

BASIC RESEARCH

Morphofunctional Characteristics of the Umbilical Vessels from Newborns Delivered by Mothers Experiencing an Exacerbation of Chronic Cytomegalovirus Infection during Gestation

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Abstract

The characteristic features of the cord blood and endothelium of the umbilical vessels from newborns delivered by mothers experiencing an exacerbation of chronic cytomegalovirus (CMV) infection during gestation has been studied. Exacerbation of the chronic CMV infection was found to increase during pregnancy and sharply affect the function of gas transport in both the maternal and fetal blood. Lipid peroxidation in the maternal blood as well as in the fetal blood was seen to increase, which was reflected in the characteristic endothelial structure and enzymatic activity in the umbilical vessels of the newborns.

Keywords: *umbilical cord, gas-transport function, lipid peroxidation.*

Introduction

The umbilical cord is a vascular-mesenchymal organ that enables the feto-maternal exchange of blood between the placenta and the fetus [1-3]. Often star-shaped in appearance, the umbilical arteries, which are muscular, reveal that the perivascular region of Wharton's jelly, instead of the adventitial region, are associated with the muscle membrane through gradual transitions. The intima includes the endothelium and a thin subendothelial layer. The subendothelial layer does not show clear boundaries and is transformed in the well-developed muscular layer [2]. The elastic membranes and fibers are observed to penetrate the entire arterial wall, from the subendothelial layer up to the perivascular regions of the Wharton's jelly. The intima of the umbilical arteries contains delicate fibrils which form dense networks. The area of the umbilical vein is larger than the area of both umbilical arteries by 30% while the lumen of the umbilical vein is much wider and

thinner [4]. Just beneath the umbilical vein endothelium, the thick coarse plexus of the elastic fibers are disposed, forming the elastic (basal) membrane [5]. The umbilical vein transports the oxygenated blood from the placenta to the growing fetus. The umbilical vein is divided into three branches: the first branch is the ductus venosus, which carries oxygenated blood into the inferior vena cava and the right heart; the second branch carries oxygenated blood into the sinus and the left liver lobe; the third branch carries the oxygenated blood into the portal vein, mixing with venous blood and moving to the right liver lobe.

Thus, 55% of oxygenated blood from the umbilical vein is diverted to the venous flow, while 45% goes to the liver [4]. It can thus be assumed that about two-thirds of the blood flowing from the placenta through the venous duct passes through the liver. The hemodynamic processes in the "mother-placenta-fetus" functional system are the prime factors that ensure a normal pregnancy, as well as fetal growth and development.

Under normal physiological conditions, the blood flow between the mother and the fetus as well as the normal morphofunctional condition of the endothelium of the vessels of the placental villi ensure a favorable gestational course. Violation of the gas-transport function of the umbilical cord blood or morphofunctional abnormalities in the walls of the umbilical vessels can occur as a result of the various metabolic disorders

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caused by the infectious processes that arose during pregnancy.

The infectious process in the mother's body, as a rule, accompanies the hypoxic conditions due to the inactivation of the enzymes of the respiratory chain and the inhibition of the activity of the dehydrogenases. The resulting tissue hypoxia leads to activation of the lipid peroxidation, which culminates in serious violations of tissue metabolism.

The aim of our study was to investigate the morphofunctional processes seen in the umbilical vessels under conditions of exacerbation of chronic CMV during gestation.

Material and Methods

The study was performed in the samples of peripheral blood and sections of the umbilical cord taken from 55 pregnant women between 18 and 25 years of age, with an exacerbation of chronic CMV infection (study group) and 30 pregnant women without CMV infection (control group). CMV infection was diagnosed in a comprehensive study of the peripheral blood to check for the presence of IgM or a four-fold or more increase in the IgG antibody titer in paired serum in the dynamics after 10 days, an avidity index reading of more than 50%, and the presence of CMV DNA. Simultaneously, a morphological study of the umbilical vessels of the same pregnant women was performed after childbirth.

The studies were conducted in line with the requirements of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" update 2000 and the Rules of Clinical Practice in the Russian Federation, approved by Order #266 of the Ministry of the Russian Federation of June 19, 2003.

