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Features of erythrocyte electrophoretic mobility in programmed hemodialysis patients

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Abstract:
Objective: to analyze the electrophoretic properties of erythrocytes in patients receiving programmed hemodialysis treatment.
Materials and Methods. The study included 100 patients with an established diagnosis of stage 5D chronic kidney disease, receiving programmed hemodialysis treatment. The control group included 17 healthy blood donors. The following indicators of erythrocyte electrophoretic activity were evaluated: oscillation amplitude, percentage of motile cells, ellipticity, and aggregation index.
Results. Compared with the control group, we revealed significant changes in the amplitude of oscillations and the percentage of motile cells ($p<0.001$). No significant gender-dependent differences were observed ($p>0.05$). Statistically significant differences between young and senile age groups ($p=0.048$) were observed in the average oscillation amplitude, whereas ellipticity differed significantly in middle-aged vs. senile patients ($p=0.040$) and elderly vs. senile subjects ($p=0.035$). As for the effect of hemodialysis treatment duration, a reduction in the oscillation amplitude was observed when comparing maximum with minimum duration: less than 1 year ($p=0.029$) and 1 year ($p=0.035$). Aggregation index values were the highest in the groups with the minimum ($p=0.044$) and maximum ($p=0.035$) dialysis duration.
Conclusion. In hemodialysis patients, the oscillation amplitude of erythrocytes was significantly reduced, with assumed tendency for further decrease associated with duration of hemodialysis treatment.

Keywords: electrophoretic mobility of red blood cells, chronic kidney disease, hemodialysis.

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Introduction

Chronic kidney disease (CKD) is a condition characterized by the presence of any markers associated with kidney damage and persisting for more than three months, regardless of the nosological diagnosis. CKD is linked to a high risk of cardiovascular diseases, premature (early) mortality, and a sharp reduction in the quality of life [1-3]; hence, it is important to use diagnostic methods that could detect the progression of pathological processes in this disease.

In recent years, progressively more attention was paid to diagnosing the condition of cell membranes in the course of investigating the pathogenetic mechanisms of many diseases. The cause lies in the fact that the erythrocyte membrane is a universal model of the cell membrane, since it possesses the general principles of organization and functioning of the membranes of other cells [4]. Among the promising research methods, the determination of the electrophoretic mobility of erythrocytes (EPME) stands out: it is capable of reflecting not only changes in the membrane charge, but also the condition of the entire human body [5]. The advantages of the method include its higher sensitivity to homeostatic changes of the body, compared with other clinical and laboratory techniques [6]; as well as its ability to assess the severity of the pathological process, based on the fact that the decrease in EPME correlates with the severity of the developing pathology [5]. Among the drawbacks, the nonspecificity of the method, in relation to any etiology, should be noted [6]. Unfortunately, there is a rather small number of published studies regarding the change in EPME in chronic diseases, including CKD. Based on the relevance of discussed problem and insufficient knowledge about it, we formulated the objective of our study.

Objective – to analyze the electrophoretic properties of erythrocytes in patients receiving programmed hemodialysis treatment.

Materials and Methods

The study included 100 patients treated with programmed hemodialysis. The criteria for inclusion in the study group were the presence of an established CKD diagnosis (stage 5D) and receiving treatment in the form of renal replacement therapy via programmed hemodialysis at selected medical institutions. The mean age of the patients was 56.5±11.8 years (ranging 30 to 80 years). The study group was divided according to several criteria: gender, age (sensu World Health Organization) and the duration of receiving dialysis. Hemodialysis procedures were carried out

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in the city of Izhevsk (Clinical Hospital No. 6 of the Ministry of Healthcare of Udmurt Republic; joint-stock company Medical Service Solutions; a separate division of Fresenius Nephrocare LLC). The procedures were performed for 4-4.5 hours 3 times a week using polysulfone dialyzers. To identify the features of cell membrane disorders, we used a comparison group, which included 17 virtually healthy individuals (blood donors of the State Healthcare Institution Republican Blood Transfusion Station of the Ministry of Healthcare of Udmurt Republic), comparable in terms of their age and gender.

The determination of EPME was conducted using the Cyto-Expert kit (open joint-stock company Axion-Holding LLC, Izhevsk, YuMGI.941413.001 2010) and WT-Cell software (Westrade LLC), which provided the capability to detect and evaluate motion parameters of living cells under the action of an alternating electric field with specified characteristics, using a video camera connected to the eyepiece of the Biolam light microscope. For the study, venous blood was taken from the patient's fistula prior to the hemodialysis procedure. Suspension medium for electrophoresis had the following composition: 1 mL of 5% glucose solution + 1 drop (0.05 mL) of native blood of the subject. An amount of 40-50 μL of the resulting erythrocyte suspension was placed in the center of the working area of the retractable platform with a dispenser. The drop was covered with a cover slip, positioned strictly symmetrically with respect to black graphite electrodes, after which a voltage of 30 V was applied to the electrodes with a frequency of changing the polarity of the current direction on the electrodes of 0.25 Hz. Accordingly, electrical current of 0.01 mA was maintained in the working area.

