Morphological and immunohistochemical analysis of placenta to establish the frequency of
villitis and its correlation with poor fetal and maternal outcome.

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Short running title: Villitis diagnosis and poor fetal outcome

Acknowledgements
This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Fundação de Ensino e Pesquisa de Uberaba (FUNEPU) and Conselho nacional de Desenvolvimento Científico e Tecnológico (CNPq).
ABSTRACT

The placental villitis has been correlated with perinatal infection, although a percentage of that remains unknown etiologically, being this the reason why it has been the object of a number of studies throughout the world. The objective of our paper was the systematic morphological study of placentas for the immunohistochemical characterization of villitis and its possible correlation with maternal and fetal intercurrences. A hundred twenty eight placentas have been studied under the HE method for morphological analysis. Immunohistochemical study was performed using monoclonal antibodies against Toxoplasma gondii, Citomegalovirus antigens, CD57, CD68, PanT and PanB cells. Villitis was identified in 11.7% of the studied cases. The intensity of the inflammatory process in the villi was discrete in 33.3% cases, moderate in 40% and severe in 26.7%. In 40% of the villitis cases the children were stillborn (p=0.003). The immunohistochemical evaluation showed one positive case for toxoplasmosis while the remaining cases were negative. Immunohistochemical staining in areas of chronic villitis showed CD68+ cells in moderated number, PanT cells in small number and the cells were negative for antibody against CD57 and PanB antigens. Moreover we described fetal and maternal alterations that are correlated with a diagnosis of placental villitis. Factors like the fetal gender, maternal age and degree of parity may be related to the etiopathogenesis of the inflammation. We have furthermore brought in new contributions concerning the relationship between the severity of the condition presented by the fetus and the severity of the placental inflammatory process.

Key words: fetal outcome, immunohistochemical, placenta, villitis.
INTRODUCTION

Chronic villositis was first mentioned by Gershon & Strauss (1961) as one of the signs of placental insufficiency. Since the detailed description by Benirschke & Altshuler (1971), this entity has been the subject of diverse studies over the years. Villositis is characterized by the infiltration of focal or multifocal leukocytes into the stroma of the chorionic villi, usually in association with fibrinoid necrosis of the syncytiotrophoblast [3,4,5]. Chronic villositis is found in approximately 10% of placentas and is associated with an increase in the risk of neonatal morbidity and mortality [5,6,7,8]. Among children who present intercurrences in the perinatal period, the placenta may be the presentation, in up to 75% of the cases, of chronic villositis [9]. The main intercurrences related to villositis are: prematurity, newborns that are small for the gestational age, congenital malformations, recurrent miscarriage, retarding of intrauterine growth [6,9,10,11,12].

The villositis may have a known etiopathogenesis when it is related to an identified infectious agent [5,13,14,15,16], or it can also appear without any agent responsible for it having been encountered, in which case it is called "villositis of unknown etiology" [5,6,13,17]. The etiopathogenesis of villositis, of both known and unknown etiological origin, is not well established [8]. In addition to the more common etiological agents for congenital infection, the mother's immune manifestations against the fetus, encountered in recurrent miscarriage, enter into the differential diagnosis of villositis of unknown etiology [7,18].

The objective of our paper was the systematic morphological study of placentas for the characterization of villositis and its possible correlation with maternal and fetal intercurrences.
METHODS

Use was made of 128 placentas from deliveries performed at the School Hospital of the Faculdade de Medicina do Triângulo Mineiro, Uberaba, Minas Gerais (HE-FMTM), collected during the years 1998 and 1999.

After macroscopic analysis, ten fragments were collected from different areas of the placenta. All the cases were firstly analyzed using the Hematoxylin-cosin method. To analyze the nature of the cells that form the villitis and research the possible etiological agents, we performed immunohistochemical reactions utilizing the monoclonal or polyclonal antibodies anti-Toxoplasma gondii, anti-Cytomegalovirus, anti-CD68, anti-CD57, anti-T lymphocytes and anti-B lymphocytes. The secondary antibodies utilized were conjugated with peroxidase or biotin (DAKO®). Warthin-Starry staining was performed for Treponema pallidum [19].

We made a review of the records of all the patients whose placentas had been examined, with the objective of gathering information relating to age and maternal parity; gender, gestational age and weight of the child; whether it was a single or twin pregnancy; and the fetal and maternal intercurrences. Fetal intercurrences and those in the neonatal period were divided into groups according to the type of problem presented, following eclectic criteria adapted from proposals by various authors [11,20,21,22,23,24].

