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Altered Placental Histologic Features In Lean And Obese Gestational Diabetic Pregnancies.

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ABSTRACT

The microscopic placental features that are found in gestational diabetes mellitus (GDM) have been widely studied. Current epidemiological trends indicate that GDM is increasingly associated with pre-gravid obesity. Reports on the histological features of placenta in pregnancies complicated by obesity are scarce. The aim of the study was to characterize the changes seen in GDM with obesity and compare them with those seen in only GDM. The placentas of 95 pregnant women were studied. Placentas were collected after delivery from three groups of pregnant women based on BMI and glycemic status. The normal body mass index (BMI) and normoglycemic women were the control group, normal BMI and GDM were lean GDM group and those who had obese BMI and had GDM belonged to the obese GDM group. The GDM groups showed increased degenerative and proliferative histopathological changes such as syncytial knots and fibrinoid necrosis as compared to control. The Obese GDM group showed increased villous fibrosis as compared to lean GDM groups, while the lean GDM group showed higher calcification. These changes are suggestive of the impact of the metabolic alterations on placenta. The interesting inference is that the placenta responds to the pathological insult by concomitant adaptive and degenerative changes.

Keywords: Gestational diabetes- Obesity- Placenta- Histopathology

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INTRODUCTION

The placenta is a feto-maternal organ that plays a vital role in maintaining a uterine environment conducive for fetal growth[1]. The placenta is subject to maternal and fetal metabolic influences that might alter its structure and function. Gestational diabetes mellitus (GDM) and obesity are two complicating conditions of pregnancy that are associated with adverse maternal and fetal outcomes[2]. It has been suggested that the obesity compounds the effect of GDM on pregnancy outcomes[2]. The altered metabolic milieu of these conditions act on the placental structure and function [3,4].

The histological changes in GDM seem to vary with the study conditions and reports list diverse microscopic alterations in the placenta[5]. The major findings in GDM placenta are increase in syncytial knots, fibrinoid necrosis, villous edema, villous fibrosis, crowding, increase in thickness of trophoblastic basement membrane , chorangiosis and villous immaturity[5–10]. The findings in obese placenta are chiefly inflammatory in character such as increased inflammatory lesions and inflammatory cells. There are very few studies that describe the effect of obesity on placental microstructure.

GDM and obesity involve metabolic disturbances[2]. In GDM, there is increased insulin resistance, hyperinsulinemia, hyperglycemia, and oxidative stress[11–13]. These changes may reflect in alterations of placental microstructure in GDM. Obesity is also characterized by glucotoxicity, lipotoxicity, increased oxidative and nitrative stress and in addition, it is an inflammatory state[14–16]. It can be proposed that due to the compounded pathologic biochemical abnormalities in obesity, the placenta may also show greater changes in GDM complicated by obesity as compared to both normal pregnancies and pregnancies with only GDM. This may in turn lead to compromise of placental efficiency which may translate to adverse pregnancy outcomes.

The study was done to evaluate and compare the microstructural changes in the placenta in obese GDM pregnancies as compared to control and lean GDM pregnancies.

MATERIALS AND METHODS:

Placentas were collected immediately after delivery from ninety-five singleton pregnancies (n=95) after obtaining written informed consent. Out of the 95 women, 30 had normal pre-pregnancy BMI and normoglycemia and formed the control group (n=30). Participants who had been diagnosed as gestational diabetics according to DIPSI criteria in the 18-24 week of pregnancy, as having more than 140mg/dl of plasma glucose 2 hours after a 75gm glucose load[17] were enrolled in the study. Pre-pregnancy BMI was calculated by the maternal height and weight recorded during the first ante-natal visit in the first trimester. The BMI cut-off values according to the Indian health ministry guidelines which were based on the WHO recommendations were used to classify women as normal (18.5-22.9) and obese (>25) BMI. The GDM women were categorized into two groups as lean GDM and obese GDM based on whether they had normal or obese BMI respectively. Participants who had pre-existing diabetes mellitus, hypertension, anemia, surgical and medical conditions, smoking habit, and who exceeded the gestational weight gain recommendations were excluded from the study.

Umbilical cord was tied and cut immediately after delivery and the placentas were washed in running tap water and collected and stored in formol saline for 24- 48 hours. The placenta was arbitrarily divided into three concentric zones from centre outward and three full thickness sections were obtained from each zone, namely central, middle and peripheral, for histopathological examination. The three blocks were processed and embedded in paraffin. Sections were cut at a thickness of 4um and stained with Hematoxylin and Eosin (H&E). Slides were stained for Masson's trichrome (MT)and Periodic Acid Schiff (PAS) for histochemical analysis.

