Pathological Changes in Placentas of Diabetic Mothers & Its Association with Fetal Outcome

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Abstract: Diabetes mellitus is one of the metabolic disorder, which can affect the pregnant mother as well as the newborn because of its effect on the placenta, the incidence of which is keep on increasing. By studying the placentas of the diabetic mothers and comparing it with the placentas of normal mothers, we can understand the changes that occur in placenta because of diabetes, like increased weight and thickness of the placenta, increase glycogen deposition or reduced VEGF expression which leads to various newborn abnormalities like increase birth weight (macrosomic babies), low APGAR score, neonatal diabetes etc. Here we compared 40 placenta of diabetic mothers with 40 placenta of normal mother and found that diabetes in mothers during pregnancy can cause various changes in the placenta like increase weight and thickness of placenta, increased syncytial knot, villous edema, villous fibrosis, increase in basement membrane thickness and reduced expression of VEGF which can affect the fetal outcome and thus requires further studies at the molecular level for the pathogenesis of these findings which might help in preventing the fetal morbidity and mortality.

Keywords: Diabetes, Immunohistochemistry, Placenta, VEGF

I Introduction

Placenta is a Latin word which literally means a flat plate or cake¹. Placenta is a very complex organ which has a very short life-span of 9 months and serves as a channel between the foetus and the mother for the selective forward transport of gases, nutrients and reverse transport of metabolic waste products². Placenta separates the fetal and maternal circulation via endothelium and syncytiotrophoblast respectively³. The development of placental villous vessels continues throughout the pregnancy and comprises of two stages, vasculogenesis and angiogenesis. The stage of vasculogenesis occurs mainly during the period of first and second trimester, in which the mesenchymal cells of the villous core differentiate into the cords of vascular cells and by the process of dehiscence, it forms the vascular lamina. The cells required for the elongation and widening of vessels and the perivascular cells – pericytes are also derived from the mesenchymal cells. In stem villi, arteries and veins are differentiated from the vessels. The surrounding supporting structures of the walls of the vessels like smooth muscle cells, myofibroblasts and fibroblasts are also recruited from the villous stroma. On the other hand, the stage of angiogenesis takes place during the third trimester. In this process, the already existing stem villous vessels sprouts and give rise to new capillaries, and thus by this way vascularizing the emerging mature intermediate and terminal villi⁴. Metabolic disease associated with pregnancy such as diabetes mellitus and hypertension can affect the components of placenta for e.g. connective tissue component in chorionic villi and the basement membrane lining the chorionic villi⁵. The Center for Disease Control and Prevention (CDC) has shown that the crude incidence of the cases diagnosed with diabetes mellitus has increased, from 3.3 per 1000 to 7.4 per 1000, i.e. 124%, from the year 1980 to 2005 and hence, diabetes mellitus is now considered to be one of the major health problem in our society. Various studies suggested that the increased prevalence of diabetes mellitus (DM) amongst the women of child bearing age is due to increase in sedentary lifestyles, changes in dietary habits and the virtual epidemic of childhood and adolescent obesity⁶.

GDM or Gestational Diabetes Mellitus is defined as variable degree of intolerance to glucose with either onset or first recognition during pregnancy. Maternal glucose intolerance occurs in 3-10% of pregnancies⁷. Pregnancy complications like gestational diabetes are reflected grossly and microscopically in the placenta. Placental examination can yield information about the existence and effects of maternal, placental or fetal disease, the cause of stillbirth, and potential risks in future pregnancies.

The various pathological changes occurring in the placenta of diabetic mothers are considered to be the important risk factors contributing to fetal anoxia and fetal compromise in pregnancy⁸.

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II Materials & Methods
The study was a prospective Case control study which was conducted in Department Of Pathology, Stanley Medical College after collecting the specimen from RSRM Govt Stanley hospital. 80 full term placentas were collected in 10% Neutral buffered formalin immediately after delivery, either following normal delivery or following caesarean section, out of which 40 placentas were from diabetic mothers and 40 were from normal mothers. The clinical history and the weight of the baby were recorded from the hospital record/casesheets. The purpose of this study was only to study our group and to obtain independent information and not to verify the existent information in the literature.

