The evidence of the processes of intraerythrocyte LPO was found in washed and resuspended erythrocytes according to the concentration of malon dialdehyde (MDA) in the reduction reaction of thiobarbituric acid and to the quantity of AHP [19]. We judged the state of erythrocytes' microrheological features by their cytoarchitecture and aggregation. We determined the quantity of erythrocytes' that is considered normal and changed forms in blood with the help of light phase-contrast microscopy [23].

The ability of erythrocytes to spontaneous aggregation was determined with the help of light microscopy by calculating the quantity of erythrocytes' aggregates, the number of aggregated and non-aggregated erythrocytes [23] in Goryaev's box.

Received results were processed by Student's tcriterion.

3. RESULTS AND DISCUSSION

The examined persons with osteochondrosis were noted to have strengthening of LPO processes (Table 1). The quantity of AHP and TBA-products in their plasma surpassed the control values by 38.4% and 37.4%, respectively (control values – 1.77 ± 0.23 D₂₃₃/1 ml and 3.26 ± 0.29 mkmol/l, respectively). It took place against the background of weakening of plasma antioxidant activity in them by 36.9% (in the control group – $32.6\pm0.49\%$).

In blood of the examined persons with osteochondrosis we noted imbalance of arachidonic acid metabolites: the level of thromboxane B_2 in their plasma turned out to be higher in comparison with the control level by 30.6%, whereas the level of its functional antagonist's derivative – 6-keto-prostaglandin $F_{1\alpha}$ – lowered by 15.9% (Table 1). It was accompanied by content lowering of summary quantity of nitric oxide metabolites by 23.6% in comparison with the control values.

In erythrocytes' membranes of persons with osteochondrosis of the 2nd degree we noted level rise of cholesterol till 1.06±0.008 mkmol/10¹² erythrocytes and CPL lowering till 0.63±0.007 mkmol/10¹² erythrocytes (in the control group 0.95±0.012 cholesterol mkmol/10¹ _ erythrocytes and CPL - 0.70±0.009 mkmol/10¹² erythrocytes). It was followed by LPO strengthening in erythrocytes (AHP up to 4.16 \pm 0.09 D₂₃₃/10¹² erythrocytes, MDA up to 1.94 \pm 0.08 nmol/10¹² erythrocytes) in comparison with the level in the control group (AHP - 3.20 ± 0.15 D₂₃₃/10¹² erythrocytes, MDA – 1.42±0.12 nmol/10¹² erythrocytes).

Indicators	Persons with osteochondrosis, n=43, M±m	Control, n=37, M±m
Acylhydroperoxides of plasma, D ₂₃₃ /I ml	2.45±0.48	1.77±0.23
		p<0.01
Thiobarbituric acid-products of plasma, mkmol/l	4.48±0.52	3.26±0.29
		p<0.01
Antioxidant activity of plasma, %	23.8±0.41	32.6±0.49
		p<0.01
thromboxan A ₂ , pg / ml	220.3±0.67	168.7±0.75
		p<0.01
6-keto-prostaglandin F _{1α} , pg / ml	82.8±0.32	96.0±0.42
		p<0.05
nitric oxide's metabolites, umol/l	29.2±0.24	36.1±0.29
		p<0.05
cholesterol of erythrocytes, mkmol/10 ¹²	1.06±0.008	0.95±0.012
erythrocytes		p<0.05
common phospholipids of erythrocytes,	0.63±0.007	0.70±0.009
mkmol/10 erythrocytes		p<0.05
acylhydroperoxides of erythrocytes, D ₂₃₃ /10 ¹²	4.16±0.09	3.20±0.15
erythrocytes	4.0.4.0.00	p<0.01
malonic dialdehyde of erythrocytes, nmol/10 ¹²	1.94±0.08	1.42±0.12
erythrocytes	75.0.0.04	p<0.01
erythrocytes-discocytes, %	75.6±0.24	85.2±0.17
	45.0.0.40	p<0.01
reversibly modified erythrocytes,%	15.2±0.12	11.2±0.09
	0.010.00	p<0.01
irreversibly modified erythrocytes,%	9.2±0.08	3.6±0.06
	40.010.44	p<0.01
sum of all the erythrocytes in an aggregate	43.0±0.11	32.8 ± 0.12
avantity of aggregates	0.4+0.07	p<0.01
quantity of aggregates	0.4±0.07	0.3 ± 0.11
quantity of free or three too	228 610 22	200 5 10 24
quantity of free erythrocytes	230.0±0.32	200.0±0.34
		µ∽0.01

Table 1. Hematologic characteristics of the examined persons second adulthood with osteochondrosis

Conventions: p – the significance of differences in the parameters of those surveyed who have osteochondrosis and control groups

The examined persons with osteochondrosis were noted to have lowering of erythrocytesdiscocytes' percentage in blood by 12.7% in comparison with the control level (Table 1). The quantity of reversibly and irreversibly changed erythrocytes' forms in blood of persons from the group of observation increased by 35.7% and in 2.5 times, respectively. At the same time, the examined persons with osteochondrosis were found to have strengthening of erythrocytes' aggregative properties. It was pointed by the index increase of erythrocytes' summary involvement into aggregates by 32.9% and quantity rise of these aggregates by 33.3% at the decrease of freely lying erythrocytes by 20.9% in comparison with the control level.