For the statistical analysis, an electronic database was created. Following this, parametric tests were utilized for comparing normal variables, with Student's "t" test for comparisons between two groups, and variance analysis for comparisons between three or more groups. When the distribution was not normal, we utilized non-parametric tests, with
the Mann-Whitney test for comparisons between two groups, and the Kruskal-Wallis test for comparisons between three or more groups. Proportions were compared using the $X^2$ test, accompanied by Fisher's exact test. Differences in which the probability of rejection of the null hypothesis was less than 5% ($p<0.05$) were considered to be statistically significant.

RESULTS

Fifteen (11.7%) placentas presented a diagnosis of villositis. The data in Table 1 represent the parameters analyzed in the cases of villositis. Placental villositis was associated with maternal or fetal intercurrences in 86.7% of the cases. In the cases of villositis, ten (66.7%) children presented intercurrences in the perinatal period, as follows: six (40.0%) cases of stillbirth ($p=0.003$), two (13.3%) cases of fetal distress, one (6.7%) case presenting serology positive for Cytomegalovirus, and one (6.7%) case presenting signs of organ immaturity. In only five (33.3%) children with villositis did we not encounter intercurrences in the perinatal period (Table 2). The immunohistochemical evaluation for infectious agents was positive in one (6.7%) case for Toxoplasma gondii (Fig. 1, E,F). The other cases (93.3%) were negative for all the agents tested, and were classified as villositis of unknown etiology.

The mothers' ages in the cases in which villositis was diagnosed were significantly greater than in the cases without placental pathological alteration ($p=0.012$). There was a statistically significant difference in relation to gender, between the cases with and without villositis ($p=0.028$). Of the fifteen children in whose placenta a diagnosis of villositis was made, twelve (80.0%) were female. The fetal weight of the full-term children in whose
placenta the diagnosis of villositis was made was less than that of the children whose placentas were considered normal.

With regard to the intensity of the inflammatory process, six (40.0%) cases were classified as mild villositis; six (40.0%) as moderate villositis; and three (20.0%) as severe villositis (Table 2).

There was a statistically significant relationship between the stillbirths and the finding of villositis in the placentas analyzed ($p = 0.003$). Among the stillbirths, sixteen cases (12.5%), the lesion most frequently encountered in the placenta was villositis (37.5%).

Among the five children who presented villositis but did not have intercurrences during the perinatal period, four were twins. There was a statistically significant relationship between gemelarity and villositis ($p = 0.017$). In the twins group (7.8%), the lesion most frequently encountered was villositis, occurring in four cases (40.0%); two cases had serious chorioamnionitis (20.0%); three cases had acute deciduitis (30.0%); and in one (10.0%) case we did not find any placental pathological alteration.

Of the cases with villositis analyzed, ten (66.6%) presented cells CD68+ inside the villi (Fig. 1; C,D) and five (33.3%) also presented CD68+ cells in the inter villous space. In five (33.3%) we don't find CD68+ cells. In one case (7.7%) we encountered cells positive to PanT in some villi affected. The other cell markers utilized, CD57 and PanB were negative in all the cases analyzed. In the normal cases we don't found CD68+ cells (Fig1., A,B).

**DISCUSSION**

Placental villositis was the second most frequent inflammatory alteration found in our sample, corresponding to 11.7% of the placentas analyzed. In the literature, the
frequency of villositis varies from 2.5% to 10.0% in routinely examined placentas and is associated with an increase in the risk of neonatal morbidity and mortality, with this relationship reaching 100% in some studies [5,8,7,25,26,27].

In 86.7% of our cases, we encountered fetal or maternal alterations associated with placental villositis. Only in one of the cases analyzed did we succeed in defining the etiological agent via immunohistochemistry. These data are in agreement with the observation that, in the majority of cases of placental villositis, a determination of the causal agent cannot be achieved, such that they are classified as villositis of unknown etiology [6,17,26].

In our sample, the maternal intercurrences that attacked the children with villositis ranged from metabolic disturbances like diabetes to infectious alterations like congenital toxoplasmosis, with a diagnosis of infection only being made in 13.3% of the cases. These data are in agreement with reports of cases of villositis of unknown etiology related to recurrent miscarriage, in which the fetuses rarely presented signs of infection but were always affected by other pathological processes [7]. Nonetheless, we had one group of children with villositis in whom we were unable to identify an infectious agent and whose mothers did not present any intercurrence during the gestational period that could explain the appearance of the villositis. What these children had in common was that their mothers were older than the mothers in whom we encountered placental alterations other than villositis. Villositis has been correlated with successive pregnancies or multiparity, and also with recurrent miscarriage [20,28]. Multiparity is commonly encountered among older women, and elevated maternal age is a risk factor for spontaneous abortion [29]. In our data, we did not find a relationship between multiparity and an increase in the diagnosis of
villositis, with the mothers in whom we found villositis having the same degree of parity as
the others. In this way we deduce that the relationship between elevated maternal age and a
diagnosis of villositis must be an additional factor to be taken into consideration in
anatomopathological examinations of placenta from spontaneous abortions or stillbirths.

