Does inflammation influence placenta shape? A morphometric analysis regarding trophoblast and intervillous space

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Abstract

The aim of this study is the morphometrics in histologic shape changes of placenta with acute and chronic inflammation in pregnant women patients and the comparison of results with normal ones, in order to observe the reaction of trophoblast and intervillous space. We conducted an observational, correlational study of 44 placenta specimens collected from the Gynecology and Obstetrics and from the Pathology departments in the Emergency University Hospital in Bucharest, processed, embedded in paraffin blocks, sectioned 3 µm thick and haematoxilin eosin stained. We found 6 cases (13.6%) with acute chorioamniotitis (CHO), 24 cases (54.5%) with chronic CHO and 14 cases (31.8%) with normal histology, with the overall mean gestational age of 30.34 weeks and mean mother age of 30.86 years old. Percentage measurements of trophoblastic areas and of the intervillous spaces were performed on scaled photographs with the aid of a morphometric software. The statistical Mann-Whitney U test applied on obtained data, proved significant differences in between groups or chronic CHO cases compared with normal ($P<0.05$), while acute CHO cases proved slight morphologic differences. The main detected feature is that the intervillous space increases and syncytiocitotrophoblastic structures reduces in quantity in chronic inflammatory states of placenta, thus, showing the more rising importance for morphometric studies in pathology and histology.
Keywords: *chorioamniotitis, placenta, hypoxia, morphometry, histology, pathology*

Introduction

Inflammation in the placenta still remains a common complication for pregnancy associated with important maternal and fetal disorders with long term outcomes. Chorioamnionitis (CHO) is an acute or chronic inflammation of the chorion and the membranes of the placenta, mostly caused by ascending bacterial proliferation, due to the premature rupture of amniotic membranes. Although this definition may be interpreted as a group of clinical symptoms for women patients that have this condition, in particular fever over 38° C (100.4° F), maternal (>100 bpm) and fetal tachycardia (>160 bpm), maternal leukocytosis (>15000-18000/mm^3) and purulent amniotic fluid, it also may exist as a "silent" CHO, the only diagnosis being made with histology sections.

In diabetes mellitus, the placentae are significantly increased in size, volume, weight, area, thickness, diameter and circumference in comparison with controls. The placental changes are positively correlated with baby weight at birth[(1)]. However, this kind of analysis in CHO placentas is still rarely used because of the paucity of control for the many obsolete factors involved in microscopic structures shape modification.

Methods

We have selected 44 placentas, from pregnant female patients admitted in the department of gynecology and obstetrics at the University Emergency Hospital in Bucharest and histologically diagnosed with CHO between 1st January 2012 and 1st February 2013, with prior consent obtained from the ethical board analysis. These tissue fragments were sampled from an intermediate position between the umbilical cord insertion and margin of the placental discus and afterwards, embedded in paraffin blocks, sectioned in 3 µm thick slides and stained H-E.

These have been studied and high quality photographs were performed with the aid of a Leica DM750 microscope, trough the 40x/0.65 objectives.

The slides were always positioned in the same way for every case, with the record number in the right side of the examiner, and the fifth field from left to right, in straight line, from the upper left corner, was photographed with the 40x optics, in order to eliminate bias as much as possible.

The images were scaled in 0.33 x 0.25 mm, and measurements were performed with the aid of ImageJ software.

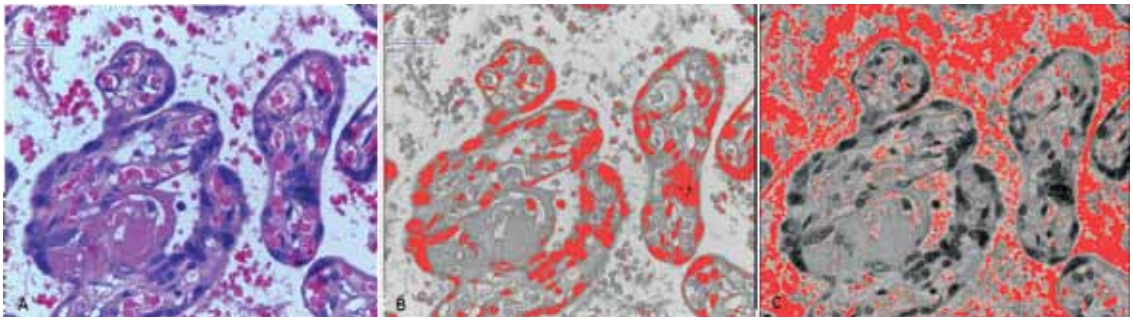

Figure 1. Image processing in a 10 weeks gestation placenta, with surface extraction and approximation for trophoblast surface and intervillous space surface (0.33 x 0.25 mm, 40x10, HE)

The entire photograph surface, which remains constant, is considered the maximum percentage surface (100%).

