EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Structural and Metabolic Status and Functional Peculiarities of Erythrocytes in Children with Insulin-Dependent Diabetes Mellitus

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Development of insulin-dependent diabetes mellitus in children is accompanied by essential disturbances in the peripheral subdivision of the erythron system, manifested in enhanced cell polymorphism, imbalance in the composition of membrane phospholipids and their increased microviscosity, reduced lipoprotein concentration, and enhanced reversible aggregation. These disturbances are most pronounced during metabolic decompensation accompanied by ketoacidosis, which agrees with the hypothesis on their role in the development of vascular complications.

Key Words: *insulin-dependent diabetes mellitus; erythrocytes*

The development of insulin-dependent diabetes mellitus (IDDM) in children is accompanied by pronounced disturbances in the erythron system [1,4,11,13]. Chronic glycemia against the background of insulin deficiency is characterized by essential disturbances in the structural and functional status of peripheral blood erythrocytes, which determines microcirculatory and rheological parameters, and probability of disseminated intravascular coagulation [5,7,14,15]. The study of lipid content and microviscosity of erythrocyte membrane, its surface architectonics, and erythrocyte aggregation parameters makes it possible to evaluate the involvement of the erythron system into the pathological process and the degree of metabolic disturbances in this process.

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MATERIALS AND METHODS

We examined 70 children at the age of 3-15 years with IDDM, patients of the First Municipal Children Hospital No. 1 in Tomsk. Thirty-five children had symptoms of diabetic angiopathy, 48 children had fatty hepatosis, lipodystrophy, sexual and physical retardation, and xanthochromia. Control group comprised 35 healthy children.

Hemoglobin concentration and erythrocyte count in the peripheral blood were routinely determined. Quantitative analysis of sulfhydryl groups and lipoproteins was performed with the help of a Lyumam I-2 cytophotometer, SH-groups were detected by ferricyanide method [11], lipoproteins were stained with Sudan black B [9]. The stained smears were tested photometrically at 590 nm. The mean concentrations of the analyzed agents were calculated per cell surface unit.

The surface architectonics of peripheral blood erythrocytes was studied by scanning electron microscopy. The samples were prepared [3] and examined

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TABLE 1. Phospholipid Composition and Microviscosity of Erythrocyte Membrane (F_m/F_e) in Children with IDDM (X±m)

Group	Phospholipid fractions of erythrocyte membrane, %								F_m/F_e, rel. units
	LPC	phosphatidylinositol	sphingomyelin	phosphatidylcholine	phosphatidylserine	PEA	polyglycerophosphates	phosphatidic acid	
Healthy children	8.54±0.81	8.07±0.65	1.01±0.78	22.22±1.20	10.20±1.10	15.63±1.21	9.16±0.90	13.59±1.35	1.64±0.06
IDDM without ketoacidosis	9.72±0.51	8.85±0.41	11.57±0.68	19.66±1.04	11.62±0.66	16.96±0.74	10.54±0.58	11.29±0.59	2.20±0.12**
with ketoacidosis	8.75±1.03	8.79±0.64	10.14±1.01	22.52±1.70	10.99±1.04	20.66±2.10*	9.06±1.27	8.79±1.01*	2.27±0.15**

Note. *p<0.05, **p<0.01 compared to healthy children.

under a REM-200 scanning electron microscope operated at 35 kV accelerating voltage, 0.63 A current, and 35° angle. The morphological forms of the cells were identified on the basis of classification [3].

The phospholipid fractions in erythrocyte membrane were determined by thin-layer chromatography on Silufol plates. The blood was drawn from a vein, erythrocyte membranes were isolated as described elsewhere [6], and lipids were extracted by the method of Folch [12]. The following phospholipid fractions were isolated: lysophosphatidylcholine (LPC), phosphatidylinositol, sphingomyelin, phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine (PEA), polyglycerophosphates, and phosphatidic acid. Identification of lipid fractions was performed using Sigma standards.

Microrheological parameters were assessed photometrically by the index of reversible erythrocyte aggregation. The reversible erythrocyte aggregation index and integral aggregation coefficient were calculated as described elsewhere [8].

Microviscosity in deep membrane layers was assessed by pyrene excimerization [2].

The data were statistically analyzed by Wilcoxon—Mann—Whitney and Kruskal—Wallis tests using SAS software.

