

Morphofunctional Characteristics of Erythrocyte Membrane at the Exacerbation of Cytomegalovirus Infection during Pregnancy

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ABSTRACT

Objective: To study the indicators of cellular immunity at the exacerbation of Cytomegalovirus Infection (CMVI) in the third trimester of gestation and to reveal the violations of erythrocyte membrane protein structure on cytomegalovirus contact with erythrocyte membrane receptor – glycophorin at 28-30 weeks of gestation.

Methods: Pregnant women who had delivery at 37-38 weeks were included into the prospective case-control study. A total of 205 pregnant women were examined including 105 CMV-seropositive at the exacerbation of CMV-infection at 25-28 weeks of gestation with IgG antibody titer to CMV 1:1600 at the time of the study and 60 CMV-seronegative at the same stage of pregnancy. The control group consisted of 40 pregnant women. The study was conducted at the Obstetric Department of Pathology of Pregnancy and in the Laboratory of Mechanisms of Etiopathogenesis and Recovery Processes of Respiratory System during the period from 2012 to 2016. Protein spectrum content in erythrocyte membrane was determined by means of vertical disk-electrophoresis in gradient polyacrylamide gel (7.5-10%) with sodium dodecyl sulfate (0.1%) according to U. Laemmli method. Microviscosity of erythrocyte membranes was measured by lateral diffusion of hydrophobic fluorescent probe pyrene on spectrophotometer “Hitachi” (Japan). Erythrocyte membrane density was measured using digital camera Bio Vision

(USA). Deformability of peripheral blood erythrocytes was studied according to Lucenko M.T., Andrievskaya I.A. formula using cytophotometric device “Mecos” (Russia).

Results: CMVI exacerbation at 25-28 weeks of gestation results in forming of a large amount of circulatory immune complexes and macrophages with CMV antigens in peripheral blood. It leads to their contacts with erythrocyte membranes through protein-receptors glycoporphines. Following erythrocyte membrane fusion with CMV membrane tegument proteins gp64 penetrate erythrocyte membrane, violate protein band 3 bond with phospholipids, and create conditions for forming of a great concentration of fatty acids peroxides (**LPO**), suppressing antioxidant activity of glutathione peroxidase and of superoxide dismutase, and also decrease hemoglobin oxygenation processes in red blood cells that results in threat of hemic anemia in the third trimester of gestation.

Keywords: Cytomegalovirus; Pregnancy; Erythrocyte membrane proteins; Anemia

INTRODUCTION

The relevance of research as established by the WHO, Cytomegalovirus (**CMV**) is the widespread type of the herpes family virus infection frequently proceeding asymptotically what compels to distinguish the latent group among the patients suffering from Chronic Cytomegalovirus Infection (**CCMVI**).

CCMVI is the persisting infection that can deteriorate owing to the force of specific circumstances so far not entirely determined. First and foremost, the reduction of immunoprotective reactions of the body comes forward [1, 9,19,21,23]. But again, it is not aimed at the components of the immune status of the body.

One thing is clear for the present that cytomegalovirus infection more often aggravates during pregnancy and this phenomenon may be expected at any stage of gestation [25,26,35]. Cytomegalovirus infection reactivation is most aggressively manifested in the first trimester of pregnancy owing to suppression of synthesis of a series of placental hormones: progesterone, estriol hormones, as well as hormones like α -fetoprotein, lactogenous hormone, in which synthesis both the pregnant woman and the germ participate at the early stage of gestation.

Cytomegalovirus propagates very quickly and being carried by maternal blood reaches the placenta and penetrates into the developing fetus. In motion it involves the immune system of pregnant women into the pathological process and certainly gets into contact with a vast area of peripheral blood cellular elements - erythrocytes that contain on their membrane surface area protein receptors capable of binding cytomegalovirus infection antigens contained in large amounts in CIC (Circulatory Immune Complexes) and macrophages of peripheral blood. The need of studying damage mechanisms of peripheral blood red cells during the exacerbation of cytomegalovirus infection, that cause the problems of lack of oxygen delivery to the organ cells of the fetus developing at the most crucial period of organogenesis, becomes apparent.

MATERIALS AND METHODS

The present study is based on the results of combined research using clinical, biochemical, morphological, histochemical, and statistical methods.

The whole study was conducted with regard to the requirements of World Medical Association Declaration of Helsinki “Ethical Principles for Medical Research Involving Human Subjects” (amended 2008)

The study was approved by the Committee on Biomedical Ethics of Far Eastern Scientific Center of Physiology and Pathology of Respiration in accordance with Principles of Convention on Biomedicine and Human Rights and Universally Recognized Norms of International Law. The informed consent was obtained from all pregnant women.

A total of 205 pregnant women were examined in the period from 2012 to 2016. The main group was formed out of 105 pregnant women seropositive to CMV (mean age 24.5 years). The control group consisted of 60 pregnant women seronegative to CMV (mean age $22,6 \pm 0,7$ years). Control group included 40 pregnant women registered in maternity consultation.

The distribution to groups was undertaken with the aim to study morphofunctional alterations arising in peripheral blood erythrocytes in pregnant women with latent cytomegalovirus infection in the third trimester of gestation.

The exacerbation of latent cytomegalovirus infection was clinically diagnosed during the combined investigation of peripheral blood for IgM and four-fold or more increase of IgG antibody titer in paired sera in dynamics after 10 days, avidity index more than 65% and also for DNA-CMV detected by PCR in blood, urine, buccal epithelium scrapings, and in the neck of the womb mucosa.

EXTRACTION OF MONONUCLEAR CELLS

Mononuclear cells were obtained from venous blood taken out of ulnar vein by means of venipuncture at 8 am on an empty stomach in amount of 5,0ml into plastic test-tubes, containing EDTA as coagulant. The extraction of peripheral blood mononuclear cells was performed using fikoll - urograffin solution (density - 1,077 g/ml (NPO “DNA-technology”, Moscow) in accordance with the manufacturer’s instructions. Obtained mononuclear cells were kept at -20°C within a month time.

Molecular Biological Method (Real - Time PCR)

To perform the real-time PCR “Reagent kit for extraction of DNA” and “Reagent kit for PCR amplification of DNA-CMV and HSV- 1,2” (NPO “DNA-technology”, Moscow) were used according to the manufacturer’s instructions. The reagent mixture comprised of: amplification mixture sealed in paraffine (20 mkl), Tag-polymerase (10 mkl), mineral oil (10 mkl), sample of DNA preparation (5 mkl). The results count was performed on the device DT-96 (NPO “DNA-technology, Moscow) permitting to analyze samples of DNA in dynamic range from 1 to 109 with

simultaneous detection with four fluorescent dyes (FAM/SIBR Green, ROX, R6G, CY5) at the target program (at 95° C for 20 sec. , at 62° C for 40 sec.). Peripheral blood herpes bodies were determined by Lucenko M.T.- Solovyova A.S. method [8]. The way of assessment of erythrocyte membrane steadiness at the exacerbation of CMVI was determined on measuring calcium ions content in peripheral blood of pregnant women by histochemical method [10].

100 (95,4%) seropositive pregnant women gave birth to their babies in time, 5 (4,6%) had premature delivery. 2 women with the exacerbation of latent CMVI had premature delivery at 25 weeks of gestation.

Inclusion Criterion for the Main Group was

Permanent remission of CCMVI induced by Herpes Simplex Virus 1-2 (HSV-1,2).

Exclusion Criteria for the Patients were

The presence of genetic, endocrinologic factors of importance in aborting, isthmic-cervical incompetence, uterus pathology (developmental defects, uterus hypoplasia, endometrial synechias).

Primary infection with CMV, HSV-1,2.

Presence at the time of the survey of other inflammatory diseases of extragenital localization and sexually transmitted infections.

The informed consent for examination and treatment was obtained from each patient, analysis and examination data and obtained results were filled in a specially developed card.

Standard Clinical Methods of Patient's Examination

1. History taking.
2. Patient's complaints (features of clinical manifestation of CMVI exacerbation).
3. Objective examination (figure type, body mass index, state of mammary glands).
4. Special gynecologic examination (examination of external genital organs, evaluation of the state of vaginal mucosa , neck of the womb, presence of trophic alterations, eruptions and ulcerous lesion, quantity and quality of vaginal discharges.
5. Clinic and laboratory investigation (determination of blood group and of Rh-factor, clinical and biochemical blood count, blood count for HIV, antibodies to Trepanema pallidum, HBs-antibodies to HCV, coagulogram, general urinalysis, immune status and hormonal blood count, bacterioscopic and bacteriological investigation of vaginal and cervical canal discharge, PCR diagnosis of sexually transmitted infections for presence of DNA-HSV-1,2 and CMV, chlamydias, ureplasma and mycoplasma.
6. Ultrasonic examination.

7. Skilled specialist consultation (therapist, otolaryngologist, ophthalmologist, stomatologist, infectionist and others due to indications).

Serologic Tests

Blood samples for serologic tests were drawn out of ulnar vein by venipuncture at 8 am on an empty stomach in amount of 5,0 ml. The testing was conducted in paired sera with 10-14 days interval. Sera samples were kept at -20°C.

IMMUNOFERMENT ANALYSIS (IFA)

I. IFA was used to determine in blood serum:

typospecific antibodies to CMV and HSV-1,2 by means of “CMV-IgM-Strip”, “CMV-IgG-Strip”, HSV-IgM-Strip” and “ HSV-1,2-IgG-Strip” test kits (CJSC “Vector-Best”, Novosibirsk) according to protocols for IFA performing as designed by producer company;

IgG avidity index to CMV and HSV-1,2 using “VectoCMV-IgG-avidity” and “ VectoHSV-1,2-IgG-avidity” test kits (CJSC “Vector-Best”, Novosibirsk) according to protocols for IFA performing as designed by producer company;

General Immunoglobulins A,M and G, using “IgA-general-IFA-BEST”, “IgM-general-IFA-BEST” and “IgG-general-IFA-BEST” (CJSC “Vector-Best”, Novosibirsk) according to protocols for IFA performing as designed by producer company ;

Immune status using “Interleukin 1- β IFA-BEST”, “Interleukin 4 IFA-BEST”, “ alpha- TNF-IFA-BEST” kits (CJSC “Vector-Best”, Novosibirsk) according to protocols for IFA performing in blood serum as designed by producer company .

