

Ultrastructural Changes in placenta and Immunity Status During Intrauterine Influenza Infection

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Abstract

The state of immunity has been studied in 380 pregnant women and their newborns, having influenza in different stages of pregnancy. The histo- and ultrastructure of 36 afterbirth have been studied. The influenza infection during pregnancy was characterized by serious oppression of all studied immunity indexes both in mothers and in their newborns. In placenta there were found considerable structural changes, also the amount of influenza virus in active state, which is certified not only by the amount of antibodies, but also by the elimination of the virus particles.

Key words: *pregnancy, influenza, Immunity, placenta*

Introduction

In recent years many investigators have been underlining the particular importance of influenza infection among the diseases of perinatal period, as one of the causes of mortinataly and high perinatal mortality.

During the morphological study of placenta, taken without selection, there have been described the placenta damage during herpes, micoplasmosis and a number of other infections (Melnikova V.F. et all 1987; Benirschke K., Kaufmann P., 200; Malassine G., 2001).

In the studies, done on the basis of materials about influenza epidemics, there have been found the role of the disease in the abortion, in the birth of low-weight newborns, and also in high perinatal mortality. In great number of studies, including our previous publications (Sigua D.S. Korsantia B.M. et al 1999), there have been pointed out at the possibility of development of intrauterine influenza. However the morphological studies have been done only in a few works. Structural changes, described there, as a rule occurred in leukocyte infiltration and discirculator shift. Fulcheri, Pantarotto (2000) mention proliferation of chronic

epithelium and an amount of the recidives of inflammatory infiltration in cells, there have been directions about transplacental penetration of influenza virus in the organs of fetus without any damage. However, in the mentioned work there has been done neither virological not immunological investigation of afterbirth.

The Aim of the Study

The goal of our investigation was to find and determine the character of structural changes of feto-placental complex components during influenza.

Materials and Methods

There have been studied 180 pregnant women having influenza infection (influenza virus A) during different periods of pregnancy. There have been studied the histo- and ultarstructure of 36 afternoons of women, whose pregnancy ended with the birth of viable babies.

There have been determined the content of immunoglobulins G, A and M both an in funic blood in mothers and in their newborns by Manchini (1965) and

also activity of α and β interferons in leukocytes (D.S.Sigua et al., 1999).

The sections from mothers and fetus parts of placenta have been fixed and elaborated according to the standard scheme for the photo optic and electro-microscopic investigation. The samples of tissue of central paraumbilical and marginal zones of placenta (2 pieces) have been fixed in 4% formalin solution on phosphate buffer (pH=7,2) with the addition of saccharose and they were included into paraffin. The sections were stained by hematoxylin and eosin, picronfuxin. PAS positive substance and glycogene have been found by Shabadash method with fermental control, confirming the amount of glycogen.

For the electro-microscopy from the sites mentioned above, and also off the flat surface of chorion, there have been cut the samples in 1mm^3 , there have been fixed in two-component fixed mixture: 0,25% glutaraldehyd and 2% formaline solution, made on 0,1m phosphatic buffer (pH=7,2%); There were elevated in 0,25ml. saccharose with the addition of 3M MgSO_4 . The postfixation was made in 2% solution OsO_4 , were added epone-aldehyd into the mixture. Ultra-thin section were obtained on ultramicrotome Reichert Om-U3, they were stained by the method of double contrast and were studied in electronic microscope Tesla BS-500 during fast intension of the set 70-80kv.

Results and Discussion

The clinical-obstetric analyses showed that among pregnant women, in 3,5% there were found the anemia

of pregnant women, in 10%-premature delivery, in 17,2%-preterm spit of amniotic fluid. 6% of the newborns were born in asphyxia.

Among 44 newborns, whose mothers were having influenza in the first trimester of pregnancy, 7 were born premature of first rate. From 52 newborns, whose mothers had influenza in the II trimester of pregnancy, 8 were born premature of first rate, two of them were diagnosed the hipoxic-ischemic encephalopathy with high excitability. In two cases there were found hipoxic-ischemic encephalopathy of slight form, in 5 cases- of middle form. From 84 newborns, whose mothers had influenza in the III trimester of pregnancy, 3 were born premature of first rate, hipoxic-ischemic encephalopathy of slight form with high excitability were found in 4 newborns, and the one of middle form with the oppression syndrome and spastic readiness were found in 2.

The influenza infection during pregnancy was characterizes by serious oppression of all immunity indexes studied by us both in mothers and in newborns (Tab.1).

