Erythrocyte Structure and Function in Fibrocavernous Tuberculosis and Tuberculous Meningitis

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Erythrocyte disintegration processes are more pronounced in meningeal tuberculosis, which is associated with a shift in isoform spectrum of hemoglobin, catalase inhibition, and a sharp aggravation of lactoacidosis. The severity of erythrocyte disintegration cannot serve as a criterion of differential diagnosis of tuberculosis severity.

Key Words: *tuberculosis; erythrocyte morphology; platelets; meningitis*

Despite etiopathogenetic and clinical differences, common features of infectious diseases are intensification of LPO, increased production of reactive oxygen species (ROS), and activation and then rapid exhaustion of tissue antioxidant defense systems [4,9-11]. The main source of ROS in the body is blood neutrophilic granulocytes. Intensive migration of neutrophils into foci of lipolythic injuries, infiltration of interacinar connective tissue and periacinar spaces creates the picture of acute purulent inflammation [11]. Neutrophils and ROS acting as mediators of destructive processes aggravate the course of tuberculosis. Systemic activation of neutrophils promotes the progress of acute tuberculosis and development of complications. Erythrocytes containing catalase can inhibit division of unicellular organisms and even cause their death. This process is based on the reaction of hydrogen peroxide degradation; hydrogen peroxide is produced by the bacterial cell, partially diffuses through the plasma membrane [2,6], and degrades under the effect of catalase located in erythrocyte membranes [5]. Contact between erythrocyte plasma membranes and bacterial cells results in a microexplosion leading to membrane destruction at the site of microexplosion and plasmolysis of the bacterial cell and erythrocyte [2,3,9]. We studied the structure and function of erythrocytes and rate of their disintegration in different forms of tuberculosis.

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MATERIALS AND METHODS

The cohort study included 30 patients (mean age 34.5±6.8 years, 17 male and 13 females) with different forms of tuberculosis without documented inflammatory diseases. The diagnosis of pulmonary tuberculosis was confirmed by anamnesis data, presence of the clinical symptoms (inflammatory intoxication syndrome), and bronchopulmonary symptoms (coughing, sputum secretion, hemoptysis, thoracic pain, dyspnea), X-ray picture of the chest in the frontal and lateral projections, 3-fold bacterioscopy of the sputum collected over 24 h for *M. tuberculosis* and inoculation of this sputum in nutrient media, results of sputum examination by direct bacterioscopy of smears stained by the Ziehl—Neelsen method, hemogram (leukocytosis, stab shift, lymphopenia, monocytosis, increase of ESR) and Mantoux tuberculin test. The presence of *M. tuberculosis* DNA was confirmed by the PCR. All patients were divided into 2 groups with different patterns of the tuberculous process. Group 1 (n=21) consisted of patients with mainly exudative disease running an acute or subacute course. Nine patients presented with fibrocavernous pulmonary tuberculosis in the infiltration phase, 12 with infiltrative tuberculosis in the degradation phase. Group 2 consisted of 10 patients with meningeal tuberculosis diagnosed from characteristic changes in the liquor. X-ray examination revealed dissemination foci, tuberculomas, or small infiltrative changes in the lung tissue with signs of

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delineation. Small cavities of tissue degradation were detected in the lungs of virtually all patients of group 1. The results of examinations of healthy volunteers served as control.

Blood was collected under standard conditions. Morphological analysis of erythrocytes was carried out in blood smears fixed and stained with Azur-II-eosin. Erythrocyte shape was analyzed under a Photomicroscope-III (Opton). Activity of plasma cationic proteins was evaluated using commercial pre-tested kits (Pharmacia) as described previously [11], catalase activity was evaluated as described elsewhere [5], lactate content and lactate/pyruvate ratio (lactoacidosis intensity indicators) were evaluated using commercial kits (Boehringer-Mannheim), hemoglobin isoform composition was evaluated as described previously [2]. The results were processed using Student's *t* test and STAT-Soft software.

RESULTS

Neutrophil activity appreciably increased in group 1 patients; the content of cationic proteins inside the cells increased from 4±2 to 27±6 arb. units. Erythrocytes mainly retained the round ellipsoid shape; only 2.0±0.5% cells were elongated, 83±4% of these normocytes, 1.0±0.1% macrocytes, and 10±1% microcytes retaining smooth surface with poorly seen contours. The number of erythrocytes with abnormal surface of the outer membrane was 0.10±0.03% (Fig. 1). The number of shadow cells was 4.2±0.3%, number of acanthocytes 2.1±0.1%. Erythrocyte catalase activity increased 2.25 times, lactate content increased 2.2 times in proportion with the increase in pyruvate content. This attests to the absence of appreciable shift towards anaerobic glycolysis (Table 1). Increased catalase activity suggests that the antioxidant system of erythrocytes retained its adaptation potential.

Changes in erythrocyte shape were much more pronounced in patients with the meningeal form of tuberculosis (Fig. 2): the percentage of elongated erythrocytes increased to 4.0±0.3, that of normocytes decreased to 70±5% ($p<0.01$), that of microcytes to 6.0±0.3 ($p<0.01$), while macrocyte content increased to 12±1% ($p<0.001$) and the content of shadow cells reached 10.0±1.0% ($p<0.001$). This was paralleled by the appearance of folds on the erythrocyte surface, their contours were better discernible, clasmatosis was noted, and the number of abnormal erythrocytes increased 20-fold in comparison with group 1 (2.2±0.5%;

