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PROTEIN COMPOSITION AND FUNCTIONAL PARAMETERS OF RBC MEMBRANES IN LIVER AND KIDNEY TRANSPLANTATION

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Organ transplantation is an effective treatment for many end-stage diseases. However, reperfusion injury constitutes a major complication of transplantation, which is associated with microcirculatory disorders and aggregation of blood corpuscles. Red blood cells (RBC) play an essential role in maintaining hemodynamic and rheological properties of the blood. Moreover, the study of mechanisms of changes in RBC functional indices is an urgent task. The main indicator of RBC functioning is the stability of RBC membrane structure. The issue of RBC membrane modification in organ transplantation has not been studied so far. **Objective:** to study the protein composition of RBC membranes, their aggregation and electrokinetic parameters in liver and kidney recipients, as well as in related kidney and liver fragment donors before and after operation. **Research materials.** Blood of 12 kidney recipients and 5 related kidney donors, 8 liver recipients and 4 related liver fragment donors – 1–2 hours before surgery, 1 week, 1, 2, 7, 10, 12 months after surgery. The control group consisted of 8 healthy volunteers. **Research methods.** Protein separation was done by Laemmli electrophoresis. RBC electrophoretic mobility, which characterizes the electrokinetic properties of cells, was measured by microelectrophoresis. Aggregation was calculated microscopically by counting unaggregated RBCs. Obtained values were compared by Mann-Whitney U test. **Results.** Examination of the RBC membrane of kidney recipients revealed a significant decrease in the amount of Band 3 protein and glycophorin before and after transplantation. Band 3 protein levels reduced at 1 month, glycophorin reduced at 7 months after surgery, with a maximum decrease in these protein fractions by more than 50% by 7 days compared with control values. There was also a decrease in spectrin content for 2 months after surgery with a maximum decrease of 30% by 1 month. In liver recipients, analysis of RBC membrane proteins revealed a decrease in the amount of glycophorin before surgery and further decrease at 2 months of post-transplant period. The maximum decrease in this index was 72% by 7 days after surgery. In addition, there was a fall in spectrin and Band 3 protein levels at 1 month by more than 60% relative to the control values. In donors, there were changes in the protein fraction of RBC membranes in the long-term post-operative period: spectrin and Band 3 protein levels reduced by 2 times at month 2 in kidney donors, while glycophorin levels reduced by 2.3 times at month 1 after operation in liver donors. Similarly, both groups of donors had increased actin levels at month 1 after surgery. The revealed changes in protein levels in the protein phase of RBC membranes were combined with functional indices of RBCs. In kidney recipients, decreased RBC electrophoretic mobility and increased aggregation were detected at 2 months. In liver recipients, the changes in these indicators were at 1 month. A decrease in RBC electrophoretic mobility was detected in donors of both groups. **Conclusion.** Changes in RBC membrane electronegativity are associated with changes in glycophorin and Band 3 protein levels, whereas in RBC aggregation process in liver/kidney recipients, the structural and functional disorders in the interrelationships of such membrane proteins as spectrin, Band 3 protein, and glycophorin, are significant factors. Alteration of actin determines inhibition of RBC aggregation growth in donors.

Keywords: kidney transplantation, liver transplantation, red blood cells.

INTRODUCTION

Organ transplantation has become a very effective method of treatment for a number of severe end-stage diseases [1]. However, transplantation is a technically complex surgical intervention that can be accompanied by massive blood loss, which causes complications in the early postoperative period [2]. Besides, with this type of surgical intervention, changes in homeostasis are associated with an imbalance in the coagulation system [3, 4]. The pathogenesis of coagulopathy is mediated through endothelial damage and activation of hypercoagulation cascade, which leads to microcirculatory disorders in the early post-transplant period [3].

Erythrocytes, in turn, have a significant impact on the rheological properties of blood and microcircula-

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tion [16]. Disturbance of the membrane structure leads to changes in their stiffness, decreases deformation, increases erythrocyte aggregation and initiates thrombosis [5, 6]. Increased cell aggregation and increased release of such procoagulants as erythrocytin and adenosine triphosphate stimulate blood clotting [7, 8]. Erythrocyte aggregation can cause tissue hypoxia, as the aggregates fill the lumen of capillaries and do not leave space for the parietal layer of plasma, causing blood stasis [9]. Thus, red blood cells (RBCs) play an essential role in maintaining the hemodynamic and rheological properties of the blood; a study of the mechanisms of changes in their functional indices is an important task. The stability of the membrane structure is taken as a major indicator of RBC functioning [10]. However, the issue of erythrocyte membrane modification in organ transplantation has not been studied so far. From this point of view, not only recipients, but also related donors, in whom the risk of hemocirculatory disorders increases manifold when a kidney or a liver fragment is removed, have not been studied postoperatively.