RESULTS

Qualitative parameters of the red blood in children with IDDM did not differ significantly from the control. The study of structural and metabolic status of peripheral blood erythrocytes from children with IDDM in the period of stage II metabolic decompensation without ketoacidosis revealed that the content of lipoproteins was significantly lower than in the control group (0.654± 0.026 *vs.* 0.488±0.029 rel. units, p<0.01). Erythrocyte population primarily (54.2%) consisted of cells with decreased or low content of the studied substrate. The content of thiol groups in erythrocytes from patients with IDDM did not differ significantly from normal (0.323±0.02 *vs.* 0.279±0.008 rel. units, p>0.05).

At the same time, a redistribution of erythrocyte population caused by accumulation of cells with decreased content of sulfhydryl groups was noted. We revealed no significant changes in the content of phospholipid fractions in erythrocyte membrane in children with IDDM in the period of stage I-II metabolic decompensation without ketoacidosis (Table 1).

Microviscosity of erythrocyte membrane in this group was characterized by a significant increase in F_m/F_e parameter (Table 1). A pronounced redistribution of erythrocyte population was observed during examination of their surface architectonics by scanning electron microscopy (Fig. 1): the content of dis-

cocytes was significantly decreased, while the content of transitional, nontransitional, and degenerative forms markedly increased. Analysis of indices of reversible erythrocyte aggregation in children with IDDM without ketoacidosis revealed that the aggregation index significantly differed from the control (2.39±0.4 *vs.* 1.65±0.19 units, $p<0.05$). The integral coefficient of erythrocyte aggregation in this group did not exceed the control values.

The development of stage III metabolic decompensation with ketoacidosis in children with IDDM was accompanied by pronounced disturbances in the structural and functional status of red blood cells. The content of lipoproteins in erythrocytes was 0.506± 0.028 rel. units, while in healthy children the corresponding value was 0.654±0.026 rel. units. In children with IDDM, most erythrocytes were characterized by low content of the studied substrate. The concentration of thiol groups in children with IDDM did not exceed the control value (0.311±0.023 and 0.279±0.008 rel. units, respectively), although a population of cells (absent in control) with a high concentration of the studied substrate appeared against the background increase in the fraction of erythrocytes with decreased content of SH-groups. Phospholipid composition of erythrocyte membrane was characterized by a significant increase in PEA and decrease in the content of phosphatidic acid in comparison with the control. Microviscosity of the lipid bilayer in children with IDDM and ketoacidosis exceeded the corresponding parameter F_m/F_e in the control (Table 1). Electron microscopy study revealed pronounced erythrocyte polymorphism in children with IDDM in the period of stage III metabolic decompensation (Figs. 1 and 2). In this period, the number of discocytes was significantly lower than in the control ($p<0.001$, Fig. 1). At the same time, the content of transitional (ellipsoid and flat discs, discocytes with single or multiple projections and a crest, and mulberry cells), nontransitional (domed, spherical, and deflated balls), and degenerative forms significantly increased in comparison with the control group and group with IDDM without ketoacidosis (Fig. 1). The indices of reversible erythrocyte aggregation significantly differed from the control.