Another alteration encountered in our study was the relationship between the child
being female and greater frequency of villositis. This relationship was also found in another
study that had the objective of correlating hemorrhagic endovasculitis and fetal and
maternal intercurrences [30]. Our study and this other one had the common feature of
finding inflammatory alterations that were more frequently related to female gender. This
may suggest some correlation between the fetal gender, the production of hormones and
inflammatory phenomena in the placenta.

Villositis of unknown etiology has been seen associated with fetal intercurrences
like prematurity, small fetal size for the gestational age, congenital malformations,
recurrent miscarriage and restricted intrauterine growth [6,9,10,11,12,31]. In our sample,
we found a statistically significant relationship between stillbirth and villositis. The
intensity of the inflammatory process has been correlated with the frequency and severity
of the fetal alterations [12,28]. The greater the intensity of the inflammatory process, the
greater the stillbirth rate was, such that among premature children presenting severe
villositis, the mortality was 100%. In cases where we did not find a fetal intercurrence
associated with villositis, the intensity of the process was mild in 80% of the cases. Such
data demonstrate that the intensity of the inflammatory process is directly related to an
increase in frequency of fetal intercurrences.
We found a statistically significant relationship between gemelarity and a diagnosis of placental villitis. One of the hypotheses for the villitis of unknown etiology is that this is secondary to an attack on the fetal tissue by maternal antibodies, as a kind of rejection [3,13,32]. The finding of a correlation between villitis and gemelarity possibly reinforces this hypothesis, in which it may be an immunological reaction by the mother against the fetus, or by one twin against the other. The intensity of the process differed between twins, and the severity of the inflammatory process presented a correlation with lower fetal weight. These findings had already been observed previously and demonstrate that villitis may affect pairs of twins with varying degrees of intensity and that it is correlated with lower fetal weight [33].

Two explanations have been put forward for the etiopathogenesis of villitis of unknown etiology: a fetal response to microbial antigens or maternal infection; and an immune attack on the placental villi by the maternal cells [32]. The cell types that make up the inflammatory infiltrate in the cases of villitis are CD68+ macrophages, histiocytes and T-lymphocytes CD3+, all having a probable maternal origin [3,13,32]. In our study, the cells that formed the villitis were macrophages CD68+ in the majority of cases, and in one case we encountered T-lymphocytes making up the infiltrate. Nevertheless, inside the villi that were attacked, we encountered other cells that were not of macrophage phenotype CD68, demonstrating that other cells may also form part of the villitis, as shown by other studies [32]. Contrary to descriptions in the literature, in only one case did we encounter cells positive to antibodies against T-lymphocytes [32,34]. Our data are insufficient to conclude whether these cells are of maternal or fetal origin. But we also investigated the presence of B-lymphocytes and "Natural Killer" cells CD57+ and LinB and did not find positivity to these cells in any of the cases, thereby advancing a little further in the search.
for the origin of the cells that form the inflammatory infiltrate in the villositis cases. In addition to this, we agree with other authors that there was no difference in the appearance and nature of the cells in the villositis, between the cases in which we were able to identify the etiological agent and those in which we were not [5,14]. However, in the unique case in which we identified the etiological agent, *Toxoplasma gondii*, we found severe intervillositis in association with the villositis. Although our sample was small, just this one case, the finding of other compartments affected by inflammatory infiltrate associated with villositis is perhaps an indication that in these cases the presence of the etiological agent may be more frequent than in the cases in which we only encountered isolated villositis.

In conclusion, we have described fetal and maternal alterations that are correlated with a diagnosis of placental villositis. The fact that infectious agents were not encountered in the majority of cases may indicate the possibility of an explanation other than infection for the chronic inflammatory infiltrate of the placental villi. Factors like the-fetal gender, maternal age and degree of parity may be related to the etiopathogenesis of the inflammation. We have furthermore brought in new contributions concerning the relationship between the severity of the condition presented by the fetus and the severity of the placental inflammatory process.
REFERENCES


Table 1. Comparison of the villositis cases in relation to cases in which there was no finding of pathological alteration, in placentas from deliveries performed at the School Hospital of the Faculdade de Medicina do Triângulo Mineiro during the period from 1998 to 1999.