The **objective** in this work was to study protein composition of erythrocyte membranes, their aggregation and electrokinetic indices in liver and kidney recipients, as well as in related kidney and liver fragment donors before and after operation.

MATERIALS AND METHODS

The blood of kidney or liver transplant recipients and that of related donors in the postoperative period was studied. Kidney and liver explantation and transplantation were performed at Volga District Medical Center in Nizhny Novgorod, Russia, where such medical interventions have been performed since 2006 [11]. All patients gave voluntary informed consent via a form approved by Order No. 517n of the Russian Ministry of Health, dated August 11, 2017. The study was approved by the local ethics committee of Volga District Medical Center. Enrolled were 12 deceased-donor kidney transplant recipients, 5 living related kidney donors, 8 deceased-donor liver transplant recipients and 4 living related fragment donors, aged from 40 to 58 years. From deceased kidney donors, the mean preservation time was 510 ± 219.33 minutes, from related kidney donors, 22 ± 2.73 minutes, from deceased liver donors, 330 ± 32.07 minutes, and from related liver donors, 26.5 ± 1.73 minutes. The control group consisted of 8 healthy volunteers. All study participants were observed at the outpatient transplant center of Volga District Medical Center according to the approved standards [12]. Blood for analysis was taken from the ulnar vein of the patients 1–2 hours before surgery, 1 week, 1, 2, and 7 months after surgery, to study protein fractions of erythrocyte membranes, and additionally 10 and 12 months after surgery to study the degree of aggregation and RBC electrophoretic mobility, which characterizes their electrokinetic properties.

Protein separation was performed via Laemmli's sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [13] using mini gel electrophoresis system Mini-PROTEAN Tetra cell (Bio-Rad, U.S.A.). The gels were prepared using a 30% acrylamide/methylene bis-acrylamide solution. The buffer used to prepare the stacking gel contained 0.5 M Tris-OH, 0.4% DSN, pH 6.8. A buffer containing 1.5 M Tris-ON, 0.4% DSN, pH 8.8 was used to prepare the separating gel. Electrophoresis chambers were filled with a buffer containing 0.025 M Tris-OH, 0.192 M glycine, 0.1% DSN, pH 8.3. Polymerization was performed in the presence of tetramethylethylenediamine (TEMED) and 10% ammonium persulfate (APS) at room temperature. The applied samples were 20 µl in volume. Before application, samples were diluted with sample buffer (0.0625 M Tris-HCl, 10% glycerol, 0.001% bromophenol blue, 5% mercaptoethanol, 2.3% DSN, pH 6.8) and heated in a water bath (100°C) for 10 minutes. While the samples were passing through the stacking gel, electrophoresis was performed at a constant current strength of 20 mA. In the separating gel, the current strength was 40 mA. At the end of electrophoresis, the gel was stained for 30–60 minutes in a solution containing Coomassie Blue R250, 40% methanol, and 10% acetic acid. Unbound dye was removed by washing the gel in a solvent (40% methanol, 10% acetic acid). The resulting gel tracks were processed using the ImageJ program. Standard protein samples (Bio-Rad, U.S.A.) were used as markers.

By electrophoresis, about 15 major membrane proteins with a molecular weight ranging from 15 to 250 kD were detected in the erythrocyte membrane. Spectrin, glycophorin, and band 3 protein account for about 60% of the membrane proteins. Given that the main cytoskeleton proteins are spectrin (band 1 and 2), ankyrin (band 2.1), band 4.1 and 4.9 proteins, and actin (band 5), while in functional and quantitative relation among integral proteins, band 3 protein or anion channel and glycophorins prevail [14, 15]. In our work, we analyzed exactly these fractions of erythrocyte membrane proteins.