The slides were observed and evaluated at 10x and 40x magnification for histological changes. The changes were evaluated chiefly under proliferative, degenerative, inflammatory and circulatory changes[6]. Chorangiosis was described as 10 fetal capillaries in ten or more terminal villi in ten or more fields under 10 x magnification[18]. The commonly reported features were determined from previous studies[6,19] and 12 histological observations were observed. If present, a score of one was assigned to the histological feature and if absent, it was scored as zero. The total histopathological score was calculated for each placenta out of a

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maximum of 12. All observations were carried out single-blind by the investigator and confirmed by an experienced pathologist.

The study was carried out after obtaining institutional ethical committee approval. The study was multicentric and was carried out in three speciality obstetric clinics in an urban city of Tamilnadu in India. Presence of lesions was expressed as percentages and data was tabulated and analysed using the SPSS software (SPSS21.0.0, IBM). Comparison of groups was carried out by the non-parametric Kruskal Wallis test for multiple groups and pairwise comparison was done. Pearson's correlation was used to study the association of total histopathological score with BMI. Differences were considered significant at p value < .05.

RESULTS

Degenerative changes: ***(Figure 1, 2 & 3)***

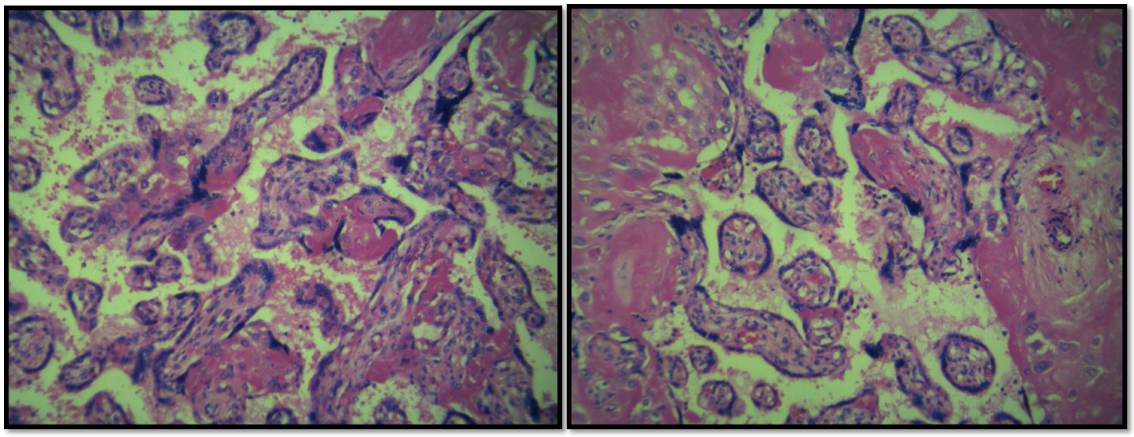

Figure 1: Increased intervillous and intravillous fibrinoid seen in the lean GDM and obese GDM group placentas

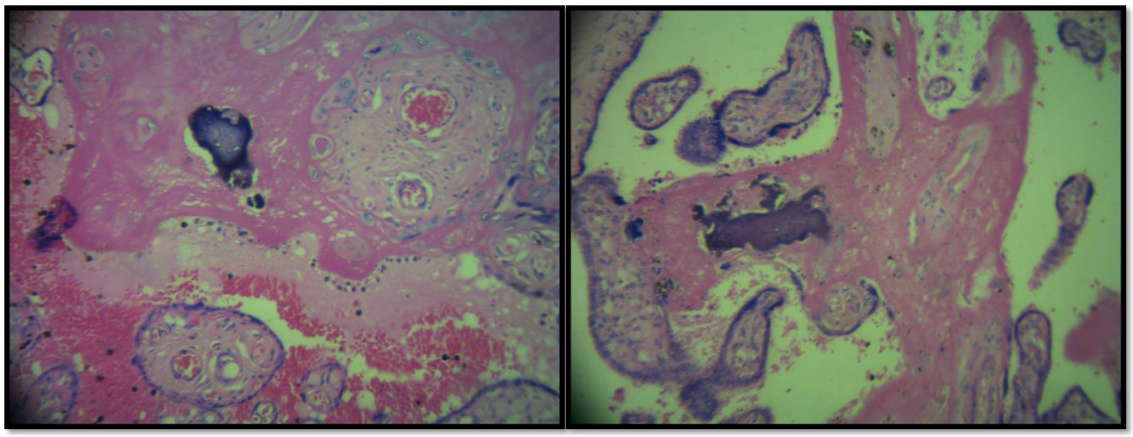

Figure 2: Lean GDM placenta showing calcification and fibrinoid necrosis (10x; H&E stain)