Working out of new effective variants of physiological optimum's lasting maintenance in a body and its rehabilitation is possible only in the course of continuation of active studying of human biology's different aspects with planned collection and systematization of information about human physiology [24,25]. It is equally right in respect of developing involuntary changes in intervertebral disks of a man while aging. These changes form the basis of osteochondrosis. It was noted long ago that gradually progressing they gave more evident clinical manifestations, and, sometimes, sharply worsened life quality [26]. Notwithstanding the efforts of medicine this state remains one of rather wide-spread among population of mature

age in the whole world during the last decades. At the same time, osteochondrosis continues to cripple greatly to economics because of high frequency of working capacity slowdown against its background [1]. It's noted that osteochondrosis formation is accompanied by worsening of not only musculoskeletal system's state but also metabolism and processes of blood circulation. It worsens the functions of spinal column.

Given situation influences negatively the functioning of the internals [27] what points at the necessity of continuation of profound studying of its progression's mechanisms.

There is some basis to suspect that great role in the formation of osteochondrosis manifestations belongs to disturbances of regular blood elements' microrheological properties [28] and, first of all, to their most multiple population erythrocytes [29]. At the same time, weakening of a body's antioxidant protection has special significance for their development. It leads to the growth of LPO intensity in plasma and cells. Surplus of lipids' peroxidation products in plasma and erythrocytes causes membranes' changes in these cells from the outside and inside what worsens their functioning. It is aggravated by development of lipid imbalance in ervthrocytes' membranes at osteochondrosis. It additionally promotes worsening of these regular blood elements' functioning [30]. Forming changes of quantity and ratio of phospholipids and cholesterol in their membranes are, evidently, functionally unfavorable. They disturb membrane's selective permeability and viscosity. They also influence negatively membrane-bound proteins in the result of their secondary structure's modification. This situation rather negatively influences the membrane state of erythrocytes' most part. As the conducted research showed, people with osteochondrosis of the 2nd degree are characterized by the increased content of erythrocytes' reversibly and irreversibly changed forms in blood against the background of lowering of their discoid forms' quantity.

Found strengthening of erythrocytes' aggregation at osteochondrosis can be estimated as the result of joint effect of its pathogenesis' elements which negatively influence microrheological processes. Reliable increase of erythrocytes' reversibly and irreversibly changed forms inevitably leads to the growth of erythrocyte aggregates' quantity in blood of these people and the degree increase of new erythrocytes' involvement into them. Found evidence' rise of erythrocytes' aggregation in conditions of osteochondrosis can be mostly explained by the increasing catecholamines' impact on them. Their level in blood reliably increases at any dysfunction [31]. The increase of catecholamines' quantity in blood of persons from the group of observation should be mostly estimated as the mechanism of metabolism intensification in tissues [32]. Given process is inevitably accompanied by activity growth of α_{2} receptors on their cells, including erythrocytes. It leads to inhibition of adenylate cyclase and, consequently, to level lowering of cyclic adenosine monophosphate [33]. Besides, the rise of erythrocytes' aggregation in the group of persons with osteochondrosis can be also connected with gradual increase of Ca²⁺ content in them.

In these conditions, vascular wall of persons with osteochondrosis is characterized by synthesis lowering of biologically active substances which can limit erythrocytes' aggregation. At the same time, proaggregates' level rises in their blood. So, noted in the group of observation intensification of thromboxane formation and production weakening of its functional antagonist prostacyclin – creates imbalance of arachidonic acid metabolites. Given disturbances are aggravated in them by developing weakening of NO production in vascular wall. May be, it takes place in the result of endothelial NO-synthase's suppression by increased LPO in plasma [34]. Forming in these conditions erythrocytes' microrheological dysfunctions can worsen microcirculation processes themselves and weaken trophism, including vascular walls and disaggregants' production in them [35].

Disturbances of erythrocytes' characteristics in persons with osteochondrosis allow us to look at the given state under a new visual angle. It becomes clear that worsening of erythrocytes' microrheological properties and manifestations of angiopathy at osteochondrosis burden each other and inevitably disturb microcirculation in tissues, including bones, cartilages and muscles. It promotes progression of the process in the spinal column. So, it is possible to weaken manifestations of osteochondrosis in case of planned impact on erythrocytes' microrheological disturbances. It can be done either with the help of medicines or without them. A hope on great availability of the given approach is suggested by received earlier results of non-pharmacological

impact's application to children with scoliosis. These means turned out to be able to optimize morpho-functional characteristics of some regular blood elements in them [36]. The authors plan to test the capacities of the designed by them medicinal-prophylactic clothes [37,38]. They are interested in erythrocytes' microrheological properties in people with osteochondrosis manifestations.

4. CONCLUSION

In conducted research it was determined that people of the second mature age with 2nd osteochondrosis of the degree are of characterized by strengthening lipid processes peroxidation in plasma and erythrocytes. It is accompanied by increase of erythrocytes' changed forms in their blood and growth of their aggregative ability. Found disturbances inevitably worsen the processes of blood rheology in capillaries. It leads to negative changes in tissue trophism, including spinal progression column. promoting of osteochondrosis. Taking into consideration the evidence of microrheological disturbances at osteochondrosis, it seems to be very important to seek correction variants of the given state in the future with regard to their ability to influence most erythrocytes' aggregation and degree of form changing. Given search must help to work out the effective inhibition variant of osteochondrosis development.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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