Electrokinetic and aggregation properties were determined by measuring RBC electrophoretic mobility and optical measurement of erythrocyte aggregation. RBC electrophoretic mobility was determined by microelectrophoresis using a cytospherometer in our modification [16]. We recorded the time of erythrocytes passing the distance of 100 µm in Tris-HCl buffer with pH 7.4 at 8 mA current strength. Erythrocyte aggregation was studied by optical microscopy by counting single RBCs and their aggregates in blue dextran T-2000 solution (GE Healthcare Company, 20 mg/mL) in a Tris HCl buffer [17].

Data obtained was statistically processed using Statistica 12, R. The distribution was checked for compliance

with the normal law using the Kolmogorov–Smirnov goodness of fit test. When analyzing differences in individual groups, statistical significance was calculated using a multiple t-test by the Sidak–Bonferroni method. Differences between recipient and donor groups were analyzed using nonparametric Mann–Whitney U test. We used the following confidence intervals to indicate statistical significance: $p < 0.05$, data differ (*); $p < 0.01$, data differ (#).

RESULTS

The study of erythrocyte membrane proteins showed a significant quantitative change in the protein fractions in RBC membrane from both kidney/liver recipients and related donors.

Analysis of erythrocyte membrane proteins in kidney recipients revealed a decrease in the main integral proteins of the erythrocyte membrane – band 3 protein and glycophorin – by 24% and 25% before surgery relative to control values (Fig. 1). In the postoperative period, there was a further 60% decrease in band 3 protein levels at day 7 after surgery relative to control values, followed by a gradual recovery to control values. By day 7, spectrin and glycophorin levels also decreased by 34% and 58%, respectively, relative to control values, after which the reduced spectrin level remained stable for two months postoperatively, and glycophorin – for the entire follow-up period.

Related kidney donors showed a 50% decrease in spectrin levels, a 65% decrease in band 3 protein by month 2 of the postoperative period, and a 78% increase in actin by month 1 relative to control (Fig. 2). At month 7 after surgery, protein composition of membranes was restored to control values.

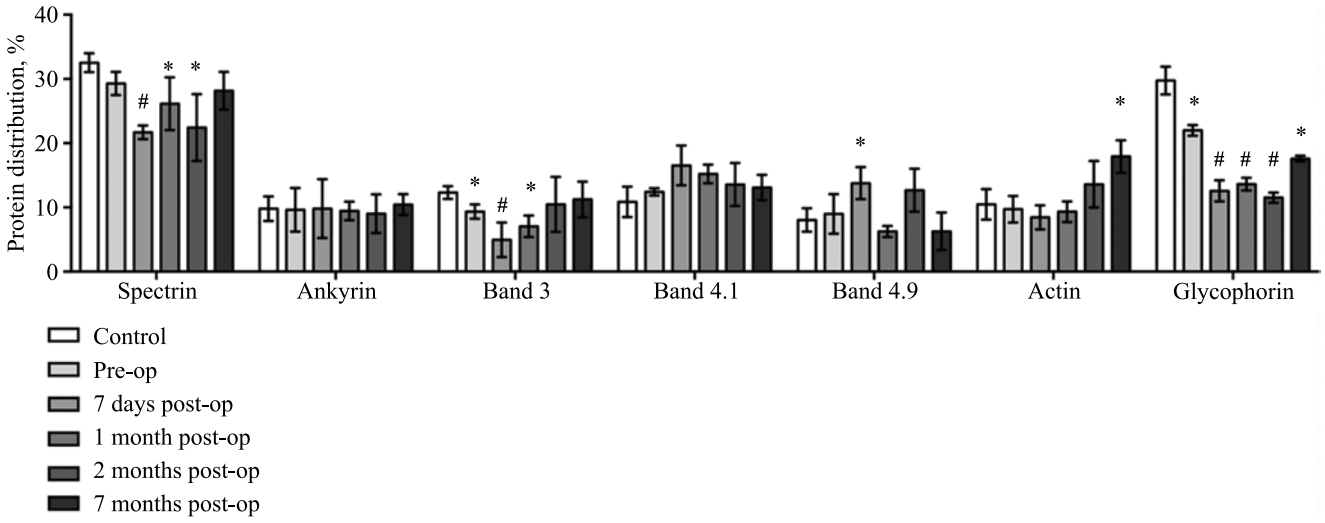

Fig. 1. Dynamics of protein composition of RBC membranes in kidney transplant recipients. Here and below in Fig.: Pre-op, before surgery; post-op, after surgery; * – statistically significant differences versus control ($p < 0.05$); # – statistically significant differences versus control ($p < 0.01$). The error bars represent standard deviations