<table>
<thead>
<tr>
<th></th>
<th>Normal placentas</th>
<th>Villosite</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>X±SD</td>
<td>M</td>
</tr>
<tr>
<td>MA (years)*</td>
<td>23.8 ± 5.7</td>
<td>19.0</td>
</tr>
<tr>
<td>GA (w)***</td>
<td>39.3 ± 1.6</td>
<td>39.5</td>
</tr>
<tr>
<td>FW (g)****</td>
<td>3144.6 ± 526.9</td>
<td>3125.0</td>
</tr>
<tr>
<td>PW (g)*****</td>
<td>444.0 ± 105.8</td>
<td>450.0</td>
</tr>
</tbody>
</table>

*T = 718.500; p = 0.012; ** T = 333.500; p = 0.004; *** T = 390.500; p = 0.036; **** t = 3.752; p < 0.001.

MA= Maternal age; GA= Gestational age; FW= Fetal weight; PW= Placental Weight; Max= Maxim Value Found; Min= Minimum value found; M±SD= Average ±Standard Deviation
Table 2. Distribution of fetal and maternal intercurrences and the intensity of the inflammatory infiltrate among the cases in which villitis was diagnosed, in placentas from deliveries performed at the School Hospital of the Faculdade de Medicina do Triângulo Mineiro during the period from 1998 to 1999.

<table>
<thead>
<tr>
<th>Placenta morphological alteration</th>
<th>Maternal intercurrence</th>
<th>Fetal intercurrence</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Twin</td>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>Premature delivery</td>
<td>Twin</td>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>Stillborn</td>
<td></td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Fetal asphyxia</td>
<td></td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Stillborn</td>
<td></td>
<td>Severe</td>
</tr>
<tr>
<td>Incompetent cervix</td>
<td>Stillborn</td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Congenital toxoplasmosis</td>
<td>Stillborn</td>
<td></td>
<td>Severe</td>
</tr>
<tr>
<td>Normal</td>
<td>Stillborn</td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Normal</td>
<td>Infection sign</td>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>Normal</td>
<td>Infection sign</td>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>Cord neck entanglement</td>
<td>Stillborn</td>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>Hypertensive syndrome</td>
<td>Twin</td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Hypertensive syndrome</td>
<td>Twin</td>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>Epilepsia</td>
<td>Fetal asphyxia</td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>Toxoplasmosis 1:32000</td>
<td>Twin</td>
<td></td>
<td>Severe</td>
</tr>
</tbody>
</table>

* Villitis X Stillborn: $\chi^2 = 9.073; p = 0.003
The manuscript entitled "Morphological and immunohistochemical analysis of placenta to establish the frequency of villitis and its correlation with poor fetal and maternal outcome." has been read by each of co-authors who approved its submission for publication. We assure that the manuscript has neither been published nor is it currently under consideration for publication in whole or in part by any other Journal. We transfer the copyright for the Pediatric and Developmental Pathology and the article can be reproduce like published material.

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Fig 1. Morphology of the placental villi after immunohistochemical analysis. The villi without alterations (A) do not present CD68+ cells in their mesoderm (B). The cells that make up the villitis (C) are macrophages in their majority CD68+ (arrow) (D). In one of the cases of villitis (E), we encountered positivity to Toxoplasma gondii (arrow) (F). All the figures on the right side are stained using HE and those on the left side using the PAP method with anti-CD68+ cells antibody (D) and anti-Toxoplasma gondii (F) (X640).
Thickening of the amnion basement membrane and its relationship to placental inflammatory lesions and fetal and maternal disorders.

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Short running title: basal membrane; placenta; fetal and maternal outcome

This work was supported by a grant from Fundação de Amparo à Pesquisa do Estado de
Minas Gerais, Fundação Ensino e Pesquisa de Uberaba, and Conselho Nacional de
Desenvolvimento Científico e Tecnológico.
ABSTRACT: Thickening of the basement membrane of the amniotic epithelium (BMAE) is associated with rhesus incompatibility and polyhydramnios, suggesting a possible relationship between this feature and maternal and fetal disorders. The aim of this study is the morphological and morphometric analysis of the BMAE of the chorionic plate to establish possible correlation between the BMAE thickening and maternal and fetal disorders. Ninety one placentas of infants delivered in Medical Hospital School were studied with Hematoxylin-eosin (HE) and Periodic Acid Schiff (PAS) methods, morphometric and ultrastructural analysis. Of the 91 placentas analyzed, 17 (18.6%) were found normal, with regards to placental morphology, fetal and maternal history. The other 74 (82.3%) had morphologic placenta abnormalities or fetal or maternal disorders. In 35 (38.5%) placentas inflammation was found in various placental compartments and the BMAE thickening was significantly greater in the cases associated with chorioamnionitis and villitis. Clinical maternal disorders were identified in 34 (37.4%) placentas and the BMAE was significantly greater (p=0.027) in mothers with infectious diseases. Fetal disorders were identified in 39 (42.9%) and the BMAE thickening significantly greater in stillborns (p=0.040) as well as congenital infected children (p=0.03). The electron microscopic examination of the BMAE identified a structural alteration and edema of the dense lamina. The thickening of the BMAE was associated with morphologic placenta abnormalities and/or fetal or maternal disorders. The BMAE was identified away from the site of placental inflammation, possibly being a consequence of cytokines, supporting more than a local effect. This could be a new insight into the pathogenesis of fetal and maternal complications associated with inflammatory placental lesions.