First of all there were found more intensive, than they are in healthy children, fall of interferon activity, especially γ i.e. of immune type. In umbilical cord were first found immunoglobulins of M classes, which are not registered in the norm. This fact shows the intrauterine antigen stimulation of fetus and the possibility of immune reaction of afterbirths.

Groups	α UF E/ml	γ Uf e/ml	T %	Th%	Ts%	UU	B%	IgG g/l	IgA g/l	IgM g/l
Control	43,4	31,8	52,6	36,6	14,9	2,5	24,5	12,5	1,88	1,26
Physiol.del. mothers	29,8	17,3	49,7	34,2	15,5	2,21	22,8	13,2	1,76	0,96
Physiol. del afterbirth	24,6	10,4	35,2	21,8	13,4	1,63	13,3	10,8		
Influenza mothers	22,8	14,5	46,4	30,8	15,6	1,97	20,1	11,8	1,83	1,56
Influenza afterbirth	10,8	4,1	27,3	15,2	12,1	1,26	11,2	10,1	0,41	0,33

Tab.1 The indices of cell and humoral immunity during physiological delivery and influenza in pregnant women.

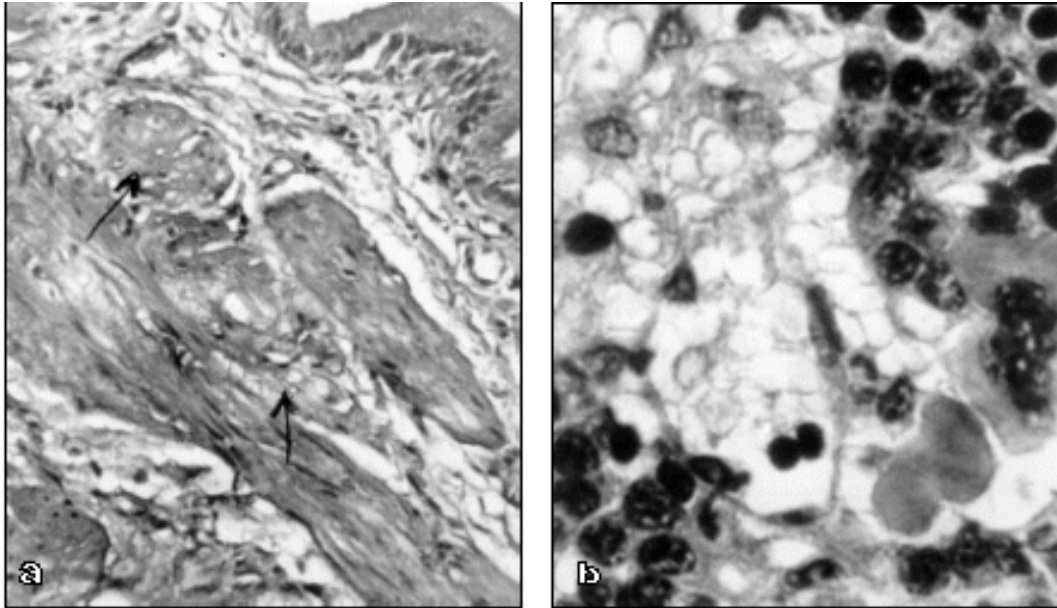


Fig.1 Change of vessels and stroma of placenta during influenza. Colouring with hemotoxilin and eosin

a-expressed fibroblastic reaction of stroma of villi (↑), X 400;
b-giant multi-nucleus cells in decidual tissue (↑), X 600.

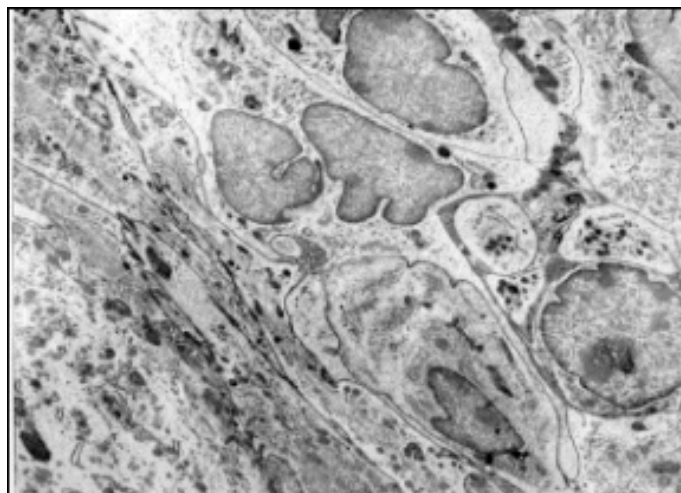


Fig.2 Multinuclear giant cells with cytoplasm areas of different density.

