Original Article

Villous Changes of Placenta in Gestational Diabetes Mellitus and Normal Pregnancy

Suchi HA[1], Sultana Z[2], Foysal AA[3], Biswas AK[4], Mansura F[5], Khatoon M[6]

Abstract:
Gestational diabetes mellitus (GDM) is an important high-risk factor for preterm birth. Again placenta is a mirror, reflects the wellbeing of the fetus and continuously undergoes a change in weight, structure, shape and function. A cross sectional descriptive study done in the department of Anatomy in collaboration with the department of Obstetrics and Gynaecology, M.A.G. Osmani Medical College and Hospital, Sylhet from July 2012 to June 2014. Study was performed on fifty (50) women with singleton term pregnancy. All of them were, had gestational diabetes mellitus, delivered baby with normal vaginal delivery or caesarean section in the Obstetric ward of Sylhet M A G Osmani Medical College Hospital (SOMCH), Sylhet. Fifty control subjects were selected from women with term pregnancy without having GDM, next to index delivery, primi parity and single pregnancy. The samples were divided into control group and study group. The present study shows statistical significant microscopic difference in placenta of mother having Gestational Diabetes Mellitus compared to normal pregnancy. The number of syncytial knots and fibrosis of villi was more in the placenta of Gestational Diabetes Mellitus in comparison with normal pregnancy.

Key words: Gestational Diabetes Mellitus, Placenta, Primipara

Received: July 10, 2018; **Accepted:** October 19, 2018

Introduction:
Gestational diabetes mellitus (GDM) is defined as glucose intolerance that is first detected during pregnancy, and it includes previously undiagnosed diabetes and impaired glucose tolerance (IGT) [1,2,3]. It is a condition in which the glucose level is elevated and other diabetic symptoms (polyuria, polydipsia, and unexplained weight loss) appear[4].

GDM usually becomes apparent during the 24th to 28th weeks of pregnancy. It is associated with both impaired insulin secretion and insulin resistance. Diabetic symptoms usually disappear following delivery[2]. The diabetic environment may have pronounced effects on placental development and functions[5]. Recently it was proposed that these specific effects critically depend on the time period of gestation when the insult of diabetic environment acts upon the placenta[6].

The placenta is a foetal organ situated between mother and foetus. It is essential for foetal growth and development; it reflects the intra-uterine status of the fetus. The architecture of the placenta has been claimed to be changed in maternal diseases like diabetes mellitus. In normal placenta protein synthesis and degradation rates progressively declines over the last weeks of gestation. In the placenta of diabetic mothers, synthesis rate of protein remained unchanged whereas protein degradation stops, as a result there is more placental tissue and thereby increase of placental weight. Degree of metabolic control of the maternal diabetes is important variables to be considered when studying the placenta[7].

Since it is one of the commonest metabolic problems of pregnancy, an accurate diagnosis of gestational diabetes mellitus is essential. High plasma glucose first identified during pregnancy, is critical for the care of pregnant women[8]. About five decades ago, GDM was used to predict pregnant women who were at a higher risk of developing type 2 diabetes mellitus after childbirth[8,9].

Currently GDM is used to predict morbidity index in pregnancy; many trials have confirmed that it is related to multiple maternal and fetal complications like pre-eclampsia, caesarean section and birth injuries and congenital anomalies[9].

[1] Dr. Halima Akther Suchi, Assistant Professor, Department of Anatomy, Mainamoti Medical College, Cumilla, Bangladesh.
[2] Prof. Dr. Zakia Sultana, Professor & Head, Department of Anatomy, Sylhet MAG Osmani Medical College, Sylhet, Bangladesh.
[3] Dr. Abdullah Al Foysal, Assistant Professor, Department of Anatomy, Eastern Medical College, Cumilla, Bangladesh.
[4] Major Anup Kumar Biswas, Assistant Professor, Department of Anatomy, Army Medical College, Bogra Cantonment, Bangladesh.
[5] Dr. Farzana Mansura, Assistant Professor, Department of Anatomy, Northern Medical College, Dhaka, Bangladesh.
[6] Dr. Mahmuda Khatoon, Assistant Professor, Department of Anatomy, Northern Medical College, Dhaka, Bangladesh.

Address of Correspondence: Dr. Halima Akther Suchi, Assistant Professor, Department of Anatomy, Mainamoti Medical College, Cumilla, Bangladesh. Mobile: +8801717540307, Email: ataul.morshed@yahoo.com

Ante-partum maternal hyperglycaemia in diabetic pregnancy is associated with impaired uterine blood flow. Uteroplacental ischaemia could be responsible for most of the observed placental changes. A long term compensatory mechanism owing to secure a sufficient nutrient supply to the foetus plays role behind these changes[7].