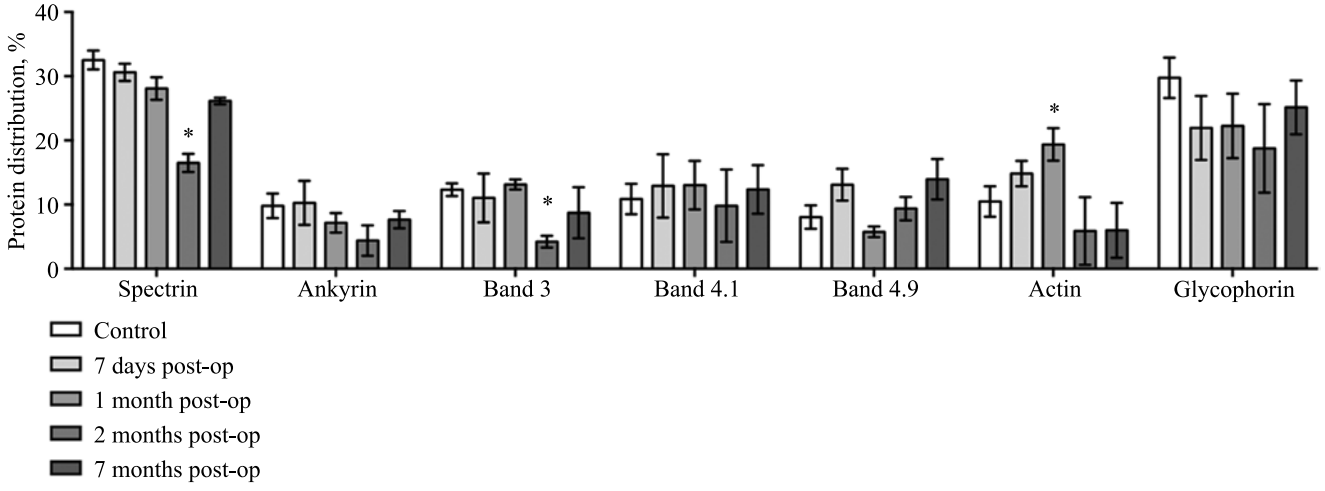

Fig. 2. Dynamics of protein composition of RBC membranes in related kidney donors

Comparison of erythrocyte protein composition between kidney recipient and donor groups revealed differences in the dynamics of spectrin, band 3 protein and glycophorin at all registration points until month 2 after surgery ($p < 0.05$), indicating more pronounced changes in recipients' RBC protein composition.

The study of erythrocyte membrane in liver transplant recipients revealed a significant decrease in glycophorin levels before and after surgery for 2 months with a maximum 72% decrease by day 7 after surgery relative to control values (Fig. 3). On day 7 after surgery, there was an 80% decrease in band 3 protein, and by month 1, there was a 66% decrease in band 3 protein and a 25% decrease in spectrin levels relative to the control. By the end of the study, the protein spectrum was restored to control.

In the related liver fragment donors, changes in the protein composition were detected only with respect to glycophorin content by month 1 of follow-up (65% decrease) and actin, whose levels increased by 49%. The levels of the other fractions were maintained at the control values (Fig. 4).

Comparison of concentrations of protein fractions of erythrocyte membranes in liver recipients and donors revealed significant differences in spectrin levels at month 1 after surgery, band 3 protein at day 7 and month 1 after surgery and glycophorin at day 7 and month 2 after surgery ($p < 0.05$).

Thus, in the postoperative period we observed changes in both peripheral and integral proteins of the erythrocyte membrane, combined with changes in functional indicators of erythrocytes: RBC electrophoretic mobility – an indicator reflecting cell surface charge and erythrocyte aggregation properties. It was shown that in kidney transplant recipients, RBC electrophoretic mobility significantly decreased in the period up to the second month after surgery (Fig. 5). Kidney donors had decreased RBC electrophoretic mobility between

