World Journal of Medical Sciences 11 (3): 320-323, 2014
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2014.11.3.852

Effect of Ammonium Load on Erythrocytes of Hemodialysis Patients

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Abstract: The aim of the study was to estimate the status of band 3 protein in erythrocytes of patients with end-stage renal failure during the hemodialysis. The objects of the study were erythrocytes of patients with chronic kidney disease 5 stage with syndrome of end-stage renal failure during the hemodialysis. The first group included patients with end-stage renal failure in the outcome of chronic pyelonephritis (27 persons), the second - patients with end-stage renal failure, developed on a background of chronic glomerulonephritis (20 persons). The status of the band 3 protein was detected following the protocol of Mindukshev under the incubation of erythrocytes with ammonium ions. The alteration of the activity of band 3 proteins depended on type of primary kidney pathology (Pyelonephritis, glomerulonephritis).

Key words: Band 3 Protein · Erythrocytes · Ammonia Load · Hemodialysis

INTRODUCTION

Program hemodialysis is the main method of treatment for patients with end-stage renal failure, which can significantly increase the life expectancy of the patients. During the last decades hemodialysis has become the object of innovative technical searches, becoming a standard medical procedure, approximated to the patient's place of residence. Enhanced capabilities for the patient at the same time leads to increase in side effects of prolonged hemodialysis respectively increased life expectancy of the patients.

Hemodialysis causes a spectrum of disorders of electrolyte balance [1] and hemodynamics, provokes the progression of cardiovascular disease and erythropoietin-deficiency anemia. Among the causes of anemia isolated the deficiency of erythropoietin and iron, reducing the lifetime of erythrocytes in conditions of uremia, chronic blood loss. Moreover, during hemodialysis hemolysis develops, which also contributes to the development of anemia.

Condition of red blood cells for patients on hemodialysis was evaluated from the perspective of the development of oxidative stress and efficiency of the components of antioxidant protection [2, 3].

Another approach includes the evaluation of physicochemical properties of erythrocytes. It was shown that hemodialysis induced decrease in osmotic resistance of erythrocytes and expressed redistribution of cholesterol and a significant decrease in the content of phosphatidylcholine and sphingomyelin in the erythrocyte membrane of patients with chronic renal failure [4]. Along with reduced ability to deformation growth of osmotic fragility of erythrocytes in patients with end-stage renal failure was recorded [5].

Prolonged program hemodialysis helped to reduce the deformability of red blood cells in patients with chronic renal failure [6- 8]. At the same time as the most likely factors determining violation of membrane structure of red blood cells isolate intensification of lipid peroxidation, carbonyl stress, damage and decomposition of erythrocyte membrane protein.

This involves the development of measures aimed at increasing the stability of erythrocytes. The basis of such studies should be representation of the character changes in biochemical and physicochemical properties of red blood cells in the dynamics of the development of chronic renal failure and under conditions of chronic hemodialysis.

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One of the most important conditions, providing the ability of red blood cells pass through the narrow tissue capillaries, is stabilization of their volume. Growth kinetics of cell volume begins to be determined of cell membrane permeability for Cl^- ions. Trigger mechanism of this process is the activation of integral band 3 protein. This protein is also involved in the formation of red blood cells architectonics and gas exchange [9].

Researches of status of band 3 protein in erythrocytes of patients with end-stage renal failure during the hemodialysis were not conducted. It determined the relevance of our research.

The aim of the study was to estimate the status of band 3 protein in erythrocytes of patients with end-stage renal failure during the hemodialysis.

MATERIALS AND METHODS

The objects of the study were erythrocytes of patients with chronic kidney disease 5 stage with syndrome of end-stage renal failure during the hemodialysis. Depending on the initiating nosological variant of disease 2 groups were formed. The first group included patients with end-stage renal failure in the outcome of chronic pyelonephritis (27 persons), the second-patients with end-stage renal failure, developed on a background of chronic glomerulonephritis (20 persons).