The underlying principle of above mentioned Test Systems is that antibodies specific to definite Igs, cytokines, are sorbed in plate wells. Binding with antibodies in plate wells occurs on adding of control blood serum samples followed by forming of enzyme complex. The named materials are removed by washing, the substrate solution is added interacting with enzyme complex followed by stain solution forming. IFA data registration was performed in microplate reader Stat-Fax -2100 (USA) at the wavelength 450 nm, comparison wavelength - 600 nm. Stain intensity measured at the given wavelength was directly proportional to the concentration of defined antibodies, immunoglobulins and cytokines.

II. To evaluate morphofunctional state of erythrocytes in latent phase and during the exacerbation of cytomegalovirus infection, blood samples drawn out of ulnar vein on an empty stomach were centrifugated at 1000g, 15 min. Erythrocyte membranes were obtained by modeling of hypoosmotic shock according to G. Dodge principle with the consequent spinning-down at 4000 g, +4°C, 10 minutes.

Protein spectrum of erythrocyte membrane was investigated using vertical one-dimensional disk-electrophoresis in gradient polyacrylamide gel (7.5-10%) with sodium dodecyl sulfate (0.1%) according to the modified method of U.Laemmli [18]. Polypeptide zones were stained in Kumassi's solution R-250 (0.1%), alcohol solution (50%) and acetic acid solution (7%). Electrophoregrams were identified with wave-length of 590-600 nm using 'BioDocAnalyze' device (Germany). Protein fragments orientation was evaluated according to Steck T. recommendations [48]. Markers used were: catalase (bovine) MW 240 kDa, aldolase (rabbit muscle) MW 240 kDa, MW 12,4 kDa ("Serwa"). Lipids were extracted by Folch method [35]. Prepared extracts were separated into individual phospholipide fractions on the plates with a thin-layer of silica gel (Woelm, Germany). Two-dimensional thin-layer chromatography and identification of individual phospholipide fractions were performed according to Kirchner Y [5].

III. Microviscosity of erythrocyte membranes was measured by method of lateral diffusion of hydrophobic fluorescent probe pyrene. Pyrene fluorescence was measured on spectrophotometer "Hitachi" (Japan). To determine microviscosity of lipide bilayer pyrene fluorescence intensity was found at the wave length of eximers 470 nm. To determine microviscosity of protein-lipide interactions - wave length of exciting is 286 nm, wave length of monomers – 395 nm.

IV. Erythrocyte membrane density was measured using digital camera "Pixera" according to Bio Vision (USA) program.

V. Deformability of peripheral blood erythrocytes during the exacerbation of cytomegalovirus infection and in latent phase was studied according to formula of Lucenko M.T., Andrievskaya I.A. [16].

VI. Different erythrocyte forms in peripheral blood were counted using automated cytophotometric device "Mekos C1" (registered certification MH RF 29/10010198/1282-01). Blood smears were prepared by means of centrifuge "StatSpin" (USA).

VII. After histochemical tests erythrocyte membrane was isolated according to cytophotometric program "Scion" (USA).

1. Ganglioside activity in erythrocyte membranes was evaluated by Bruckner method [6].
2. Restored form of glutathione in erythrocytes was estimated in accordance with Lucenko M.T., Andrievskaya I.A. method [12,14,15].
3. Histidine concentration in erythrocytes was determined by Lucenko M.T., Andrievskaya I.A. method [11].

VIII. Spectrophotometric reactions:

1. Oxyhemoglobin level – according to Evelini and Melloy [20].
2. 2,3 – DPG concentration was measured in accordance with Luganova I.S. and Blinova M.N. method [7].

3. TNF α level in blood serum was determined by means of "CYTOKIN Co. Ltd." kits (St.Petersburg).
4. Activity of restored glutathione in peripheral blood erythrocytes was estimated on the content of glutathione peroxidase and glutathione reductase using reagent kits of "Sentinel diagnostics" (Italy).
5. Superoxide dismutase activity was evaluated using "Randox Laboratories Ltd." kits (England).
6. Calcium concentration on erythroid membranes at various pH-values was determined according to Lucenko M.T., Andrievskaya I.A. [17].
7. Localization of H³ - labeled cholesterol in erythrocytes was investigated in accordance with Sarkisov D.S. and Perov Y.S method for suspension of single cells [22]. Incubation – 15 min. Monolayer smears of erythrocytes prepared in centrifuge "DiffSpin 2" were fixed in formal-calcium solution (Becker) for 10-12 hours, then dehydrated and immersed at 37°C into photoemulsion M. After emulsion drying slides were kept at room temperature for 5 days, developed, washed and studied using digital microscope (Japan).

STATISTICAL METHODS

Mathematical and statistical processing of obtained data was performed by means of Kolmogorov-Smirnov evaluation tests using various parametric and nonparametric statistical methods at equation significance less or equal to 5% ($p \leq 0,05$). Calculated were arithmetic mean (M) and standard error of arithmetic mean (m). Analysis of between-groups differences for independent samples on each of normally distributed features was performed via parametric criteria: Student's t-test and Fisher's Exact test (F-test) for normally distributed, and via nonparametric criteria: by Mann-Whitney test (U-test). Analysis of within-group interrelation of qualitative features was performed by Pirson's correlation coefficient (for normally distributed values with the construction of Gauss curve). Linear correlation coefficient was estimated as follows: $r=1$ – very high dependence; $r > 0,7$ – good dependence; $r > 0,4 - 0,7$ – moderate dependence; $r > 0,5-0,4$ – low dependence; $r > 0,05$ – no dependence. Differences in all cases were considered statistically significant at $p < 0,05$.

Authenticity of obtained data was studied by means of [24]:

1. Multiple linear regression model;
2. Correlation analysis;
3. Multiple discriminant analysis;

Variables comparison by Student's t-test.

FEATURES OF CELLULAR LINK OF IMMUNITY IN PREGNANT WOMEN AT THE EXACERBATION OF CYTOMEGALOVIRUS INFECTION IN THE THIRD TRIMESTER OF GESTATION

At the exacerbation of the latent phase of cytomegalovirus infection in pregnant women in the third trimester of gestation high concentrations of proinflammatory cytokines of Th1-type were found : thrice-fold increase in IL-1 β ,2,5-fold increase in IFN γ and 15-fold increase in TNF α against the group of the pregnant women with latent cytomegalovirus infection at 22-23 weeks of gestation.

The integral estimation of intersystem interrelations in values of cytokine status in seropositive pregnant women has revealed the high correlation dependence between TNF α and IL-4 ($r=0,79$; $p< 0,001$), medium – between IFN γ and IL-4 ($r=0,66$; $p< 0,05$), as well as between IL-1 β and IL-4 ($r=0,69$; $p< 0,05$), which is indicative of disbalance in production of proinflammatory cytokines during the exacerbation of cytomegalovirus infection. It has been confirmed when calculating the ratio between the interdependent values TNF α -IL-4; IFN γ -IL-4; IL-1 β - IL-4. The ratio IFN γ /IL-4 – $22,5 \pm 0,35$, in control group – $4,7 \pm 0,25$, the ratio IL-1 β /IL-4 – $15,3 \pm 1,3$, in control group – $1,6 \pm 0,65$, that is 2,44 times more compared to the control group ($p< 0,001$).

The changes revealed in the intersystem interrelations structure can be estimated as follows.

The high concentration of proinflammatory cytokines in peripheral blood of seropositive pregnant women at the exacerbation of cytomegalovirus infection in the third trimester of gestation was indicative of the violation of locality principle of cytokine system and was due to the general activation of immune system cells what has manifested in the accumulation of great amount of macrophages containing causative viruses of cytomegalovirus infection in peripheral blood (figure 1) and also in building up of the bodies with the products of active replication of viral elements.

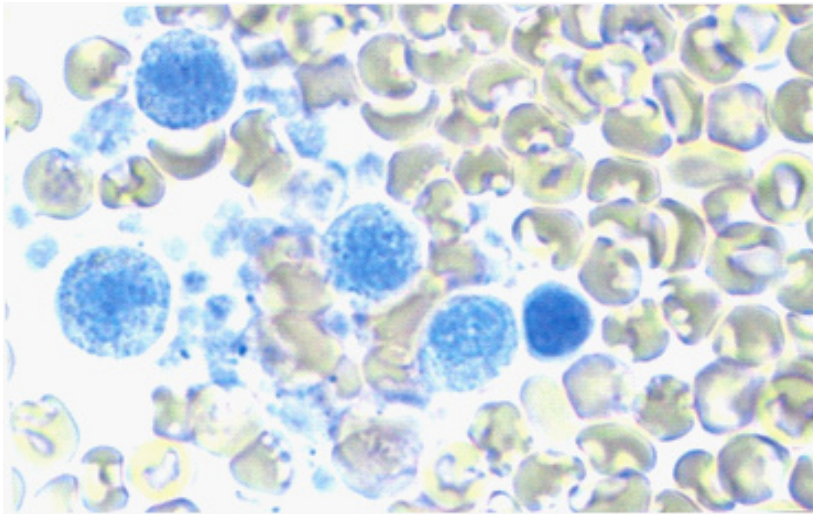


Figure 1: Peripheral blood obtained from a pregnant woman in acute phase of cytomegalovirus infection in the third trimester of gestation. There is a great amount of macrophages containing elements of the causative agent of cytomegalovirus infection: a- normal lymphocyte; b - macrophages saturated with the cytomegalovirus pathogene. Peripheral blood smears staining with methylviolet. Magnification 10 x 100.