Through the algorithms available with threshold processing (Figure 1) carefully made in the same way for all cases, percent of measurements of trophoblastic surface and intervillous space were taken, because the use of absolute metric data becomes unreliable, as absolute values are very small, thus hard to use without error with a standard statistical computer.

The data obtained was centralized in a database form and statistical calculations were performed, manually, in an Excel spreadsheet.

Results

Features of the studied lot

The mean gestational age of the 44 cases was 30.34 weeks and of pregnant women 30.86 years old. We found 6 pregnant women (13.6%) with acute CHO, 24 (54.5%) with chronic CHO and 14 (31.8%) with normal.

The trophoblast surface in normal placenta and placenta with CHO

As it is visible in Table 1, the trophoblastic surface has a higher average value in normal placentas (13.283%), while in the chronic inflammation placentas, the trophoblast shows a small average surface (8.58%). The cases with acute CHO histology proved an "in-between" mean value (8.96%).

The intervillous space area in normal placenta and CHO lots

The intervillous space surfaces were higher in small age gestations for normal placentas; chronic CHO placentas proved a larger mean value for all cases (39.88%), while normal histology tissue samples have a smaller average value (33.3%, Table 1).

Discussion

It seems that the overall trophoblastic surface becomes smaller as the inflammation continues to be active in an acute and chronic pattern, at least, on the

Table 1 Average values for trophoblastic surface and intervillous space areas for all categories (acute, chronic and normal placentas) independent of gestational age - shown in percentages

Trophoblast surface		Acute inflammation placentas (%)	No inflammation placentas (%)	Chronic inflammation placentas (%)
	Average	8.9697	13.2833	8.5795
	Stdev	4.1845	4.9378	3.9007
Intervillous space surface	Average	35.4728	33.3009	39.8848
	Stdev	13.5186	11.1720	13.9062

syncytiotrophoblast. While the gestational age increases, the intervillous space becomes smaller (data not showed). Therefore, we have detected that, in placental inflammations, the intervillous space becomes wider than normal controls on the same gestational age as the gestation evolves, the space between the villi still becomes smaller, but in a smaller rate in those placenta with chronic CHO than controls. In acute CHO this issue is not significantly modified. The trophoblast, in acute and chronic CHO, seems to have almost no influence from the gestational age, as we couldn't reject the null hypothesis. A larger study regarding this issue is needed, as many other unknown factors may become involved. The two-sided Mann-Whitney U test proves significant statistical difference between the three categories - acute, chronic and control - as this reflects changes in shape and quantity.

The gold standard in diagnosis for CHO remains the culture of amniotic fluid[2]. In this matter, clinical silent CHO becomes a dangerous risk factor in increasing the possibility for respiratory distress syndrome, retinopathy of prematurity and necrotizing enterocolitis disorders in preterm babies. Some studies suggest a strong link between gram positive bacteria CHO and necrotizing enterocolitis[3]. Also, it seems that recurrent otitis media with effusion, a leading cause of acquired hearing loss in very low weight newborn babies during the first 3 years of life, seems to be significantly correlated with histological confirmed CHO[4]. Morphometric models have been used for placental shape variance assessment, especially in animals, like one study that investigated placental basement membrane thickness correlation with maternal leptin blood concentrations in a baboon[5]. An increased stromal volume density, increased villus size and trophoblastic thickness were also discovered in smoking mothers, due to morphometric stereology techniques[6].

Conclusions

In inflammation, intervillous space increases and syncytiocitotrophoblastic structures reduce, more obviously in chronic inflammatory states of placenta than in acute CHO, probably due to the amount of time necessary for lesion induction. This is, probably, an adaptive modification to biological stress agents, in order to reduce chances of hypoxia to fetus or maternal-fetal blood circulation insufficiency.

Therefore, appropriate multidisciplinary management, regarding morphometric analysis with computer assisted algorithms, could help pathology researchers in developing new means of treating more placenta biology mysteries. ■

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