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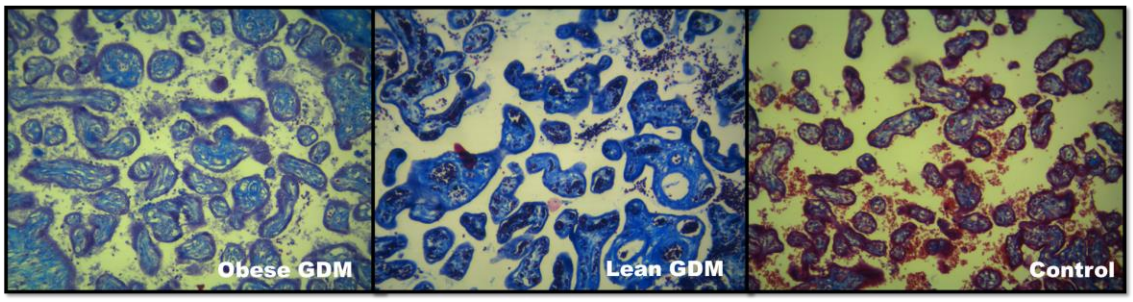

Figure 3: Increased villous fibrosis in obese GDM placenta as compared to control (10x; Masson trichrome)

Hematoxylin and Eosin: There was increased percentage of villous edema and extra and intra-villous fibrinoid necrosis in both the lean GDM and obese GDM groups as compared to the control group (Figure 1). The increase in fibrinoid necrosis was significant. Calcification was significantly increased in the placentas of lean GDM groups, seen in 8 placentas (26.66%), as compared to control group where calcification was noted in 1 placenta (3.33%)(Figure 2). The obese GDM group showed calcification in 5 placentas (14.29%). This was higher than the controls, though the difference was not significant.

Masson's trichrome (Figure 3): Villous fibrosis was seen to be significantly increased in the obese GDM group as compared to the control group, but not in the lean GDM group.

Periodic Acid Schiff Stain: The thickening of the trophoblastic basement membrane was seen in 7 (23.33%) of lean GDM placentas, 5 (14.28%) of obese GDM placentas as compared to 1 (3.33%) control placenta. However, the difference was not statistically significant. There was no incidence of increase in glycogen deposits in the placenta of cases and as well as controls.

Inflammatory changes:

There were no instances of chronic villitis or increase in Hofbauer cells among the groups.

Proliferative changes: ***(Figure 4 & figure 5)***

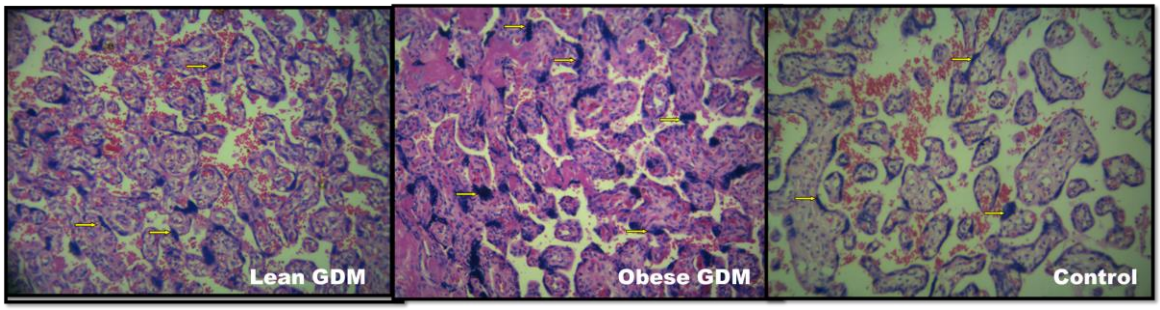

Figure 4: Increased syncytial knots (yellow arrows) from a lean GDM, obese GDM and control group placentas(10x; H& E stain)

