MORPHOLOGICAL AND MICROMETRICAL CHANGES OF THE PLACENTAL TERMINAL VILLI IN NORMAL AND PREGNANCIES COMPLICATED WITH GESTATIONAL DIABETES MELLITUS

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ABSTRACT

AIM & OBJECTIVES

Incidence of gestational diabetes mellitus (GDM) is escalating in Indian women amounting to 10% of the total pregnancies mainly due to the diet, obesity and sedentary life style. Placenta is a crucial organ of intrauterine life. The functional units of the placenta such as chorionic villi, foetal blood vessels, and the syncytial knots are altered histologically in gestational diabetic condition. The present study is undertaken to observe the morphological and micrometrical changes of the GDM and normal placenta.

MATERIAL AND METHODS

Total number of 96 placentas, out of which 48 are GDM and 48 from control were procured for the present study. The placentas were collected from our General Hospital, Nellore, AP. Morphology and micrometry of the placentas were studied.

RESULTS

The shape of placenta was similar in both groups, but the weight in GDM (537.27±131.97 g), diameter (168.2±13.23 mm) and thickness (29.9±3.45 mm) were significantly (P<0.005) increased when compared to control. The mean number (9.01±2.25 mm^3) and diameter (0.08±0.03 mm) of the terminal villi and number of foetal blood vessels (21.76±8.52 mm^3) were found to be increased in GDM, but the diameter of the blood vessel (0.04±0.02 mm) was decreased and highly significant (P<0.001). The syncytial knots and fibrinoid necrosis were also observed in GDM when compared to the normal placenta.

CONCLUSION

The placentas from GDM were observed with significant morphological and histological changes as compared to controls, which may alter the perinatal outcome resulting in macrosomia, congenital malformations and intrauterine growth retardation.

KEYWORDS

Gestational Diabetes, GDM, Placenta, Terminal Villi, Micrometry, Pregnancy.

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INTRODUCTION: Placenta is a complex transient organ interposed between the mother and foetus and is vital for the transfer of nutrients and waste products between them through placental circulation.[1,2] It plays a major role in the foetal development. The architecture of placenta is altered in many maternal diseases such as diabetes mellitus,[3] hypertension,[4] pre-eclampsia[5] and eclampsia.[6] Because of embryonic transitory organ, placenta's systemic study has been abandoned; however, in recent years, it has evoked great interest and much work is being conducted to understand the distinctive biological status of this complex organ.[7]

Diabetes mellitus (DM) is a major health concern in our society which is revealed to be increased from 4.7% in 1980 to 8.5% in 2014.[8] while gestational diabetes mellitus (GDM) in women is known to be increased due to late pregnancy, sedentary life styles, diet and obesity.[9] DM in pregnant women may categorise into pre-gestational diabetes (Previously diagnosed with type-1 or 2 diabetes) and gestational diabetes mellitus (GDM)[10] described as glucose intolerance of varying severity with the onset or first recognition during pregnancy.[11,12,13,14] GDM complicates approximately 2-4% of pregnancies and it is the major cause of prenatal mortality and may increase maternal long term risk of developing type-2 diabetes mellitus (T2DM).[15]

Abnormal glucose tolerance during pregnancy causes numerous placental disturbances resulting in retarded foetal

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growth and development, congenital malformations and intrauterine growth retardation and foetal morbidity and mortality.[16] Other recognised risk factors of GDM are maternal age, parity, obesity (BMI>27), family history of T2DM or GDM, polycystic ovary disease and previous history of macrosomic infant or stillbirth.[17]

So far GDM cases have been studied more enough with the blood samples. Placental tissue study in relation to the micromorphometry seemed to be less in literature though normal foetal growth and development depend largely on placental function. Generally, the terminal villi (TV) of placenta are the final branches which establish the foetal contact for maternal circulation.[18] So studying the placental TV and its surroundings could probably be evaluated accurately through micromorphometry. The aim of the present work is to investigate the morphological and micromorphometrical changes in the GDM and normal pregnancies (controls).