In GDM, when the intra-uterine environment for fetus become hostile, the placenta tries to exert its reserve capacity by changing its histomorphological structure, as well as some pathological changes occur that are compounded principally of some disturbances in its normal rate of maturation[10], produces variety of placental abnormalities such as significant thickening of basal membranes of trophoblast, separation of basal membranes in basal capillaries[11] distension and proliferation of endothelial cells, disarrangements of perivascular space and decrease of vascular surface of terminal villi[12,13].

So, gross histomorphological study of the placenta in GDM may provide information of certain alterations which could reflect the fetal condition and which would be of value in terms of predicting fetal outcome.

Materials and Methods:
In this cross sectional observational study 100 human placenta of full term pregnancy having Gestational Diabetes Mellitus delivered in the department of Obstetrics and Gynaecology, M.A.G. Osmani Medical College Hospital, Sylhet; during the study period from 1st July 2012 to 30th June 2014 and fulfilling the inclusion and exclusion criteria included as the study population in this study.

Data were collected by using pre designed questionnaire prepared for the study. Consecutive sampling technique was applied to collect sample. Diagnosis of term pregnancy was established with the help of history of last menstrual period in regular menstruating woman or by ultrasound in early pregnancy. After selection of patient informed written consent was taken from the patient after explaining the purpose of study. Permission from ethical committee of M.A.G. Osmani Medical College Hospital was also obtained.

For each case, maternal age, parity, gestational period will be recorded. After delivery the fetal end of umbilical cord was cut at 5cm away from the umbilicus and ligated. Then the specimen was placed on a flat tray and wash with tap water. After proper washing the specimen was preserved in 10% formal saline in a labeled plastic bucket. Then they were brought to the Department of Anatomy, M.A.G. Osmani Medical College, Sylhet.

Among those 50 placenta of GDM and 50 placenta of normal pregnancy 10 placenta of each group were selected and one tissue block was taken from each of 10 placenta for histological study with a measurement of about 1 cm X 0.5 cm. Tissue blocks were taken from the central portion of cotyledon, in a plane parallel with the maternal and fetal surface, and the area which appeared normal and of least pathologic.

Tissue blocks were fixed in 10% formal saline in a plastic container, after 48 hours the tissue blocks were washed in running tap water, and then all tissue blocks were fixed in Carnoy's fluid overnight. Then the tissue blocks were embedded in paraffin after dehydrating in ascending grade of alcohol and clearing in xylene. Thus paraffin blocks were prepared and were cut at 6-micron thickness with a rotary microtome and mounted on a slide and stained with hematoxylin and Eosin stain. The prepared slides were examined under light microscope and one good slide was selected from each block for final study.

The stroma of mature placental villi usually contain little collagen, in all placenta; some villi contain much collagen are fibrotic villi. The first change in the mechanism of formation of fibrosis appears to be a contraction of the villous capillaries with subsequent loss of their normal sinusoidal pattern. This leads to a reduction of the fetal blood flow, followed by ischemia causes appearance of large amount of glycogen in stromal connective tissue cells, increase in the intercellular ground substance and deposition of collagen, the fetal capillaries of the villi disintegrate and disappear with development of stromal fibrosis.

Diagnosis of GDM:
A 75-g Oral Glucose Tolerance Test (OGTT) was performed in the morning after an overnight fast of at least 8 h, with plasma glucose measurement fasting and at 1 and 2 h, at 24–28 weeks of gestation in women not previously diagnosed with overt diabetes. The diagnosis of GDM is made when any of the following plasma glucose values are exceeded a) Fasting: ≥92 mg/dL (5.1 mmol/L), b) 1h: ≥180 mg/dL (10.0 mmol/L) and c) 2h: ≥153 mg/dL (8.5 mmol/L)[14].

The variables were studied in one hundred terminal villi, one slide was prepared from each blocks of placenta and was examined under light microscope at 40 objectives and 10 eyepieces and expressed in percentage. In some of the slides 100 terminal villi could not be found. In these cases the values were converted into percentage.

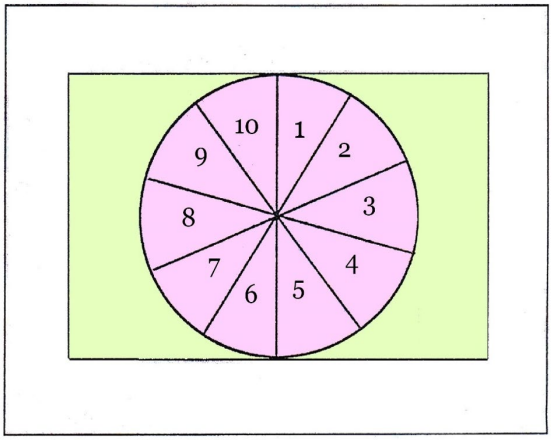

Figure-1: Diagrammatic representation of the method of examining 100 terminal villi on histological slides. Placental tissue section of the slide was divided into 10 divisions with an extra cover slip marked with 10 lines. From each of the 10 divisions, 10 terminal villi were studied.