Key words: placenta; basement membrane; fetal; maternal; disorders; perinatal
INTRODUCTION

The thickening of the glomerular basement membrane has been correlated with metabolic disease. More recently, thickened basement membrane of the vocal cords has been associated with expected and unexpected infant death and its etiopathogenesis have been study in many different organs [1,2,3].

In the placenta, the thickening of the basement membrane has most frequently been studied in the trophoblast of the chorionic villi in diabetes [4], smoking [5]; prolonged pregnancies [6]; hypertension [7,8,9] and malaria [10]. Thickening of the trophoblast basement membrane has been interpreted as a consequence of hyperplasia of the cytotrophoblast due to hypoxia. In this hypothesis, the cells would increase in number, leading to greater deposition of basement membrane component, thereby provoking the thickening [11].

Few studies reporting the thickening of the basement membrane of the amniotic epithelium (BMAE) in humans were found in the literature. Abnormalities in the amniotic epithelium has been reported in diabetic patients, hydrops fetalis and polyhydramnios [12, 13,14,15].

In diabetic rabbits the thickening of the basement membranes observed in capillaries of the muscle tissue was considered to be due to a process of replacement of dead cells. The basement membrane of the dead cell would serve as a frame for the cell replacement, without the basement membrane being destroyed afterwards, but rather being retained under the basement membrane of the newly formed cell. In this way, the thickening would represent several cell membranes left behind by successive episodes of cell death and replacement [16]. The same authors later demonstrated that the cell replacement occurs by
proliferation or migration of the cells along the pre-existing structure of basement membranes, which may or may not be destroyed afterwards [17].

The aim of this study was to characterize the normal amnion basement membrane of the chorionic plate and correlate thickening with placental abnormalities, maternal and fetal disorders.

METHODS

During one year (1999), ninety-one placentas from deliveries at the School Hospital of the School of Medicine of Triângulo Mineiro, Uberaba, Minas Gerais were sent to the Pathology Department. They are received labeled with mother identification in a saline solution. The placentas that were sent to Pathology were pre selected by the physicians, as they were concerned about some fetal or maternal disorders similar to the recommendation of the College of American Pathologist (CAP). We performed the gross exam and collected ten tissue specimens, two from the margins, one near the umbilical cord insertion, one from the free membranes, two from the umbilical cord extremities and four from random area of the placenta disc. The specimen collected near the umbilical cord insertion was sectioned and a small part was fixed with glutaraldehyde for the ultrastructural analysis, the remainders of the tissues were fixed 10% buffered formalin.

All the cases were first analyzed using the H&E stain. Shiff Periodic Acid was used to measure the thickness of the basement membrane of amniotic epithelium of the chorionic plate by utilizing the morphometry instrument KS 300, Kontron Eletronik ©, Germany. The section utilized to morphometric analysis was taken from the central section, near the umbilical cord, in each placenta. We made five measurements in each field, for a total of one hundred fields measured. The measurements were distributed around the field in the
following manner: one measurement in the center of the field, two at the extremities of the field and the other two at the halfway point between the center and the extremities. The measurement utilized was the arithmetic mean of the measurements made in the 100 fields.

The tissue fixed in glutaraldehyde was processed for analysis under a transmitting electron microscope. Ultrastructural analysis was done in two cases where we encountered thickening of the basement membrane of amniotic epithelium on H&E and in two Normal cases.

The medical records of all the patients were reviewed, with the objective of gathering demographic information relating to maternal age and parity; fetal gender, gestational age and birth weight (Table 1). Fetal and maternal disorders were divided into groups according to the type of problem presented, following eclectic criteria adapted from proposals by many authors [18,19,20,21]. The asphyxia at perinatal period was correlated with low Apgar scores and as we did not performed an autopsy in most of the cases, the cause of perinatal mortality remains unknown (Table 2).

For the statistical analysis, we used Student's "t" test, variance analysis, Mann-Whitney and the Kruskal-Wallis test. Proportions were compared using the \( \chi^2 \) test, followed by Fisher's exact test. The level of significance at the 5% (\( p<0.05 \)) was considered to be statistically significant.

