Protein Spectrum and State of Lipid Bilayer in Erythrocyte Membranes in Children with Insulin-Dependent Diabetes Mellitus: Polyacrylamide Gel Electrophoresis and Fluorescence Analysis Data

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Erythrocyte membranes in children with insulin-dependent diabetes mellitus are characterized by a decreased content of high-molecular-weight proteins and increased content of low-molecular-weight proteins and fractions of aggregated material against the background of increased microviscosity of deep membrane layers and changes in the protein-lipid interactions. These abnormalities were found in phases of clinical metabolic decompensation and subcompensation.

Key words: *erythrocyte; membrane protein spectrum; lipid bilayer microviscosity; insulin-dependent diabetes mellitus; children*

Abnormalities of peripheral blood erythrocytes, such as composition of membrane proteins and lipids, functional characteristics of the ion-transporting systems, biophysical characteristics of lipid bilayer, rheological properties, *etc.*, play an important role in the development of diabetic microangiopathies in children with insulin-dependent diabetes mellitus (IDDM) [3,4,10]. Sustained metabolic and structural disturbances in functional activity of mature erythrocytes aggravate tissue hypoxia, which to a certain extent determines the prognosis and the course of the primary disease [2]. Detailed study of the peripheral element of the erythron in children with IDDM allow to optimize monitoring systems and enhance the efficacy of traditional endocrinological therapy in children.

MATERIALS AND METHODS

Seventy-nine children with IDDM aged 4-15 years were examined. Diagnosis was based on medical history, physical examination, blood biochemical tests, and ultrasound investigation of the abdominal organs. The control group included 35 healthy children. Blood was collected from the vein. Erythrocyte membrane proteins were analyzed by disk-electrophoresis in PAAG according to Laemmli. Stained gels were scanned, and the images were processed with a computer software, which allows averaging across the row and to calculation of the area of each peak. Protein fractions were classified according to Fainbanks and Steck [12]. Intrinsic fluorescence (FL) of erythrocyte ghosts and fluorescence of probes incorporated into the erythrocyte membranes were recorded on a Hitachi-MPF-4 spectrofluorimeter. Pyrene (Sigma) was used as a fluorophore [7]. The data were analyzed by ANOVA statistics. Student *t* test was used to compare the groups of parameters with normal distribution, otherwise nonparametric tests were used.

RESULTS

In children with decompensated IDDM with ketoacidosis the content of spectrin during treatment was significantly decreased compared to the control. At the same time, the level of α-spectrin did not differ from

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the respective value in healthy children, while the content of β-spectrin was considerably below the control (Table 1). In children with decompensated IDDM with ketoacidosis, the level of band 7 protein and content of aggregated proteins in erythrocyte membrane were significantly higher than in the control (Table 1). Other protein components did not differ from the control (data not shown).

Before treatment, the content of band 7 protein and aggregated proteins in the erythrocyte membrane markedly increased in children with decompensated IDDM without ketoacidosis. During clinical metabolic subcompensation, the total content of spectrin in this group was far below the control (Table 1): the content of β-spectrin decreased significantly compared to healthy children, while α-spectrin remained nearly unaffected. The content of band 2 protein also significantly decreased, band 2.1 fraction decreased and band 2.2 fraction remained near the normal. The levels of band 7 and 8 proteins far surpassed the control. Other protein components of erythrocyte membrane did not differ from the control (data not shown).

Membrane protein composition in children with decompensated IDDM and ketoacidosis did not differ significantly from children without ketoacidosis. At the same time, in patients with clinical metabolic subcompensation the levels of band 7 protein and β-spectrin significantly differed from the corresponding values in decompensated patients without ketoacidosis before treatment (Table 1).

It is suggested that this phenomenon observed during chronic hyperglycemia under the conditions of insulin deficiency is determined by pronounced activation of lipid peroxidation [6], which results in the formation of lipid-lipid and lipid-protein crosslinks and produces changes in physicochemical properties of the lipid matrix (increased bilayer viscosity, decreased rotational and lateral mobility of membrane proteins, facilitated phospholipid flip-flop, oligomerization and aggregation of membrane proteins, structural modifications of membrane lipid vesicles). Moreover, enhanced protein glycosylation during IDDM [9,15], disturbances of the tertiary structure of membrane erythrocyte proteins, cleavage of disulfide bonds, exposure of SH-group, and rearrangement of the protein-lipid interactions (both integral and superficial proteins, and glycoproteids are involved in structural rearrangements) can also contribute to this phenomenon [10,11,14]. These factors can promote cell hemolysis, presumably due to changes in the parameters of thermal transitions in the erythrocyte membrane proteins (spectrin, membrane domain of band 3 protein), aggregation of membrane vesicles, and their enlargement during pathology [1].

According to our data obtained with the use of fluorescence, microviscosity of deep structures of the erythrocyte lipid bilayer increased and protein-lipid interactions are disturbed in erythrocyte membranes from patients with clinical metabolic decompensation with and without ketoacidosis before and after traditional treatment (Fig. 1). Laser therapy in combination with traditional treatment did not completely normalize these parameters. However, some positive tendencies appeared: normalization of pyrene FL at excitation wavelength of 340 nm (total pyrene FL), while pyrene FL at 285 nm changed insignificantly (marginal zone, Fig. 1).

