A study of histological changes of human placenta in rural population of eastern India

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ABSTRACT

Background: Placenta is essential for maintenance of pregnancy and for promoting normal growth and development of fetus. It forms the morphological record of anatomical condition, intrauterine events and intrapartum events of gestation. Present study has been undertaken to record the data on the morphology and histology of placenta from mothers with hypertension and diabetes.

Methods: This study showed several significant morphological and histological differences in the placenta of the mother with GDM and hypertensive placenta. The histological study of the placenta was done under microscope and number of syncytial knots, cytotrophoblastic cellular proliferation, fibrinoid necrosis, endothelial proliferation, calcified and hyalinised villous spots were noted per low power field in the diabetics and hypertensive group in comparison to control group.

Results: All other parameters including area, thickness, diameter, and circumference of GDM placenta show a significant increase when compared with normal placenta. The gross anatomic features of placenta e.g. infarcted areas, calcified areas and marginal insertion of the umbilical cord in the study group show significant increase in value (p<0.01) in diabetic and hypertensive groups when compared to that of the control or normal group.

Conclusions: In present study we found that hypertensive placentae tend to be slightly smaller in size, weight, volume, area, thickness, diameter, circumference and feto-placental ratio than normal placentae but the parameters were found to be significantly greater than that of normal placentae in case of diabetic placentae. No significant differences were found in umbilical cord insertion. In normal pregnancy cases we found several histological findings which were increased in hypertensive and diabetic cases.

Keywords: Diabetes mellitus, Histology, Morphology, Placenta, Pathology, Pregnancy induced hypertension.

INTRODUCTION

The human placenta is an intrauterine fusion of fetal and maternal tissues for the purpose of physiological transfer of nutrients and oxygen from mother to fetus and transfer of waste products of metabolism from fetus to mother for continuation of fetal life. Placenta is the vital organ for maintaining pregnancy and promoting development of the fetus. The intrauterine existence of fetus is dependent on this vital organ.¹ Placenta is the mirror of maternal and fetal status. Hypertensive disorders in pregnancy like
pregnancy induced hypertension (PIH); preeclampsia; eclampsia; or chronic hypertension aggravated by pregnancy leads to intrauterine growth restriction (IUGR) of fetus, fetal distress or even intrauterine death due to compromised utero-placental blood flow.\textsuperscript{2, 3}

In normal pregnancy the cytotrophoblastic invasion into the spiral arteries of intervillous spaces occur in a retrograde fashion deep into the myometrium, the muscular and elastic tissue coats of arteries and these are replaced by fibrinoid substances which lead to funnelling and dilatation of spiral arteries thus increasing the utero-placental blood flow Broens et al.\textsuperscript{4} However these changes are confined to the decidua only in patients having pre-eclampsia and IUGR which results in varying grades of placental ischemia Grannum PA.\textsuperscript{5}

Pregnancy is also diabetogenic state by virtue of various physiological changes which causes insulin resistance. During normal pregnancy despite increase in plasma levels of insulin, there occurs decreased glucose tolerance by third trimester.

Mothers at risk are those who have positive family of diabetes, history of previous stillborn or previous large baby and polyhydramnios. In gestational diabetes usually, there is delivery of large babies and large placentas. The mother also has high risk of hypertensive disorder, abortion, stillbirth, preterm labour, puerperal sepsis etc.\textsuperscript{6}

Diabetic mother’s placenta is larger in size, pale appearing due to villous edema and it may also have some histological changes commonly found in PIH as diabetes is associated with maternal hypertension too. Neonatal complications like hypoglycemia, respiratory distress syndrome, hyperbilirubinaemia etc may be also found in these cases.\textsuperscript{6, 7, 8}

In conclusion it will be of great clinical importance since if the variations in dimensions such as weight, volume, thickness, diameter and area of placentas are studied along with the birth weight, birth length of babies and neonatal complications if any and a relation established between the placental and fetal parameters. This study will serve greatly the interest of both the mother and her baby.

The information gathered by this study will certainly help obstetricians and pediatricians to take further precautions before, during, and after labour to modify the course of pregnancy as well as the perinatal outcome. The aim and objective of the present study was to study of relevant morphology and histopathological changes in normal, hypertensive and diabetic placenta.

**METHODS**

After acquiring local ethical approval and informed patient consent, the materials of the present study i.e. placentae were collected at random from pregnancy cases attending the department of Obstetrics and Gynaecology at MGM medical college and LSK Hospital, Kishanganj, Bihar, during the period from Jan-2012 to Dec-2012.

**Selection of cases**

A total number of 150 cases were included in this study. All the cases included in this study were booked cases. In each case antenatal check up was done routinely. The cases were then divided into three main groups.