RESULTS

We analyzed ninety-one placentas with regard to the thickness of BMAE (Table 3; 4). Of these placentas analyzed, seventeen (18.6%) did not have placental morphologic or function abnormalities and maternal or fetal disorders during the gestational or perinatal periods and were considered normal. Seventy-four (81.3%) were considered to be altered in
within one or more of these parameters. There was a statistically significant difference in the thickness of BMAE between the cases considered to be normal and altered (p=0.030) (Table 3; Fig. 1).

Of the altered placentas with fetal and/or maternal disorders, we encountered 35 (38.5%) cases of inflammatory infiltrate in one of the placental compartments. There was a statistically significant difference in the thickness of BMAE between the placentas of the group with inflammatory infiltrate and those considered to be normal (p=0.029) (Table 4). Within the compartments where we encountered inflammatory infiltrate, we observed a statistically significant difference in the average thickness of BMAE between the placentas with chorioamnionitis (p=0.013) and those with villitis (p =0.04). We found 4 (4.4) cases, with meconium alterations of the membranes. None of these cases were associated with increase at the basement membrane thickness.

The thickness of BMAE was greater in the placentas associated with fetal disorders in comparison with the Normal group. Nevertheless, it was only in the stillborn group (p= 0.040) that the difference in relation to the Normal group reached statistical significance (Table 5).

With regard to the placentas associated with maternal disorders, the thickness of BMAE was also greater in all groups when compared with the Normal group. In the group with morphological alterations compatible with low placental blood flow (p = 0.006) and in the group with maternal or fetal infection (p= 0.027), the difference was statistically significant (Table 6).

Ultrastructural analysis was done in two cases with thickening of the BMAE, and in two cases from the Normal group with normal thickness of the BMAE on H&E examination. The thickened basement membrane demonstrated increased space between
collagen fibrils. The increased space was focal, and we could find thickened areas intersperse with areas without alterations within a single case (Fig. 2)

DISCUSSION

The aim of this study was to characterize the normal thickness of the amnion basement membrane of the chorionic plate and correlate thickening with placental abnormalities, maternal and fetal disorders. The most frequent lesion we found in the placenta was inflammation. The other groups only had few cases, which did not allow for definition conclusions.

The study showed that thickening of BMAE was significantly correlated with maternal and fetal infection and with hypertensive disorders during pregnancy. Episodes of cell death and lamellar appearance of the trophoblast basement membrane has been reported in ultrastructural studies, both in placental inflammatory processes and in placentas from patients with pregnancy induced hypertension [7,8,10,9]. In patients with malaria, necrosis of trophoblast basement membrane has been described [22]. One possible explanation for the thickening of the basement membrane in our cases, is that the basement membrane of the dead cell is retained and serves as the support for the cell replacement, thereby increasing the thickness of the basement membrane [16,17]. As the thickening encountered in our study was not continuous but localized, we believe that the above explanation could be the etiology of BMAE thickening. However, in our ultrastructure studies we did not find a lamellar appearance but rather “edema” located between the fibrils that make up the dense lamina at the thickened locations.

According to the literature, the basement membrane thickening is the consequence of cell death, frequently secondary to an inflammatory process [10,22]. Edema is one of the initial phenomena in an inflammatory reaction; therefore our findings maybe from an
earlier stage than those previously reported. The evolution of the inflammation in our cases would perhaps culminate in cell death and the formation of the lamellar thickening described in the literature.

Thickening of the basement membrane was associated with inflammatory reaction of the amnion as well as away from the amnion, supporting more than only a local effect. The changes at the amnion basement membrane were the consequence of the action of cytokines released at the inflammatory site, rather than only a local effect of inflammatory cell infiltration itself. The thickening of the BMAE could be a part of the inflammatory placenta lesions and maybe correlated with a bad fetal outcome.

The exchange areas between the mother and the fetus are made up of amnion and chorion in contact with the maternal decidualized endometrium [23], and the syncytiotrophoblast covering the chorionic villi in contact with the maternal intervillous space. This latter is the main exchange route between the child and the mother [24, 25]. We found a statistically significant relationship between villitis, chorioamnionitis and thickening of the amnion basement membrane. In addition to this, the babies with villitis and chorioamnionitis had lower weights than the babies in the Normal group. In previous reports, an association was described between the cytotrophoblast thickening basement membrane and the villitis and villous edema [26]. One possible explanation for the pathogenesis of decrease fetal weight is that inflammation of the exchange areas between the mother and the fetus leads to the accumulation of fluid in the basement membrane, as demonstrated in our ultrastructural results, and thus a decrease in nutritional support to the babies.

