Histopathological Changes in the Placenta of Pregnant Women with Gestational Diabetes Mellitus and Fetal Outcome

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ABSTRACT

Background: Proper fetal growth depends on proper development and function of the placenta. Gestational diabetes mellitus (GDM) lead to an abnormal placental development and affect its function.

Objective: To study histopathological changes in the placenta of pregnant women with GDM and to compare these changes with that of women of normal pregnancy.

Methods: This was a case-control study carried out at Al-Yarmouk Teaching Hospital, department of obstetrics and gynecology between January 1st 2016 and January 1st 2017. The study included 120 pregnant women, 60 with gestational diabetes mellitus (study group), and 60 women with normal pregnancy (control group), the placental tissues of all pregnant women were examined both grossly and histologically after delivery. The fetal outcomes were noted.

Results: Gross examination of the diabetic placenta showed increased weight, diameter, number of cotyledons, p value (<0.001). Microscopic examination of the diabetic placenta showed the following changes: villous edema, villous fibrin (focal and perivillous) and increased syncytial knotting, p value (<0.001). Fetal outcome include increased birth weight, macrosomia, p value (<0.001), and low Apgar score at 1 minute, p value (0.043) and low Apgar score at 5 minutes, p value (0.022).

Conclusion: Gestational diabetes mellitus lead to changes both grossly and histologically in the placenta and may affect its function and consequently fetal growth and development.

Keywords: Gestational diabetes mellitus, Placenta, Pregnancy.

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Gestational diabetes mellitus (GDM) is defined as impaired carbohydrate tolerance resulting in hyperglycemia, which first develops or becomes diagnosed during pregnancy. Some of these women will, in fact, have previously undiagnosed diabetes, usually type 2. Women developing GDM face similar increased risks as diabetic women in terms of macrosomia and its associated complications[1]. During pregnancy, as gestational age progresses, the size of the placenta increases. There is a rise in the levels of pregnancy-associated hormones[2], accompanied by an increasing insulin resistance[3].

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Adipose tissue produces adipocytokines, including leptin, adiponectin, tumor necrosis factor-α (TNF-α) and interleukin-6, as well as the newly discovered resistin, visfatin and apelin[4,5]. Evidence suggests that one or more of these adipokines might impair insulin signaling and cause insulin resistance[6,7]. Specifically, TNF-α has a potential role in decreasing insulin sensitivity[8].

The placenta at term displays a round disc-like appearance. The average measures of a delivered placenta at term are as follows: diameter 22 cm, central thickness 2.5 cm, and weight 450–500g[9]. Sonographically, the normal placenta is homogenous and 2 to 4 cm thick, lies against the myometrium, and indents into the amniotic sac. The retroplacental space is a hypoechoic area that separates the

myometrium from the placenta's basal plate and measures less than 1 to 2 mm[10]. Like the knots and branches of a tree, the fetal tissues repeatedly branch into smaller and slender villi[9].

Histopathologically, the layer of mononucleated *villous cytotrophoblast* cells is the basal layer of the tree that proliferate throughout gestation and resting on the basal lamina underneath the multinucleated cells of syncytiotrophoblast[11]. Villous cytotrophoblasts do not normally come into direct contact with maternal blood, unless focal damage occurs to the overlying syncytiotrophoblast. If focal areas are lost, for example due to focal necrosis, the deficit is filled with fibrin-type fibrinoid (maternal blood clot product) that covers the exposed cytotrophoblasts[12]. The *syncytiotrophoblast* is a multinucleated layer without lateral cell borders, hence there is a single syncytiotrophoblast covering all villi of a single placenta[11]. Growth and maintenance of the syncytiotrophoblast is dependent on the fusion with the underlying cytotrophoblasts, since syncytial nuclei do not proliferate. Within the syncytiotrophoblast the nuclei, and during maturation, become smaller and denser. These are the typical features of apoptosis, a physiological process in the normal placenta[13].

Syncytial fusion remains critical for maintaining the functional and structural integrity of the syncytiotrophoblast. Although certainly the placenta and cord should be examined in the delivery room , the decision to request histopathological examination should be based on clinical and placental findings[10].