The verification of CMV, the definition of type-specific antibody, and the avidity index were determined by ELISA on the spectrophotometer Stat Fax 2100 (USA) using the sets of CC "Vector-Best" (Novosibirsk) and by PCR on the machine DT-96 using sets of "DNA-Technology" (Moscow).

Blood gas analysis was performed on the biochemical analyzer "IRMA TruPoint" (USA), a well-recognized blood analysis system.

The intensity of lipid peroxidation in the peripheral blood was investigated by I. Stalnaya & T. Garishvili [6]. Umbilical cord was fixed in 10% neutral-buffered formalin and embedded in paraffin. The structure of the endothelium of the umbilical vessels was examined on the umbilical cord sections and studied after staining with H&E and Van Gieson.

The histochemical reaction to determine the lipid peroxidation activity in the endothelium of the umbilical cord blood vessels was performed according to Winkler-Schulze [7]. The activities of NOS and alkaline phosphatase were recorded according to D. Korzhovsky [8] and Gomory [9], respectively. The number of the histochemical reaction products was determined using computer-cytophotometry.

Statistical data processing was performed using the software package Statistica 6.0 for Windows. Analysis of the distribution of values obtained was performed using the Kolmogorov-Smirnov test. For data with normal distribution, inter-group comparisons were performed using Student's t-test and F-test. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups (for nonparametric data). P value less than 0.05 was considered significant.

Results and Discussion

First, in the case of an exacerbation of chronic CMV infection, attention should be directed towards the increase in the fatty acid peroxides and impairment of the gas transport functions in the maternal and fetal peripheral blood. Thus, an increase in the values of the CMV antibody titer up to 1:400 was associated with an increased level of the fatty acid peroxides (0.45 ± 0.02 mmol/mL in control) in the peripheral blood of the pregnant women to 0.96 ± 0.04 mmol/mL ($p < 0.01$) and to 1.22 ± 0.05 mmol/mL ($p < 0.01$) at an antibody titer value of 1:800. This was reflected in the circulating fetal blood. The fatty acid peroxide level (0.40 ± 0.04 in control) in the arterial blood of the newborns was 1.01 ± 0.03 ($p < 0.01$) at the antibody titer value of 1:400 and 1.40 ± 0.06 ($p < 0.01$) at the antibody titer value of 1:800. The gas-transport function of the maternal and fetal peripheral blood was greatly changed. Thus, the blood pH of the pregnant women was 7.30 ± 0.2 ($p < 0.01$) at the antibody titer value of 1:400 and 7.20 ± 0.1 ($p < 0.001$) at the antibody titer value of 1:800. The fetal blood pH, however, was 7.29 ± 0.2 at the positive CMV antibody titer (7.40 ± 0.04 in control). The oxygen tension in the maternal peripheral blood (39.22 ± 0.6 in control) decreased to 29.98 ± 4.0 ($p < 0.001$) at the positive CMV antibody titer and to 27.68 ± 0.7 ($p < 0.001$) at the antibody titer value of 1:800. This condition dramatically affected the oxygen saturation of the hemoglobin ($96.0 \pm 5.1\%$), which dropped to $93.30 \pm 2.5\%$ ($p < 0.001$) at the antibody titer value of 1:400 and to $90.20 \pm 1.95\%$ ($p < 0.001$) at the antibody titer value of 1:800. The oxygen saturation of the hemoglobin ($95.0 \pm 5.7\%$) in the newborn umbilical cord arterial blood decreased to 91.0 ± 3.1 ($p < 0.01$) at the antibody titer value of 1:400 and to $88.50 \pm 3.4\%$ ($p < 0.01$) at the antibody titer of 1:800. It can be assumed that such a state of the gas transport process and lipid peroxidation process in the peripheral blood of the mother and the fetus can lead to severe violations of the metabolic processes in several organs in the fetus.