- Pregnancy cases without any complication or = 50
- Normal pregnancy
- Pregnancy cases with PIH = 50
- Pregnancy cases with gestational diabetes = 50

Each group comprised of patients having known LMP (last menstrual period), EDD (Expected date of delivery), blood pressure, blood sugar levels and gestational period ranging from 36 to 40 weeks. In each case antenatal record having patient’s name, identification, age, parity, height and weight was recorded.

**Method of the Study**

Clinical studies were done during pregnancy as well as in labour and post-partum period. At labour room placentae were collected as fresh specimen after delivery and then examination was carried out in the following way by morphological and histological. In some cases, few sections of placentae were kept in 10% buffered formal saline (initial fixation). These specimens were sent to histopathology laboratory for preparing slides. The slides then examined microscopically.

**Histology of placenta**

After external examination was done, multiple sections of placenta were taken by cutting it at an interval of 3 cm. These cut sections were examined for the presence of infarcts, calcification, intervillous thrombosis and cysts. Some of the cut sections were selected and kept for histological examination and fixed in 10% formal saline solution.

The cut sections were again cut in small pieces of 5 mm x 3 mm x 2 mm. These pieces were kept in running water overnight for better fixation. Then dehydration of the fixed tissues was done by dipping them serially in increasing strength of alcohol i.e. 70%, 90% and absolute alcohol for 24 hours and 1 hour respectively. In absolute alcohol 3 changes were to be given each last for 1 hour.

Then tissues were treated with xylene as the de-alcoholising agent ½ an hour each change. The translucency is used as a guide to indicate dealcoholisation. Then the tissues were kept overnight in a paraffin pot at 58°C to 60°C, then 2nd change in paraffin is given for 1 hour. Blocks were then made from
which sections were cut at 5-micron thickness using a rotary microtome. These were fixed a clean glass slides by albumin. The slides were dipped in hot water bath, dried and again placed on a hot plate. After melting of wax the slides were removed from hot plate and then stained.

**The method of staining of slides**

De-paraffinized the section and treat the section with xylene. Immediately after taking it out from the drying oven / hot plate, repeat xylene treatment with agitation (3 to 4 minutes in each) was done.

Then take the section to water through downgraded alcohol bath (100% - 90% - 70%) for 30 to 60 second in each and wash in tape water, rinse in distilled water drain well. Stain with Harris’s haematoxylin solution for 3 to 5 minutes was done and then wash it in running tap water. Quickly dip slides in and out of 0.5% hydrochloric acid and then wash briefly for 30 to 60 seconds. Then dip several times in dilute ammonia water. After that the section was changed to a blue color. Then wash in water and rinse in 95% alcohol.

After that agitate it in eosin staining solution for 10 to 60 seconds and placed it in 70% alcohol (30 to 60 seconds). Finally, clear in xylene (2 changes 30 to 60 seconds in each) and allow the excess xylene to drain out and mount Canada balsam with a cover slip.

**RESULTS**

The collected placentae (for study) were divided into three groups-
- Group A: Normal uncomplicated pregnancy [n=50]
- Group B: Pregnancy associated with pregnancy induced hypertension [n=50]
- Group C: Pregnancy associated with diabetes mellitus [n=50]

Mother included in this study was from different age groups starting from 19 to 38. They were divided into three groups. Percentage of participants in age groups 19-24 yrs, 25-30 yrs and 31-38 years were 30%, 46.66% and 23.33% respectively.

Majority of the study subjects 60 (40%) were between 50-59 kg followed by 33.33% in 60-69 kg category. Mean gestational age in normal, hypertensive and diabetic groups were 38.4, 36.1 and 37 weeks respectively and their percentage was equally 33.33% (Table 1).

**Table 1: The mean gestational age at time of delivery among three groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Types of patient</th>
<th>Mean gestational age at the time of delivery</th>
<th>% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Normal</td>
<td>38.4 weeks</td>
<td>33.33</td>
</tr>
<tr>
<td>Group B</td>
<td>Hypertensive</td>
<td>36.1 weeks</td>
<td>33.33</td>
</tr>
<tr>
<td>Group C</td>
<td>Diabetic</td>
<td>37 weeks</td>
<td>33.33</td>
</tr>
</tbody>
</table>

**Table 2: Placental morphometry in case of normal uncomplicated pregnancies along with birth weight and feto-placental ratio.**