The aims of this study were to study histopathological changes in the placenta of pregnant women with GDM and to compare these changes with that of women of normal pregnancy.

Methods

This study included 120 pregnant women, sixty of them (the study group), diagnosed as having gestational diabetes mellitus by abnormal oral glucose tolerance test (OGTT) another 60 pregnant women with a match gestational age with normal OGTT (the control group) at Al-Yarmouk Teaching Hospital, the department of obstetrics and gynecology, from January 1st 2016 to January 1st 2017. All pregnant women gave their inform consents prior to study. The study was approved by ethical committee in Al-Yarmouk Teaching Hospital.

The inclusion criteria for both groups is singleton viable pregnancy, gestational age from 24 weeks to 40 weeks according to an accurate menstrual period and early ultrasound and GDM diagnosed by OGTT for the study group. While exclusion criteria involved pre-existing diabetes, multiple pregnancy, smokers, placenta previa or accreta and any medical condition that affect placentation and fetal outcome, as chronic hypertension, preeclampsia and renal disease.

The sample of study were collected from outpatient clinic of obstetrics at Al-Yarmouk Teaching Hospital, where full history was taken and a thorough examination was done. Investigations were done including, full blood count, blood group and Rh. The control group as well as those with established diagnosis of gestational diabetes were followed until time of delivery. Some of the later group were controlled by diet and some by medical treatment (antidiabetic agent).

Mode of delivery and fetal outcomes were recorded, including gender, birth weight, Apgar score at 1 and 5 minutes, any obvious abnormality or need for neonatal care unit admission. Cord blood sample was taken for blood sugar, total serum bilirubin, and hemoglobin concentration. Placentae of the two groups were collected after delivery, the umbilical cord was cut close to the placental surface, the placenta was evacuated from excess blood and

dried, the shape, cord implantation site, any abnormality were noted, then the placental weight was measured with a scale and recorded in grams. The placenta was kept in a container with formalin 10%, and send to the histopathology department at Al-Yarmouk Teaching Hospital where examination was done by two consultant histopathologists.

The tissue specimens were dissected from the placental sub-chorionic zone corresponding to the umbilical cord insertion, collected and floated in ice-cold phosphate-buffered saline (PBS), cleaned of blood, and immediately cut into double four 1 × 1 × 1 cm fragments, which were fixed with 10% formalin for further light microscopy (LM) examination.

For LM examination, three paraffin-embedded blocks from four formalin-fixed tissues and from each placenta were randomly selected. Sections were taken from each block and stained with hematoxylin and eosin (HE). The slides were examined under light microscope (Olympus CX 31) with 40 X and photographed.

Discrete variables presented using their number and percentage used to present the data, chi square test used to analyze the discrete variable or Fisher exact test used to analyze the distribution between two groups (used instead of chi square for 2x2 table, if total sample <20 and if two or more with expected frequency less than 5). Two samples t test used to analyzed the differences in means between two groups (if both follow normal distribution with no significant outlier).

SPSS 20.0.0 software package used to make the statistical analysis, p value considered when appropriate to be significant if less than 0.05.

Results

There was no significant difference between control and GDM group in maternal age 24.1 ± 4.2 years versus 24.3 ± 4.8 years and height (1.65 versus 1.60), while weight and BMI was significantly different in which GDM had higher weight (72.0 ± 3.7 kg versus 66.0 ± 1.9 kg), and higher BMI (28.6 ± 3.7 kg/m^2 versus 24.5 ± 2.7 kg/m^2), (Table 1).

Placental weight, cotyledons number and placental diameter were significantly higher in GDM compared to control, (Table 2). No significant differences were observed between GDM and control in umbilical cord insertion and shape of placenta.

Table 3 demonstrates microscopic findings of diabetic placentae and normal women placentae, with significant differences between the two groups (P value <0.001), where 85% of diabetic women had syncytial knots while only 25% of control group showed syncytial knots, (Figures 1 and 2).

All other microscopic abnormalities like focal and diffuse villous edema and focal and diffuse perivillous fibrin deposition were seen only in GDM group while no women in control group had such changes, P value <0.001, (Table 3 and Figures 3 and 4).