In our study, we have demonstrated that the thickening of the basement membrane of amniotic epithelium is associated with inflammation, infection and stillbirths. The
ultrastructural study demonstrated edema as a possible explanation for the thickening, probably an inflammatory reaction, due to release of cytokines at the inflammatory site.
Our acknowledgment for Dr Edwina Popek for the kind review of our translation and for her support during this period.
REFERENCES


Table 1. Demographic analysis.

<table>
<thead>
<tr>
<th>Demographic Patterns</th>
<th>X ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age</td>
<td>37.7 ± 4.5 weeks</td>
</tr>
<tr>
<td>Maternal Age</td>
<td>23.8 ± 3 years</td>
</tr>
<tr>
<td>Fetal weight</td>
<td>2837.0 ± 863.7g</td>
</tr>
<tr>
<td>Primipara</td>
<td>15 (16.48%)</td>
</tr>
</tbody>
</table>

X ± SD = Average ± Standard Deviation; g = grams.
Table 2. Placental morphology categories in relation to fetal and maternal disorders.

<table>
<thead>
<tr>
<th>Placental Disorder</th>
<th>Maternal Disorder</th>
<th>Fetal Disorder</th>
<th>GA</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>39.7</td>
<td>17 (18.7)</td>
</tr>
<tr>
<td>Edema</td>
<td>Normal</td>
<td>Asphyxia</td>
<td>39.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>VDRL +</td>
<td>Stillborn</td>
<td>27.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Chorangiosis</td>
<td>Normal</td>
<td>Normal</td>
<td>40.9</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Malformations</td>
<td>35.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Decreased Placenta Blood Flow</td>
<td>Normal</td>
<td>Normal</td>
<td>35.2</td>
<td>3 (3.3)</td>
</tr>
<tr>
<td></td>
<td>PIH</td>
<td>Normal</td>
<td>38.7</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>PIH</td>
<td>Stillborn</td>
<td>39.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>PIH</td>
<td>Asphyxia</td>
<td>34.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Normal</td>
<td>Cardiopathy</td>
<td>Asphyxia</td>
<td>37.0</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
<td>Normal</td>
<td>40.0</td>
<td>3 (3.3)</td>
</tr>
<tr>
<td></td>
<td>VDRL +</td>
<td>Normal</td>
<td>39.5</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Urinary Tract Infection</td>
<td>Normal</td>
<td>39.0</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Bleeding</td>
<td>Normal</td>
<td>40.2</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Bleeding</td>
<td>Asphyxia</td>
<td>41.3</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Bleeding</td>
<td>Malformation</td>
<td>36.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>PIH</td>
<td>Prematurity</td>
<td>36.5</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Normal</td>
<td>Asphyxia</td>
<td>Normal</td>
<td>40.0</td>
<td>8 (8.8)</td>
</tr>
<tr>
<td></td>
<td>VDRL +</td>
<td>Normal</td>
<td>39.8</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Prematurity</td>
<td>Normal</td>
<td>35.2</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Intervillosis</td>
<td>Normal</td>
<td>Normal</td>
<td>39.5</td>
<td>3 (3.3)</td>
</tr>
<tr>
<td></td>
<td>Treated Toxoplasmosis</td>
<td>Normal</td>
<td>39.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Asphyxia</td>
<td>41.0</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>PIH</td>
<td>Asphyxia</td>
<td>41.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Acute Deciduitis</td>
<td>Normal</td>
<td>Normal</td>
<td>38.7</td>
<td>4 (4.4)</td>
</tr>
<tr>
<td></td>
<td>PIH</td>
<td>Normal</td>
<td>39.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Asphyxia</td>
<td>35.0</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Pneumoniae</td>
<td>Normal</td>
<td>39.6</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Villitis</td>
<td>Normal</td>
<td>Normal</td>
<td>38.3</td>
<td>3 (3.3)</td>
</tr>
<tr>
<td></td>
<td>Abortion</td>
<td>Stillborn</td>
<td>22.0</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>VDRL +</td>
<td>Prematurity</td>
<td>37.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Stillborn</td>
<td>23.0</td>
<td>3 (3.3)</td>
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<tr>
<td></td>
<td>PIH</td>
<td>Normal</td>
<td>39.3</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>CMV IgM +</td>
<td>39.0</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>Normal</td>
<td>Normal</td>
<td>38.1</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td></td>
<td>Pneumoniae + UTI</td>
<td>Normal</td>
<td>38.4</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Treated Toxoplasmosis</td>
<td>Normal</td>
<td>40.6</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>HIV+</td>
<td>HIV+</td>
<td>34.3</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>VDRL+</td>
<td>VDRL+</td>
<td>40.2</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Stillborn</td>
<td>39.1</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>Others</td>
<td>Others</td>
<td>Others</td>
<td>39.0</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>91 (100)</td>
<td></td>
</tr>
</tbody>
</table>

GA = Average of gestational age; UTI = Urinary Infection; HIV = Human Immunodeficiency Syndrome; VDRL = syphilis serology; PIH = Pregnancy Induced Hypertension.
Table 3. Basement membrane amniotic epithelium thickness in the normal cases in relation to altered cases.