During the exacerbation of cytomegalovirus infection in the third trimester of gestation and the amplification of proinflammatory processes the peripheral blood of a pregnant woman contains a great amount of causative viruses of cytomegalovirus infection. Simultaneously, in acute latent course of cytomegalovirus infection in seropositive pregnant women at 22-23 weeks of gestation the decrease of Th-2 cellular stimulation and of regulatory cytokines production, including IL-4, is observed, resulting in violation of interrelations between T and B-cells.

The high reverse correlation dependence between IL-4 and IgM ($r=-80$; $p< 0,01$) and direct dependence between IL-4 and IgG ($r=0,90$; $p< 0,01$) was estimated in seropositive pregnant women at 26-27 weeks of gestation after exacerbation of cytomegalovirus infection. Thus, at the exacerbation of cytomegalovirus infection in the third trimester of gestation IgM level decreases, IgG concentration increases and the deficiency of IL-4, produced by Th-2 lymphocytes, occurs.

Due to accumulation of cytomegalovirus containing macrophages, ganglioside production in cellular membranes is stimulated promoting the unification of macrophages followed by forming of complexes out of the cells containing causative virus (figure 2). This phenomenon can be detected by means of devised in our laboratory method permitting to detect these bodies while keeping to the neutral medium of incubation solution.

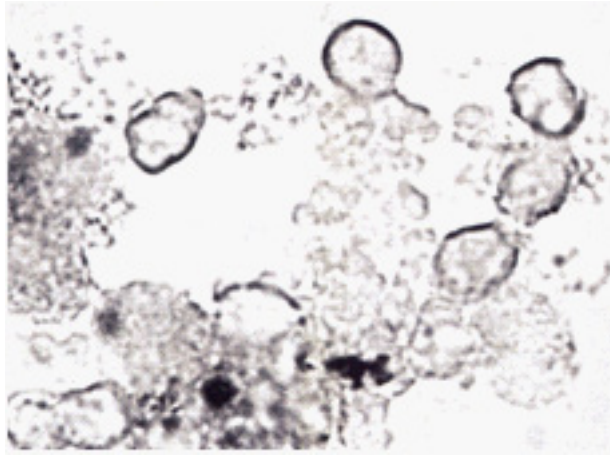


Figure 2: Peripheral blood obtained from a pregnant woman during the exacerbation of cytomegalovirus infection in the third trimester of gestation. Peripheral blood macrophages contain a large amount of gangliosides. Histochemical staining for gangliosides by Bruckner method. Magnification 10 x 100.

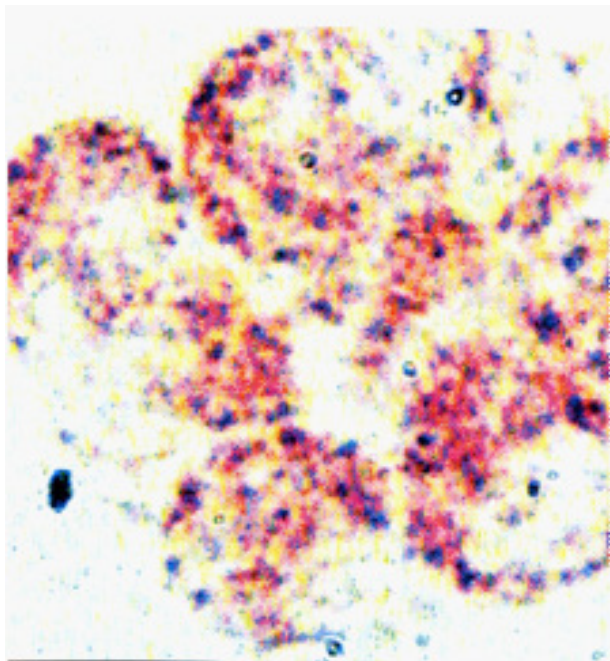


Figure 3: Peripheral blood obtained from a pregnant woman in acute phase of cytomegalovirus infection in the third trimester of gestation. Formation of complex herpetic bodies. Gangliosides permitting macrophages approach each other are produced on macrophage surface. Complex histochemical test by Lucenko M.T, Solovyova A.S. method [8]. Gangliosides are detected by Bruckner method. Staining for herpetic body's with neutral red. Magnification 10 x 100.

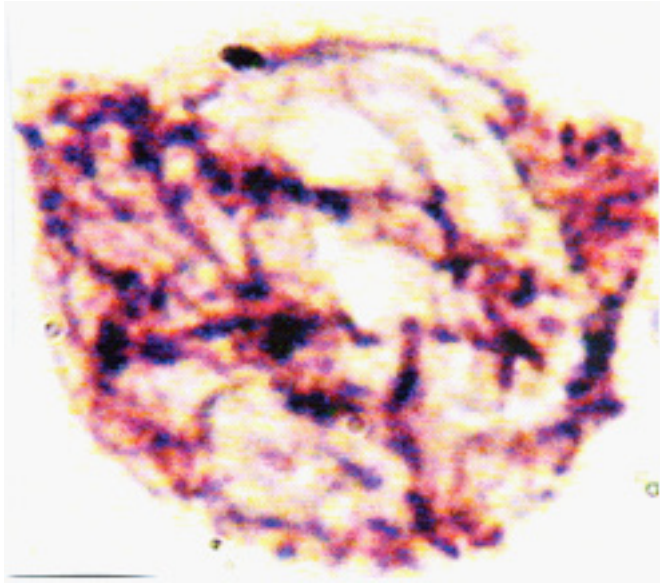


Figure 4: Peripheral blood obtained from a pregnant woman in acute phase of cytomegalovirus infection in the third trimester of gestation. Herpetic bodies are formed. Staining for gangliosides by Bruckner method, herpetic bodies were detected with neutral-red. Complex histochemical test by Lucenko M.T and Solovyova A.S. method [8]. Magnification 15 x 100.

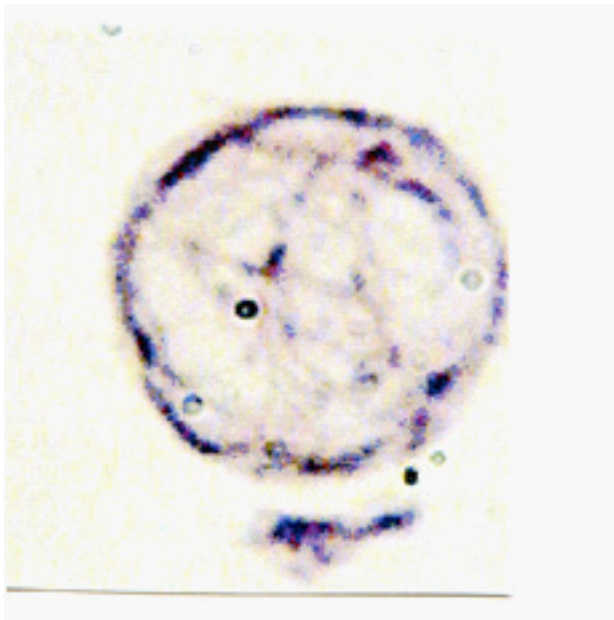


Figure 5: Herpetic body in peripheral blood at the exacerbation of cytomegalovirus infection [8] Magnification 10 x 100.

Accumulation in peripheral blood of great amount of causative agent results in a rapid development of virus contact with the membrane of erythrocytes making up in peripheral blood the vast area for contiguity and containing protein-receptor glycoporphin with high concentrations of sialic acids.

MORPHOFUNCTIONAL STATUS OF ERYTHROCYTE MEMBRANES AT THE EXACERBATION OF CYTOMEGALOVIRUS INFECTION DURING PREGNANCY

The structural status of erythrocyte membranes and its effect on hemoglobin oxygenation at 25-28 weeks of gestation have been analyzed to study the morphofunctional status of erythrocytes.

At an exacerbation of the latent course of cytomegalovirus infection in the third trimester of gestation it was observed that antibody titer to cytomegalovirus increased to 1:1600, a great amount of macrophages (figure 1) and complex herpetic bodies became apparent (figures 2-5). On gel electrograms glucophorin concentration in erythrocyte membranes increased to $10,09 \pm 0,02\%$ (latent stage – $7,73 \pm 0,31\%$; $p < 0,01$). It was indicative of intensification in peripheral blood of cytomegalovirus antigene getting into contact with the receptor of erythrocyte membranes.

Having analyzed the activity of CIC, $TNF\alpha$, IgM and LPO at the peak of cytomegalovirus infection exacerbation, when antibody titer to cytomegalovirus made up 1:1600, the high correlation dependence was revealed between CIC and glycoporphin ($r=81$; $p < 0,001$), medium dependence occurred between $TNF\alpha$ and glycoporphine ($r=65$; $p < 0,05$).

On defining the multiple linear regression we are convinced that CIC in the first instance become apparent when getting into contact with erythrocyte membrane glycoporphin.

In addition, the normality of glycoporphin series and CIC has been estimated both at the latent stage and during the exacerbation of CMVI.



Figure 6: Peripheral blood erythrocytes obtained from a pregnant woman in the third trimester of gestation at the exacerbation of cytomegalovirus infection. A great amount of gangliosides becomes apparent in erythrocyte membrane stained for haemoglobin by Lepene method and for gangliosides – by Bruckner method. Staining for haemoglobin - by Lepene method, for gangliosides - by Bruckner method. Magnification 15 x 100.