MATERIAL AND METHODS: For the present study, 96 placentas (48 from GDM and 48 form controls) were collected from Obstetrics and Gynaecology Department and were sent to Anatomy Department, Narayana Medical College and General Hospital (NMCGH), Nellore, and the gestational age of the women was between 36-40 weeks. GDM cases were identified if they had two or more blood glucose values greater than or equal to the defined threshold levels (plasma glucose level of ≥140 mg/dL is taken as cut-off for diagnosis of GDM) on a 100-g oral glucose tolerance test (OGTT). As a criterion, only those who delivered full term, singleton live births were selected for this study (n=96) and mothers in the age group of 21-39 years. The exclusion criteria are Type-1 DM, combined diabetes and hypertensions, positive VDRL and severe anaemia were excluded from the study. All participants gave informed written consent to encompass this study, and the study protocol was approved by the Institutional Ethical Committee.

Immediately after the delivery, the placenta with attached membranes and umbilical cord was collected; membranes were trimmed and blood coagulants were removed. The placental weight, diameter and thickness were recorded. For histological studies, full depth tissue samples were placed in 10% formal-saline for 24-48 hours and were subsequently embedded in paraffin. The 4-µm thick sections were stained with Haematoxylin & Eosin, Masson's and Martius Scarlet Blue (MSB) Trichrome. Microscopic examinations were carried out on placental TV randomly on both groups. TV was recognised as smallest villi containing capillary loops without any histological artefacts, as observed under a trinocular microscope (CX31, Olympus, Tokyo, Japan) with 40x objective.

In addition to TV, other pathological findings of the placental villi were also noted. For micrometry, the following parameters were quantified in TV from control and GDM placentas: 1) Numerical density of TV and its blood vessels (BV).[19] and 2) Diameter of TV and its BV[20] were measured using stage, ocular and reticule micrometres. The data were analysed using the computer program, SPSS ver. 17 (SPSS Inc., Chicago, IL, USA). The statistical significance of difference between the two groups was evaluated using the Student's paired t-test.

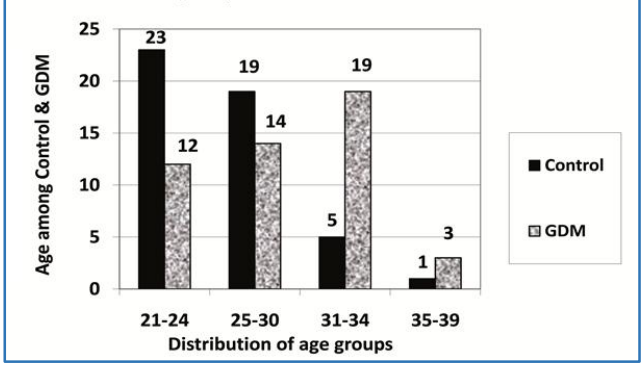
Data were presented as mean±SD. P-value less than 0.05 was considered statistically significant.

RESULTS: In control group, 87.5% in age range of 21-30 and in GDM 54.2%. Age group of 31 to 39 found to be 45.8% in GDM mothers and 12.5% control mothers (Graph 1), showed the GDM mothers were older than the control mothers and was significant (P>0.004). In control group (n=48), the mean placental weight was 412.08 g (range 280-520 g); mean placental diameter was 156.4 mm (range 147-182 mm); mean placental thickness was 18.92 mm (range 14-25 mm); and mean birth weight of babies were 2527.50 g (range 1500-3800). The foetoplacental index was calculated by dividing the mean baby birth weight by placental weight - 6.11 (Table 1).