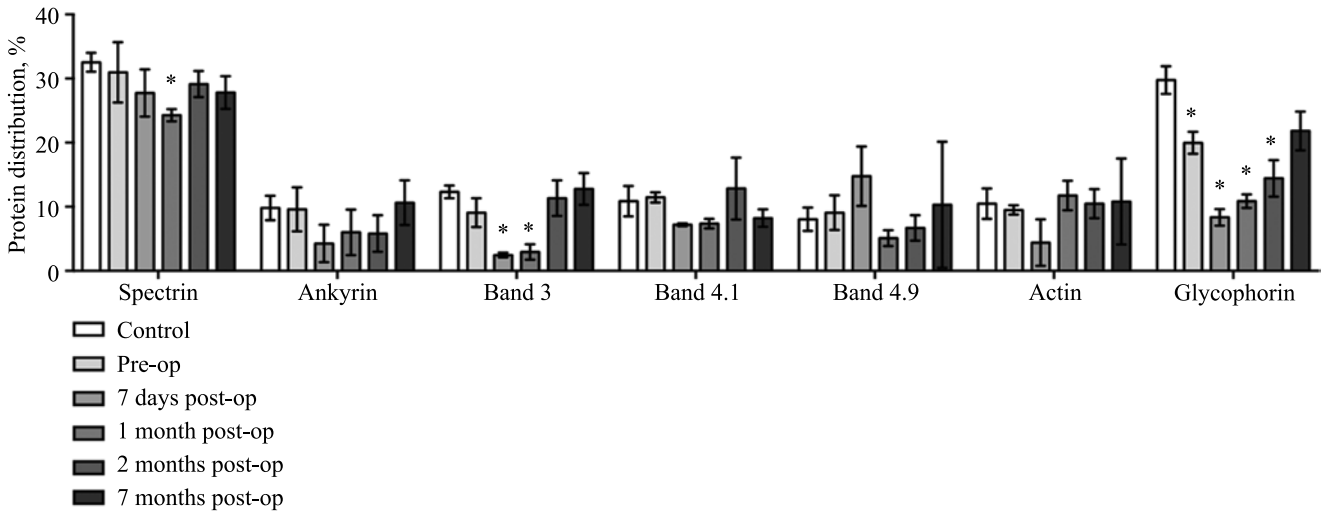

Fig. 3. Dynamics of protein composition of RBC membranes in liver transplant recipients

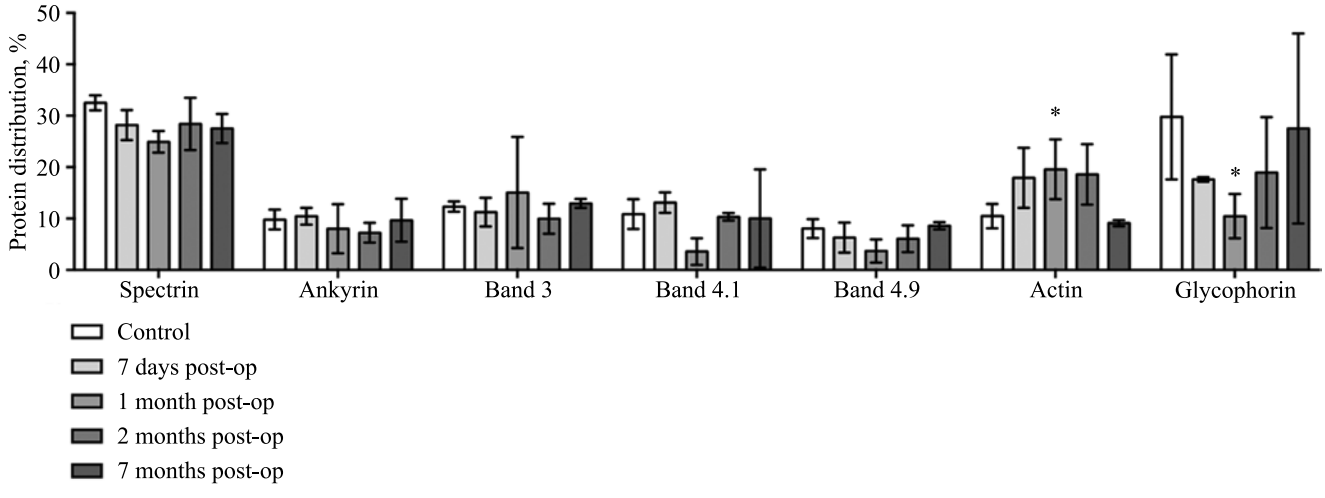

Fig. 4. Dynamics of protein composition of RBC membranes in related liver donors

months 1 and 2 after surgery (Fig. 6). RBC electrophoretic mobility was restored after month 2. The study of erythrocyte aggregation properties revealed an increase in erythrocyte aggregation in kidney transplant recipients, which is consistent with a decrease in RBC electrophoretic mobility in this patient cohort (Fig. 7). No significant change in aggregation was observed in related kidney donors (Fig. 8).