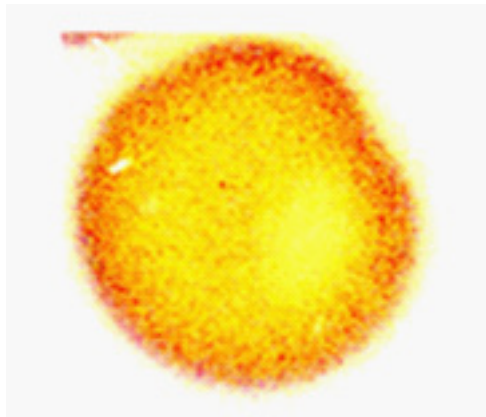


Figure 7: Peripheral blood erythrocytes obtained from a pregnant woman in the third trimester of gestation. A pregnant woman without a history throughout the gestation. Gangliosides in erythrocyte membranes are not found. Erythrocyte staining for haemoglobin – by Lepene method. Staining for gangliosides is negative. Magnification 15 x 100.

Proceeding from the calculation of the multiple linear regression CIC are the main factor getting into contact with the membrane receptor glycopherin. It has been confirmed that macrophages containing a large number of $TNF\alpha$ and cytomegaloviral proteins are the main damage factors for the erythrocyte membranes. Ganglioside synthesis in erythrocyte membranes amplifies (figure 6,7) alleviating their link to the surface area of cytomegalovirus.

Cytomegalovirus invasion into the erythrocyte membrane violates its structure resulting in a series of conformational alterations in membrane proteins and in their link to each other.

Most distinctively expressed is the increase of fatty acids peroxides concentration up to $80,35 \pm 1,9$ pixels/mm² (in control – $25,2 \pm 0,8$ pixels/mm²) revealed by means of cytophotometric analysis in erythrocyte membranes according to “Scion” program. The violation of correlation with the membrane phospholipide complex results in decrease of phosphatidyl ethanolamine level to $20,03 \pm 0,35\%$ (in latent period – $23,95 \pm 0,75\%$; $p < 0,05$; in control – $24,25 \pm 0,41\%$ compared with the acute period – $p < 0,01$).

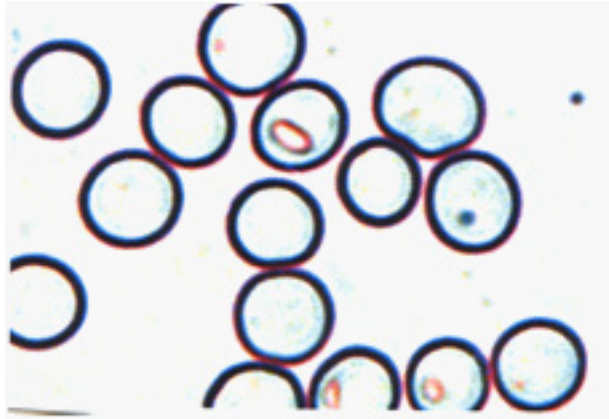


Figure 8: Peripheral blood erythrocytes of a pregnant woman in the third trimester of gestation at the exacerbation of cytomegalovirus infection, antibody titer to Cytomegalovirus 1:1600. Increase of fatty acids peroxides concentration in erythrocyte membranes up to $80,35 \pm 1,9$ pixels/mm². Winkler-Schultze test for fatty acids peroxides. Magnification 10 x 100.



Figure 9: Peripheral blood erythrocytes obtained from a pregnant woman not being sick throughout the gestation. Cytomegalovirus infection in latent phase. Fatty acids peroxides concentration in erythrocyte membranes – $25,2 \pm 0,8$ pixels/mm². Magnification 10 x 90.

Notwithstanding the accumulation of phosphatidylcholine in erythrocyte membranes at the exacerbation of cytomegalovirus infection to $29,00 \pm 0,35\%$ against the control values - $26,43 \pm 0,37\%$ ($p < 0,05$) it practically has not altered at the latent stage - $27,05 \pm 0,40\%$; compared with the acute phase its authenticity equals $p < 0,05$.

During the exacerbation of cytomegalovirus infection phosphatidylinositol concentration in erythrocyte membranes slightly increases up to $9,82$ against the indices in the latent period ($8,40 \pm 0,04\%$, $p < 0,05$). Difference between the control values and the level of phosphatidylinositol in erythrocyte membrane in latent phase is not found - $8,80 \pm 0,07\%$. Phosphatidylserine content in erythrocyte membranes remains identical both in control - $9,92 \pm 0,3$, in latent period - $9,90 \pm 0,5\%$, and during the exacerbation of Cytomegalovirus infection in the third trimester of gestation - $9,71 \pm 0,60\%$.

Distinct alterations take place in erythrocyte membranes with regard to lysophosphatidylcholine concentration. Its level during the exacerbation of cytomegalovirus infection in the third trimester of gestation significantly increases up to $8,00 \pm 0,4\%$ compared with the latent stage - $5,20 \pm 0,27\%$ ($p < 0,001$) and the control - $5,00 \pm 0,45\%$ ($p < 0,001$). Alteration of the ratio between phosphatidylethanolamine and phosphatidylcholine content results in decrease of unsaturated fatty acids concentration in erythrocyte membranes to $21,00 \pm 0,4$ pixels/mm² (latent phase - $62,0 \pm 1,3$ pixels/mm²; control - $61,90 \pm 2,8$ pixels/mm²; $p < 0,001$) (fig.10) which becomes a cause of erythrocyte membrane microviscosity violation. In the lipid bilayer - to $0,60 \pm 0,005$ Fe / Fm (latent phase - $0,84 \pm 0,008$ F / F; $p < 0,005$; control - $0,85 \pm 0,006$ Fe / Fm; $p < 0,001$) and in the zone of lipid-protein interactions - to $0,79 \pm 0,04$ Fe / Fm (latent phase - $1,15 \pm 0,008$ F / F; control - $1,09 \pm 0,004$ Fe / Fm; $p < 0,001$). It is manifested as erythrocyte membrane fluidity (figure 11). Simultaneously, reaction for SH-groups in erythrocyte membranes reduces to $15,10 \pm 0,75$ pixels/mm² (figure 12).

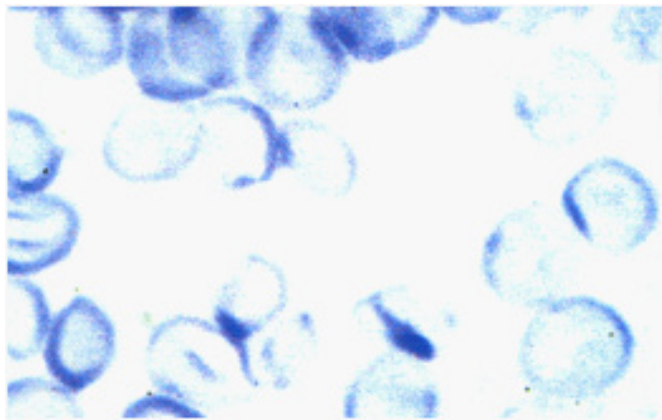


Figure 10: Peripheral blood obtained from a pregnant woman during the exacerbation of cytomegalovirus infection in the third trimester of gestation. Concentration of unsaturated fatty acids in erythrocyte membranes is about $21,00 \pm 0,4$ pixels/mm². Histochemical test for unsaturated fatty acids by R. Lillie method. Magnification 10×100 .

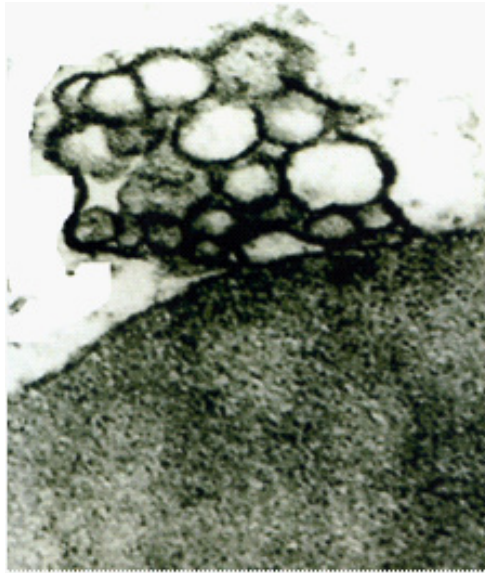


Figure 11: Peripheral blood erythrocyte of a pregnant woman in the third trimester of gestation during the exacerbation of cytomegalovirus infection. Lysophosphatidylcholine concentration in erythrocyte membranes increases up to $6,2 \pm 0,08\%$, erythrocyte membrane becomes “fluid”. Electronic microscopy. Magnification 18000x.



Figure 12: Peripheral blood erythrocytes of a pregnant woman during the exacerbation of cytomegalovirus infection in the third trimester of gestation with antibody titer to cytomegalovirus 1:1600. Test for SH-groups in erythrocyte membranes by Barnett and Seligman’s method. SH-groups content reduces to $15,10 \pm 0,75$ pixels/mm². Magnification -10 x 100.

The contact of circulatory immune complexes and $\text{TNF}\alpha$ with erythrocyte membrane is followed by the launching of chain reaction involving the entire number of protein ingredients of red blood cell membrane.

In our experiments we observed [4] that at the exacerbation of cytomegalovirus infection in the third trimester of gestation with antibody titer to cytomegalovirus 1:1600 histidine concentration in red blood cells in the area of $13,8\pm 1,2 \text{ mmk}^2$ had reduced to $19,5\pm 1,20 \text{ pixels/mmk}^2$ (latent phase – $62,17\pm 2,50 \text{ pixels/mmk}^2$) ($p < 0,001$) (figure 11,12). Against this background ankyrine content in red blood cell is observed to decrease to $2,32\pm 0,02\%$ (latent stage – $4,16\pm 0,08\%$) (figure 13,14), and protein band 4.1 reduces to $3,28\pm 0,05\%$ (latent phase – $5,10\pm 0,07\%$). Of special interest is protein band 3, which previously has been considered to be an erythrocyte membrane structure regulating the transport function of Na^+/K^+ canals and associated specific ATP-ases only.