In GDM group (n=48), the mean placental weight was 537.27 g (range 330-890 g); mean placental diameter was 168.2 mm (range 147-186 mm); mean placental thickness was 29.9 mm (range 24-38 mm) and mean birth weight of babies were 3040 g (range 2500-4000). The foetoplacental index was 6.70 (Table 1). The foetoplacental weight ratio was significantly increased in GDM group than in the control group (P<0.02). Microscopic findings of the placenta between control and GDM were illustrated in Table 2. In control, the mean number of terminal villi was 5.94 with standard deviation of ±1.73 and diameter of the villi was 0.07±0.02. The density of BV and its diameter was found to be 10.70±4.66 and 0.05±0.03 respectively (Table 2).

In GDM cases, villi density was 9.01±2.25 while its mean diameter was 0.08 with ±0.03 standard deviation. The number of BVs were increased which was 21.76±8.52, whereas the mean diameter was 0.04±0.02 and found to be significantly decreased (Table 2). The GDM placenta also showed increased syncytial knots, thickening of vasculosyncytial membrane, vast fibrinoid necrosis and chorangiosis (Fig. 1a-f).

Graph 1 - Distribution of age among the control and gestational diabetes mellitus (GDM).



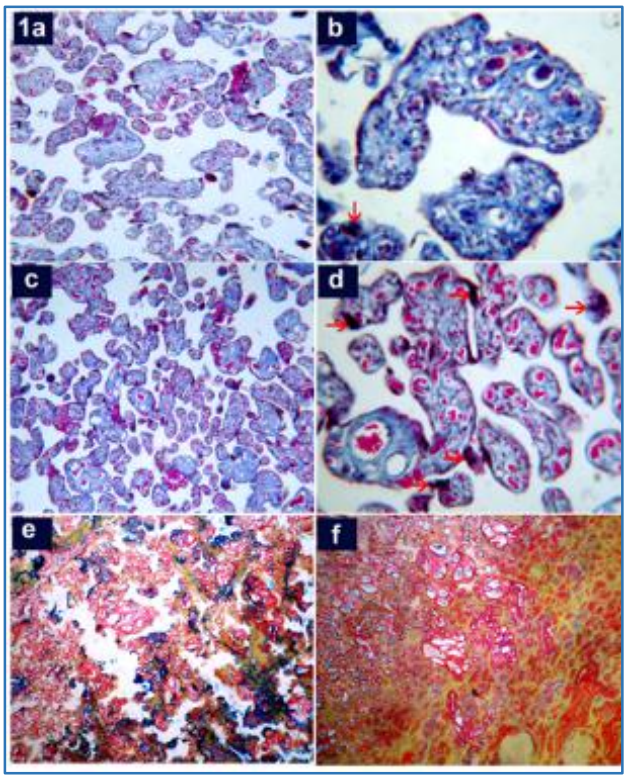


Figure 1a, b, c, d, e, f

Figure 1: Photomicrograph of placental terminal villi (TV) from control and GDM. A - b - low (10X) and high power (40X) magnification of TV from control showing the less number of blood vessels and syncytial knots (Arrow); c - d - low and high magnification of TV from GDM placenta showing the increased number of blood vessels (Chorangiosis) and syncytial knots (Arrows); e - f - low magnification of placental TV from control and GDM respectively showing the fibrin deposition more in GDM than the control, red and yellow colour indicates the fibrin deposition. (Stain: Mallory's trichrome - a - d; MSB trichrome - e - f).

Parameter	Control (n=48)	GDM (n=48)	P-value
Placental Weight (g)	412.08±54.03	537.27±131.97	0.0001**
Placental Diameter (mm)	156.4±6.64	168.2±13.23	0.0001**
Placental Central Thickness (mm)	18.92±4.68	29.9±3.45	0.0001**
Baby's Birth Weight (g)	2527.50±516.05	3040.00±464.09	0.0001**
Foetoplacental Weight Ratio	6.11±0.92	6.70±1.62	0.02*

Table 1: Macroscopic Findings of Maternal, Neonatal and Placental Parameters of Control and Gestational Diabetes Mellitus (GDM)