In the course of the examination, the following indicators were identified, recorded by the program during the analysis of the suspension medium: mean oscillation amplitude of erythrocytes, mean percentage of motile cells, mean ellipticity of erythrocytes, and mean aggregation index.

Statistical data processing was carried out using Statistica 13 (2018, version 13.5.0.17) and Microsoft Excel 2016. The data were presented as M±σ (M is the arithmetic mean, σ is the standard deviation). The normality function of the distribution of parameter values was evaluated via the Kolmogorov-Smirnov test. Statistical significance of differences in sample means was assessed via the Mann-Whitney test (T). Significant differences were assumed at $p \leq 0.05$.

Results

Mean oscillation amplitude of blood erythrocytes in patients receiving hemodialysis was 10.2±4.7 μm vs. 21.3±8.5 μm in the comparison group ($p<0.001$). Mean percentage of motile cells in the study group was 69.5±18.6% vs. 89.8±9.9% in the comparison group ($p>0.05$). Mean ellipticity in the study group (70.2±3) differed slightly from the control group (68.6±2.9) ($p<0.05$). Mean aggregation index in the study group was 24.8±16.2 vs. 20.9±11.7 in the comparison group ($p>0.05$).

At the next stage, patients receiving programmed hemodialysis treatment were divided into groups by their gender, age and dialysis duration, and differences in these indicators were analyzed within those groups.

As for gender-dependent differences in the study group (*Table 1*), 46 patients were women aged 57.8±13.9 years and 54 subjects were men aged 55.4±13.9 years, the duration of the dialysis was on average 4.0±4.3 years for women and 3.5±3.7 years for men. The analysis revealed that there were no significant gender-based differences in all four indicators in the study group.

Age groups were arranged according to the criteria of the World Health Organization (*Table 2*). The first group (young age, 25-44 years old) comprised 25 patients, including 14 men (56%) and 11 women (44%) 37.2±4.48 years of age. The second group (middle age, 45-60 years old) encompassed 29 patients, including 19 men (65.5%) and 10 women (34.5%) 53.7±4.8 years of age. The third group (elderly, 61-75 years old) contained 40 patients, including 18 men (45%) and 22 women (55%) 67.2±3.7 years of age. The fourth group (senile age, 76-90 years) enrolled 6 patients, including 3 men (50%) and 3 women (50%) aged 79.5±2.9 years. Statistical analysis demonstrated that statistically significant difference ($p<0.05$) in the average oscillation amplitude was detected solely between the patients of young and senile groups. When comparing the age dynamics of erythrocyte ellipticity, its increase was revealed in senile hemodialysis patients, compared with all other age groups, except for the young ($p<0.05$). Age differences in the percentages of motile cells and aggregation index values were not detected ($p>0.05$).

As far as the duration of the dialysis treatment goes, five groups were distinguished. Group I (dialysis duration less than a year) enrolled 14 patients, including 10 men (71.4%) and 4 women (28.6%) aged 63.9±13.1 years; mean duration of the dialysis period was 0.5 ±0.2 years.

Group II (dialysis duration of 1 year) involved 23 patients, including 10 men (43.5%) and 13 women (56.5%) aged 59.4±14.5 years; mean dialysis duration was 1.1±0.2 years. Group III (dialysis duration of 2 to 5 years) encompassed 40 patients, including 22 men (55%) and 18 women (45%) aged 53.2±13.8 years; mean dialysis duration of 2.8±0.9 years. Group IV (dialysis duration of 6 to 10 years) comprised 16 patients, including 9 men (56.3%) and 7 women (43.7%) aged 55.1±10.8 years; mean dialysis duration of 7.8±1.3 years. Group V (dialysis duration of over 10 years) contained 7 patients, including 3 men (42.9%) and 4 women (57.1%) aged 54.7±15.9 years, the mean duration of dialysis treatment was 14,9±2.8 years. The statistical analysis demonstrated that an increase in the dialysis duration led to progressive deterioration in EPME indicators (*Table 3*): mean oscillation amplitude was reduced in group V against groups I and II by 1.5 times (from 11.7±5.4 μm to 7.4±2.5 μm, $p<0.05$). There were differences in the mean aggregation index between groups I and III ($p<0.05$) and groups III and V ($p<0.05$). Ellipticity and the proportion of motile cells did not undergo significant changes with an increase in the duration of hemodialysis.