Electronogram, X 4000.

From 36 samples of the placenta tissues in 14 cases there was found the influenza virus, considered as an influenza A (H3N2), and in 8 cases influenza B. In 9 cases influenza was recognized as mono-infection, and in other cases there were found the mixture of influenza

with other respiratory viruses (3 observations) and bacterial infection (2 observations). In 20 placentas during IF-investigation there were found influenza antigens both in the structures of chorion and in decidual cells.

On the surface of villi the analogue changes developed in chorionic epithelia. Its cytoplasm was increased, nucleus, cyto- and syncytiotrophoblasts were getting bigger and were located in several rows, and some of them were effected by necrosis. Groups of villi with a lack of epithelial plate on considerable expansion. Stroma of villus was getting edematous, its fibers were swelling and were in danger of reorganization. In villus like this you could find a number of glycosaminoglycans and formation of fibrinoids.

A considerable change occurred in the villus vessels: the endothelium was swelling followed by the growth of cells measures and dystrophic changes of basal membrane. In addition, there occurred the paralytic expansion of capillary lumen, thinning of syncytiotrophoblast membrane in stroma of villus, the hemorrhage occurred in intervillous space. Together with these changes in villi you could see a fibroblastic reactivity (*Fig. 1a*).

It is quite evident, that is caused by the reproduction of influenza virus here, moreover the possibility of such kind of reproduction in fibroblasts of lungs in embryos of a man is described by virusologists (Dumont, 1989).

In addition, in villi there were found not big flocks of lymphoid cells and macrophages. In base plate decidual cells had large sizes, weak-acidophilic or paucicystoplasm, some of them had necrosis. The necrosis foci sometimes reached considerable sizes and occupied almost the whole thickness of base plate, there were also discovered few lymphoid infiltrates. During the counting of different forms of lymphoid cells (lymphocytes, blasts, plasmocytes) there was found a definite dependence of their amount on the time of having ARVD according to clinical indexes. The changes characteristic for influenza, found in different structures and places of placenta and tunic had foci

character, there appeared multi-nucleus decidual cells. They reached the biggest degree of expression in those cases, in which from the afterbirth tissue was distinguished the influenza virus. Together with changes described above, in placenta there were found hemorrhage of different localization, "infarcts", villi fibrosis.

During the ultra structure there were distinguished the necrosis foci of villus epithelium, thickening of basal membranes of capillaries of truncal villi in the persistence of Langhans layer. Our findings certify that in uteroplacental area there appear multi-nucleus cells, which contain fragments of cytoplasm of different thickness (*Fig. 2*), which in Milovanova A.P and Kirichenko's (2001) opinion, are the recognized markers of cytotrophoblast invasion and are regulated by hypoxic stimulus of epithelium.

Conclusion

The done investigation showed, that the influenza of pregnant women may be accompanied with generalization, which is confirmed by the elimination of the viruses from the parts of investigated placenta, by the amount of characteristic changes, identical with those which appear in other organs, including lungs, formation of interferon and antibodies. We must underline, that the influenza viruses were eliminated from women's placenta, having influenza 10-12 weeks before delivery. It allows us to consider that in placenta, probably because of long vital cycle of cells, it is possible the longer persistence and reproduction of influenza virus, that it is found in respiratory organs. By the moment of delivery the influenza virus were in active state, which is certified not only by amount of antibodies, but also by the elimination of virus particles.

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Ультроструктурные изменения в плаценте и иммунный статус при гриппозной инфекции беременных

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Р Е З Ю М Е

Изучено состояние иммунитета у 180 беременных и их новорожденных, перенесших в различные сроки беременности грипп. Исследована гисто- и ультроструктура 36 последов. Гриппозная инфекция во время беременности характеризовалась серьезным угнетением всех изученных показателей иммунитета как у матерей, так и у их новорожденных. В плаценте обнаружены значительные структурные изменения, а также наличие вируса гриппа в активном состоянии, о чем свидетельствует не только наличие их антигенов, но и выделение самих частиц вирусов.

Ключевые слова: *беременность, грипп, иммунность, плацента*