Values are presented as mean±SD. * significant; **highly significant

Parameter	Control (n=48)	GDM (n=48)	P-value
Density of TV/unit area (mm^3)	5.94±1.73	9.01±2.25	0.0001*
Diameter of TV (mm)	0.07±0.02	0.08±0.03	0.0001*
Density of Blood Vessels in TV/unit area (mm^3)	10.70±4.66	21.76±8.52	0.0001*
Diameter of Blood Vessels in TV (mm)	0.05±0.03	0.04±0.02	0.0001*

Table 2: Micrometrical Findings of Placental Terminal Villi of Controls and Gestational Diabetes Mellitus (GDM)

Values are presented as mean±SD. *highly significant.

DISCUSSION: Placenta is a specialised organ of pregnancy that supports normal growth and development of the foetus.[21] The foetus, placenta and mother form a triad of equilibrium. Disturbances in any of these affect the others.[22] The placenta provides interface between maternal and foetal circulations, and plays a crucial role in protecting the foetus from adverse effects of the maternal diseases. Placenta derived data for subsequent maternal disease is another opportunity to inform risk stratification in GDM population. In the present study, the gross anatomic features of placental weight, thickness and diameter were significantly greater in GDM ($p<0.0001$) as compared to normal and coincides with earlier studies.[23,24]

Weight of the babies was found to be significantly high when compared to the normal cases which can be attributed to one of the impact of the hyperglycaemia in GDM conditions. In the present study, the same has been revealed with the foetoplacental index which was calculated from weight of the baby/weight of the placenta and was found to be significant ($p<0.02$). Histologically, GDM placenta showed increase in villous density (villous hyperplasia) and diameter of TV. The densities of BV were increased (Vascular Hyperplasia or Chorangiosis) than the control.[25,26] But diameters of BV were found to be decreased in TV of GDM than control. According to Calderon.,[21] the increased number of TV could be due to low oxygen (O_2) tension in TV and villous blood. In response to this low O_2 transport, the TV hyperplasia may be partially responsible for the increase in weight of placenta in diabetic group.[27,28] Or it may be attributed to the compensatory mode to macrosomia of the foetus.[29,30] The increased number of BV may be due to the TV hyperplasia and thrive for compensation to the low O_2 environment prevailing in the placenta.

In the present study, as the number of TV was increased, its diameter was also increased. In contrast, when the number of BV increased, its diameter was found to be decreased in GDM when compared to normal. This may lead to the insufficient perfusion of the placental tissues. These changes of the structure and function of placenta in particular to villous capillaries of the TV in placenta would disrupt the environment of foetal development.[31] resulting in hypoxia, the state of OS and consequently lipid peroxidation resulting in cellular damage during diabetic gestation.[32] The weight of placenta is an important and functionally significant parameter as it is related to villous area and foetal metabolism. In this study, the mean placental weight in GDM group was more as compared to control group, and this difference was found highly significant. Similar findings were reported in previous studies of GDM in populations Pakistan,[27] Bangladesh[33] and in India (New Delhi[34] and Kolkata[35]).

In the present study, in addition of increased number of capillaries (Chorangiosis), thickened vessel walls due to endothelial proliferation and thickening of the basement membrane were also identified. Also infraction of the villi, fibrin depositions were found in the placenta on histological observations.[26] The decreased elasticity of vessel wall leads to vascular hardening and increased susceptibility to arteriosclerosis. It has been seen that increased blood glucose levels induce oxidative stress (OS) and subsequent changes of the placental architecture.[36] essentially the vascular properties, which are apparent in GDM.

CONCLUSION: In summary, placental architecture is severely altered in GDM, which reflects over the maternal morbidity and perinatal outcome. Though hypoxic state prevailing in GDM may be one of the degrading factor of the development and function of the placenta, tight glycaemic control can benefit both preconceptionally and during pregnancy in GDM cases. Further exploration in relation to the hypoxia rendering oxidative stress and its underlying pathophysiology are necessary to understand severity and its maternal and foetal outcome in GDM.

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