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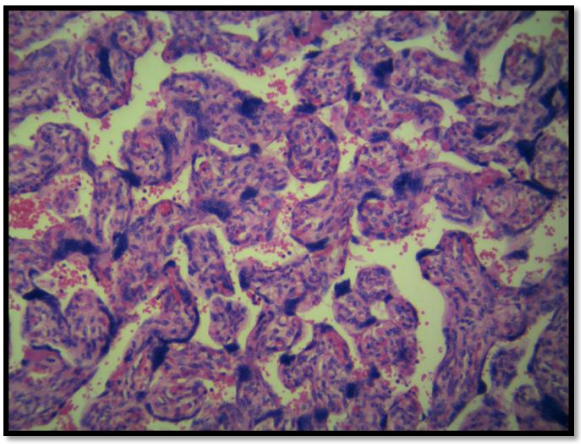

Figure 5: GDM placenta showing hypovascular and cellular villi (10x; H& E stain)

Statistically significant change was seen in the increased syncytial knots were seen in 20 lean GDM placentas (66.67%) and 22 (62.86%) of obese GDM placenta as compared to 10 (28.57%) of control placentas (Figure 3). Villous maldevelopment, showing hypovascular villi with few vasculo-syncytial membranes, was noticed in 2 (6.67%) placentas of lean GDM pregnancies and 5 (14.29%) placentas of obese GDM as compared to 0% of controls (Figure 4). 3 (10%) of the placentas from lean GDM group and 5 (14.29%) of the placentas from obese GDM group showed chorangiosis as compared to 0% of placentas of control group. Difference in villous maldevelopment and chorangiosis were not statistically significant.

Circulatory changes:

Congestion of the fetal capillaries was seen in 7 (23.33%) of the placentas from lean GDM group and in 14 (40%) of placentas of obese GDM group as compared to 4 (13.33%) of control group. There was no incidence of nucleated RBCs as seen under high magnification in any of the groups. Interstitial hemorrhage was seen in 5 (16.66%) and 5 (16.66%) of the lean and obese GDM placentas respectively and in 2 (6.67%) of the normal placentas.

Table 1: Summary of the chief histopathological changes seen in comparison of GDM and control placentas and in between the lean GDM and obese GDM group placentas

Parameter	Observations (Significance at p < .05)
Syncytial knots	Increased in both lean GDM and obese GDM groups
Fibrinoid necrosis	Increased in both lean GDM and obese GDM groups
Calcification	Increased in lean GDM group
Fibrosis	Increased in obese GDM group
Congestion, Chorangiosis, Villous edema, Villous maldevelopment Trophoblastic membrane thickening	Increased from control group in the GDM placentas, but not significant

Comparison of total histopathological scores for each group by Kruskal Wallis, two-tailed test showed significant difference between groups ($p < .05$). Pairwise comparisons showed significant differences in the total histopathological score of lean GDM and control ($p<.05$) and in the scores of obese GDM and control groups ($p<.05$). Pearson's test of correlation showed a weak but significant and positive correlation($r = .261$; $p=.011$) with BMI.

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DISCUSSION

Placental alterations in structure and function occur based on the duration of the maternal disease and the underlying pathogenesis[5,20]. GDM has been associated with histological changes in placenta, though the type of lesions reported vary based on the timing of onset of GDM and the criteria that were adopted in methodology[5,20]. Studies show increase in the prevalence of GDM and this increase has been associated with the increase in obesity, especially in women of reproductive age group. Obesity and GDM are associated and independent risk factors for adverse pregnancy outcomes [2]. Both the conditions are characterized by glucotoxicity, lipotoxicity and increased insulin resistance[2].

A study on women with diabetes classified histopathologica placental changes as inflammatory, circulatory, proliferative and degenerative in nature and listed 22 different types of histopathological lesions under these headings[6]. They concluded that GDM placenta commonly featured 9 of these findings, most of which were degenerative, proliferative and circulatory[6]. A study on gestational diabetes also found predominant circulatory, proliferative and degenerative changes in GDM[19]. In our study, we evaluated changes under the same criteria but included 12 criteria to encompass the other changes such as basement membrane thickening that are often associated with GDM. The commonly reported histological changes seen in obese placenta are seen to be characteristic of inflammation[21–23].

Degenerative changes:

Villous edema has been said to represent fetal ischemia. Several authors have reported increased villous edema in villi of GDM placenta[6–8,24–26]. Some authors did not find any increase in villous edema among villi[9,19]. In this study, villous edema was increased in GDM but not significantly so. Fibrinoid necrosis is a feature of term placenta that is limited to less than 10 percent of the villi. Increased amounts of fibrinoid deposition were seen in GDM placentas[6–9,19,24,27]. The results of this study are in agreement with these findings. Fibrinoid deposition is representative of increased degenerative changes or a result of immunological responses[28]. The presence of fibrosis has been variably reported by authors. There has been report of increased fibrosis as seen by special histochemical staining[8,24]. This is in partial agreement with the findings of our study. The fibrosis was significantly increased in the placentas of obese GDM but not in placentas of lean GDM. Villous fibrosis is associated with hypoxic condition in the placenta[29]. The findings of the study may indicate that the obese GDM placenta are exposed to vascular functional disturbances that may have led to increased hypoxic conditions as compared to the control placentas. The trophoblastic membrane thickening has often been described in gestational diabetes as a result of abnormal mucopolysaccharide synthesis and deposition[9,24,25]. Our study results show an increase in the trophoblastic membrane thickness in a few placentas of the GDM groups as compared to normal, but the finding did not reach statistical significance. Increased calcification was reported in GDM placentas[6,19,26]. Calcification in term placenta has been viewed as a sign of maturation. In the present study, there was significantly increased calcification seen in placenta of lean GDM as compared to controls but this was not seen in obese GDM placentas.

Inflammatory changes:

An increase of Hofbauer cells was discussed by an author in type I and gestational diabetes[20]. In maternal obesity, features of inflammatory change such as increased neutrophils and increase in macrophages was found in the placenta [22,23,30–32]. On the other hand, a report noted the absence of vascular or inflammatory findings from placenta of women who had obese pre-pregnancy BMI[33]. Our findings do not demonstrate any increase in chronic villitis or hofbauer cells in the lean and obese GDM placentas and in the control placentas.

Proliferative changes:

The normal term placenta demonstrates syncytial knots in less than 30% of the placental villi. Increased syncytial knots have been an often reported histopathological finding associated with GDM[8–10]. However, some authors did not report increased syncytial knots in GDM[6,24]. In our study, both the lean GDM and the obese GDM groups show an increase in syncytial knots. Syncytial knots have been attributed to either increased trophoblast synthesis or degenerative changes of the syncytiotrophoblast [34,35]. Villous maldevelopment is characterized by the increased cytotrophoblasts, and decrease in vasculo-syncytial

membranes in terminal villi[1]. Several studies have referred to gestational diabetic placentas as showing features of dysmaturity or abnormality of villous development[25,26,36]. Placentas from GDM pregnancies show villous maldevelopment as compared to control placenta, though the differences were not significant. Chorangiosis has been described in GDM placenta[25,27,37]. Few studies did not find significant changes in chorangiosis in GDM placentas[6,8,19]. The present study shows marginal increased chorangiosis in GDM placenta which was not statistically significant.

Vascular changes:

The study finding reports increased percentage of congestion of fetal capillaries. This is similar to findings of increased congestion of fetal capillaries from gestational diabetic placentas[7,26]. The increased congestion might be a response of the placenta to altered vaso-active molecules seen in hypoxic placenta [38]. Though nucleated RBCs, which are interpreted as a sign of fetal hypoxia, was reported in GDM placenta [27], the present study did not find any instances of fetal nucleated red blood cells.

Studies have established that maternal obesity complicates pregnancy outcomes in GDM patients[2]. Both the conditions are marked by increased insulin resistance glucotoxicity and lipotoxicity[2]. There are a few variations in the pathologic mechanisms underlying GDM and obesity. There is evidence of increased oxidative stress in GDM[39]. In obesity, increased nitrative stress is seen[40,41]. There is increased activation of inflammatory pathways that is associated with increasing BMI[42,43]. The present study shows increase in the total histopathological score in obese and lean GDM placentas as compared to control placentas and positive and weak but significant correlation with BMI.

In summary, the chief findings in lean GDM group and in obese GDM group placentas are degenerative, proliferative and circulatory. Differences between the lean GDM and obese GDM groups are seen in the increased calcification in lean GDM and in the increased fibrosis in obese GDM. The findings suggest that the mechanisms that influence the placenta and more significantly, the nature of the placental responses to the increasing BMI are different in both lean and obese GDM. Further this is supported by the increase in the total histopathological changes seen in lean GDM and obese GDM placentas and the weak positive correlation of the histopathological score with BMI. Further studies that study the biochemical and molecular changes that are associated with increasing BMI in obesity would shed light on the pathological mechanism that contribute to the placental changes and in turn, the adverse pregnancy outcomes.

CONCLUSION

The histological changes are seen in the placentas of lean GDM pregnancies and in the obese GDM pregnancies. The specific histopathologic changes, however, vary between the groups. There is a weak positive correlation of the histologic changes with body mass index. These findings might reflect the multiple mechanisms of pathogenesis that are present in GDM and in obesity and the varied response of the placenta thereof that might explain the increased adverse effects in GDM complicated by obesity.

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