The clinical picture before the first hemodialysis sessions in both groups was characterized by expressed syndrome of uremic intoxication. Patients complained about lack of appetite, nausea, significant reduction in body weight over the past few months preceding of the identification of end-stage renal failure. 79% of surveyed patients had dizziness and headaches. Decrease in memory and cognitive disorders were observed in 49% of patients. Insomnia, dysthymic disorders were observed in 84% of patients in both groups. Anemic syndrome was diagnosed in all patients of the 1st and 2nd groups. Degree of anemia was evaluated as the second, level of hemoglobin in the average was 82 ± 85 g/l, color index 0,68 ± 0,11. Arterial hypertension 2 and 3 degrees was recorded in all patients of the 1st group and in 79% of patients of the 2nd group. On the background of hemodialysis arterial hypertension in the first weeks of treatment normalized only in 36% of patients.

Duration of the disease (Chronic pyelonephritis) in the 1st group was on average 17 ± 5,3 years, duration of disease (Chronic glomerulonephritis) in the 2nd group was 92 ± 17 years.

Creatinine level in the serum of patients of both groups reached 0,78 ± 0,09 mmol/l, glomerular filtration rate - 9,5 ± 1,2 ml/min. Oliguria was observed in 36% of the patients in the 1st group and 47% in the 2nd group; in other patients diuresis has been saved. The relative density of urine in patients with chronic pyelonephritis was 1,006 ± 0,0012 while in patients with chronic glomerulonephritis it was 1,010 ± 0,0023.

Determination of the indicator of creatinine in serum was performed in all patients after each hemodialysis session. Decrease in serum creatinine at 50% was observed after the second and third hemodialysis sessions in 74% of patients in both groups. Subjective improvement noted in 69% of patients. Deterioration of feeling indicated 13% of patients in the 1st group and 9% of patients in the 2nd group by the end of the first and second hemodialysis sessions. It is expressed in severe headaches, weakness and fever. Stabilization of the general condition of these patients and normalization of their feeling fixed to the fifth and sixth sessions of chronic hemodialysis.

All examined persons gave informed consent to participate in the survey. Blood volume in 3 ml was stabilized by heparin. Plasma was separated from erythrocytes by centrifugation at three thousand revolutions per minute.

Activity of band 3 protein was detected following the protocol of Mindukshev *et al*. [10]. Washed erythrocytes were placed in isotonic environment in which sodium ions have been replaced by ammonium ions (140 μM NH_4Cl, 5 μM KCl, 5 μM glucose, 1 μM $CaCl_2$). Under these conditions, the alkalization of intracellular pH by the penetration of NH^{4+} leads to the activation of exchanger, regulating entrance of chloride anions, which leads to swelling of the cells.

Kinetics of cell volume changes in conditions of ammonium load recorded on a hematology analyzer BC-3200 (Mindray, China) for 15 minutes and expressed in fL.

Comparison the results obtained was performed using non-parametric Mann-Whitney U-test (For independent variables) and Wilcoxon matched pairs test.

RESULTS

As a result of the study, it was found that in patients of the 1st group increase in the volume of erythrocytes occurs within 5 minutes of incubation (Figure 1). Thereafter, the hemolysis has begun. An increasing of

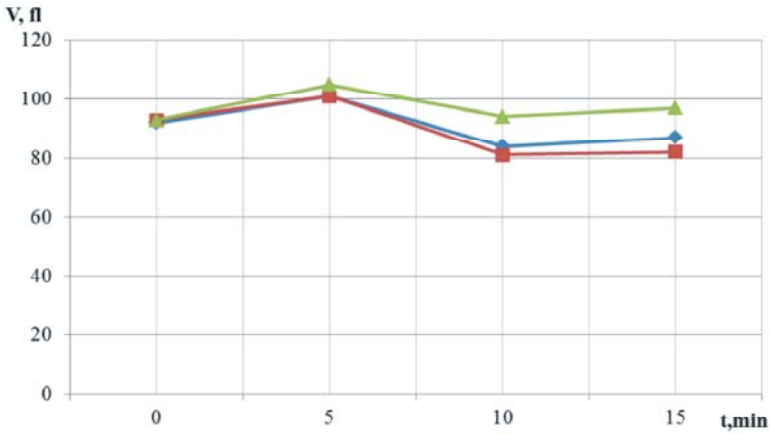

Fig. 1: Three variants of the change in the volume of erythrocytes of patients in the 1st group under ammonium load.