Table 1. Erythrocyte Membrane Proteins (%) in Healthy Children and in Children with IDDM ($X \pm m$)

Proteins		Healthy children (control)	Children with IDDM		
			phase of decompensation		phase of clinical metabolic sub-compensation after treatment
			without ketoacidosis before treatment	with ketoacidosis during treatment	
Spectrin		18.52	16.31	14.61***	13.22**
	a	6.69±0.58	7.75±0.48	7.18±0.61	6.65±0.87
	b	9.82±0.54	8.56±0.43	7.42±0.49*	6.57±0.65***+
Protein bands					
	2	4.06	3.84	4.09	3.49***
	2.1	2.87±0.12	2.78±0.22	2.86±0.25	2.38±0.16***
	2.2	1.19±0.07	1.07±0.05	1.24±0.10	1.11±0.08
	7	5.07	7.26***	8.01**	9.48*
	8	4.13	5.18	5.58	6.39***
Aggregated protein		1.06	1.81***	1.66***	1.30

Note. *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared with control; +$p<0.05$ compared with the phase of decompensation without ketoacidosis before treatment.

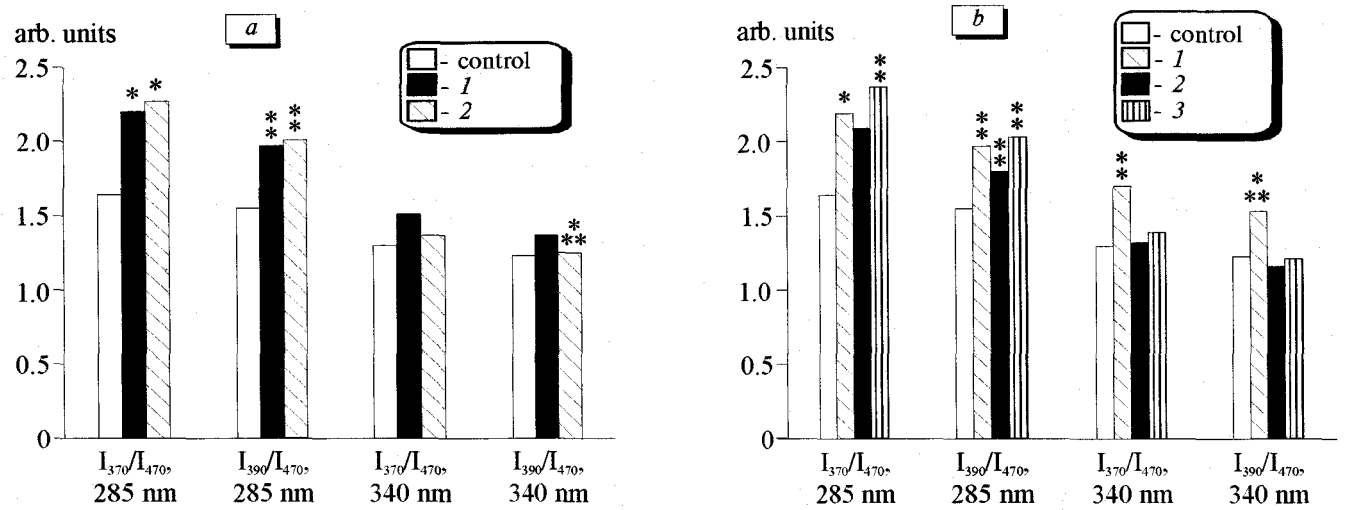

Fig. 1. Microviscosity of the erythrocyte membrane proteins in healthy children (control) and children with IDDM (fluorescence analysis). Ordinate: fluorescence index. *a)* phase of decompensation before therapy without ketoacidosis (*1*) before treatment and with ketoacidosis (*2*); *b*) phase of clinical and metabolic subcompensation (3) after traditional (*1*) and combined (*2*) therapy. *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared to the control.

Membrane fluidity depends on mobility of carbon atoms in fatty acid hydrocarbon chains, length of hydrocarbon chain in phospholipids, saturation of fatty acids, concentration of bivalent cations, and the content of cholesterol. Loose packing of phospholipids enriched with unsaturated fatty acids increases membrane fluidity due to more rigid steric structure. Ca and Mg ions diminish electrostatic repulsion of charged phospholipid heads. This results in more compact packing of the bilayer, reduceds tail mobility, and decreases membrane fluidity [7,13]. Lipids take part in structural arrangements of the bilayer and preserve conformational changes of membrane enzymes, control interaction within oligomer complexes. At the same time, physicochemical properties of lipids depend on whether or not they contact with protein in the bilayer [4,5].

Thus, erythrocyte membrane in children with IDDM is characterized with increased microviscosity of deep layers, disturbed protein-lipid interactions, decreased concentration of spectrin, and increased levels of bands 7 and 8 proteins and protein aggregates. This can serve as a measure of disturbances in the peripheral element of erythron and to some extent determines the prognosis and complications of the disease.

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