Microrheology Disorders and Their Biochemical Correlates in Patients with Diabetes Mellitus and Initial Manifestations of Hypertension

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Similar disorders in microrheology manifesting in increased rigidity of leukocytes and correlating with high blood levels of globulins and C-reactive protein were detected in patients with diabetes mellitus and initial manifestations of hypertension. It was found that statistical relationships between aggregate properties of erythrocytes and biochemical parameters of the plasma are not universal and depend on the disease.

Key Words: *hemocyte adhesion; leukocyte plasticity*

Previous experimental and clinical studies showed that changes in the contact and elastic characteristics of cells are universal constituents of the adaptation and compensatory reactions in dysfunctions of different origin and severity [1-4,8,10].

We studied the microrheology of blood cells and their biochemical correlates in patients with type I diabetes mellitus (DM1) and patients with spontaneous episodes of arterial hypertension (AH).

MATERIALS AND METHODS

Blood parameters of diabetics with angiotrophic disorders (n=12) and patients with initial manifestations of hypertension presented by spontaneous AH elevation (n=12) were studied. Twelve age- and sex-matched donors comprised the control group. Erythrocyte aggregation was studied by direct microscopy of cell suspension in autologous plasma [7]. Aggregation index (ratio of erythrocyte count in aggregates to total erythrocyte count) and aggregate size coefficient (ratio of small to large aggregates) were estimated. Adhesion characteristics of leukocytes were studied by cell incubation in a glass capillary at 37°C for 60 min, after which the capillary was perfused with Dulbecco's solution at a shear stress of 30 N/m^2. The number of adherent leukocytes was estimated by the difference between cell counts in the initial and final suspension. Reserve potentialities of leukocyte membrane were studied as described elsewhere with some modifications [9]: cell suspension (10 μl) was placed into 4 wells of a plate for microbiological studies and 100 μl NaCl solution of different concentrations was added into each well: 0.9% in well 1, 0.45% in well 2, and 0.2% in wells 3 and 4. After incubation (60 sec for wells 1, 2, and 3 and 1 h for well 4) the cells were fixed with glutaraldehyde (Merck). Smears were prepared from fixed cells and stained with azure and eosin. The diameters of at least 100 cells on each slide were measured. Osmotic stability was evaluated by counting leukocytes in 0.9% NaCl solution and after 1-h exposure in 0.2% solution. Membrane function was evaluated by the kinetics of swelling (60 sec in 0.45% and 0.2% solutions). Biochemical parameters were evaluated by the standard methods used for laboratory diagnosis (electrophoretic separation of blood proteins on acetate cellulose membranes on an Astra device, measurement of total cholesterol by the enzymatic colori-

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TABLE 1. Blood Rheology Parameters (*M*±*m*)

Group	Aggregation index, rel. units	Aggregate size coefficient, rel. units	Osmotic resistance, %	Adherent leukocytes, %	Mean volume of erythrocytes, μ^3
Control	0.71±0.06	1.6±0.2	45±9	51.60±4.06	85.8±2.0
Diabetes	0.79±0.03*	1.2±0.2*	64±18*	42.60±6.35	83.3±1.1*
Hypertension	0.56±0.06*	2.2±0.3*	58±21*	57.10±4.63*	84.7±1.1

Note. *p<0.05 compared to the control.

metric method, measurement of C-reactive protein by latex agglutination, and of fibrinogen by Claus' method) [5].

The results were compared using Student's t and Wilcoxon's tests.

RESULTS

The functional and pathological shifts of different etiology were paralleled by changes in the blood cell rheology. The predominating components of microhemorheological profiles of diabetics were high erythrocyte aggregation with higher percentage of large aggregates and reduced plasticity of leukocytes, seen from the significant increase of osmotic stability and lower utilization of the membrane reserve in media with low osmolarity (Tables 1, 2). A decrease in erythrocyte and leukocyte size and a trend to a reduction of while blood cell adhesion in comparison with the control were noted. The main manifestations of microrheology disorders in patients with initial stage of hypertension were increased adhesion and reduced plasticity of leukocytes in parallel with less pronounced increase in osmotic resistance and significant limitation of the membrane reserve utilization in hypotonic media. Low aggregation of erythrocytes with predominance of small aggregates were noted in this group of patients. The counts and size of blood cells did not differ from the parameters of controls (Tables 1, 2).

Similar changes were noted for the majority of biochemical characteristics in patients with dysfunctions of neuroendocrine regulation: increased levels of globulins, cholesterol, C-reactive protein, and reduced percentage of albumins (Table 3). Reduced plasticity of leukocytes was the common cellular reaction associated with the detected shifts in plasma composition. Similar changes in the biophysical characteristics of leukocytes were detected under conditions of acute and autoimmune inflammation and during exposure to extreme environmental factors [1,8,10]. It seems that the increase in white blood cells rigidity is a universal reaction associated with a variety of functional and pathological changes in the body. Contact characteristics of the blood, regulated by numerous genes and mediated by respective adhesion molecules, are largely specific [6]. Changes in erythrocyte aggregation and leukocyte adhesion were opposite in the studied patient populations. The universal assumption according to which globulins and fibrinogen are aggregating proteins was confirmed only for diabetics. Lower content of albumin and higher level of fibrinogen in patients with the initial manifestations of hypertension were paralleled by low aggregation of erythrocytes in comparison with the controls.

TABLE 2. Diameter of Leukocytes (*M*±*m*, μ) Incubated in Media of Different Osmolarity

Group		Solution concentration, duration of incubation		
		0.9%	0.45%, 60 sec	0.2%, 60 sec
Control	neutrophils	10.65±0.46	11.15±0.34* (9.6)	11.88±0.30* (24.4)
	lymphocytes	6.77±0.16	7.11±0.18* (10.3)	7.49±0.37* (22.4)
Diabetes	neutrophils	10.06±0.77$^+$	10.43±0.67$^+$ (7.5)	10.92±0.83* (17.8)
	lymphocytes	6.55±0.29$^+$	6.84±0.40$^+$ (9.1)	7.11±0.23*$^+$ (17.8)
Hypertension	neutrophils	10.21±0.54	10.44±0.62$^+$ (4.5)	11.16±0.29* (19.5)
	lymphocytes	6.54±0.37	6.61±0.40$^+$ (2.2)	6.98±0.46*$^+$ (13.9)

Note. Here and in Table 3: *p<0.05 *vs.* *isotonic solution, $^+$control. Changes in cell surface area (%) in comparison with the isotonic medium is shown in parentheses.

TABLE 3. Blood Biochemistry (*M*±*m*)

Group	Albumins, %	Albumin-globulin index, rel. units	Fibrinogen, g/liter	Cholesterol, mmol/liter	C-reactive protein, rel. units
Control	58.6±1.0	1.37±0.08	2.6±0.2	3.8±0.2	0
Diabetes	48.6±2.2*	0.56±0.07*	2.8±0.3	5.5±0.2*	0.67±0.14*
Hypertension	53.7±1.2*	1.16±0.06	3.1±0.2*	4.3±0.3*	0.33±0.14*

We conclude that systemic dysfunctions of different etiology are paralleled by changes in the blood microrheology. The earliest nonspecific sign of microhemorheological disorders associated with high blood levels of globulins, C-reactive protein, and cholesterol is high rigidity of leukocytes. The dynamics of contact characteristics of blood cells is determined by specific pathogenetic mechanism, does not directly depend on the direction of changes in the plasma biochemistry, and can be a factor inhibiting or facilitating bloodflow in the capillaries.

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