INFECTION BY CORONAVIRUS IN THE PLACENTAL VILLI

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RESUMEN El síndrome respiratorio agudo y severo provocado por el coronavirus 2(SARS-CoV2), un nuevo agente zoonótico brotando actualmente, ha sido estudiado por su efecto sobre la vellosidad placentaria en paciente de 29 años de edad quien tuvo Covid-19 a las 36 semanas de embarazo y cuya placenta fue descrita con microscopía de luz.

Deciduitis, villitis, desorganización de la región estromal de la vellosidad, citotrofoblastos hipertrofiados fusionados, calcificación distrofica, deposición de fibrinoide, necrosis celular, vellosidades destruidas, restos de vellosidad troncal fibrotica en el espacio intervelloso, numerosos nódulos sincitiales y extensas zonas de sincitio con numerosos núcleos fueron encontrados.

Este ataque viral contra el árbol veloso puede resultar en una aumentada morbilidad y mortalidad entre las mujeres embarazadas con el potencial para adversamente afectar al feto y neonato en desarrollo.

PALABRAS CLAVE: SARS-CoV2. Cambio placentario.

ABSTRACT: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) a newly emerging zoonotic agent, has been studied by their effect on the placental villi, in patient of 29 years old who had Covid-19 at 36 weeks of pregnancy whose placenta was described with light microscopy.

Deciduitis, villitis, disorganization of the stromal region of the villi, fusioned cytotrophoblasts hypertrophied, distrophic calcification, fibrinoid deposition, cellular necrosis, destroyed villi, fibrotic rest of stem villi in the intervillous space, numerous syncytial knots and extensive zones of syncytium with numerous nuclei were found.

This viral attack against the villous tree can result in increased morbidity and mortality among pregnant women with the potential to adversely affect the developing fetus and neonate.

KEY WORDS: SARS-CoV2. Change Placental.

INTRODUCTION: The coronaviruses are virus pleomorphic or spherical of 80-220nm, with an icosahedral core structure within which is a helical nucleocapsid. Their genome consists of a single molecule of linear positive sense, single-stranded RNA, 23-31 kb in size and is very infectious. Replicate in the cytoplasm and are released by exocytosis1.
It is known that viruses circulating in the maternal bloodstream enter the placenta from uterine arteries, circulate in the intervillous space, and can pass to the fetus through the chorionic villi tree where they eventually enter the fetal circulation. It appears that the absence thus far of maternal-fetal transmission of the SARS-CoV2 virus during the covid-19 pandemic is similar to other coronaviruses, and is also consistent with the extreme rarity of suggested or confirmed cases of intrauterine transmission of other respiratory RNA viruses. If intrauterine transmission of SARS-CoV2 eventually occurs, it will be a rare event².

There is evidence of SARS-CoV2 vertical transmission when the infection occurs in the third trimester of pregnancy and an increased risk for premature delivery. It is of 3.2% (23/936) by infant nasopharyngeal swab testing. Although it has been a point of a recent debate concluding that there is no evidence of vertical transmission and no known cases have been noted in similar coronavirus as SARS and MERS. The coexpresion of ACE2 and transmembrane serine protease2 (TMPRSS2), receptors of coronavirus in the placenta, are in a minimal number of placental cells. The chorioamniotic membrane of the third trimester exhibits minimal coexpresion of both proteins. Nonetheless, others entry mediators could to be active³.

The RT-PCR which was positive in the amniotic fluid and throat swab at 24 hours, for the baby born at 32 weeks gestation to the mother with symptomatic Covid-19 strongly raises the possibility of vertical transmission⁴.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) formerly known as 2019 novel coronavirus (2019-nCoV) is a newly emerging zoonotic agent that appeared in December 2019 and causes the coronavirus disease 2019 (COVID-19)⁵.

This emerging disease course with fever, cough, dyspnea, shock, lymphopenia, high erythrocyte sedimentation rate and can to be fatal ⁶.

The preliminary findings in covid-19 positive mothers describe fetal vascular malperfusion provoked by intervillous thrombus, villous stromal-vascular karyorrhexis, avascular villi, intramural fibrin deposition in stem vessel, decidual vasculopathy, chronic villitis and focal chorangiosis⁷.

Numerous placental lesions have been associated with SARS-CoV2 infection during third trimester of pregnancy as fetal vascular malperfusion, maternal vascular malperfusion, perivillous fibrin deposition, increased syncytial nodules, chorioamnionitis, chronic villitis, intervillousis, chorangiosis, delayed villous maturation, deciduitis, villous oedema, placental abruption, and infarction⁸.