Table 4 demonstrates neonatal characteristics. There are significant differences regarding Apgar score in 1 and 5 minutes, P value > 0.05 also the difference in birth weight is highly significant P value < 0.001 while there are no significant differences in gestational ages between the two groups P value =0.8.

Table 5 demonstrates gender and mode of delivery.

Table 6 is demonstrating the association between management of GDM and microscopic findings of the placentae where all microscopic changes were seen more in diabetic patient treated by insulin comparing to those treated by diet alone, P value < 0.05.

Table 1: Maternal characteristics.

		Control	GDM	All	P value
Number of patients		60	60	-	-
Maternal age (years)		24.1 ± 4.2	24.3 ± 4.8	-	0.810
Weight (kg)		66.0 ± 1.9	72.0 ± 3.7	-	0.005
Height (m)		1.65 ± 0.09	1.60 ± 0.2	-	0.081
BMI kg/m^2		24.5 ± 2.7	28.6 ± 3.7	-	<0.001
Parity	1	26 (43.3%)	23 (38.3%)	49 (40.8%)	0.953
	2	25 (41.7%)	27 (45.0%)	52 (43.3%)	
	3	6 (10.0%)	7 (11.7%)	13 (10.8%)	
	4	3 (5.0%)	3 (5.0%)	6 (5.0%)	
Post prandial blood sugar (1hr)		120.4 ± 10.2	147.3 ± 10.3		<0.001

Table 2: Placental gross morphology between gestational diabetic and normal pregnancies.

	Control	GDM	All	P value
Number of patients	60	60	120	-
Weight of placenta	404.6 ± 31.2	546.4 ± 31.9	-	<0.001
Cotyledons number	17.1 ± 1.3	18.2 ± 1.7	-	<0.001
Placental diameter	15.6 ± 0.9	17.1 ± 0.8	-	<0.001
Shape of Placenta				0.699
Round	21 (35.0%)	19 (31.7%)	40 (33.3%)	
Oval	39 (65.0%)	41 (68.3%)	80 (66.7%)	
Umbilical cord insertion				0.999
Eccentric	20 (33.3%)	19 (31.7%)	39 (32.5%)	
Central	40 (66.7%)	41 (68.3%)	81 (67.5%)	

Table 3: Microscopic finding of diabetic placenta and normal women.

	Control	GDM	All	P value
Number	60	60	120	-
Syncytial knots				<0.001
Negative	45 (75.0%)	9 (15.0%)	56 (46.7%)	
Positive	15 (25.0%)	51 (85.0%)	64 (53.3%)	
Villous edema				<0.001
Negative	60 (100.0%)	5 (8.3%)	65 (54.2%)	
Positive	0 (0.0%)	55 (91.7%)	55 (45.8%)	
Focal Villous edema				<0.001
Negative	60 (100.0%)	15 (25.0%)	75 (62.5%)	
Positive	0 (0.0%)	45 (75.0%)	45 (37.5%)	
Fibrin deposition (perivillous)				<0.001
Negative	60 (100.0%)	10 (16.7%)	70 (58.3%)	
Positive	0 (0.0%)	50 (83.3%)	50 (41.7%)	
Fibrin deposition (focal perivillous)				<0.001
Negative	60 (100.0%)	5 (8.3%)	65 (54.2%)	
Positive	0 (0.0%)	55 (91.7%)	55 (45.8%)	

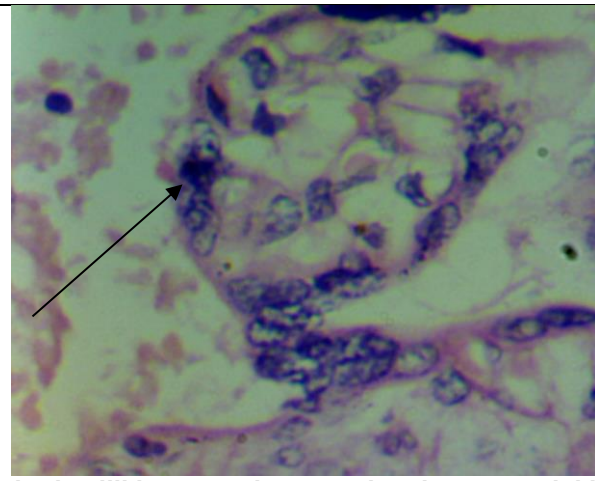

Figure 1: Chorionic villi in GDM placenta showing syncytial knot, H&E 40X.