Liver recipients had decreased RBC electrophoretic mobility during the first month after surgery (Fig. 9). In related liver fragment donors, there was decreased RBC electrophoretic mobility at day 30 after surgery (Fig. 10). A significant increase in erythrocyte aggregation up to 1 month was shown in liver recipients (Fig. 11). No significant changes in the studied index were observed in related liver fragment donors (Fig. 12).

DISCUSSION

It is known that structural changes in erythrocyte membranes in pathological processes play a crucial role in the functional activity of cells. In this pilot study, we have shown that changes in the protein composition of the membrane had an influence on RBC electronegativity and RBC aggregation in recipients, and on RBC electronegativity in liver and kidney donors. It should be noted that reperfusion injury, which is associated with microcirculatory disorders and cell aggregation, is a major complication of transplantation [18].

Results show that liver and kidney recipients, as well as related donors had unidirectional dynamics in the form of decreased number of integral proteins. Moreover, in recipients, changes in integral protein, glycophorin, were registered before surgery and persisted in the postopera-

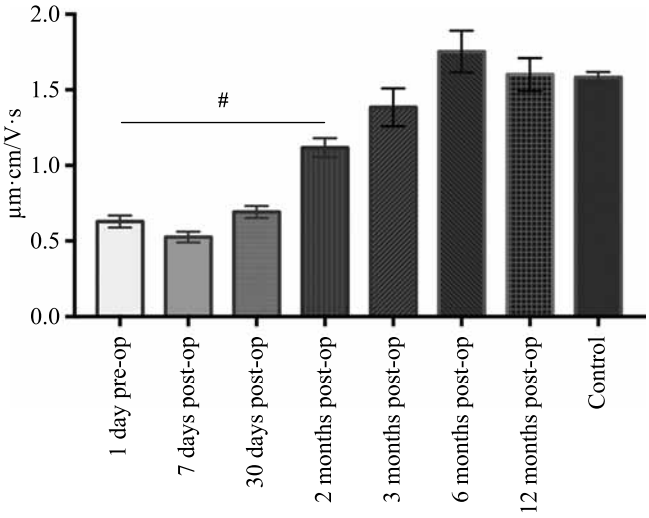

Fig. 5. Dynamics of changes in RBC electrophoretic mobility in kidney transplant recipients

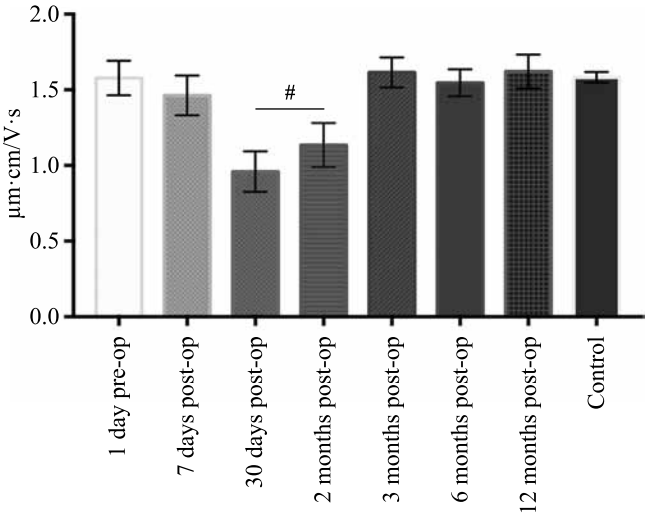

Fig. 6. Dynamics of change in RBC electrophoretic mobility in related kidney donors