SARS-CoV2 induce syncytial cell formation and cell tight junction destruction, extensive cell death caused by apoptosis or necrosis, formation of numerous pleomorphic double-membrane vesicles in the cytoplasm of infected cells and aggregation of organels close to the apical surface⁹.

Pathologic studies on biopsy samples of lung, liver, heart, obtained of death Covid-19 pacients have revealed that the lung is the main affected tissue with pathological changes as hyperplasia of type II pneumocytes, damage to the alveolar epithelial cells, formation of hyaline membrane and diffuse alveolar damage¹⁰.

Thrombotic microangiopathy, accumulations of CD4 mononuclear cells around small thrombotic vessels and notable hemorrhage as cause of death in these pacients, presence of megacariocytes in the lung, platelet aggregation, fibrin deposition and clot formation have been found¹¹.

Others morphological changes observed in the placenta are:accelerated villous maturation, distal villous immaturity, vasculitis, intervilous hematoma and subchorial hematoma¹². Fibro-myxoide exudates, mononuclear inflammatory infiltrates dominated by lymphocytes, large nuclei, prominent nucleoli. No obvious intranuclear or intracitoplasmic viral inclusions, over activation of T cells and lymphopenia¹³.

In the placentas of two women who were convalescing from SARS-CoV2 infection in the third trimester of pregnancy, these were highly abnormal with extensive fetal thrombotic vasculopathy, with areas of avascular chorionic villi as chronic findings of fetal vascular malperfusion. These pregnancies were complicated with IUGR¹⁴.

However, a characteristic placental pathology has not been clearly demonstrated in placentas exposed to SARS-CoV2 since ACE2 viral receptor in the infection is localized with their expression highest on the stromal side of the syncytium away from the maternal blood being the infection an event rare¹⁵. To describe the histopathological changes provoked by the coronavirus on the structure of the placental villi is our proposal.

**CASE REPORT**

Pacient of 29 years old, with 38 weeks of pregnancy, who had Covid-19 at 36 weeks and whose placenta presented zones of...
hemorrhage, hematomas, infarcts, sclerosis of vessels and calcifications. With fever during a day, anosmy and hipogeusy
and was recupered, live newborn, of 3050gr and puerpery normal.

The infected woman pregnancy had knowledge of informed consent and approval by the ethical committee of the
hospitalary institution for the realization of this investigation according to the Helsinki declaration.

Five zones of the maternal-decidual region were taken: Four peripheric and one central. Small pieces of placenta were fixed
in 10% formaldehyde, dehydrated in absolute alcohol, included in parafine, cuted in the microtome at 4-5um, desparafined
in xilol, colored with H&E stain, deshidrated and aclared with Canada Balsam.

Regions of interest were photographed with a light photomicroscope MC63A Zeiss Clinic Standart (Carl Zeiss,
Oberkochen, West Germany), observed at 400x, compared with normals control samples of patients without Covid-19 or
another disease and prepared with similar procedures.

Cells of the placental decidual region are invaded by mononuclear cells. This region contains areas of cellular necrosis (Fig.
1). Some placental villi were infiltrated by numerous mononuclear cells in the intervillous space. The cytoarchitecture of
the villi exhibits death cells with picnotic nucleus and their internal organization has been lost (Fig. 2).

Numerous stem villi have lost their endothelium and edematous region can to be observed in the stroma which is not noted
in control sample (Fig. 3).

Under the syncytium some hypertrophied cytotrophoblasts are fusioned and have originated a small syncytium or
multinucleated giant cell (Fig. 4). Any stem villi are observed degenerated with lost of syncytium, fibrotic stromal region in
their inferior zone and deposition of fibrinoid that contains death cells (Fig. 5). Others are seen destroyed in their stromal
region with calcium deposition (Fig. 6).
These figures were not seen in normal placenta. In the intervillous space can to be observed several villi destroyed (Fig. 7) and with deposition of calcium (Fig. 8). Rest of stem villi were seen with frecuency between damaged villi (Fig. 9). In regions of infarct numerous syncytial knots are noted (Fig. 10). Mature intermediate villi contains in the syncytiunm a double file of nuclei (Fig. 11) and extensive zones of syncytium contain numerous nuclei in others placental villi (Fig. 12).
Fig. 9. Rest of stem villi. H&E. 400x

Fig. 10. Region of infarct with numerous nuclei in syncytiun and syncytial knots. H&E. 400x

Fig. 11. Double file of nuclei in syncytiun of mature intermediate villi. H&E. 400x

Fig. 12. Extensive zones of syncytiun contain numerous nuclei. H&E. 400x

DISCUSSION:

The presence of villitis here described is evidence of a possible vertical transmission which could to spread the virus to the fetal bloodstream and to affect to the fetus. Papain-like protease 2 (PL2pro) and the 3C protease (3CLpro) as viral proteins present in the coronavirus could be contributing with the interruption of membranes and cytoplasmic lysis in the cells of the placental villi provoking their disorganization.