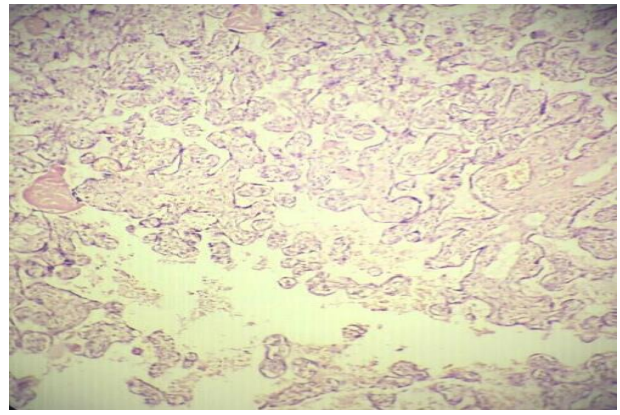

Figure 2: Chorionic villi in the control placenta, H&E 40X.

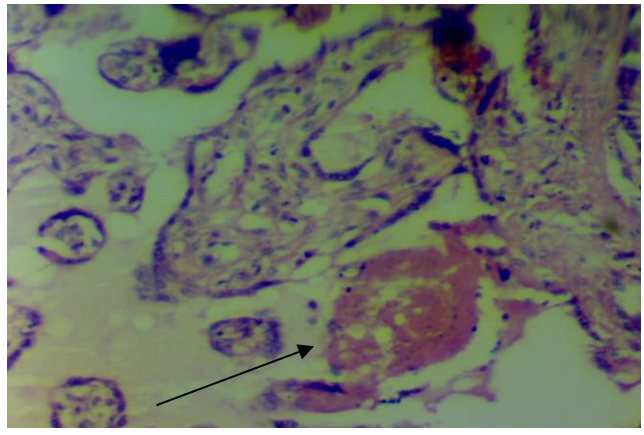

Figure 3: Chorionic villi in GDM placenta showing fibrin deposition, H&E 40X.

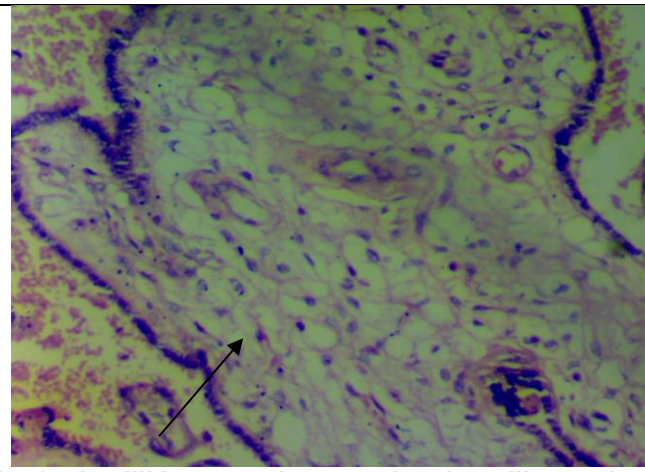

Figure 4: Chorionic villi in GDM placenta showing villous edema, H&E 40X.

Table 4: Neonatal characteristics.

	Control	GDM	P value
Number	60	60	-
Apgar score in 1 minutes	8.2 ± 1.0	7.6 ± 1.8	0.043
≥7	55 (91.7%)	45 (75.0%)	
<7	5 (8.3%)	15 (25.0%)	
Apgar score in 5 minutes	8.6 ± 1.2	8.0 ± 1.3	0.022
≥7	57 (95.0%)	52 (86.7%)	
<7	3 (5.0%)	8 (13.3)	
Birth weight (Kg)	3.2 ± 0.2	4.8 ± 0.3	<0.001
Gestational age(weeks)	38.7 ± 1.1	38.7 ± 1.0	0.867

Table 5: gender and mode of delivery.