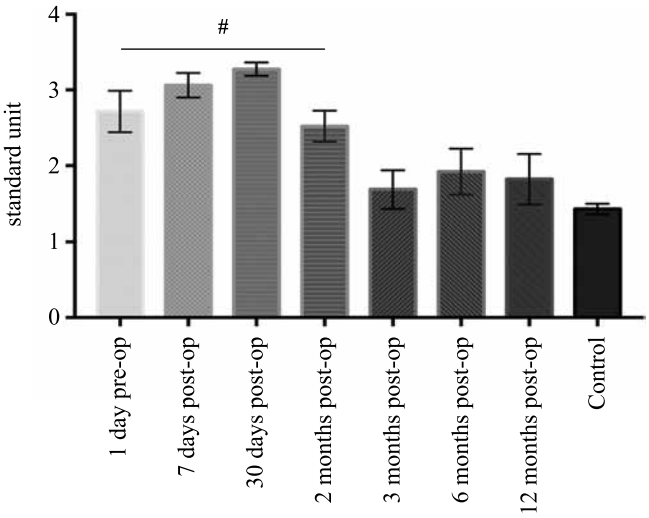

Fig. 7. Dynamics of RBC aggregation in kidney transplant recipients

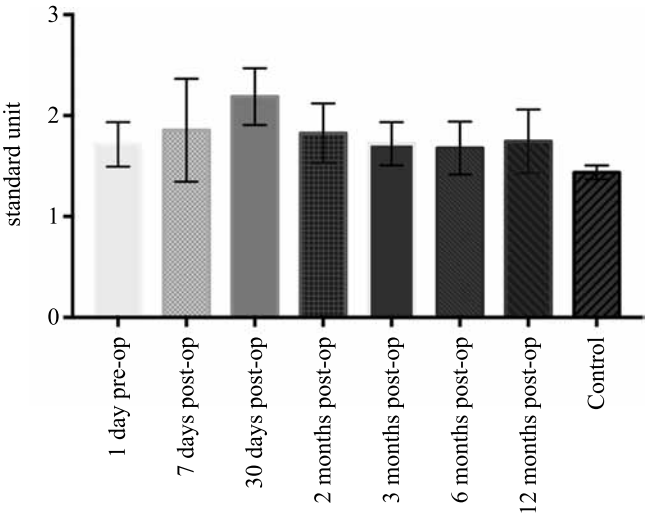

Fig. 8. Dynamics of RBC aggregation in related kidney donors

tive period. Given the high correlation of RBC electrophoretic mobility with deviation of glycophorin and band 3 protein, we can assume that the above dynamics of changes in the level of these membrane proteins is one of the leading factors determining the change in RBC membrane electronegativity. For instance, it is known that band 3 and glycophorin proteins belong to sialoglycoproteins and strongly promote the creation of a negative surface charge [19, 20]. Surface charge modification can promote erythrocyte aggregation, but is not the underlying factor, which proves the identified decrease in RBC electrophoretic mobility and the absence of aggregation in the donors of the studied groups.

The change in aggregation appears to be of a more complex nature and depends on multiple interactions between both integral and peripheral proteins. Cytoskeleton proteins, which determine membrane plasticity, may act as significant factors in the aggregation process: when spectrin levels fall, there is reduced ankyrin binding sites and membrane surface viscosity [21]. Probably, the band 3 protein contributes in a way to such characteristics as erythrocyte plasticity and aggregation. Thus, the cytoplasmic region of band 3 protein has binding sites for a number of glycolysis enzymes [22], decreased glycolysis activity reduces ATP concentration, Na^+/K^+-ATPase activity and erythrocyte plasticity [23]. Inhibition of Na^+/K^+-ATPase activity leads to increased intracellular Ca^{2+} levels [24]. Accumulation of Ca^{2+} ions activates calmodulin, which determines the growth of erythrocyte aggregation [22, 25].

Thus, the totality of results obtained indicates that in the process of erythrocyte aggregation in liver/kidney recipients, structural and functional disorders, determined by spectrin, band 3 protein and glycophorin,

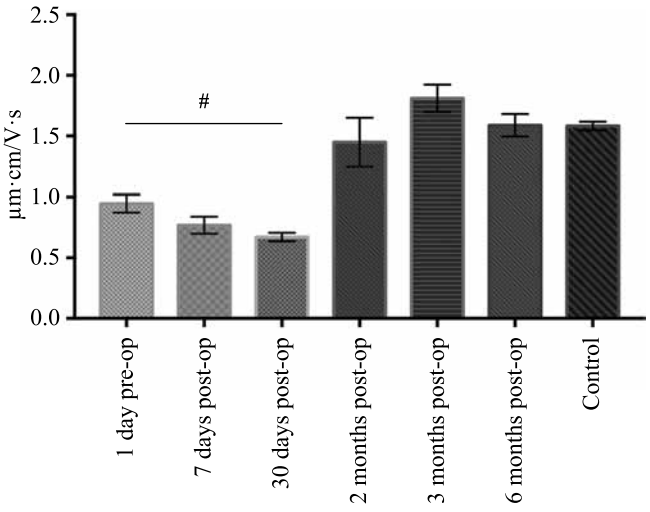

Fig. 9. Dynamics of changes in RBC electrophoretic mobility in liver transplant recipients