This villitis although very scanty, is the microscopic finding of inflammation of the chorionic villi that is the histologic hallmark of many maternal hematogenous infections that are transmitted through the placenta to the fetus which has been identified. Their scarcity could be correlated with the negligible presence of ACE2 and TMRSS2 described by Pique et al.

By other hand, the vaculitis of stem villi, in these cases of placental insufficiency, can induce in the ramifications of the placental tree events of thrombosis and subsequent fetal vascular malperfusion, extensive fetal thrombotic vasculopathy, hypoxia and complications in the IUGR.

The betacoronavirus have a glycoprotein, the HE protein, with capability of haemagglutinating and binding to erythrocytes, an esterase activity, correlated with events of thrombosis.

Hypertrophy cellular also has been seen in type II neumocytes and cell fusion was provoked by Chikungunya virus to produce an enlarged and multinucleated cell.

Death of the syncytiun and stromal region with numerous death cells could be provoked by the non-structural protein 10 (nsp10), protein viral of the SARS coronavirus which interact with the cellular oxido-reductase system causing an

http://biomed.uninet.edu/2019/n3/castejon.html
extensive cytopathic effect since this molecule interrumpts the physiological function of mitochondria and cause severe damage to the cells19.

Pathological study suggest that there are not morphological changes related to infection in three placentas and no evidence for intrauterine vertical transmission16. Similar results have been found by He et al20. However, Patané et al using advanced cell diagnostic with a ProbeV-nCov2019-S and automated equipment, visualizing the virus directly, has found the possibility of vertical transmission21.

But the majority of the literature has reported healthy neonates born to mothers with Covid-19 and the most frequently reported pathological findings are the fibrosis, maternal vascular malperfusion, intervillous thrombi and increased wall of vessels in the chorionic plate22.

Pathological examinations have demonstrated that syncytium are often infected with SARS-CoV2, but fetuses are not always infected23. Maternal vascular malperfusion, injured maternal vessels, intervillous thrombi and villous edema, may reflect a systematic inflammatory or hypercoagulable state influencing adverse perinatal results during second and third trimester of pregnancy22. Although during the first trimester does not seem to predispose to early pregnancy loss, having a favorable maternal course 23.

Fibroblastic proliferation in fibrotic pneumonia has been observed in coronavirus infection of late stage. The incremented observation of nucleus here reported in the syncytium or syncytial knots could to be induced by these viruses24. The cytokine storm induced by SARS-CoV2 has provoqued this attack against the villous tree and can result in increased morbidity and mortality among pregnant women with the potential to adversely affect the developing fetus and neonate25.

In a pregnant woman with mild Covid-19 disease, with maternal vascular bad perfusion and fetal vascular good perfusion, the formation of microthrombi, accelerated villous maturation, infarction, intervillous thrombi, extravillous trophoblastic lesions, subchorionic necrosis, villous sclerosis and vascular kariorrhexis have been found in the placenta26.

Recently Schwartz and Moretti have found that placentas from infected maternal-neonatal dyads are characterized by the finding of mononuclear cell inflammation of the intervillous space, termed chronic histiocytic intervillosites and that together with syncytial necrosis, in co-occurrence, appears to be a risk factor for maternal -fetal viral transmission27. Our samples have not this rare event possiblely by early treatment of the patient.

The presence of SARS-CoV2 has to be confirmed by placental sections, amniotic fluid or cord blood in order to investigate whether the placenta is infected and of this manner using transmission electron microscopy single virions were detected in the syncytium and stromal fibroblasts of a woman with severe Covid-1928.

An early treatment has been indicated for Covid-19 with azithromycin and hidroxychloroquine29 since azithromycin inhibits SARS-CoV2 in vitro30.

Autophagy inhibitors as chloroquine, hidroxicloroquine, mefloquine, clomipramine, and others have suppressed the viral attack in culture of Vero-E6 cells inhibiting release of the viral genome and reducing the cytopathic effect. A viable target pathway for Covid-19 drug discovery according to a non-peer-reviewed pre print31.

In conclusion, Coronavirus have provoqued a strong attack to the placental villi disorganizing their structure which indicate that the placenta is not in their best condition for the interchange of gases and nutrients which could affect notably the fetal growth.

REFERENCES


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