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Morphological characteristics of erythrocytes at undifferentiated connective tissue dysplasia in children

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Examination of 73 children of 8–12 years of age was conducted in order to study morphological characteristics of erythrocytes at undifferentiated connective tissue dysplasia (UCTD). Scanning electron microscopy revealed significant alterations of surface architectonics of red blood cells, characterized by reduction in the number of discocytes and increase in the number of transformed and degenerative cell forms in the group of children with UCTD without development of anemic syndrome. The most intensive morphological presentation disorders of red blood cells were registered in children with UCTD and anemic syndrome. The article shows that structural inferiority of erythrocyte membranes is a pathogenetic anemia development factor in children with connective tissue dysplasia.
***Key words**: children, connective tissue dysplasia, erythrocytes, electron microscopy.*

INTRODUCTION

Hematologic disorders are frequent pathological conditions secondary to hereditary connective tissue disorders in children. Numerous trials demonstrated interconnection between collagen proteins of connective tissues and hemostatic system [1]. Best studied are peculiarities of hemorrhagic syndrome secondary to thrombocytopathies, deranged end stage of coagulation, decrease in activity of coagulation factors secondary to hemorrhagic mesenchymal dysplasias and Ehlers-Danlos syndrome [2-5]. According to N.N. Gladkykh et al., genetically defined maturation and differentiation defects of mesenchymal derivatives affect structure and functional entirety of cell membranes at hematomesenchymal dysplasias, thus causing platelet and endothelial dysfunction [6]. The older publications also feature data on development of anemic syndrome and erythrocyte structural-metabolic disorders in children in the event of undifferentiated connective tissue dysplasia (UCTD) [7]. However, there has been no common opinion on the assessment of pathogenetic mechanisms of anemic syndrome formation in children in the event of UCTD; this considerably complicates management of such patients.
The objective of this work is to study morphological characteristics of erythrocytes in the event of undifferentiated connective tissue dysplasia in children.

PATIENTS AND METHODS

The trial involved 73 8-12-year-old children registered for pediatric surveillance at ambulatory departments of Tomsk pediatric hospital No. 1. The main group consisted of 44 children with undifferentiated connective tissue dysplasia without anemic syndrome.
Criteria of inclusion to the main group:

- UCTD confirmed by clinical laboratory methods;
- no anemia, hemostatic system diseases or acute diseases;
- examination performed during remission of the chronic pathology;

- no drug intake at the moment of laboratory examination.

The comparison group consisted of 7 children with anemic syndrome secondary to UCTD. 22 children without connective tissue dysplasia comparable with the main group patients in terms of sex, age, health groups comprised the control group.

Quantitative red blood parameters (erythrocyte, reticulocyte and hemoglobin count), erythrocyte indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, erythrocyte distribution width) were determined with standard hematologic methods. Surface architectonics of erythrocytes was analyzed using scanning electron microscopy (electron microscope "REM-20", Russia), preparing samples according to the method of G.I. Kozinets et al. (1982). Morphological forms of erythrocytes were calculated according to the classification by G.I. Kozinets et al. (1982) distinguishing between the following cell forms: discocyte, discocyte with a single outgrowth, discocyte with a ridge, discocyte with multiple outgrowths; "mulberry"-shaped erythrocytes, dome-shaped erythrocytes, spherical erythrocytes, "deflated-ball"-shaped erythrocytes and degeneratively altered forms.

Statistical data manipulation was performed using software package Statistica 6 for Windows. We used Student's t-test for normal distribution of data and non-parametric Mann-Whitney U test for deviant distribution. Differences of compared groups' variables were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Quantitative analysis of red blood parameters revealed significant hemoglobin level and erythrocyte count decrease down to 127.98±1.17 ($p < 0.05$) and 3.88±0.03 x 10^{12}/l ($p < 0.01$), respectively, in the main group children in comparison with these parameters in the control group children. Although these parameters were within the average age norm, a tendency to anemia development in children with UCTD is observed. Values of the other parameters (reticulocyte and hematocrit count, erythrocyte indices and color index) were similar to those in the control group children. Analysis of surface architectonics of red blood cells in children with UCTD with scanning electron microscopy revealed significant changes in quantitative distribution of erythrocyte cell forms (tb.). Thus, the main group children featured significant ($p < 0.001$) decrease in the amount of normal disc-shaped erythrocytes down to 84.18±0.17% and, at the same time, increase in the share of reversibly transformed cells (ellipses, flat discs, discocytes with a single outgrowth, a ridge, multiple outgrowths and "mulberry"-shaped erythrocytes) in comparison with the control group children. The amount of pre-hemolytic forms of red cells exceeded their count in the control group ($p < 0.001$): 2 times more dome-shaped erythrocytes and 3 times more "deflated-ball"-shaped erythrocytes. The number of degenerative forms of erythrocytes in children of this group reached 0.4±0.03% - twice as many as in the control group children. In terms of microcirculation, oxygen transport function and deformation potential, transformed forms of erythrocytes are less adequate than discocytes, which is why increase therein within the erythrocyte population is an unfavorable symptom [8].