After thoroughly washing the specimens in tap water, weight of the placenta is taken with the help of spring balance weighing scale measuring the weight accurate to grams and thickness of placenta is measured at 3 positions, at center, at the periphery and between center and periphery, with the help of a knife which is then measured with measuring tape and an average value is taken. 2 sections were taken from each placenta and processed with conventional histological technique followed by staining of one section with Hematoxylin & Eosin stain, one with Periodic Acid Schiff (PAS) stain to highlight the thickness of the basement membrane and one section was used for immunohistochemistry using monoclonal antibody against VEGF.

All the slides were examined under light microscope by double blinding the observer. Hematoxylin & Eosin slides were examined for the features like Synctial knots, villous edema, villous fibrosis and fibrinoid necrosis. There is no specific/strict criterias given when to call an increase in Synctial knots, villous edema, villous fibrosis and fibrinoid necrosis, and hence each case was studied and percentage of findings seen in 10 HPF were taken as average number of finding in that case. The PAS slides were examined to assess the thickness of the basement membrane of the villi which was categorized into 5 categories, Hazy (±), trace (+), mild (++), moderate (+++) and severe (++++) . The immunohistochemistry slides were analyzed according to a subjective evaluation of intensity of reaction of VEGF into 4 categories negative (-), mild (+), moderate (++) and severe (+++) in the trophoblastic cells and in endothelial cells.

III Result
On gross examination, we found out that the placentas from diabetic mothers were heavier and thicker than those of normal placenta. The maximum weight of placenta in diabetic mother was 800 gms while in case of normal mother it was 750 grams and the difference between the mean weights of placenta in 2 groups were statistically significant (Table1). The maximum thickness of placenta in diabetic mother was 4.0cms , while in normal mother it was 2.5 cms with a statistically significant difference between the mean thicknesses of placenta in 2 groups (Table1). The difference of average weight of the newborn in diabetic mothers and the normal mothers were also statistically significant (Table1).

Histopathological examination of the sections from the placentas of diabetic mothers showed increased villous edema (Fig.1), villous fibrosis (Fig.2), syncytial knots (Fig.3) and fibrinoid necrosis (Fig.4) as compared to the placentas of normal mother which was found to be statistically significant with a value of p <0.05 using Mann-Whitney U test (Table2).

There is an increase in glycogen deposition in placentas of diabetic mothers with basement membrane thickening as demonstrated by PAS staining, with 15% of diabetic cases showing trace staining (Fig.5), 50% showing mild staining (Fig.6), 30% showing moderate staining (Fig.7) and 5%cases showing strong staining (Fig.8). (Table3).

There is reduced expression of VEGF in the fetal endothelial cells and trophoblastic cells in placentas of diabetic mother as compared to the normal placentas (Table 4 & 5).
IV Figures

Fig. 1 - Villous edema (10x)
Fig. 2 - Villous fibrosis (10x)
Fig. 3 - Increased syncytial knot (10x)
Fig. 4 - Fibrinoid deposition (10x)
Fig. 5 - Trace staining of basement membrane by PAS (10x)
Fig. 6 - Mild Staining of basement membrane by PAS (40x)
Fig. 7 - Moderate staining of basement membrane by PAS (40x)
Fig. 8 - Strong staining of basement membrane by PAS (40x)
Fig. 9 - Strong staining of VEGF in trophoblastic cells (40x)
Fig. 10 - Moderate staining of VEGF in trophoblastic cells (40x)
Fig. 11 - Weak staining of VEGF in trophoblastic cells (40x)
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V. Tables

