Histomorphological Changes in Placentae of Pre-Eclamptic Mothers with Reference to Vasculosyncytial Membrane Thickness and Syncytial Knot Formation

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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.
Methods: In this comparative study, hundred placentae were taken and divided into two groups. Normotensive group included placentae from mothers having normal blood pressure and hypertensive group included placentae from mothers having pre-eclampsia. Placentae were fixed in normal saline for 48 hours. 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 terminal villi were increased in hypertensive group. The quantitative difference between number of syncytial knots and villous membrane thickness in normotensive and hypertensive groups was statistically significant.
Conclusion: Increased number of syncytial knots was observed along with increased thickness of vasculosyncytial membrane in hypertensive group as compared to normotensive group that may be the cause or effect of placental hypoxia.
Key Words: Placenta; Pre-eclampsia; Syncytial knots; Vasculosyncytial membrane.

Introduction
Pre-eclampsia is pregnancy-induced hypertension in association with significant amounts of protein in the urine with or without pathological edema. Pre-eclampsia affects approximately 3% of all pregnancies worldwide. Although much research into the etiology and mechanism of pre-eclampsia has taken place, its exact pathogenesis remains uncertain. Some studies support notions of inadequate blood supply to the placenta making it release particular hormones or chemical agents that, in mothers predisposed to the condition, leads to damage of the endothelium (lining of blood vessels), alterations in metabolism, inflammation, and other possible reactions.
Syncytiotrophoblastic knots or syncytial knots are aggregates of syncytial nuclei at the surface of terminal villi. In the term placenta, most syncytial knots are thought to be artifacts from tangential sectioning while the minority are syncytial sprouts, bridges, or apoptotic knots. Syncytial knots are consistently present, increasing with increasing gestational age, and can be used to evaluate villous maturity. Increased syncytial knots are associated with conditions of uteroplacental malperfusion and are important in placental examination. The reference data can facilitate histologic assessment of normal placental maturation as well as evaluation of placental morphology in placental malperfusion.
Vasculo-syncytial membranes are localized areas of the placental villous membrane where the thickness of the barrier separating the maternal and fetal circulations is reduced to as little as 1-2 microns. Consequently, they are believed to be important sites for diffusional exchange. The morphological appearances suggest that they are caused by the obstruction of locally dilated segments of the fetal capillaries into the trophoblast layer.

Patients and Methods
Fifty mothers with uncomplicated pregnancy (Normotensive Group) and same number with pre-eclampsia (Hypertensive Group) were selected from indoor patients of Gynaecology/Obstertrics Department of Holy Family Hospital, and Combined Military Hospital Rawalpindi. The criteria for
hypertensive group was blood pressure 140/90 mm of Hg to 160/100 of Hg at two different occasions six hours apart with gestational age 34-38 weeks and for normotensive group was blood pressure 120 to 130/80 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 placentae were collected for gross and morphometric study. Terminal villi were recognized as smallest villi containing capillaries and stroma. In the study of Syncytiotrophoblast, number of syncytial knots was counted randomly in three high power fields per slide from regions A and B at 40X magnification. In the study of vasculosyncytial membrane, thickness of vasculosyncytial membrane was measured in cross sections of three terminal villi per slide in A and B regions under 40X magnification and mean was calculated. For this purpose ocular micrometer was used which was calibrated with standard stage micrometer.

**Results**

In microscopic appearance, arrangement of the placental tissue was similar in both normotensive and hypertensive groups. The number of terminal villi per low power field in hypertensive group was increased and had atrophic capillaries in their core. Increased number of syncytial knots were observed. Complete circular cross-sections of the terminal villi were selected and number of syncytial knots were counted in three different fields in each section.

In hypertensive group, the mean number of syncytial knots in A and B regions was 62.62 + 0.802 and 63.68 + 0.905 respectively (Table-1). The quantitative difference between the mean value of villous membrane thickness in A and B region was statistically insignificant (P >0.05). The mean value of villous membrane thickness in normotensive Group in A and B regions was 2.716 + 0.04 um and 2.844 um respectively (Fig 3&4; Table-2).

On pooling the data from two regions, the mean value of villous membrane thickness in normotensive group and hypertensive group was 2.78 + 0.029 and 3.16 + 0.075 um respectively. The quantitative difference between the mean value of villous membrane thickness in normotensive group and hypertensive group was statistically significant (p<0.05, Table-3)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Regions of Placenta</th>
<th>Normotensive Group Mean ± SE</th>
<th>Hypertensive Group Mean ± SE</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Syncytial Knots</td>
<td>A</td>
<td>35.90 ± 0.973</td>
<td>62.62 ± 0.802</td>
<td>P&lt;.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>34.06 ± 0.755</td>
<td>63.68 ± 0.905</td>
<td>P&lt;.001</td>
</tr>
<tr>
<td>Thickness of Vasculosyncytial</td>
<td>A</td>
<td>2.716 ± 0.0415</td>
<td>3.24 ± 0.775</td>
<td>P&lt;.001</td>
</tr>
<tr>
<td>Membrane (um)</td>
<td>B</td>
<td>2.8440 ± 0.0396</td>
<td>3.08 ± 0.068</td>
<td>P&lt;.001</td>
</tr>
</tbody>
</table>