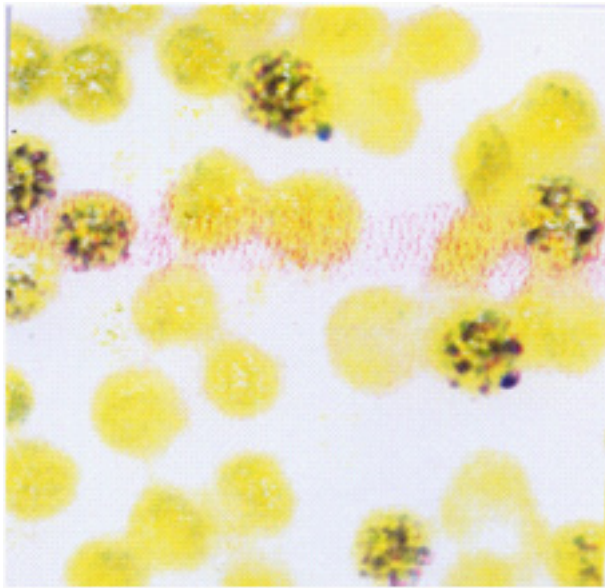


Figure 13: Histidine activity in peripheral blood erythrocytes of a pregnant woman in the third trimester of gestation without any disease throughout the gestation. Test by Lucenko M.T., Andrievskaya I.A. method [13]. Magnification 10 x 100.

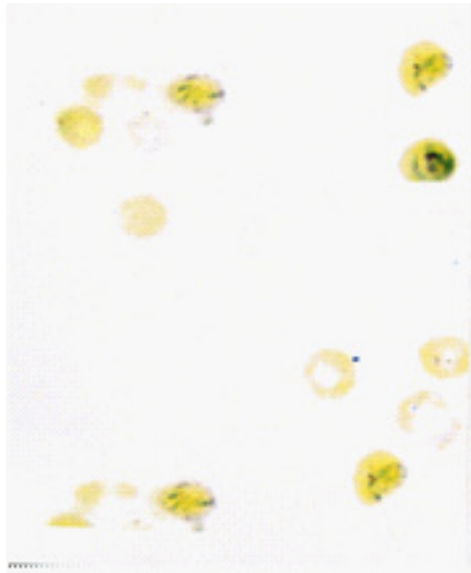


Figure 14: Histidine activity in peripheral blood erythrocytes in the third trimester of gestation at the exacerbation of cytomegalovirus infection with antibody titer to cytomegalovirus 1:1600. Histochemical test for histidine in integral erythrocytes - by Lucenko M.T. - Andrievskaya I.A. method [13]. Reaction intensity - by “Scion” program- $19,5 \pm 1,20$ pixels/mm² (latent phase $-62,17 \pm 2,5$ pixels/mm²). Magnification – 10 x 100.

During the exacerbation of cytomegalovirus infection circulatory immune complexes in the first instance come in contact with erythrocyte membrane. At the exacerbation of cytomegalovirus infection after cytomegalovirus contact with erythrocyte membrane the latter including ankyrin also becomes damaged by TNF α .

During the process of exacerbation of cytomegalovirus infection the decrease of protein band 3 content down to $15,75 \pm 1,2\%$ (latent phase – $17,8 \pm 0,78\%$; control – $18,72 \pm 0,25\%$; $p < 0,001$) is observed. Simultaneously, in the biconcave hollow of erythrocyte the amount of H3- labeled cholesterol decreases what enables to assume close connection of protein band 3 with cholesterol (figure 15). This site can be regarded as the centre spreading protein threads (spectrin α and β mainly) all over the inner surface area of erythrocyte membrane (figures 16,17). The suppression of protein band 4,1 , 3 and ankyrine synthesis at the exacerbation of cytomegalovirus infection also decreases the content of basic proteins in erythrocyte skeleton. The level of spectrin – α decreases during the exacerbation of cytomegalovirus infection down to $7,22 \pm 0,09\%$ (latent period – $7,95 \pm 0,08\%$; control – $8,34 \pm 0,104\%$; $p < 0,05$), and that of spectrin – β down to $7,32 \pm 0,06\%$ (latent phase – $9,0 \pm 0,02\%$; control – $8,9 \pm 0,11\%$; $p < 0,05$). Conformational cytoskeleton system is determined by force of energetic processes in erythrocyte membrane owing to protein actin, which content during the exacerbation of cytomegalovirus infection reduces to $7,31 \pm 0,08\%$ (latent phase – $11,50 \pm 0,3\%$; control – $11,0 \pm 0,33\%$; $p < 0,05$).

Exacerbation of cytomegalovirus infection in the third trimester of gestation suppresses protein band 4,9 synthesis to $5,49 \pm 0,069$ % compared to the latent period – $8,23 \pm 0,25$ %; $p < 0,01$ and to the control – $8,7 \pm 0,25$ %; $p < 0,01$ what threatens to form erythroid series in an organism of a pregnant woman by lowering erythropoietin production activity to $5,49 \pm 0,09$ mE/ml; control – $31,60 \pm 0,73$ mE/ml; $p < 0,001$; latent phase – $30,10 \pm 2,1$ mE/ml; $p < 0,001$.

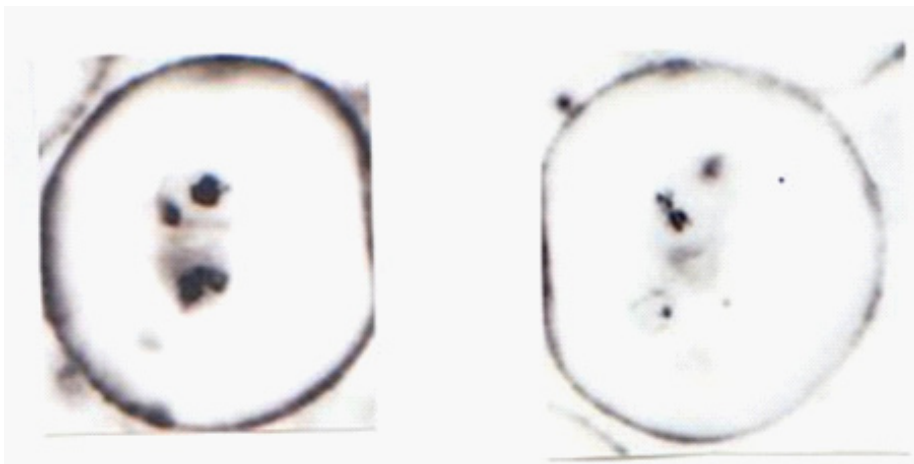


Figure 15: Erythrocyte incubated in the solution containing H3-labeled cholesterol. In the normal state cholesterol is evenly distributed on the erythrocyte lateral surface area and in the hollow depth (a). At the infectious process cholesterol level decreases both in the centre and on the lateral surface area (b). The method of autoradiography enabling to determine H3- labeled cholesterol content in erythrocyte. Magnification 15 x 100.



Figure 16: Erythrocyte inner surface area. Close-up of erythrocyte protein skeleton structure. Scanning electron microscopy. Magnification - 22000x.

The investigations have demonstrated that protein band 4.9 distinctively correlates with erythropoietin ($r = 7,8$; $p < 0,001$). In seropositive pregnant women in the third trimester of gestation protein band 4.9 content in erythrocyte membranes decreases to $5,49 \pm 0,09\%$ (latent stage – $8,23 \pm 0,25\%$; control – $8,7 \pm 0,25\%$; $p < 0,01$). The obtained figures confirm that protein band 4.9 in erythrocyte membranes is closely related to erythropoietin and according to the principle of reverse bond is the regulator of erythropoiesis in an organism.

The alteration of protein structure of erythrocyte membranes and of erythrocyte skeleton sharply changes the protein – lipide interrelation in it on the whole. Conformational alterations in erythrocyte membrane protein elements acutely make it thick. On using Bio Vision method enabling to distinguish between the sites of tissue structures of greater density and those of lesser density according to their composition we observed that with erythrocyte protein stroma alteration at the invasion of cytomegalovirus into the membrane the latter becomes more thick. While in a normal state erythrocyte membrane is characterized by isolation of integral proteins only (figure 17), then during the protein composition disintegration at the cytomegalovirus invasion into the erythrocyte and the violation of microviscosity sharp thickening of the membrane and erythrocyte shape alteration are noticed (figures 18,19). Some outgrowths, inside of which proteins spectrines predominate, arise out of the membrane and proteins band 3 are disposed on their surface area (figures 20,21,22). At the high antibody titer to cytomegalovirus (1: 1600) in peripheral blood there is not only the increase of echinocytes concentration (table 1), but also there appear up to 12-15% of degenerative forms with greatly condensed membrane (figure19).

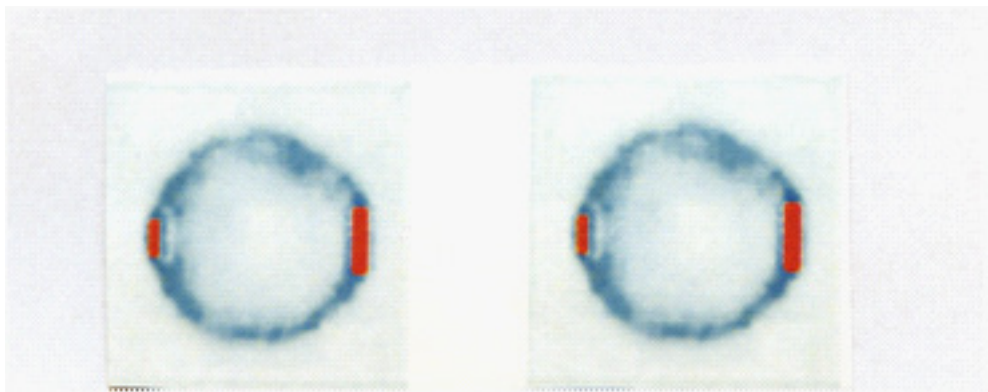


Figure 17: Peripheral blood discocytes of a pregnant woman in the third trimester of gestation without a history during the entire the gestation. There are solitary sites in the membrane visualized by treatment according to Bio Vision program – condensed ranges - 65 pixels.