Results:

Total 100 human placenta were studied in the present study. Fifty placenta were from GDM and 50 placenta were from normal pregnant women. All the placenta were examined under microscope to detect the histological parameters.

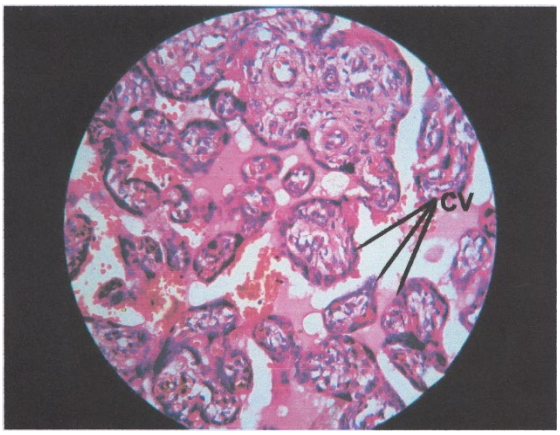

Figure-2: Photomicrography of histological section of placenta showing chorionic villi (black arrow) (X40 objective X10 eyepiece). H & E stain.

Comparison between histology of placenta and maternal age was shown in table-I (figure-4). The maternal age of the studied placenta was ranged from 19 to 30 years with the mean age of 24.64 (SD ± 3.30) years in GDM group; while the age of the patients ranged from 19 to 29 years with the mean age of 24.54 (SD ± 2.56) years in normal pregnancy group. The age of the patients did not differ significantly between GDM group and normal pregnancy group (t=–1.523; p=0.131).

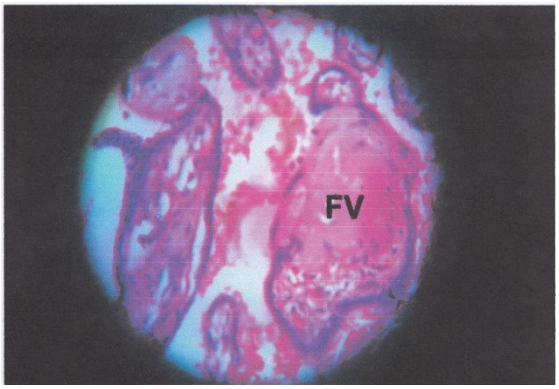

Figure-3: Photomicrography of histological section of placenta showing fibrosis villi (FV), (X40 objective X10 eyepiece). H & E stain.

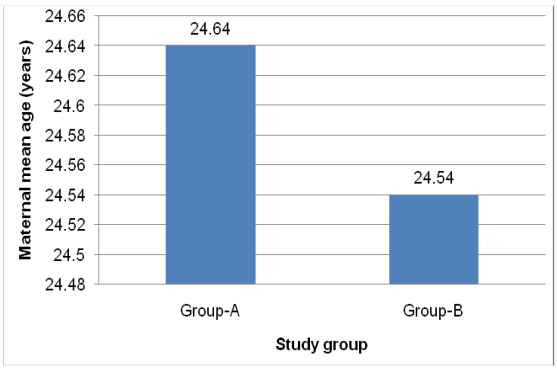

Figure-4: Bar diagram showing Comparison of age of the patients between two study groups

Table-I: Comparison of age of the patients between the two study groups

Maternal age in years	Study group		*p-value
	Group-A (n=50)	Group-B (n=50)	
Mean	24.64	24.54	p=0.131
Standard deviation	± 3.30	± 2.56	
Range	19-30	19-29	

Data were presented as mean ± Standard deviation (SD).
*Unpaired t test was applied to test the level of significance.

Comparison between histology of placenta and Gestational age of mother ranged from 37 to 40 weeks with the mean gestational age of 38.08 (SD ± 1.00) weeks in GDM group; while the gestational age ranged from 37 to 40 weeks with the mean gestational age of 38.34 (SD ± 0.87) weeks in normal pregnancy group. The gestational age did not differ significantly between GDM group and normal pregnancy group (t=–1.371; p=0.171). Comparison of gestational age of the patients between the two study groups was shown in table-II and figure-5.

Table-II: Comparison of gestational age of the patients between the two study groups

Gestational age in weeks	Study group		*p-value
	Group-A (n=50)	Group-B (n=50)	
Mean	38.08	38.34	p=0.171
Standard deviation	± 1.00	± 0.87	
Range	37-40	37-40	

Data were presented as mean ± Standard deviation (SD).
*Unpaired t test was applied to test the level of significance.