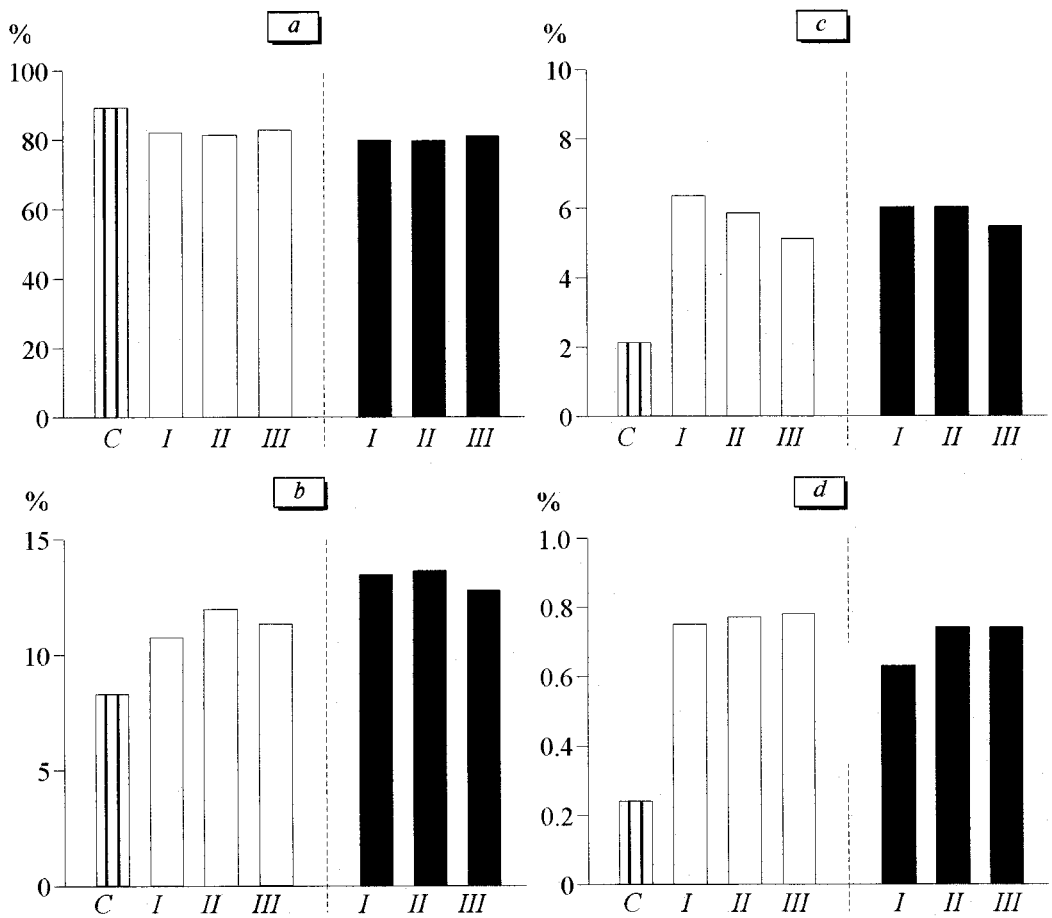

Fig. 1. Distribution of morphological forms of peripheral blood erythrocytes in children with insulin-dependent diabetes mellitus inthe period of stage I-II metabolic decompensation without ketoacidosis (light bars) and in the period of stage III metabolic decompensation with ketoacidosis (dark bars), scanning electron microscopy. Discocytes (*a*), transitional (*b*), nontransitional (*c*), and degenerative (*d*) forms. Control (C); before (*I*), during (*II*), and after (*III*) therapy.

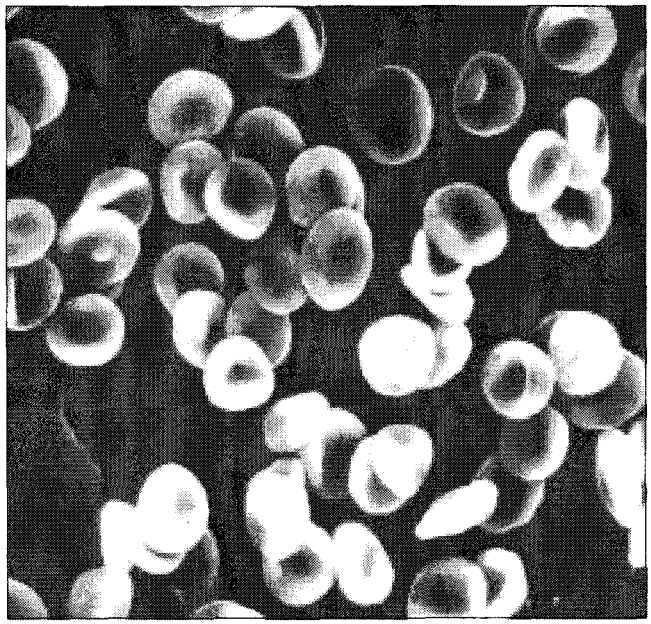

Fig. 2. Electron microscopy of peripheral blood erythrocytes in patients with insulin-dependent diabetes mellitus in the period of stage III metabolic decompensation with ketoacidosis, ×2000.

Therefore, the development of IDDM in children is characterized by pronounced disturbances in structural and functional status of peripheral blood erythrocytes: the percentage of prehemolytic, hemolytic, and degenerative forms increased against the background decrease in the number of biconcave discocytes; accumulation of lipoprotein-deficient cells was accompanied by redistribution of erythrocyte population in respect to the content of sulfhydryl groups; the increase in microviscosity of the lipid bilayer was associated with changes in their phospholipid composition and activation of reversible erythrocyte aggregation. These changes in the structural and functional status of erythrocytes in children with IDDM depend on the stage of metabolic decompensation and are most pronounced in patients with ketoacidosis.

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