<table>
<thead>
<tr>
<th>Total no. of normal pregnancies</th>
<th>Placental parameters</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Placental weight (gm)</td>
<td>570</td>
<td>340</td>
<td>510.1</td>
</tr>
<tr>
<td></td>
<td>Placental volume (cc)</td>
<td>515</td>
<td>300</td>
<td>431.84</td>
</tr>
<tr>
<td></td>
<td>Placental area (sq.cm)</td>
<td>211.2</td>
<td>112.2</td>
<td>183.65</td>
</tr>
<tr>
<td></td>
<td>Placental thickness (cm)</td>
<td>2.2</td>
<td>0.9</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>Placental diameter (cm)</td>
<td>17.6</td>
<td>12</td>
<td>15.44</td>
</tr>
<tr>
<td></td>
<td>Placental circumference (cm)</td>
<td>55.4</td>
<td>37</td>
<td>48.36</td>
</tr>
<tr>
<td></td>
<td>Birth weight of baby (kg)</td>
<td>3</td>
<td>1.7</td>
<td>2.58</td>
</tr>
<tr>
<td></td>
<td>Feto-placental ratio</td>
<td>5.76</td>
<td>4.76</td>
<td>5.07</td>
</tr>
</tbody>
</table>

**Table 3: Number of cases in each group with percentages in relation to insertion of umbilical cord on the fetal surface.**

<table>
<thead>
<tr>
<th>No. of cases in group</th>
<th>Central type 76-100%</th>
<th>Eccentric type</th>
<th>Medial type 51-75%</th>
<th>Lateral type 26-50%</th>
<th>Marginal type 0-25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal 50 cases</td>
<td>15 (30%)</td>
<td>16 (32%)</td>
<td>3 (6%)</td>
<td>16 (32%)</td>
<td></td>
</tr>
<tr>
<td>Hypertensive 50 cases</td>
<td>16 (32%)</td>
<td>13 (26%)</td>
<td>2 (4%)</td>
<td>19 (38%)</td>
<td></td>
</tr>
<tr>
<td>Diabetic 50 cases</td>
<td>10 (20%)</td>
<td>20 (40%)</td>
<td>5 (10%)</td>
<td>15 (30%)</td>
<td></td>
</tr>
<tr>
<td>Total =150 cases</td>
<td>41 (27.33%)</td>
<td>49 (32.66%)</td>
<td>10 (6.66%)</td>
<td>50 (33.33%)</td>
<td></td>
</tr>
</tbody>
</table>

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The Table 2 shows average placental weight, placental volume and placental diameter in normal uncomplicated pregnancies was 510.1 gms, 431.84cc and 15.44 cm respectively.

The Table 3 shows insertion of umbilical cord on the fetal surface was noted central type in normal pregnancy, hypertensive and diabetic cases with pregnancy by 15 (30%), 16 (32%) and 10 (20%) respectively.

A total of 41 (27.33%) was noted as central type insertion of umbilical cord on the fetal surface.

All other parameters including area, thickness, diameter, and circumference of GDM placenta show a significant increase when compared with normal placenta in Table 4.

<table>
<thead>
<tr>
<th>Placental parameters</th>
<th>Normal cases</th>
<th>Hypertensive cases</th>
<th>Diabetic cases</th>
<th>P-value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental weight (gm)</td>
<td>510.1</td>
<td>493.18</td>
<td>577.9</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Placental volume (cc)</td>
<td>431.84</td>
<td>406.8</td>
<td>494.1</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Placental area (sq cm)</td>
<td>183.65</td>
<td>179.54</td>
<td>219.3</td>
<td>&lt;0.003</td>
<td>HS</td>
</tr>
<tr>
<td>Placental thickness (cm)</td>
<td>1.68</td>
<td>1.54</td>
<td>2.32</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Placental diameter (cm)</td>
<td>15.44</td>
<td>15.08</td>
<td>16.74</td>
<td>&lt;0.018</td>
<td>HS</td>
</tr>
<tr>
<td>Placental circumference (cm)</td>
<td>48.36</td>
<td>46.58</td>
<td>52.06</td>
<td>&lt;0.019</td>
<td>HS</td>
</tr>
<tr>
<td>Birth weight of baby (kg)</td>
<td>2.58</td>
<td>2.37</td>
<td>3.44</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Feto-placental ratio</td>
<td>5.07</td>
<td>4.82</td>
<td>5.96</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
</tbody>
</table>

The Table 5 shows majority of perinatal outcome was term and live deliveries of babies. Early neonatal death was observed two cases each in hypertensive and diabetic group associated with pregnancy.

The gross anatomic features of placentae e.g. infarcted areas, calcified areas and marginal insertion of the umbilical cord in the study group show significant increase in value ($p>0.01$) in diabetic and hypertensive groups when compared to that of the control or normal group (Table 6).