Table 1 - Mean and p value for the placental weight, central thickness and weight of the baby in normal and diabetic mothers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Mean±SD</th>
<th>Diabetic Mean±SD</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Placental weight</td>
<td>469.63±88.39</td>
<td>563.75±96.78</td>
<td>.000</td>
</tr>
<tr>
<td>Central thickness</td>
<td>1.79±0.37</td>
<td>2.51±0.57</td>
<td>.000</td>
</tr>
<tr>
<td>Baby weight</td>
<td>2.82±0.36</td>
<td>3.14±0.35</td>
<td>.000</td>
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</tbody>
</table>

Table 2 – Mann Whitney U test for comparison of histopathological changes in placenta of diabetic and normal mother

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Mean of rank</th>
<th>Diabetic Mean of rank</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villous edema</td>
<td>25.44</td>
<td>55.56</td>
<td>0.000</td>
</tr>
<tr>
<td>Villous fibrosis</td>
<td>28.58</td>
<td>52.43</td>
<td>0.000</td>
</tr>
<tr>
<td>Syncitial knots</td>
<td>24.15</td>
<td>56.85</td>
<td>0.000</td>
</tr>
<tr>
<td>Fibrinoid necrosis</td>
<td>22.53</td>
<td>58.48</td>
<td>0.000</td>
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</table>

Table 3 – PAS staining in placentas of diabetic mother and diabetic mother

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PAS</th>
<th>HAZY</th>
<th>Count</th>
<th>% within GROUP</th>
<th>DIABETES</th>
<th>NORMAL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trace</td>
<td>0%</td>
<td>17</td>
<td>0%</td>
<td>42.5%</td>
<td>21.3%</td>
<td></td>
<td></td>
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<tr>
<td>Mild</td>
<td>20%</td>
<td>20</td>
<td>100.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>8%</td>
<td>5</td>
<td>100.0%</td>
<td></td>
<td></td>
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</tr>
</tbody>
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Table 4 – VEGF staining in trophoblastic cells of placentas of diabetic mother and normal mother

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DIABETES</th>
<th>NORMAL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEEK INTENSITY (0.5)</td>
<td>Count</td>
<td>% within GROUP</td>
<td>Count</td>
</tr>
<tr>
<td>Strong Intensity</td>
<td>12</td>
<td>30.0%</td>
<td>3</td>
</tr>
<tr>
<td>Moderate Intensity</td>
<td>15</td>
<td>37.5%</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.0%</td>
<td>40</td>
</tr>
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</table>

Table 5 - VEGF staining in endothelial cells of placentas of diabetic mothers and normal mothers

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DIABETES</th>
<th>NORMAL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF IN ENDOTHELIAL CELLS</td>
<td>NEGATIVE (0)</td>
<td>Count</td>
<td>% within GROUP</td>
</tr>
<tr>
<td>Week Intensity (0.5)</td>
<td>27</td>
<td>67.5%</td>
<td>1</td>
</tr>
<tr>
<td>Moderate Intensity</td>
<td>3</td>
<td>20.0%</td>
<td>1</td>
</tr>
<tr>
<td>Strong Intensity</td>
<td>1</td>
<td>2.5%</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.0%</td>
<td>40</td>
</tr>
</tbody>
</table>