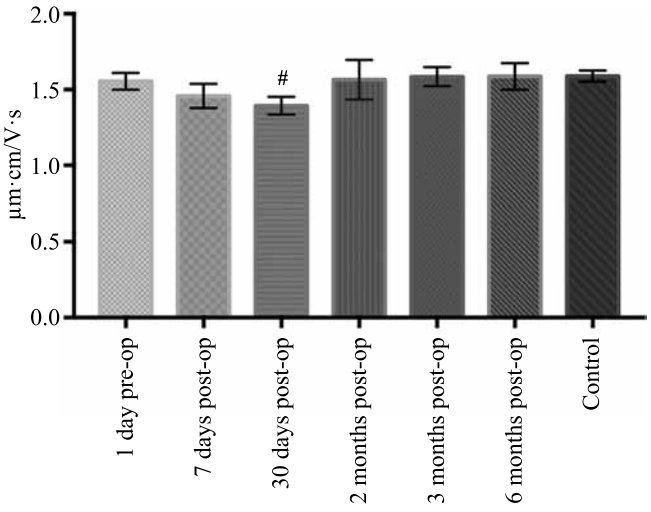

Fig. 10. Dynamics of changes in RBC electrophoretic mobility in related liver donors

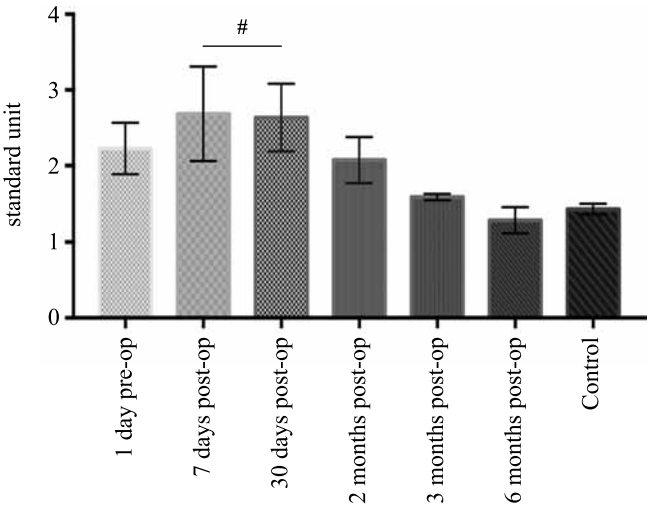

Fig. 11. Dynamics of RBC aggregation in liver transplant recipients

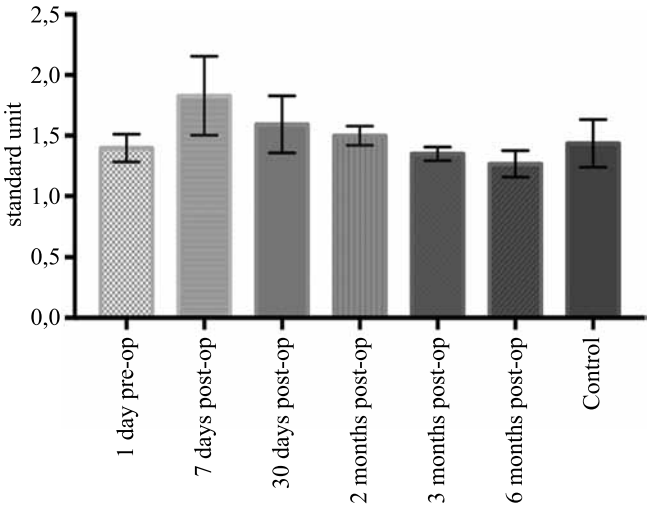

Fig. 12: Dynamics of RBC aggregation in related kidney donors

are significant factors. Analysis of the dynamics of the protein composition of donor erythrocytes show that increased actin levels restrain an increase in erythrocyte aggregation.

CONCLUSION

During organ transplantation, in particular liver and kidney, the protein structure of erythrocyte membranes undergoes damage, expressed both in the recipient and in the related donor. This can initiate a decrease in erythrocyte electronegativity and an increase in erythrocyte aggregation.

The authors declare no conflict of interest.

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