Quantity– 15 pixels/mm². Magnification 10 x 100.

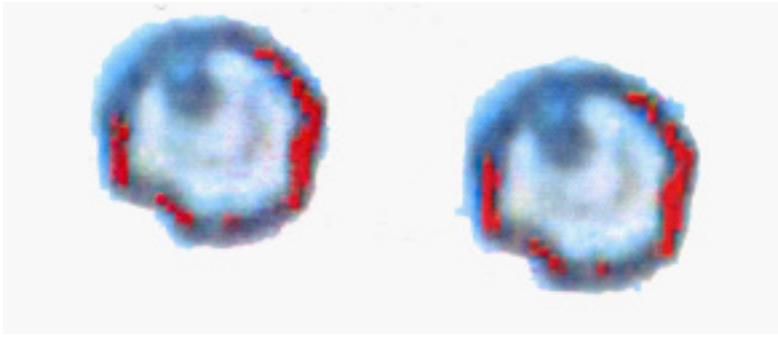


Figure 18: Peripheral blood erythrocytes of a pregnant woman in the third trimester of gestation. Echinocytes series. According to Bio Vision program up to $52 \pm 1,2$ pixels of condensed sites are visualized. Magnification 10×100 .

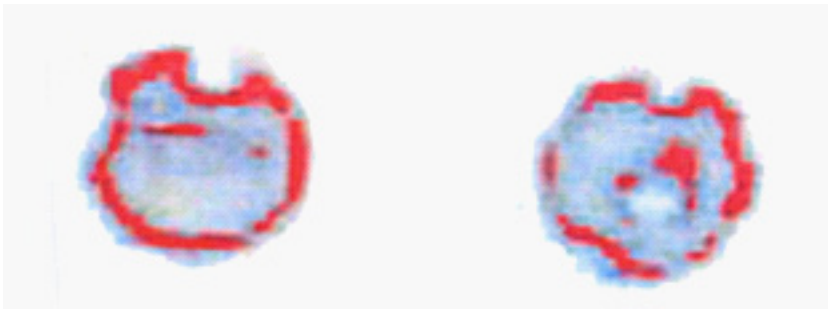


Figure 19: Erythrocytes series in peripheral blood of a pregnant woman in the third trimester of gestation during the exacerbation of cytomegalovirus infection up to antibody titer to cytomegalovirus 1:1600. Erythrocyte membrane is highly thickened. Up to 120 pixels of thickened membrane sites are counted. These erythrocytes are attributed to degenerative ones. Magnification 10×100 .

Table 1: Count of variously shaped erythrocytes in healthy pregnant women and in patients at the exacerbation of cytomegalovirus infection in the third trimester of gestation by means of automated cytophotometric device "Mekos".

Erythrocyte types studied according to Bio Vision program at the identical range – 65 pixels	Control	Latent stage	The third trimester of gestation , exacerbation of cytomegalovirus infection up to antibody titer to cytomegalovirus 1: 1600
discocytes	$9,3 \pm 1,3'$	$9,2 \pm 2,1^\circ$	$79,0 \pm 1,7$
echinocytes	$2,5 \pm 0,3'$	$3,0 \pm 0,2^\circ$	$8,0 \pm 0,07$
degenerates	$4,5 \pm 0,05'$	$5,0 \pm 0,4^\circ$	$13,0 \pm 0,09$

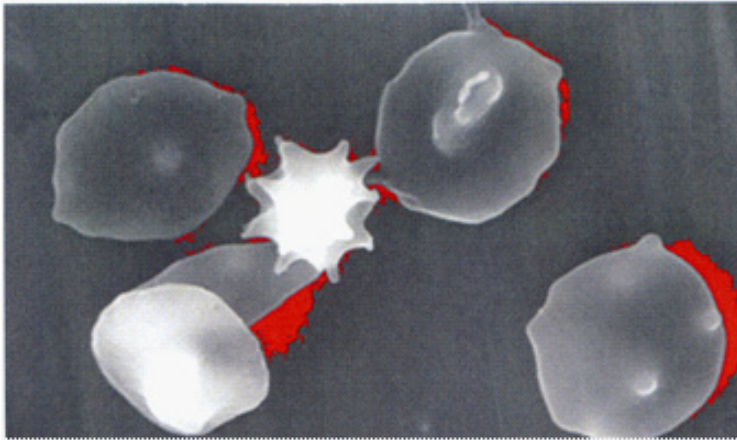


Figure 20: Peripheral blood of a pregnant woman in the third trimester of gestation during the exacerbation of cytomegalovirus infection up to antibody titer to cytomegalovirus 1:1600. A smear section with distinctively visualized various stages of echinocytes forming. Scanning electron microscopy. Magnification 5000x.

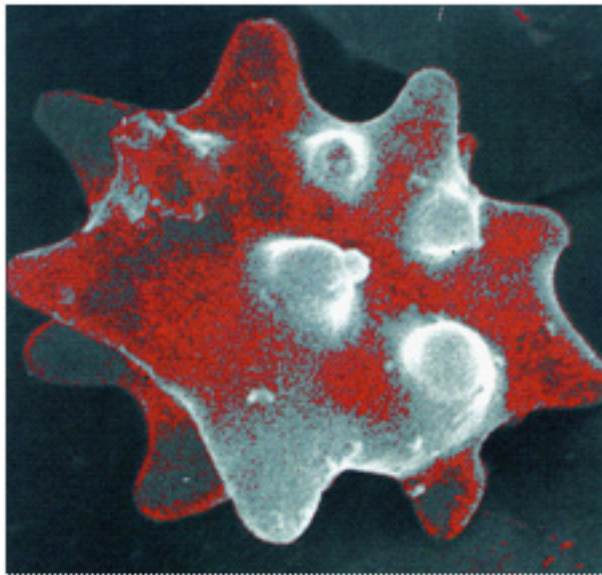


Figure 21: Peripheral blood of a pregnant woman in the third trimester of gestation at the exacerbation of cytomegalovirus infection to antibody titer to cytomegalovirus 1:1600. Echinocytes outgrowths become large size and off the surface area are covered with protein band 3. At the sites of just starting forming outgrowths the amount of protein band 3 is just starting to increase. Scanning electron microscopy accompanied by studying using Bio Vision program. Magnification 10000x.

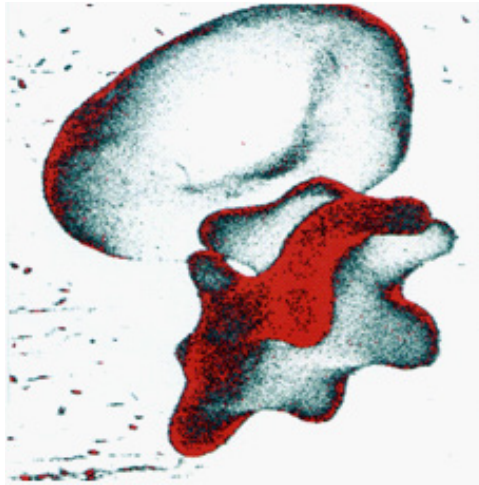


Figure 22: Peripheral blood of a pregnant woman in the third trimester of gestation during the exacerbation of cytomegalovirus infection to antibody titer to cytomegalovirus 1:1600. In the figure the discocyte structure (a) is compared to that of echinocyte (b). In case of discocyte only the lateral surface area of erythrocyte (torus) is covered with protein band 3. Scanning electron microscopy followed by studying using Bio Vision program. Magnification 10000x.

Metabolic processes violation in erythrocytes during the exacerbation of cytomegalovirus infection.

Expressed morphofunctional alterations in protein structure of erythrocyte membranes during the exacerbation of cytomegalovirus infection induce the violation of enzymatic processes in erythrocytes, of hemoglobin oxygenation and of gas transport function.

First and foremost, note should be taken that the violation of erythrocyte cytoskeleton elastic features at the exacerbation of cytomegalovirus infection leads to appearance in peripheral blood of great amount of degenerative forms and echinocytes (table 1) sharply decreasing erythrocyte deformability. The penetration of such erythrocytes forms through the narrow capillaries in diameter of about 4-5 m km becomes extremely embarrassing. We have developed the formula to determine erythrocyte deformability in peripheral blood (table 2).

Table 2:

$$ID = \frac{NS}{NV}$$

Fully expanded formula can be expressed as follows:

$$ID = \frac{NS}{Nm(Dcp)^3 \times (1 + 3k^2)}$$

- ID – deformability index,
- N – erythrocytes count under study,
- S – one erythrocyte average square in mkm,
- V – erythrocyte volume,
- m – standard deviation,
- Dcp – erythrocyte average diameter,
- k – erythrocyte diameter coefficient of variation.

The formula is developed by Lucenko M.T., Andrievskaya I.A. [16].

The investigations of erythrocyte deformability during the exacerbation of cytomegalovirus infection in the third trimester of gestation have demonstrated that erythrocyte deformability index makes up $0,132 \pm 0,002$ conventional units, in latent period – $0,88 \pm 0,004$ conventional units ($p < 0,001$). In the control group patients – $0,07 \pm 0,003$ conventional units.