VI Discussion

Diabetes is now-a-days one of the leading metabolic disorder in the world which has the effect on all the organs of the body, including the temporary organ like placenta and thus can have adverse fetal outcome and thus is of serious concern. The increased placental weight and thickness in diabetes may be because of reactionary hyperglycemia in fetuses of diabetic mothers which leads to compensatory hyperplasia of the villous structure and fetal macrosomia. Another factor which leads to villous hyperplasia could be because of vascular compromises in diabetes mellitus which causes low oxygen tension in chorionic villous blood. Weight of the newborn baby depends directly on the environment it experienced during the intrauterine life. In case of gestational diabetes mellitus, glucose crosses the placental barrier and causes fetal hyperglycemia which in turn stimulates the pancreatic islet cells and leads to fetal hyperinsulinemia, and as insulin itself is an anabolic hormone can lead to fetal morbidity and mortality, mainly in association with fetal macrosomia. Burstein et al in the year 1963, observed that placentas from the diabetic patients had marked increase in syncytial knotting. In 2014, Rafah Hady Lateef Al-Mamori in his study showed that there was an increased number of syncytial knots in the terminal villi in the placentas of diabetic mothers controlled by insulin. Villous edema is defined as accumulation of fluid in the interstitium of the villi with disruption and replacement of intravillous cellular architecture. As hyaluronic acid molecules have the property to retain water, it was concluded that, the presence of abnormal deposits of mucopolysaccharides in the villous stroma can lead to the appearance of the true villous edema in placentas of diabetic mothers. Burstein et al, in 2012, Lavinia Gheorman et al, and in 2014, Rafah Hady Lateef Al-Mamori in their respective studies showed that there was an increased incidence of villous edema in the placentas of diabetic patients as compared to normal patients. In 2015, Soad A. Treesh et al also mentioned in their study, an increase in the incidence of villous stromal fibrosis which was demonstrated with the help of Masson Trichrome stain.

Fibrinoid is a non-cellular homogenous eosinophilic material seen in placenta. Fibrinoid necrosis also referred as intravillous fibrinoid is a fibrinoid patch that replaces the villous stroma predominantly, the chorionic villi. In the year 2012, Lavinia Gheorman et al noticed an increased incidence of fibrinoid necrosis in 47% of diabetic cases. Liebhart, in the year 1971, noted that there was marked thickening of basement membrane in diabetic placentas. There as on for this thickening of basement membrane was probably because the secretory products
of trophoblastic cells constitute the basal lamina. The only study in the literature which has given descriptive analysis of the PAS staining, was that of Tewari et al in the year 2011. In the present study only few cases showed strong and moderate staining in diabetic group, as compared to the study done by Tewari et al but the difference between the diabetic group and control group was significant and thus present study showed there was basement membrane thickening in diabetic group because of increased glycogen deposition. The accumulation of glycogen in the placenta of diabetic mothers occurs in marked contrast to other tissues, such as maternal liver, from which glycogen disappears. Glycogenesis and glycogenolysis occurring in the muscle and the liver are under the control of insulin, which regulates the activity of phosphorylase and glycogen synthase. However, in diabetic mothers the glycogen accumulation in the placenta is not dependent on insulin and is related to the extent of maternal hyperglycemia. The increased capacity of placental cells for glucose uptake in diabetes could be related to the expression of GLUTs (glucose carrier transporter isofoms), especially GLUT1. In the year 1996, Carmeliet et al observed in their study that VEGF and its receptors are essential for the development of embryonic vasculature as embryonic death can result from the loss of even a single VEGF allele. VEGF is a potent inducer of endothelial cell proliferation, activation and migration. L. Pietro et al in the year 2010 found out that VEGF was generally detected in muscle cells and vascular endothelial cells of the intermediate villi, in the cytoplasm of the syncytiotrophoblast, in the mesenchymal cells and in the capillary endothelial cells and cytotrophoblastic cells in the basal decidua proximity to the maternal vessels. The women with gestational diabetes showed somewhat different pattern of staining of VEGF. In the present study the expression of VEGF was reduced at both the sites in diabetic patients with the trophoblastic cells showing strong positivity in 32.5% of cases, and the fetal endothelial cells showing strong positivity in only 10% of cases.

VII Conclusion

Diabetes mellitus represents the most common metabolic complication of pregnancy and is associated with increased maternal and foetal morbidity and due to recently adapted western lifestyle; people are more prone for diabetes and related complication. Histopathology along with immunohistochemistry using various new developing markers, would be of great help for fetal well being in diabetic mothers, and might help in preventing potential negative outcomes in pregnancy, moreso if we are able to do study these changes in vivo, which can be a future perspective.

VIII Acknowledgement

I acknowledge the help and support of all the technicians from the Department of Pathology, Stanley Medical College and the postgraduates of Obstetrics and gynaecology of RSRM Govt. Stanley hospital without whom this work would have been impossible.

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