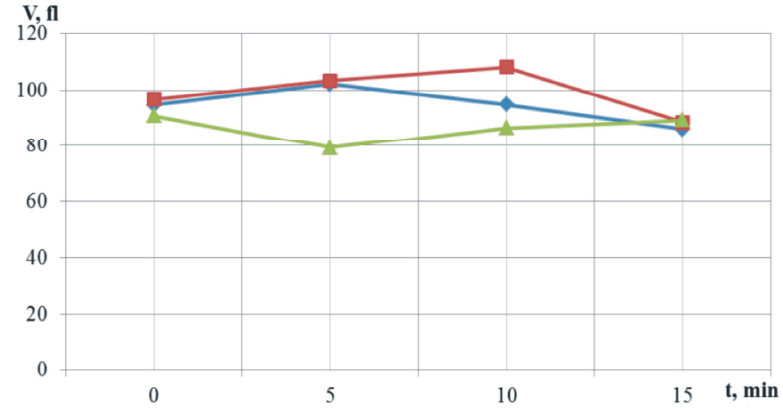

Fig. 2: Three variants of the change in the volume of erythrocytes of patients in the 2nd group under ammonium load.

erythrocytes volume under incubation in ammonium environment was caused by the change of ammonium and chloride ions current in the cell, which is accompanied by a decrease in the emission of sodium from the cell.

Under the conditions of the ammonium load enhanced osmotic hemolysis of low resistant erythrocytes and by the end of the experiment only erythrocytes with high adaptive-compensatory status of ion exchange systems stay in the environment.

Answer of erythrocytes on ammonium load in patients of the 2nd group had a number of features. At placing cells in isotonic environment with ammonium ions, the degree of changes in the activity of the band 3 protein was varied. In the part of patients (36%) (Cluster 1) erythrocytes increased in volume, which reached maximum after 5 minutes. In the other part of patients (45%) (Cluster 2) erythrocytes showed high speed of primary swelling and increased instability to ammonia load, i.e. early transition to hemolysis. In the remaining part of patients (18%) (Cluster 3) recorded by prolonging of the time to reach maximum volume of the cells, i.e. increased resistance to ammonium load (Figure 2).

Formation of three different forms of erythrocytes response to ammonium load is determined by different ability of ion exchange systems to compensate for the volume of red blood cells.

DISCUSSION

Erythrocyte population is not homogeneous and consists of subpopulations of low resistant, medium resistant and high resistant erythrocytes (By the resistance to osmotic hemolysis).

Under pathological conditions the ratio of red blood cells subpopulations may change. We have previously shown an increase in medium resistant subpopulation of erythrocytes in patients with chronic pyelonephritis. This trend was uniform in all patients of this group.

Within the group of patients with chronic glomerulonephritis, apparently, change of erythrocytes subpopulations varied. In the part of patients with chronic glomerulonephritis, it was observed an increase in the proportion of rigid erythrocytes, for which the rate of hemolysis is reduced. Such increase in rigidity can be

regarded as state of the cells prior to apoptosis. In the other part of patients, it was observed an increase in the proportion of low resistant erythrocytes, at which reduced the transport characteristics of ion exchange.

Naturally, violation of the ability of erythrocytes to regulate their volume is associated not only with working of band 3 protein. Previously, we have found increase in the water content in erythrocytes of patients with chronic renal failure in the predialysis period [11].

Violation of ability of erythrocytes to regulate their volume may be due to dysfunction of water channels and transporter proteins involved in the transport of ions. Moreover, the most probable cause is oxidative modification of transporter proteins. This is evidenced by increase in the content of carbonyl derivatives of proteins in erythrocytes of patients with chronic kidney disease [12]. Oxidative damage of membrane proteins and lipids in red blood cells of patients with chronic renal failure leads to reduction of macroergs, contributes to shutdown ion pumps and increase level of $Ca^{2+,}$ which creates conditions for the inclusion of apoptotic program of erythrocytes [13].

Disturbance of regulation by erythrocytes of own volume leads to dysfunction of the cytoskeleton and loss of the ability of cells to deformation, which provokes hemolysis.

CONCLUSION

Thus, individual variation in subpopulations of erythrocytes in the blood of patients with chronic renal failure, defined by the status of systems involved in the regulation of cell volume, to a large extent affect to the adaptation of dialysis patients.

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