The best marked structural disorders of erythrocyte membranes were observed in children with anemic syndrome secondary to UCTD. Significant decrease in hemoglobin concentration and erythrocyte count down to 104.9±1.3 g/l ($p < 0.001$) and 3.57±0.07 x 10^{12}/l ($p < 0.01$), respectively, was registered in this group of patients. The peripheral blood reticulocyte count increased to 8±0.44‰ ($p < 0.05$), apparently, by way of compensation. Anemia was characterized as normocytic normochromic in most cases. Scanning electron microscopy data revealed that development of anemia in children with connective tissue dysplasia is accompanied by alteration of morphological characteristics of erythrocytes: decrease in the count of normal disc-shaped forms of red cells down to 83.3±0.21%, increase in the count of reversibly transformed (13.12±0.07%), pre-hemolytic (3.0±0.14%) and degenerative (0.56±0.05%) forms of erythrocytes. At the same time, the count of pre-hemolytic (dome-shaped, spherical and "deflated-ball"-shaped erythrocytes) and degenerative forms of erythrocytes was significantly

different from the count thereof not only in the control group ($p < 0.01$), but also in the group of children with UCTD without anemia ($p < 0.05$).
Results of the electron microscopic analysis of erythrocyte peculiarities in children with UCTD revealed surface microrelief deviations of peripheral blood erythrocyte membranes, characterized by decrease in discocyte count and increase in the amount of transition, pre-hemolytic and degenerative forms of cells. We may assume that genetically determined connective tissue defects of mesenchymal structures comprising the hematopoiesis-inducing microenvironment may cause erythropoiesis inefficiency and entry of red blood cells with primarily modified membranes to blood channels. In view of the concept formulated by V.V. Novitskiy et al. on universal response of plasma membranes to the development of pathological processes of varying genesis, erythrocyte structure alterations may be extrapolated on the other membrane systems [9].

CONCLUSION

Results of the electron microscopic analysis of erythrocyte peculiarities in children with UCTD without development of anemic syndrome revealed significant alterations of surface architectonics of red blood cells, characterized by decrease in discocyte count and increase in the amount of transformed and degenerative forms of cells. The best marked morphological deviations of red blood cells were registered in children with UCTD and anemic syndrome. We may assume that structural inferiority of erythrocyte membranes is one of the important pathogenetic factors of anemia development in children with connective tissue dysplasia; this needs to be taken into account when performing therapeutic correction in patients of this category.

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Table. Morphological description of erythrocyte population in children according to the scanning electron microscopy data

Type of erythrocyte surface (per 100 cells)	**Control group** M±m	**Main group** M±m	**p**
Discocytes	88.89±0.16	84.18±0.17	$p < 0.001$
Reversibly transformed erythrocytes	9.37±0.17	12.81±0.1	$p < 0.001$
• Ellipses;	0.17±0.06	0.56±0.03	$p < 0.001$
• Flat discs;	0.2±0.03	1.38±0.03	$p < 0.001$
• Discocytes with a single outgrowth;	4.22±0.02	5.64±0.04	$p < 0.001$
• Discocytes with a ridge;	4.19±0.07	4.62±0.04	$p < 0.001$
• Discocytes with multiple outgrowths;	0.53±0.03	0.53±0.02	$p > 0.5$
• “Mulberry”-shaped erythrocytes;	0.06±0.02	0.08±0.01	$p > 0.5$
Irreversibly transformed erythrocytes:	1.54±0.08	2.61±0.07	$p < 0.001$
• Dome-shaped;	0.53±0.03	1.13±0.03	$p < 0.001$
• Spherical;	0.84±0.04	0.96±0.03	$p > 0.5$
• “Deflated-ball”-shaped;	0.17±0.02	0.52±0.02	$p < 0.001$
Degenerative forms	0.2±0.04	0.4±0.03	$p < 0.001$

Note. The reached difference significance level (p) in comparison with the control group.