**Table 1:** Number of syncytial knots & thickness of vasculosyncytial membrane

<table>
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<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of syncytial knots</td>
<td>A</td>
<td>P&lt;.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>P&lt;.001</td>
</tr>
<tr>
<td>Thickness of vasculosyncytial</td>
<td>A</td>
<td>P&lt;.001</td>
</tr>
<tr>
<td>Membrane (um)</td>
<td>B</td>
<td>P&lt;.001</td>
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</table>

**Table 3:** Quantitative difference between syncytial knots & vasculosyncytial membrane thickness in Normotensive and hypertensive groups

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Hypertensive Group Mean ± SE</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synctial Knots</td>
<td>69.96 ± 1.395</td>
<td>126 ± 1.378</td>
<td>P&lt;.001</td>
</tr>
<tr>
<td>Vasculosyncytial membrane</td>
<td>2.78 ± 0.029</td>
<td>3.16 ± 0.075</td>
<td>P&lt;.001</td>
</tr>
</tbody>
</table>
Fig.1. Human placental tissue from normotensive group showing terminal villi (A), Syncytial knots (B) and intervillous space (C)

Fig.2. Human placental tissue from hypertensive group showing terminal villi, Syncytial knots and intervillous space.

Fig 3: Thickness of vasculosyncytial membrane in normotensive group.

Fig 4: Thickness of vasculosyncytial membrane in hypertensive group.

Discussion

Although the pathophysiology of preeclampsia remains undefined, placental ischemia is widely cited as a key factor. During early human pregnancy, cytotrophoblast cells invade the uterine spiral arteries, replacing the endothelial layers of these vessels with the subsequent destruction of the medial elastic, muscular, and neural tissue. By the end of the second trimester of pregnancy, the uterine spiral arteries are lined exclusively by cytotrophoblast, and endothelial cells are no longer present in the endometrial or superficial myometrial regions. This remodeling of the uterine spiral arteries results in the formation of a low resistance arteriolar system with a dramatic increase in blood supply to the growing fetus. In preeclampsia, invasion of the uterine spiral arteries is limited to the proximal decidua, with 30% to 50% of the spiral arteries of the placental bed escaping endovascular trophoblast remodeling. Myometrial segments of these arteries remain anatomically intact and undilated, and adrenergic nerve supply to the spiral arteries is not affected. The mean external diameters of the uterine spiral arteries in women with preeclampsia are less than one half of the diameters of similar vessels from uncomplicated pregnancies. This failure of vascular remodeling prevents an adequate response to increased fetal demands for blood flow that occur as gestation progresses.

The main feature of abnormal placentation is inadequate trophoblastic invasion of the maternal spiral arteries. This results in persistence of muscular and elastic tissues of the media of spiral arteries. As a result, the vessels fail to dilate and remain responsive to vasomotor influences that lead to high resistance low flow chorio-decidual circulation.

The histological examination of chorionic villi, showed an increase in number of syncytial knots, which is a feature of normal pregnancy and syncytial knots bulges slightly from the surface of the terminal villi. Due to high degree of differentiation, the syncytiotrophoblast loses its power of division, so throughout pregnancy, it depends upon cytotrophoblast. This continuous input is counterbalanced by continuous extrusion, by apoptosis of this material in the maternal circulation. This material is packed into membrane sealed structures called syncytial knots.

As oxidative stress has been implicated in the pathophysiology of these disorders, an increased number of syncytial knots were observed when tissue is cultured in hypoxia, hyperoxia or in the presence of Reactive oxygen species (ROS). The increased number of syncytial knots in placentae from pregnancies complicated by pre-eclampsia can be replicated in vitro by ROS or hypoxia, supporting their involvement.
in the pathogenesis of these conditions. 8,9

Studies revealed that immuno-histochemistry for alpha smooth muscle actin showed few contractile cells in normal terminal villi stroma, localized around the fetal capillaries and showed processes in vasculosyncytial membrane. Pre-eclamptic placentae exert increase in number of capillaries presenting alpha SM action advential positive cells. Ultrastructure observation confirmed in pre-eclampsia terminal villi the presence of these processes in vasculosyncytial membrane and also showed thickened trophoblastic membrane. It was demonstrated that important myofibroelastic system is present in terminal villi and that this system is remodeled in pre-eclampsia. 10

Marked thickening of trophoblastic basement membrane is associated with various pathologic conditions such as pre-eclampsia.11 Vasculosyncytial membranes are result of sinusoidal dilatation of the terminal villous capillaries, which bulge against the trophoblastic surfaces and alter them to thin lamellae; the incidence is closely related to fetal villous vascularization. Immature placenta and cases of persisting immaturity show reduced villous capillarization, a paucity of vasculosyncytial membrane. Thickened membrane is found in normal placenta but increased foci occur in pre-eclampsia.12

References

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