Histomorphological Changes in Placentae of Pre-Eclamptic Mothers with Reference to Number of Villous Capillaries

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Abstract
Background: To study the histomorphological changes in placentae of pre-eclamptic mothers and to compare them with placentae of normotensive mothers with reference to number of villous capillaries.

Methods: In this comparative study, one hundred placentae were divided into two groups. Normotensive (N) group included placentae from mothers having normal blood pressure and Hypertensive (H) group included placentae from mothers having pre-eclampsia. After fixation the placentae were divided into four quadrants and 5mm tissue was taken from the center of upper right and lower left quadrants. After tissue processing and staining, the histomorphological changes were studied in both Normotensive and Hypertensive groups.

Results: The number of villous capillaries was increased in hypertensive group. The quantitative difference between number of villous capillaries in normal and hypertensive groups was statistically significant.

Conclusion: Increased number of villous capillaries was observed in (H) group as compared to (N) group that may be the cause or effect of placental hypoxia.

Key Words: Placenta; Pre-eclampsia; Terminal villi.

Introduction
The placenta and umbilical cord form a transport system for substances passing between the mother and fetus. Pregnancy complications like hypertension or gestational diabetes are reflected in placenta Pre-eclampsia develops during pregnancy and remits after delivery, implicating the placenta as a central culprit. Pre-eclampsia is a leading cause of maternal and perinatal mortality. It is an important factor in foetal growth retardation as it is commonly associated with placental insufficiency. It has been recorded that maternal utero-placental blood flow is reduced in pre-eclampsia because of maternal vasospasm. Blood pressure elevation is the most visible sign of the disease, it involves generalized damage to the maternal endothelium, kidneys, and liver, with the release of vasoconstrictive factors being secondary to the original damage. Presumably, there are substances from the placenta that can cause endothelial dysfunction in the maternal blood vessels.

Hypoxia resulting from inadequate perfusion upregulates sFlt-1, a VEGF and PI GF antagonist, leading to a damaged maternal endothelium and restriction of placental growth. Endoglin, a TGF-beta antagonist, is elevated in pregnant women who develop pre-eclampsia. Soluble endoglin is likely upregulated by the placenta in response to an upregulation of cell-surface endoglin produced by the maternal immune system and endothelium. Levels of both sFlt-1 and sEng increase as severity of disease increases, with levels of sEng surpassing levels of sFlt-1 in HELLP syndrome cases. Recent data indicate that Gadd45a stress signaling regulates elevated sFlt-1 expression in pre-eclampsia. Another VEGF antagonist implicated in pathogenesis of preeclampsia is soluble fms-like tyrosine kinase-1.

As natural killer cells are closely involved in placentation and as placentation involves maternal immune tolerance for a foreign placenta, it is not surprising that the maternal immune system might respond negatively to the arrival of some placentae under some circumstances, such as a placenta which is more invasive than normal. Initial maternal rejection of the placental cytotrophoblasts may be the cause of the inadequately remodelled spiral arteries in those cases of pre-eclampsia associated with shallow implantation, leading to downstream hypoxia and the appearance of maternal symptoms in response to upregulated sFlt-1 and sEng. The chorionic villi emerge from the chorion plate and invade the endometrium. They are first seen around the 12th day after fertilization. They destroy...
the uterine decidua and at the same time absorb nutritive materials for the growth of the embryo that allow transfer of nutrients from maternal blood to fetal blood. The chorionic villi are at first small and non-vascular but over time, in normal placenta show gradual but recognizable alterations, from basophilic/hypocellular and nonvascular stroma to basophilic/cellular stroma containing angiogenic cell cords (immature blood vessels), and then to loose, edematous/reticular stroma with mature blood vessels containing vascular lumina and hematopoietic components. Blood is carried to the villi by the paired umbilical arteries. The umbilical arteries are the only arteries in the human body, aside from the pulmonary arteries, that carry deoxygenated blood. The pressure inside the umbilical artery is approximately 50mm Hg.

Inside the placenta, the umbilical arteries connect with each other at a distance of approximately 5mm from the cord insertion in what is called the Hyrtl anastomosis. Subsequently, they branch into chorionic arteries or intra placental fetal arteries. After circulating through the capillaries of the villi, the blood is returned to the embryo by the umbilical veins. Until about the end of the second month of pregnancy, the villi cover the entire chorion, and are almost uniform in size but after this, they develop unequally.