<table>
<thead>
<tr>
<th>Group</th>
<th>N (%)</th>
<th>M (μm)</th>
<th>Min (μm)</th>
<th>Max (μm)</th>
<th>X±SD (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered</td>
<td>74 (81.3)</td>
<td>6.6</td>
<td>1.9</td>
<td>16.9</td>
<td>6.6 ± 4.6</td>
</tr>
<tr>
<td>Normal</td>
<td>17 (18.7)</td>
<td>2.2</td>
<td>1.1</td>
<td>9.3</td>
<td>3.9 ± 2.7</td>
</tr>
</tbody>
</table>

T = 522.000; p = 0.030; M= Median; Max= Maximum Value Found; Min= Minimum value found; X±SD= Average ±Standard Deviation
Table 4. Basement membrane amniotic epithelium thickness in the cases with and without inflammation.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N (%)</th>
<th>X±SD(µm)</th>
<th>M(µm)</th>
<th>Min(µm)</th>
<th>Max(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>17 (18.7)</td>
<td>3.9 ± 2.7</td>
<td>2.2</td>
<td>1.1</td>
<td>9.3</td>
</tr>
<tr>
<td>Intervillositis</td>
<td>7 (7.7)</td>
<td>7.4 ± 5.9</td>
<td>6.5</td>
<td>1.5</td>
<td>16.0</td>
</tr>
<tr>
<td>Deciduitis</td>
<td>8 (8.8)</td>
<td>5.5 ± 4.1</td>
<td>4.6</td>
<td>1.2</td>
<td>15.0</td>
</tr>
<tr>
<td>Villitis*</td>
<td>12 (13.2)</td>
<td>6.6 ± 3.8</td>
<td>6.9</td>
<td>1.2</td>
<td>17.0</td>
</tr>
<tr>
<td>Chorioamnionitis**</td>
<td>8 (8.8)</td>
<td>9.5 ± 5.0</td>
<td>8.3</td>
<td>1.7</td>
<td>17.0</td>
</tr>
</tbody>
</table>

*t = 2.162; p = 0.040; ** T = 141.000; p = 0.013; M= Median; Max= Maximum value found; Min= Minimum value found; X±SD= Average ± Standard Deviation; N= number of cases. The percentage is related to the total amount of placentas (N=91; 100%).
Table 5. Basement membrane amniotic epithelium thickens in the groups with and without fetal/neonatal disorders.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>X±SD (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.9 ± 2.7</td>
</tr>
<tr>
<td>Fetal asphyxia</td>
<td>5.6 ± 4.3</td>
</tr>
<tr>
<td>Stillborn*</td>
<td>6.5 ± 3.0</td>
</tr>
<tr>
<td>Premature delivery</td>
<td>2.9 ± 2.3</td>
</tr>
<tr>
<td>Infection sign</td>
<td>8.8 ± 6.7</td>
</tr>
<tr>
<td>Death at the first week of life</td>
<td>5.2 ± 5.5</td>
</tr>
</tbody>
</table>

* t = 2.172; p = 0.040; X±SD = Average ± Standard Deviation
Table 6. Basement membrane amnion epithelium thickness with and without maternal disorders.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>M (μm)</th>
<th>Max (μm)</th>
<th>Min (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.9</td>
<td>9.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Low placenta blood flow**</td>
<td>5.1</td>
<td>10.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Infection Disease *</td>
<td>7.1</td>
<td>17.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Teste de Dunn: p<0.05; M = Median; Max = Maximum value found; Min = Minimum value found; *p=0.027; **p=0.006.
Fig. 1. Morphology of the basal membrane of the amniotic epithelium. The normal aspect of the basal membrane (A) and the thickening of the basal membrane (B) (arrow). All the figures are stained using Peridic Acid Schiff method (X640).
Fig. 2. Ultrastructural analysis of the basal membrane of the amniotic epithelium. The normal aspect of the basal membrane (A), showing the normal dense lamina (*) and the thickening of the basal membrane (B). The fibrils that make up the dense lamina in cases where we encountered thickening were distributed further from each other in comparison to basal membrane of the normal cases (arrow; X 60,000).