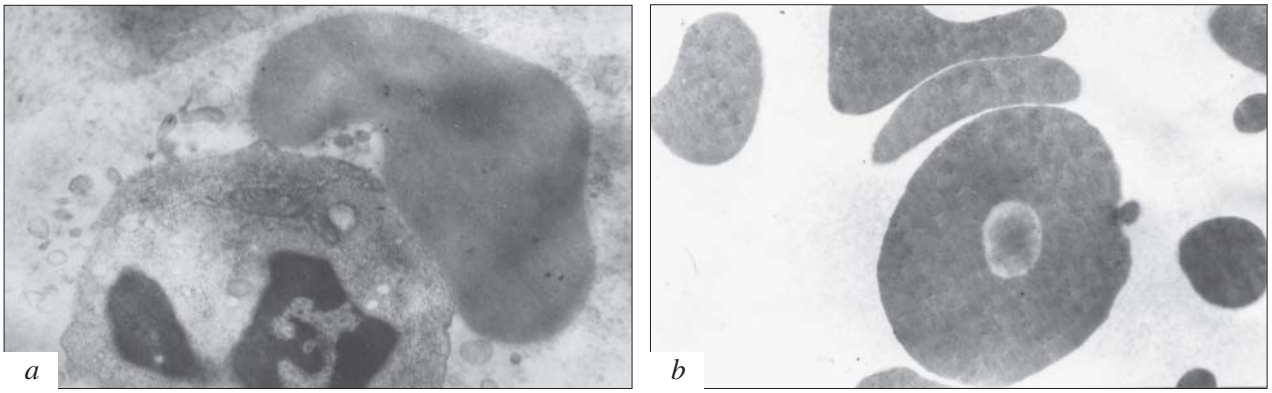

Fig. 1. Blood electronograms of a patient with fibrocavernous tuberculosis, ×12,500. *a*) acute and *b*) subacute forms.

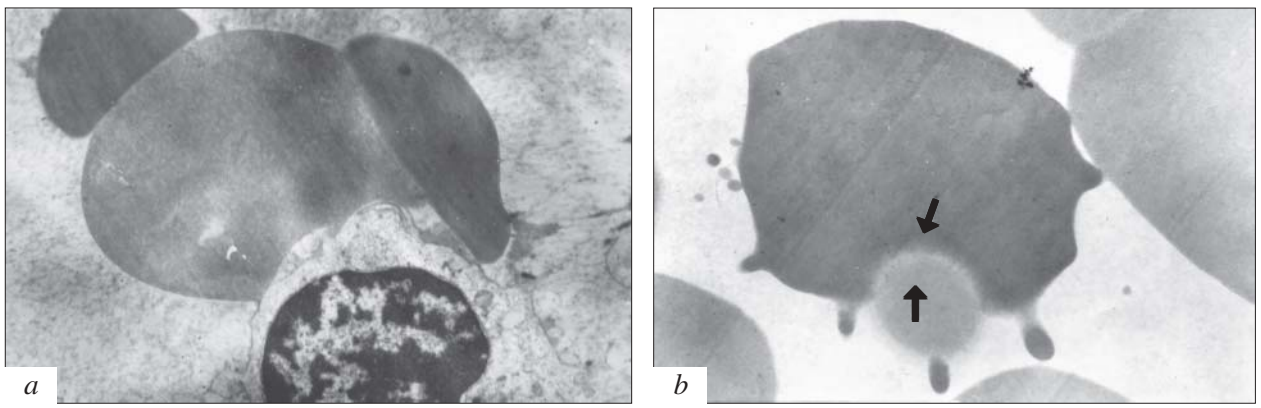

Fig. 2. Blood electronograms of a patient with meningeal tuberculosis, ×12,500. *a*) multiple adhesion of erythrocytes on platelets; *b*) clasmatosis.

TABLE 1. Content of Hemoglobin Isoforms, Lactate, Pyruvate, and Catalase Activity in Fibrocavernous Pulmonary and Meningeal Tuberculosis (*M*±*m*)

Parameter	Healthy volunteers	Tuberculosis form	
		fibro-cavernous	meningeal
Deoxyhemoglobin, %	47.8±4.4	48.2±3.4	59.5±5.28*+
Oxyhemoglobin, %	50.9±4.5	47.8±3.9	36.8±4.1
Methemoglobin, %	0.79±0.12	2.51±0.45	3.67±0.57*
Deformability, arb. units	0.95±0.13	0.73±0.12	0.61±0.12*
Catalase activity, H_2O_2/mg protein/min	12±3	27±4	7±2*+
Lactate, mmol/liter	1.2±0.2	2.6±0.4*	3.8±0.4*+
Pyruvate, μmol/liter	24±5	36±4*	12±4*+
Lactate/pyruvate	50±5	72±9*	317±25*+

Note. $p<0.05$ **vs.* healthy volunteers; *+vs.* patients with fibrocavernous tuberculosis.

$p<0.001$). Catalase activity decreased in comparison with the control, which attests to exhaustion of the adaptation potential of the erythrocyte antioxidant system, while sharp lactoacidosis and increased lactate/pyruvate ratio indicated reduced glycolytic activity of erythrocytes. Isoform composition of hemoglobin was shifted towards deoxy- and methemoglobin (Table 1).

In group 2 the number of nonadhesive erythrocytes in the microscopic field 1.53 times surpassed that in group 1 (Fig. 2). Platelets more often adhered to erythrocytes, lymphocytes and neutrophils being less adhesive. In meningeal tuberculosis platelet shape was degenerative and cells adhered to erythrocyte surface individually. The results indicate changed structure and functions of erythrocytes, intensification of disintegration processes in these cells, which was paralleled by their oxygen-transporting dysfunction and impairment of their elastic characteristics in chronic pulmonary tuberculosis. These phenomena were still more pronounced in meningeal tuberculosis.

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