Activity of Histidine in Peripheral Blood Erythrocytes of Pregnant Women during Exacerbation of Cytomegalovirus Infection
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We studied the effect of active cytomegalovirus infection on histidine content in peripheral blood erythrocytes of pregnant women at gestation weeks 20-22 and its involvement into hemoglobin oxygenation. Using the histochemical technique developed by us, we studied the distribution of products of specific reaction for histidine in peripheral blood erythrocytes of pregnant women. The percentage of histidine-positive erythrocytes and their area were evaluated. The relationship between the distribution of the products of the reaction for histidine in peripheral blood erythrocytes of pregnant women and the titer of anti-cytomegalovirus IgG was revealed. The histidine content in peripheral blood erythrocytes of pregnant women with active cytomegalovirus infection was reduced, which impaired heme binding to globin and decreased the formation of oxyhemoglobin.

Key Words: erythrocytes; histidine; oxyhemoglobin

Histidine is one of 20 proteinogenic amino acids taking part in the biosynthesis of histamine [2], growth and regeneration of tissues, and hemoglobin oxygenation in blood erythrocytes [4]. Upon binding oxygen, hemoglobin is transformed into oxyhemoglobin, but iron valency remains unchanged [6]. Hemoglobin is a tetrameric protein formed by four polypeptide chains presented by α,β-subunits; α-polypeptide chain ends with valine-leucine and β-polypeptide chain with valine-histidine-leucine [6]. The advantage of histidine over other amino acids is that it promotes fixation of heme in globin preserving the Bohr effect [3,4,7]. Stabilization of heme iron is provided by hydrogen bonds formed between the tertiary imidazole nitrogen of histidine and an oxygen molecule. Due to specific location of the surface imidazole groups in globin and specific orientation of hemes relative to the globin surface, the probability of formation of new bonds upon interaction with oxygen sharply increases. The formation of new bonds upon interaction with oxygen increases stability of the molecule and forces the heme plane to approach the protein surface, which manifests in modifications of the molecule macrostructure.

In abnormal globin chains resulting from substitution of histidine with other amino acids, heme iron remains in oxidized state and loses its capacity to bind oxygen [5,6]. The contribution of herpesviruses [2] and free radical oxidation products to disturbances in heme synthesis has been demonstrated, which led to irreversible oxidation of Fe^{2+} to Fe^{3+} with the formation of methemoglobin incapable of carrying oxygen.
and development of oxygen deficiency in pregnant women [1]. However, the influence of cytomegalovirus infection (CMVI) on the peripheral compartment of the erythron and oxygen metabolism was never studied.

Here we evaluate the effect of active CMVI on histidine levels in the peripheral blood erythrocytes of pregnant women on gestation weeks 20-22 and its involvement to hemoglobin oxygenation.

**MATERIALS AND METHODS**

We examined 75 pregnant women aged 18-25 years (gestation weeks 20-22). The main group included 55 pregnant women with chronic CMVI during exacerbation, the control group comprised 20 pregnant women without this pathology. CMVI was symptomatically manifested as an acute respiratory disease accompanied with rhinopharyngitis. Clinical diagnosis of CMVI exacerbation was made according the results of complex testing of the peripheral blood for CMV DNA, IgM or 4-fold and higher increase in IgG titers in paired serum samples within 10 days, as well as avidity index >65%. The study was carried out in accordance with the requirements of the Helsinki Declaration of the World Medical Association “Ethical Conduct for Research involving Human Subjects” amended in 2000 and the Rules of Clinical Practice in the Russian Federation approved by the Order No. 266 of the Ministry of Health of the Russian Federation from June 19, 2003.

Verification of CMV, measurement of type-specific antibodies and avidity index were carried out by ELISA on a Stat-Fax-2100 spectrophotometer using Vector-Best test systems. CMV DNA was detected by PCR on a DT-96 instrument using DNA-Technology kits. Oxygenated hemoglobin ($HbO_2$) was determined by the method of Evelyn–Malloy.

Histidine in peripheral blood erythrocytes was detected histochemically. The method implied incubation of whole blood erythrocytes with Pauli’s reagent consisting of equal volumes of solution 1 (1% sulfanilic acid in 1 M hydrochloric acid) and solution 2 (5% aqueous solution of sodium nitrite). Solutions 1 and 2 were kept in a refrigerator at 4°C before use. Equal volumes of solutions 1 and 2 were mixed (also cold) 5 min prior to use of the reagent. The blood was taken from the cubital vein in the morning on an empty stomach into standardized vacuum tubes PUTH with K3 EDTA. A 200-μl aliquot of whole blood and Pauli’s reagent (500 μl) were mixed in the test tube, incubated for 10 min at room temperature, and monolayer smears were prepared using a Diff-e Spin-2 centrifuge. The smears were dried and examined under MT digital microscope operated in immersion mode connected with Scion Corporation hardware-software system providing selection of individual cells in smear image and determining its area with identified grains of reaction product. For calculations, we used area of

Fig. 1. Reaction to histidine in peripheral blood erythrocytes of pregnant women with CMVI (gestation week 20-22), ×1000. a) Control; b) anti-CMV IgG titer 1:800; c) titer 1:1600.