On cytomegalovirus contact with erythrocyte membrane and violation of protein stroma bond with phospholipids a great amount of fatty acids peroxides arise in erythrocyte, the concentration of which, as mentioned above, increases up to $80,35 \pm 1,9$ pixels/mm² that is much more than in latent phase – $25,2 \pm 0,8$ pixels/mm² ($p < 0,001$). In the control group the level of peroxides is slightly less than during the exacerbation of cytomegalovirus infection – $15,5 \pm 0,7$ pixels/mm² what enables to assume that LPO is one of the main factors having a damaging effect on erythrocyte membrane proteins at the cytomegalovirus infection.

The investigations have shown that upon the exacerbation of cytomegalovirus infection in the third trimester of gestation in erythrocytes of seropositive pregnant women the content of restored glutathione acutely decreases down to $18,0 \pm 0,35$ pixels/mm²; latent stage – $49,5 \pm 0,95$ pixels/mm²; ($p < 0,001$), and in the control group – $50,8 \pm 0,45$ pixels/mm² ($p < 0,001$) (figure 23 a,b,c).

The process of glutathione restoration depends on glutathione reductase, which level in erythrocytes at the exacerbation of cytomegalovirus infection in the third trimester of gestation reduces to $4,20 \pm 0,18$ units/rHb, in latent period glutathione reductase level remains high – $8,29 \pm 0,15$ units/rHb ($p < 0,001$) and is little different from its content in the control group – $8,15 \pm 0,12$ units/rHb ($p < 0,001$). The decline of restored glutathione level violates the whole glutathione cycle. Its forming is decreased due to lack of H⁺ and glutathione peroxidase, which is the damaging factor for H₂O₂ in erythrocytes ($2\text{GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{glutathione peroxidase}} 2\text{GSSG} + 2\text{H}_2\text{O}$). H₂O₂ removing out of the erythrocytes is extremely important for it is able to damage compound elements of erythrocyte including those ones of membrane. This process will manifest because of OH⁺ forming out of H₂O₂, which exhibits high toxicity for the structural elements of the cells.

The exacerbation of cytomegalovirus infection not only suppresses glutathione reductase activity but also reduces the glutathione peroxidase content in erythrocytes. At the exacerbation of cytomegalovirus infection in the third trimester of gestation glutathione peroxidase level in erythrocytes of seropositive pregnant women decreases to $5,90 \pm 0,16$ units/rHb compared to the latent stage of gestation – $13,95 \pm 0,55$ units/rHb ($p < 0,001$). In the control group glutathione peroxidase content in erythrocytes in the third trimester of gestation is practically equal to this enzyme content in healthy pregnant women – $14,30 \pm 0,4$ units/rHb9 ($p < 0,001$).

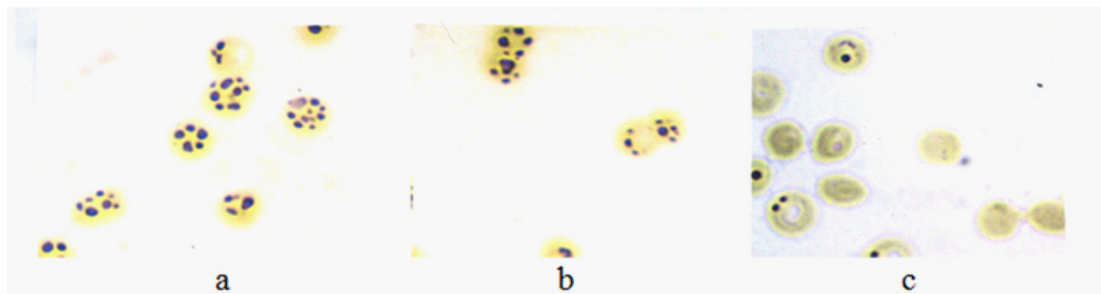


Figure 23: Restored glutathione activity in peripheral blood erythrocytes obtained from pregnant women in the third trimester of gestation. a – control; b – latent stage; c – erythrocytes of seropositive pregnant women. The test is developed by Lucenko M.T. and Andrievskaya I.A. [12].

Simultaneously, antioxidant function of superoxide dismutase, which is the leading factor at this area of erythrocyte, is reduced as well. In a normal state superoxide dismutase level in healthy pregnant women without a history during the entire gestation was determined within the limits of $390,0 \pm 15,2$ units/rHb. In seronegative pregnant women superoxide dismutase content has not been changed significantly and made up $385,75 \pm 14,7$ units/rHb ($p < 0,001$). However, at the exacerbation of cytomegalovirus infection superoxide dismutase content in erythrocytes acutely decreased to $233,0 \pm 11,5$ units/rHb ($p < 0,001$).

Thus, glutathione cycle functional activity and the superoxide dismutase antioxidant function in erythrocytes of pregnant women in the third trimester of gestation at the exacerbation of cytomegalovirus infection to antibody titer to cytomegalovirus 1:1600 were acutely suppressed due to the increase of fatty acids peroxides level and cytomegalovirus tegument proteins invasion.

The reduction of the heme iron - histidine bond at the exacerbation of cytomegalovirus infection had profound impact on hemoglobin oxygenation as it was histochemically tested that Fe²⁺ concentration in erythrocytes reduced to $36,5 \pm 1,8$ pixels/mm²; latent stage – $62,3 \pm 2,8$ pixels/mm²; $p < 0,001$, and in the control group – $63,5 \pm 2,5$ pixels/mm²; $p < 0,001$ with respect to the acute period.

The decrease of Fe²⁺ level (figure 24) in erythrocytes at the exacerbation of cytomegalovirus infection induces Fe³⁺ increase, methemoglobin formation and hemoglobin oxygenation violation

- $94,02 \pm 2,9$ % (latent phase - $97,0 \pm 3,2$ %; $p < 0,05$, control - $98,0 \pm 2,7$ %; $p < 0,05$ in regard to the period of cytomegalovirus infection exacerbation).

One of the important processes participating in hemoglobin oxygenation adjustment is its bond with 2,3-diphosphoglycerophosphate (2,3 - DPG). In a normal state in peripheral blood erythrocytes of pregnant women in the third trimester of gestation without a history during the entire gestation 2,3-DPG concentration is observed within the limits of $4,90 \pm 0,085$ mmol/mL. In seronegative pregnant women 2,3-DPG level does not change significantly and was registered at $5,9 \pm 0,09$ mmol/mL ($p < 0,05$). Erythrocyte 2,3-DPG concentration in pregnant women during the exacerbation of cytomegalovirus infection increases up to $6,1$ mmol/mL; $p < 0,01$. It strengthens the process of hemoglobin oxygenation lowering during the exacerbation of cytomegalovirus infection what is confirmed by the decrease of oxyhemoglobin amount in seropositive pregnant women to $94,0 \pm 2,9$ %.

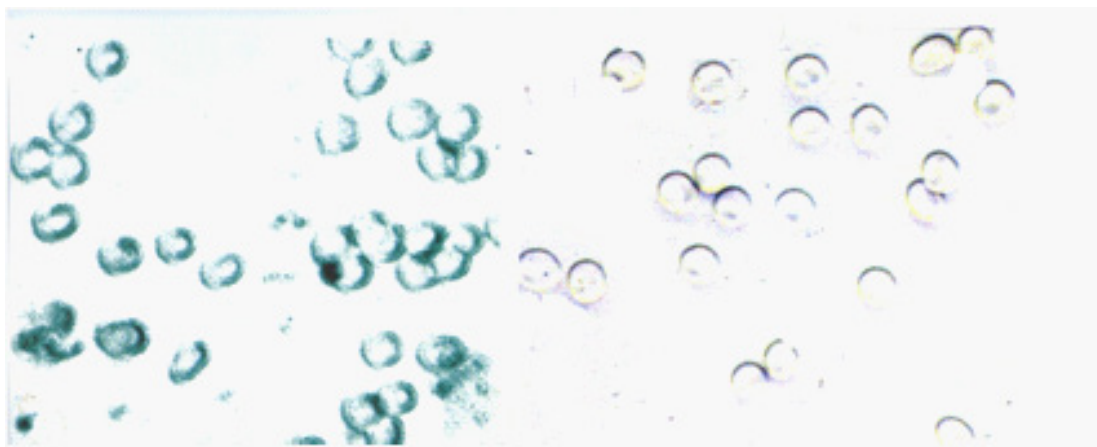


Figure 24: Peripheral blood erythrocytes of pregnant women in the third trimester of gestation during the exacerbation of cytomegalovirus infection to antibody titer against CMV 1:1600. Decrease of Fe^{2+} content to $36,5 \pm 1,8$ pixels/mmk². Reaction by Pierson E. Magnification 10 x 90.

DISCUSSION

Erythrocyte membrane structure like its functional activity is easily violated on contact with viruses getting into peripheral blood of the body. The exacerbation of persistent infections frequently occurs in the organism of a pregnant woman during the gestation. The conducted research has shown that cytomegalovirus infection comes in contact with the vast area of peripheral blood elements - erythrocytes, which membranes contain proteins capable of binding with the cytomegalovirus antigen. Such kind of protein in erythrocyte membrane is glyophorin. When analyzing statistically we have established that the circulating immune complexes and interleukin $TNF\alpha$ are the most aggressive elements for the erythrocyte membrane. It is confirmed by the multiple discriminant analysis.

All these elements being concentrated can freely circulate in blood plasma when in macrophages (figure 1) and in herpetic bodies. The production of leucocyte series of gangliosides in macrophages and in other cells precedes the herpetic bodies forming. It promotes their unification with each other and alleviates the contact with villus placenta syncytiotrophoblast or with erythrocyte membrane (Figure 2). Cytomegalovirus contains at least 33 structural proteins, including phosphoprotein pp65 antigen which is the product of UL83 [30,31]. And though anti-pp65 antibodies and pp65 – antigens are revealed in immunosuppressed patients with active virus infection [52], antibody response to pp65 antigen in infected individuals is not always detectable (immunoblotting) [4].