Table 7 shows that common histological findings syncitial knots formation, cytotrophoblastic cellular proliferation and hyalinization of villi was noted 22%, 10% and 12% in pregnancy with hypertension. Similar pattern was also observed in diabetic group.

The important microscopic findings are depicted in Figure 7 of diabetic placenta showed increased syncytial knots, fibrinoid necrosis, decreased VSM, and chorangiosis.
Table 7: Common histological findings in different group.

<table>
<thead>
<tr>
<th>Histological changes in placenta (at term)</th>
<th>Normal cases (%)</th>
<th>Hypertensive cases (%)</th>
<th>Diabetic cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syncitial knots formation</td>
<td>18</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Cytotrophoblastic cellular proliferation</td>
<td>12</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Villous fibrinoid deoosition</td>
<td>14</td>
<td>08</td>
<td>06</td>
</tr>
<tr>
<td>Villous fibrinoid necrosis</td>
<td>04</td>
<td>04</td>
<td>04</td>
</tr>
<tr>
<td>Stromal fibrosis</td>
<td>04</td>
<td>06</td>
<td>04</td>
</tr>
<tr>
<td>Thickening of basement membrane</td>
<td>06</td>
<td>10</td>
<td>08</td>
</tr>
<tr>
<td>Calcification of villi</td>
<td>06</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Hyalinization of villi</td>
<td>08</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Villous solidification</td>
<td>04</td>
<td>08</td>
<td>12</td>
</tr>
<tr>
<td>Hofbauer cells</td>
<td>-</td>
<td>-</td>
<td>02</td>
</tr>
<tr>
<td>Endothelial proliferation</td>
<td>-</td>
<td>06</td>
<td>08</td>
</tr>
</tbody>
</table>

Figure 1: Normal chorionic villi in uncomplicated pregnancy.

Figure 2: Cytotrophoblastic cellular proliferation in placenta of normal pregnancy.

Figure 3: Placental infarction in PIH.

Figure 4: Thickening of basement membrane in placenta of PIH.

Figure 5: Syncitial knots and villous fibrinoid necrosis --pregnancy in placenta of PIH, H and E x 100

Figure 6: Placental infarction in a case of gestational diabetes.
In a diabetic placenta, H and E x 100. The important microscopic findings are depicted in figure 7 of diabetic placenta showed increased syncytial knots, fibrinoid necrosis, decreased VSM, and chorangiosis.

DISCUSSION

The present study was carried out in the department of anatomy as well as in the department of obstetrics and gynecology, MGM Medical College and LSK Hospital over a period of one year (from January 2012 to December 2012). Subjects of this study were selected at random from mothers attending antenatal clinic and also coming for delivery at the department of obstetrics and gynecology. For the purpose of this research works 150 cases of placentae were collected from the labour room and operation theatre as well. Out of these specimens, 50 were collected from normal uncomplicated pregnancy cases, 50 from cases complicated by hypertension and remaining 50 cases were complicated by gestational diabetes mellitus.

It is found that the mothers included in present study were from various age groups, of which the youngest mother was 19 years old and the oldest was 38 years old. For present study purpose, they were broadly classified into three age groups. In the 19-24 years age group there were 45 mothers (30% of cases). In 25-30 years age group there were 70 mothers (46.66% of cases) and in 31-38 years age group there were 35 mothers (23.33% of cases). So, we find that maximum number of cases between 25-30 years age groups mother, which indicates number cases coming for delivery in the hospital in these age groups.

It was shown that average gestational age at the time of delivery was 38.4 weeks in case of normal uncomplicated pregnancy. 36.1 weeks in case of hypertensive mothers and 37 weeks in case of diabetic mothers group. This indicates that normal mothers were delivered closer to term than hypertensive and diabetic mothers.

A study by Ahmed suggests that despite similarities in placental abnormalities, differences in placental pathology may reflect differences in pathophysiology among different types of diabetes. These changes were absent in normal placenta.9 Low oxygen tension is physiological for organogenesis and is a key regulator of cellular events in early trophoblast differentiation.

We came to know the perinatal outcome in different groups of pregnancy cases. Here we studied 150 cases in all. In normal case groups out of 50 babies 45 were born in term and 05 were born in preterm. In hypertensive cases, out of 50 cases 45 were born in term, 03 were born in preterm and 03 were still born all were preterm. In diabetic cases, out of 50 cases 40 were born in term, 06 were born in preterm and 04 were stillborn (01 term, 03 preterm).