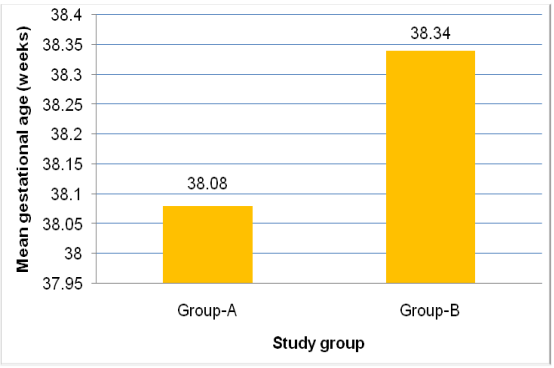

Figure-5: Comparison of gestational age of the patients between the two study groups.

The number of fibrosis of villi ranged from 4 to 16 with the mean fibrosis of villi of 9.80 (SD ± 3.68) in placenta of GDM; while the fibrosis of villi ranged from 2 to 12 with the mean number of fibrosis of villi of 7.90 (SD ± 3.45) in placenta of normal pregnancy. The fibrosis of villi of the placenta of GDM was significantly more than that of normal pregnancy (t=2.662; p=0.009).

Table-III: Comparison of the number of fibrosis of villi of the placenta between two study groups

Number of fibrosis of villi	Study group		p-value
	GDM Group-A (n=50)	Normal pregnancy Group-B (n=50)	
Mean	9.80	7.90	p=0.009
Standard deviation	± 3.68	± 3.45	
Range	4 to 16	2 to 12	

Data were presented as mean ± Standard deviation (SD).
*Unpaired t test was applied to test the level of significance.

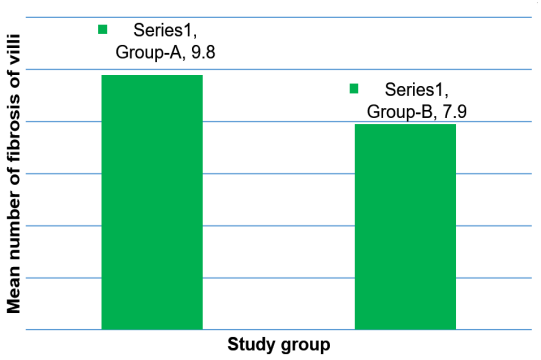

Figure-6: Bar-diagram showing Comparison of number of fibrosis of villi between two study groups

Discussion:

Human placenta is a highly vascular organ. The volume and flow of blood through placenta is kept adequate by its characteristic vascular arrangement. As placenta performs essential abortive, endocrine, metabolic, exchange and excretory functions during pregnancy; and many vascular changes are very likely to affect placental functions and thereby, fetal wellbeing[15].

The present study was carried out on placenta from mothers with gestational diabetes and those of non GDM. The study was aimed at to observe microscopic changes in human placenta of GDM and those of normal pregnant woman. In this study the age of the patients did not differ significantly between GDM group and normal pregnancy group (p=0.131).Other studies suggested that the mean age little bit higher in GDM women than the present study[16,17]. Difference may be due to inclusion of all primi patients in the present study.

In present study the gestational age of the patients did not differ significantly between GDM group and normal pregnancy group (p=0.171). So, in this study gestational age matched. This study correlated with the other studies[16,17].

In this study the fibrosis of villi of the placenta of GDM was significantly more than that of normal pregnancy (p=0.009).So it could be assumed that the number of fibrotic villi was not enough to produce any ill-effect in the placental function in diabetic subjects. Increase in the number of fibrotic villi was observed by various workers in diabetic placenta[18,19,20]. Fox H, stated that fibrosis of the villi in the diabetic placenta may be due to fetal stem artery thrombosis but suggested that the villous fibrosis was without any deleterious effect on the growth and wellbeing of the fetus[18].

This result was correlated with a study done previously, that the fibrosis of villi of the placenta of

GDM was significantly more than that normal pregnancy ($p<0.05$)[17]. But some study reported that the fibrosis of villi of the placenta of GDM did not differ significantly from that of control subject ($p>0.05$)[15,16].

Conclusion:
Microscopic changes like the number of Fibrosis of Villi are significantly more in placenta of gestational diabetic mother.

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Citation of this article:
Suchi HA, Sultana Z, Foysal AA, Biswas AK, Mansura F, Khatoon M. Villous Changes of Placenta in Gestational Diabetes Mellitus and Normal Pregnancy. Eastern Med Coll J. 2019; 4 (1): 26-30.