Table 1. Gender-dependent distribution of mean values of electrophoretic mobility indicators of erythrocytes, M±σ

Indicator	Women	Men	*p*
Oscillation amplitude, μm	10.0±5.0	10.2±4.4	>0.05
Percentage of motile erythrocytes, %	68.8±20.7	70.2±16.8	
Ellipticity	70.0±2.8	70.4±3.2	
Erythrocyte aggregation index, %	25.1±16.8	24.5±15.9	

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Table 2. Age-dependent distribution of mean values of electrophoretic mobility indicators of erythrocytes, M±σ

Indicator	Age group				*p*
	I (Young)	II (Middle age)	III (Elderly)	IV (Senile)	
Oscillation amplitude, μm	9.0±4.9	9.9±4.6	10.9±4.9	11.7±1.8	p_{1-4}= 0.048
Percentage of motile erythrocytes, %	65.5±21.0	73.4±15.6	68.6±19.5	73.4±15.0	>0.05
Ellipticity	70.4±3.0	69.8±2.5	69.9±3.1	73.4±3.3	p_{2-4} =0.040 p_{3-4}= 0.035
Erythrocyte aggregation index, %	22.2±17.1	24.3±13.7	26.7±16.7	25.5±22.6	>0.05

Table 3. Age-dependent distribution of mean values of electrophoretic mobility indicators of erythrocytes vs. dialysis duration, M±σ

Indicator	Group (dialysis duration)					*p*
	I (<1 yr.)	II (1 yr.)	III (2-5 yrs.)	IV (6-10 yrs.)	V (>10 yrs.)	
Oscillation amplitude μm	11.7 ±5.4	11.2 ±4.3	9.9 ±4.6	9.4 ±5.4	7.4 ±2.5	p_{1-5} =0.029 p_{2-5} =0.026
Percentage of motile erythrocytes, %	69.5 ±18.0	74.4 ±17.8	69.8 ±17.7	66.1 ±22.0	59.5 ±18.6	>0.05
Ellipticity	70.2 ±4.3	70.83 ±2.9	70.0 ±2.6	69.7 ±2.1	70.8 ±4.2	
Erythrocyte aggregation index, %	32.5 ±19.4	23.3 ±14.3	21.6 ±17.7	25.6 ±10.5	30.6 ±13.8	p_{1-3} =0.044 p_{3-5} =0.035

Discussion

Available published scientific journal articles predominantly presented the studies on the effect of fairly short-term negative factors on the change in EPME, such as reactive oxygen species [4] or acute inflammatory diseases (viz., pneumonia) [5]. At the same time, there are much fewer data on changes that occur against the background of chronic diseases [7, 8], including those regarding patients with CKD receiving programmed hemodialysis treatment [9]. In our paper, we presented data on EPME differences between healthy patients and subjects with CKD receiving hemodialysis, as well as on the gender-dependent, age-specific, and hemodialysis duration-based differences within the sample of patients. The obtained data serve an addition to existing knowledge about the issue under study. The limitation of our study is related to the small sample size in some groups of patients; hence, it is desirable to conduct a more extensive study dealing with this issue.

Regarding the revealed reduction in the mean erythrocyte oscillation amplitude in patients receiving hemodialysis vs. the control group, it can be assumed that such change was due to an involvement of stress response systems as a reaction to both the disease per se and the hemodialysis procedure. According to a number of authors, in such scenario, the normal condition of cytoplasmic membranes is disturbed, which is why their charge changes [5, 10]. Changes in the ellipticity of erythrocytes could be caused by the changes in the patient cytoskeleton associated with the hemodialysis procedure [11].

The lower mean value of the erythrocyte oscillation amplitude in young vs. elderly patients could be explained by the fact that subjects who acquired stage 5D CKD at a younger age have a faster rate of disease progression and its higher severity. However, an alternative explanation is that this anomaly may be due to an error caused by a small sample size of elderly patients. An increase in the ellipticity value in elderly patients, compared with the rest (except young), could be associated with age-specific changes in erythrocyte membranes, viz., an increase in its stiffness and morphometric parameters [12].

Progressive deterioration of the average oscillation amplitude with an increase in the duration of dialysis is presumably associated with the effect of chronic stress on erythrocyte membranes in the form of an augmented lipid peroxidation, changes in Na^+/K^+-ATPase, and depletion of the glutathione system [7]. The revealed differences in the erythrocyte aggregation index between groups I-III and III-V can be caused by various factors, possibly complementary to each other. E.g., some published sources demonstrated directly proportional relationship between the aggregation index and the severity of the course of some diseases [13, 14], which may imply that during the first year, as well as after 10 years of programmed hemodialysis, the pathological process caused by stage 5D CKD manifests itself most actively. Also, it was shown that aggregation properties were associated, among other things, with the condition of erythrocyte membranes [15], which presumably indicated especially pronounced changes in their structure during the aforementioned periods. However, the observed changes could result from the bias caused by small sample sizes.

Conclusion

Hence, according to the results of conducted analysis, it could only be stated with certainty that EPME indicators change significantly solely in the group of patients with stage 5d CKD on hemodialysis, while there is presumably a tendency towards a progressive reduction in the average amplitude of erythrocyte oscillations with an increase in dialysis duration. However, the revealed differences among patients, when they were divided into groups in accordance with the indicated characteristics, with the current sample size could be considered an indication for a further investigation with an increase in the sample size.

Conflict of interest: None declared.

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