Mirchandani et al, Masodkar et al and Avasth et al observed 12%, 11.9% and 12.5% still births associated with PIH.10-12 In present study, IUDs were noted in PIH cases (Table 5). Placental inadequacy and altered placental function may result from primary pathological alterations in the mother, fetus or the placenta ultimately leading to intrauterine growth retardation and IUDs.13

Woods and Malan studied 940 placentae and found no correlation between the birth weight and site of cord insertion.14 But Rath showed statistical significant between marginal attached of cord and low birth weight. From table 7 we find that the average numbers of cotyledons were 16 in the normal groups, 15 in hypertensive groups and 18 in diabetic groups. Infarcted region was 3 in normal groups, 15 in hypertensive groups and 17 in diabetic groups. Area of calcified region was 5 in normal cases, 26 in hypertensive cases and 37 in diabetic cases. Presence of hemorrhage was 3 in normal groups, 19 in hypertensive groups and 03 in diabetic groups.

From table 7 we find the common histological findings which are more or less similar as observed by H Fox.15 Syncitial knots were found 14% in normal, 22% in hypertensive and 20% in diabetic cases. Cytotrophoblastic cellular proliferation was found 12% in normal 10% in hypertensive and 10% in diabetic groups. Villous fibrinoid deposition was found 14% in normal, 08% in hypertensive and 06% in diabetic groups. Villous fibrinoid necrosis was found 04% in normal, 04% in hypertensive and 4% in diabetic groups. Villous solidification was found in 4% in normal, 8% in hypertensive and 12% in diabetic cases. Stromal fibrosis was found 4% in normal, 6% in hypertensive and 4% in diabetic cases. Thickening of basement was found 6% in normal, 10% in hypertensive and 8% in diabetic cases. Calcification of villi was found 6% in normal, 12% in hypertensive and 14% in diabetic cases. Hyalinization of villi was found 8% in normal, 14% in hypertensive and 14% in diabetic cases. Endothelial proliferation was found 6% in hypertensive and 8% in diabetic cases. Hofbauer cells were found in 4% of diabetic cases. Histologically, the placentae from pregnancy complicated
with GDM showed increased incidence of syncitial knots, cytotrophoblastic cell proliferation, and calcification of villi, fibrinoid necrosis, hyalinisation, basement membrane thickening and thickening of the wall of stem arteries. Also, we have seen Hofbauer cells in these placentae. These findings corroborate those of Fox and Perrin Eugene. Daskalakis et al. noted that the presence of degenerative lesions such as fibrinoid necrosis and vascular hyperplasia (chorangiosis) was apparent mainly in the diabetes group.15–18

Syncytial knots are focal aggregation or clumping of syncytial nuclei on the surface of a tertiary placental villous, forming a multinucleated protrusion from the villous face of a tertiary placental villous, forming a multinucleated protrusion from the villous surface. These are small collections of structureless, homogenous, and eosinophilic material within the villous.6 Rath in 1994 stated that in hypertension arrangement of the intracotyledonous vasculature is altered; resulting in low birth weight of the babies.19

A significant increase in syncytial knot formation in placental villi indicates the disturbance in the hormonal factors, which may probably lead to altered blood flow. According to Robertson, the cause of reduction in blood flow is due to vasculopathies of spiral arteries, which in turn causes reduction in the weight of placenta. It has been recorded that maternal utero-placental blood flow is decreased in preeclampsia because of maternal vasospasm. Reduced maternal utero-placental blood flow indirectly leads to constriction of fetal stem arteries.20 Study by Salmani D et al, the histology revealed various structural changes such as significant number of syncytial knots, areas of fibrinoid necrosis, areas of medial coat proliferation of medium sized blood vessels, areas of calcification, and areas of hyalinization (Figure 3, 4, 5, 7).21

A significant increase in syncytial knot formation in placental villi indicates the disturbance in the hormonal factors, which may probably lead to altered morphometry of placenta resulting in PIH in the mother and to low birth weight babies. Microscopic findings of localized fibrinoid necrosis, medial coat proliferation of arteries, and hyalinization depict the mosaicism of placenta and probably the aftermath of hypertension.22,23 Again the mosaicism of the placenta probably leads to placental insufficiency and ultimately to fetal growth retardation, thus creating a vicious cycle.24

On histological observation of placentae, evidence of cytotrophoblastic cellular hyperplasia and patchy necrosis of the villous syncytiotrophoblastic cells are obvious in the study group in comparison to the control group. This is also very much in accordance with the previous studies conducted by Jones and Fox.25 Microscopic findings of localised fibrinoid necrosis, endothelial proliferation of arteries and hyalinisation depict the mosaicism of placenta and probably the aftermath of hypertension. Teasdale and Udainia et al.22,21

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**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the Institutional Ethics Committee

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