Unlike pp65-antigen, HCMV (human cytomegalovirus) contains pp150, which is stored in another viral membrane phosphoprotein provoking the profound humoral response in most infected individuals [40].

Most researches detected pp150 protein in HCMV sera irrespective of their clinical conditions [40]. At the same time, it was noticed that the humoral response in the individuals under the CMVI exacerbation against pp65-antigen was variable and was not always detectable with immunoglobulin [40].

PP65 may compose epitopes, which are capable of triggering the Th1-predominant reaction in nonautoimmune patients. Therefore, the prevailed over the T-lymphocytes reaction to HCMV pp65 antigen in control and seronegative population may display its protective effect to avoid the dangerous Th-2 autoimmune reaction.

Cytomegalovirus membrane protein gp64 [28,42,43] actively participates in fusion and in pH changing down to lower values (pH5 – pH6). Histidine becomes a sensor of low pH values and a trigger off conformational alterations in gp64. Three of histidine residues (H152, H155, H156) are disposed in a fusion loop 2 and were identified to be of great importance for cytomegalovirus membrane fusion [32,41,45,47,49,51]. These three histidine residues were substantial for the effective dilation of the erythrocyte membrane pores [38,39]. Three histidine residues (H245, H304, H430) generate a node, which is the cause for pH factor forming. Our investigations have demonstrated that during the exacerbation of cytomegalovirus infection the histidine content in peripheral blood erythrocytes decreases [53] that leads to the lowering of pH value to 5,2 and results in membrane pores dilation revealed through the distance enlargement up to 10 nmk between the points saturated with sialic acids. The membrane pores dilation creates favourable conditions for calcium ions transfer inward the erythrocyte from its outer surface area [17]. The increase of calcium concentration in erythrocyte adversely influences its enzymatic activity and conformational structure of membrane proteins. Viral fusion protein fixed in the viral covering then deepens its penetration into the erythrocyte membrane forming the bridge between the viral covering and the cellular membrane. It causes the destruction of the outer surface area of the erythrocyte membrane, while viral proteins penetrate further deep into and form a fusion pore [38, 50]. This process occurs in common with the erythrocyte glycoporphin and other viral protein

receptors and at the histidine proteins level decrease the forming of low pH level is initiated [50]. Histidine residues become trigger off conformational changes in fusion protein. At low pH value histidine becomes uncharged.

At present three classes of viral membrane fusion proteins have been identified.

Proteins removing the protective covering from orthomyxoviruses, paramyxoviruses, retroviruses, and coronaviruses are believed to relate to the first class. The second class is represented by alphaviruses utogaviruses proteins. Herpes virus proteins are related to the third class. The leading of them is gp64 protein [27, 50]. The availability of this protein (gp64) makes it be the universal damaging protein, which is the member of the whole herpes group of pathogens.

Thus, the data obtained of late are indicative of that the protein gp64 fusion with the membrane proteins demands histidine to be involved into the given process [32-37,44-47,49] to induce pH lowering down to 5 and to be a trigger off membrane pores dilation [36,37], it is of special concern to H152, H156.

Of viral surface proteins acting on the erythrocyte membranemarked out are group III proteins, which are related to herpes virus proteins gp64. Owing to this protein a rapid virus penetration inward the organism cells, including erythrocytes, occurs. The interaction with the histidine labeled membrane proteins first and foremost decreases the intracellular H₂ level down to pH5 what acutely changes canalicular system state of membranes making them well-permeable for Ca⁺⁺ inside the erythrocyte. Simultaneously, it becomes a cause of the violation of cytoskeleton proteins conformation and of their interaction with each other. H₂ donors and triggers off starting these processes are H152 and H156 clusters bearing histidine [13]. In addition, proteins containing histidine provide the normal cycle of hemoglobin oxygenation and the suppressive influence of cytomegalovirus infection on the histidine proteins content is one of the factors acting negatively on the oxygen adherence to deoxyhemoglobin in erythrocytes. On studying this point at great length we have established the oxyhemoglobin – histidine dependence with its maximum manifestation at the antibody titer to cytomegalovirus 1:1600 when the correlation coefficient was at the level 0,76 (p < 0,001).

The first stage of cytomegalovirus interaction with erythrocyte membrane enables it to advance deep down the erythrocyte by its proteins and affect the erythrocyte enzymatic system and hemoglobin oxygenation due to the toxic effect of its tegument proteins.

First and foremost, cytomegalovirus protein invasion into the erythrocyte membrane violates phospholipide bond with skeletal proteins and results in the alteration of phospholipide structure: phosphoethanolamine concentration decreases and through increasing of phosphoethanolcholine concentration lysophosphatidylcholine synthesis is activated, and as a result erythrocyte membranes acquire low microviscosity followed by the stormy process of erythrocyte oxidant elements forming, which, parallel with cytomegalovirus tegument proteins, act toxically on the basic enzymatic processes of erythrocytes.

First and foremost, influenced by peroxides and toxic effect of cytomegalovirus proteins it becomes suppressed the activity of glutathione reductase, which is the main element providing the presence in erythrocyte the restored glutathione performing the antioxidant function in the erythrocyte owing to the glutathionperoxydase level support that has a neutralizing effect upon H₂O₂ by converting it into H₂O+O₂ and not permitting extremely toxic OH⁻ to be formed out of H₂O₂. Simultaneously, suppression of the main antioxidant enzyme – superoxide dismutase- is observed. Proceeding from the forming of great amount of peroxides at the cytomegalovirus protein penetration in erythrocyte, it is generated the centre extremely toxically operating with regard both to the stroma proteins and simultaneously to hemoglobin oxygenation suppression. The violation of erythrocyte stroma proteins conformation and of their interrelations makes the erythrocyte membrane condensed, its microviscosity becomes violated, and up to 15% of discocytes acquire the shape of degenerative elements not capable of deformability enough to transfer through the narrow capillaries to the developing fetus organs and to supply them with the oxygen. On the other hand, peroxides and toxically acting virus tegument proteins suppress the glutathione cycle, decrease the antioxidant activity and glutathione bond with hemoglobin heme iron (F₂) what inevitably leads to the F₃ level increase incompetent to oxygenate, unfavourable conditions for the developing fetus tissues supply with oxygen are aggravated. During the oxygenation the iron atom transfers to the plane of the heme and pulls the proximal histidine. Herewith saline bonds are splitted and hemoglobin equilibrium displacement from T-form to R-form occurs. 2,3DPG associates with the positive charged groups of these two chains. 2,3-DPG binding stabilizes T-form and thereby lowers the hemoglobin affinity with the oxygen. 2,3-DPG level increase over the optimal threshold – 5 mkmol/mL- lowers hemoglobin oxygenation [29]. These displacements occur due to heightened erythrocyte content of fatty acids peroxides appeared owing to the toxic effect of cytomegalovirus proteins when penetrating into the erythrocyte membrane.

During the exacerbation of cytomegalovirus infection the main factors having effect on the 2,3-DPG are LPO and CIC. Therefore, at the exacerbation of cytomegalovirus infection in pregnant women the decrease of oxyhemoglobin level to $94 \pm 2,9\%$ (control – $p < 0,001$); (latent phase - $86,0 \pm 2,5\%$ ($p < 0,001$) is observed.

In the third trimester of gestation during the exacerbation of cytomegalovirus infection to antibody titer to cytomegalovirus 1:1600 there is the threat of developing anemia.

CONCLUSION

During the exacerbation of latent cytomegalovirus infection in the third trimester of gestation the alteration of immunity indices in the organism of a pregnant woman was observed: 2,1-fold increase in circulating immune complexes ($p < 0,014$); 4,2-fold increase in TNF α ($p < 0,021$); 2,3-fold increase in interleukin 8 ($p < 0,013$).

Herpes virus invasion into the erythrocyte membrane occurred owing to the high correlative value of protein glycophorin and circulatory immune complexes with the probability 97,74%.

Herpes virus proteins gp64 invasion into the erythrocyte membrane resulted in pore dilation and in the violation of erythrocyte membrane conformation followed by the appearance in erythrocytes of great amount of fatty acids peroxides - 4,3-fold increase ($p < 0,001$) compared to latent period.

Cytomegalovirus proteins invasion into the erythrocyte membrane results in the violation of phospholipide bonds with erythrocyte proteins, promotes the increase of lysophosphatidylcholine concentration in the membrane and leads to its microviscosity lowering.

Erythrocyte degenerative forms percentage in peripheral blood increases up to $15,0 \pm 0,09\%$ and that of echynocytes – up to $8,0 \pm 0,07\%$, what acutely lowers the erythrocytes deformability at the exacerbation of cytomegalovirus infection in pregnant women to $0,132 \pm 0,002$ conventional units compared to latent period – $0,088$ conventional units.

The increase of fatty acids peroxides level in peripheral blood erythrocytes of pregnant women at the exacerbation of cytomegalovirus infection in the third trimester of gestation results in suppression of antioxidant activity of superoxide dismutase, glutamate reductase, and peroxidase in erythrocytes provoking conformational displacements in hemoglobin erythrocytes and leading to the lowering of its oxygenation.

Conformational displacements in hemoglobin intensificate 2,3-DPG bond with hemoglobin up to $6,2 \text{ mkmol/mL}$ and induce Fe² into Fe³ oxidation what acutely lowers hemoglobin oxygenation to $94,0 \pm 2,9\%$ offering the conditions for erythrocyte anemia developing.

Consequences of CMVI effect on the erythrocyte membrane;

LPO increase;

Disintegration of protein-lipide bonds;

Violation of erythrocyte membrane microviscosity;

Erythrocytes deformability lowering.

Dyscocytes concentration decrease and echynocytes and degenerative forms concentration increase in blood.

Total: HbO₂ decrease within the limits to 86,0%.

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