The endothelium of the umbilical vessels during physiological pregnancy is found on the elastic membrane, although not completely damaged (Figure 1). We measured the length of the umbilical vein endothelium in arbitrary units using the cytophotometric method. In fact, 5 (11%) of 45 arbitrary units of the endothelial length showed some damage at the CMV antibody titer of 1:400, and more than 20 (40%) of 50 arbitrary units at the CMV antibody titer of 1:800 (Figures 1a-c). Endothelial cells showed increased size, with compacted nuclei

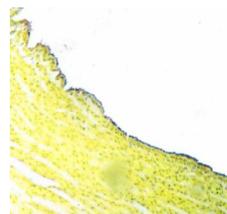


Fig 1 b
Exacerbation of the chronic CMV infection (antibodies titer – 1:400). Stained by Van Gieson $\times 240$

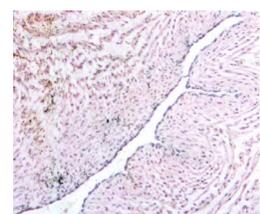


Fig. 1 a
Control. Stained with Hematoxylin and Eosin $\times 240$.



Fig. 1 c
Exacerbation of the chronic CMV infection at 21 weeks of gestation (antibodies titer – 1:800). Stained by Van Gieson $\times 260$

(Figures 1d-e), and fine foamy cytoplasm; also, in most cases, they contained large vacuoles (Figure 1f).

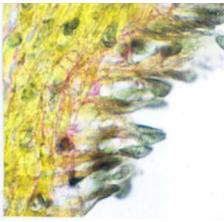


Fig. 1 d
Exacerbation of the chronic CMV infection (antibodies titer – 1:1600). Stained by Van Gieson ×1000



Fig. 1 e
Exacerbation of the chronic CMV infection (antibodies titer – 1:800). Stained by Van Gieson ×1000

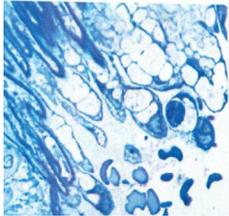


Fig. 1 f
Exacerbation of the chronic CMV infection (antibodies titer – 1:800). Semithin sections, stained with toluidine blue × 1000.

The cytoplasm of the endothelial cells of the umbilical vessels demonstrated an intense reaction to the fatty acid peroxides (Figure 2a). Single cells maintained contact with the basal layer through a thin cytoplasmic stem (Figure 2b).

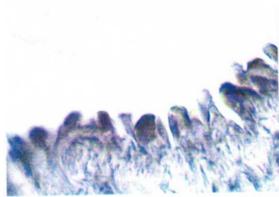


Fig. 2 a
Fatty acid peroxides in the endothelial cells. The reaction according to Winkler-Schulze ×400

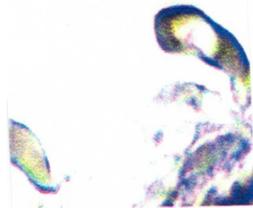


Fig. 2 b
Fatty acid peroxides in the endothelial cells. The reaction according to Winkler-Schulze ×1000

The vast majority of the deformed endothelial cells demonstrated an intense reaction to nitric oxide synthase (Figure 2d) and alkaline phosphatase (Figure 2c).

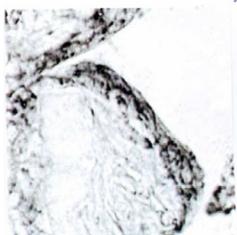


Fig. 2 c
The deformed endothelial cells demonstrated an intense reaction to alkaline phosphatase. The reaction according to Gomory ×400

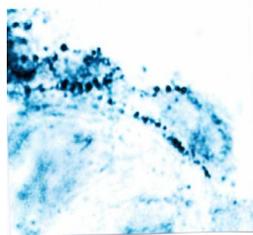


Fig. 2 d
The deformed endothelial cells demonstrated an intense reaction to NOS. The reaction according to D. Korzhevsky ×1000

It is well known that the presence of excess free radical products and NO, damages the proteins and unsaturated fatty acids, reduces the activity of most enzymes, and disrupts oxidative phosphorylation [10,11]. The increase in the alkaline phosphatase activity in the endothelium of the umbilical vessels may indicate preeclampsia and the formation of placental insufficiency.

Conclusion

The study of the endothelium of the umbilical vessels from the newborns delivered by mothers experiencing an exacerbation of chronic CMV infection during gestation reveals significant damaging effects of CMV infection. This suggests that serious complications can occur during the first month of the newborn's life.

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