Patients and Methods

Fifty mothers with uncomplicated pregnancy [Normotensive (N) Group] and same number with preeclampsia [Hypertensive (H) Group] were selected from indoor patients of Gynaeology/Obstertrics Department of Holy Family Hospital, Rawalpindi. The criteria for (H) group was blood pressure 140/90 mm of Hg to 160/100 of Hg at two different occasions two hours apart with gestational age 34-38 weeks and (N) group was blood pressure 120 to130/80 throughout pregnancy at gestational age 34-38 weeks. Mothers having history of pre-gestational hypertension and diabetes and babies with congenital abnormalities were excluded. After delivery placenta were collected for gross and morphometric study (Fig. 1). For normotensive group, the bottles were labelled as N/X-Y, where N stands for normotensive, X for case number and Y denotes the region. Similarly for hypertensive group, bottles were labeled as H/X-Y and here H stands for hypertensive group, X for case number and Y for the region of placenta. After fixation, tissue processing was done. Before sectioning, the block trimming was done with sharp knife till the specimen was just exposed. Then it was placed in block holder of rotary microtome and 4-5μm thick sections were made. Sections were taken on slides for H &E stain and PAS stain. Microscopic study was carried out on sections of placenta. Terminal villi were recognized as fringing villi containing capillaries and stroma, completely surrounded by blood. Complete circular cross sections were selected. Detailed examination was done for syncytiotrophoblast, cytotrophoblast, connective tissue stroma and foetal capillaries. Number of capillaries was counted in complete circular cross sections of terminal villi visible in the field (Fig. 2 & 3). Three fields were randomly selected in each slide from A and B regions under X40 magnification and total number of capillaries were noted.

Results

In normotensive group the mean blood pressure of subjects was 115.20±0.6/75.20±0.8 mm of Hg. The subjects were 10 primigravida and 40 multigravida. The mean age of subjects was 25.14±0.429 years. On microscopic examination general arrangement of the placental tissue was similar in both N and H groups. In normotensive group the chorialic plate was made up of simple squamous epithelium, a connective tissue layer having blood vessels and the syncytiotrophoblast facing the intervillus space. The decidual plate had large number of cells with eosinophilic cytoplasm and basophilic nuclei, these were recognized as decidual cells. Mean number of capillaries in A and B regions was 70.00±1.164 and 63.84±1.269 respectively (Fig. 2; Table 2). The quantitative difference between the number of capillaries in A and B region was statistically significant (p<0.001).

In hypertensive group the mean blood pressure was 165.30±0/99.50±0.6mm of Hg. The subjects were 23 primigravida and 27 multigravida. The mean age of the subjects in H group was 23.90±0.376 years and the mean age of the subjects in N group was 25.14±0.429 years. The mean gestational age in H group was 37.22±0.736 weeks and mean gestational age in N group was 38.38±0.124 weeks. The quantitative difference between the age and gestational age in N and H group was statistically significant (p=0.03 and p<0.001 respectively(Table 1).

In microscopic examination the appearance and arrangement of the placental tissue was similar in both groups. The number of terminal villi per low power field in H group was increased and had atrophic capillaries in their core. Increased number of syncytial knots was observed. Sections from two regions A and
B were examined using X 40 objective. Number of capillaries was counted in three different high power fields per slide from two regions A and B (Fig. 3). Mean number of capillaries in H group in A and B regions was 73.18±0.939 and 73.78±1.086, respectively (Table 2). The quantitative difference between the mean number of capillaries in A and B region was statistically insignificant (P = 0.679). In N group mean number of capillaries in A and B regions was 70.00 ±1.164 and 63.84±1.269, respectively (Table 2). On pooling the data from two regions the mean number of capillaries in N group and H group was 133.84±1.875 and 146.96±1.431 respectively (Table 4) (Fig. 4). The quantitative difference between total number of capillaries in N group and H was statistically significant (p<0.001).

**Table 1: Maternal parameters in normotensive (N) and hypertensive (H) group**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N Group Mean±SE (n=50)</th>
<th>H Group Mean±SE (n=50)</th>
<th>Statistical Significance of difference N and H group(p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age (years)</td>
<td>25.14±0.429</td>
<td>23.90±0.37607</td>
<td><strong>p=0.032</strong>*</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>38.38±0124</td>
<td>37.22±0.736</td>
<td><strong>p&lt;0.001</strong>*</td>
</tr>
<tr>
<td>B.P (mm Hg) systolic</td>
<td>115.20±0.699</td>
<td>165.30±0.896</td>
<td><strong>p&lt;0.001</strong>*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>75.20±0.868</td>
<td>99.50±0.642</td>
<td><strong>p&lt;0.001</strong>*</td>
</tr>
</tbody>
</table>

**Table 2: Mean number of villous capillaries in normotensive (N) and hypertensive (H) groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Regions of Placenta</th>
<th>N Group Mean±SE (n=50)</th>
<th>H Group Mean±SE (n=50)</th>
<th>Statistical Significance between N and H groups(p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of villous Capillaries</td>
<td>A</td>
<td>70.00±1.164</td>
<td>73.18±0.939</td>
<td><strong>p=0.036</strong>*</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>63.84±1.269</td>
<td>73.78±1.086</td>
<td><strong>p&lt;0.001</strong>*</td>
</tr>
</tbody>
</table>

**Table 3: Quantitative difference between number of villous capillaries in normotensive (N) and hypertensive (H) groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Regions of Placenta</th>
<th>Statistical Significance between N and H groups(p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of villous Capillaries</td>
<td>A</td>
<td><strong>p=0.036</strong>*</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td><strong>p&lt;0.001</strong>*</td>
</tr>
</tbody>
</table>

*** Statistical difference between the two groups is highly significant with p<0.001; ** Statistical difference between the two groups is moderately significant with p<0.01; * Statistical difference between the two groups is significant with p<0.05.
Discussion

Mean number of villous capillaries in N and H groups were 133.84±1.875 and 146.96±1.431 and were statistically significant (p<0.001). The histological examination of chorionic villi showed an increase number of terminal villi with narrow intervillous spaces and hence in number of capillaries per field. Ishihara has reported increase in the number of terminal villi (p< 0.05) with increase in blood pressure from 100-110mm Hg. She observed short knob like villi which were smaller in diameter and had increased villous branching as well as increased angiogenesis in placenta of pre-eclamptic mothers as a result of utero-placental hypoxia produced by hypertension. 12

There is an immaculate coordination of trophoblast and endothelial cell proliferation and differentiation during early stages of placental development. This is considered to be regulated by locally acting growth factors, that themselves are regulated by partial pressure of oxygen and mechanical stimuli. In pre-eclampsia, the uterine blood vessels do not undergo adequate vascular transformation, so that rate of delivery of oxygenated blood to the fetus falls. It comes to an uterine insufficiency that lead to the seem of placental hypoxia which leads to compensatory mechanisms like increased angiogenesis that leads to increased number of villous capillaries of narrow lumen. 13

Histological examination of terminal villi in H group showed increased number of villous capillaries but number of atrophic capillaries was also high. Atrophic capillaries were found even in placenta with mean diastolic blood pressure of 90mm Hg. The number of atrophic capillaries in terminal villi progressively increased with the increase in severity of pre-eclampsia. Salafia in her study of 75 cases of preeclampsia with a range of blood pressure between 150-110mm Hg has reported increase number of terminal villi with atrophic capillaries. 14 Vascular endothelial growth factor A (VEGF-A) produced by trophoblast and its receptor VEGFR-2 are responsible for the aggregation of haemangiogenic progenitor cells and growth of capillaries. In later stages of preeclampsia, VEGF-A is reduced which may lead to atrophy of capillaries in the core of terminal villi. Barut also mentioned that placental hypoxia and increased VEGF cause increase branching angiogenesis in conditions such as in pre-eclampsia. 15 Increased capillarization of the terminal villi was also reported by Saga at high altitude where cause for this enhanced angiogenesis was hypoxia that does occur in pre-eclampsia. 16

Conclusion

Placenta tries to compensate for deficit caused by hypertension by increasing the villous proliferation as well as by increased vasculogenesis within the villous tree but these compensations fail to cope with increasing demand as pregnancy advances and this adversely affects the pregnancy outcome leading to low birth weight babies.

References