	Control	GDM
	60	60
Gender		
Female	23	27
Male	37	33
Mode of delivery		
Vaginal delivery	38	13
CS	22	47

Table 6: The association between management of GDM and microscopic findings of the placentae

		Diet	Insulin	P value
Syncytial knots	Negative	7 (26.9%)	2 (5.9%)	0.024
	Positive	19 (73.1%)	32 (94.1%)	
Villous edema	Negative	5 (19.2%)	0 (0.0%)	0.012
	Positive	21 (80.8%)	34 (100.0%)	
Focal Villous edema	Negative	10 (38.5%)	5 (14.7%)	0.035
	Positive	16 (61.5%)	29 (85.3%)	
Fibrin deposition (perivillous)	Negative	9 (34.6%)	1 (2.9%)	0.001
	Positive	17 (65.4%)	33 (97.1%)	
Fibrin deposition (focal perivillous)	Negative	5 (19.2%)	0 (0.0%)	0.012
	Positive	21 (80.8%)	34 (100.0%)	

Discussion

Gestational diabetes mellitus (GDM) have been associated with alterations in placental anatomy and physiology. To compensate the hyperglycemic blood from the mother, there is islets cell hypertrophy and beta cell hyperplasia of fetal pancreas with the release of excessive amounts of insulin in the fetal body[14]. In GDM the increased oxygen consumption along with the placental abnormality can lead to chronic fetal hypoxia[15].

In the current study, there was no significant difference between the study and control groups regarding maternal age, height and parity. There was significant difference regarding maternal weight, BMI, and postprandial blood sugar (1hr) in study group in comparison to the control. In the current study, gross placental morphological changes in the GDM group were noted, the placenta showed increased weight, placental diameter and increased number of cotyledons.

Regarding increased Placental weight and diameter similar results were found in studies which were conducted by Ashfaq M, et al (2005), Saha S. et al (2014), Rafah Hady (2014) where they studied the effect of gestational diabetes and maternal hypertension on gross morphology of placenta and concluded that diabetic's placentae showed increase in weight and diameter[16-18].

On the other hand, no major gross changes was observed by some studies like Ranjana Verma et al (2010), in India. In that study there was no major difference in the placenta of gestational diabetes and the control, The weights of diabetic placentae were similar as compared to the control group[19].

Regarding microscopical features in the current study, the placenta of the control group showed normal branching of villi while the gestational diabetes group showed peri-villous edema, villous edema, villous fibrin(focal and perivillous) and increased syncytial knotting. This findings in the placenta of gestational diabetes supported by the study conducted by Saha S et al (2014) in India, that investigated the light microscopic appearance of gestational diabetes placenta and compare it to the control. This study revealed an increase in villous edema and fibrin deposition[17].

The above studies as well as the current study are comparable to a study conducted by Augustine G. et al (2016), in India, where GDM placentae showed increased syncytial knots[20].

In the current study, the histological features already mentioned above were noted more in those receiving insulin treatment greater than those controlled by diet alone which suggest changes correlated with severity of the condition.

This finding supported with the study of Abeer M. Hafez,et al (2015), in Zagazig University hospitals, which is similar to findings in Verma, R. et al (2010), the study conducted that several differences were identified in the terminal villi from the placentae of GDM group controlled with insulin; large number of syncytial knots and villous edema. While placentae of GDM group controlled with diet alone showed minimally increased number of syncytial knots and none of later showed villous edema[19,21].

Therefore, even if we control hyperglycemia we cannot totally prevent the effect of hyperglycemia on the placenta, and eventually fetal outcome.

In the current study, fetal outcome was noted, examination of newborns of diabetic mothers showed increased in the birth weight as compared to the control group. Macrosomia affect fetal parts of the placenta, it is contributing factor for increased diameter of the diabetic placenta. Fetal hyperinsulinemia due to gestational diabetes is associated with macrosomia, more birth injuries, higher caesarean section rate and neonatal hypoglycemia as noted in the current study as compared to the control group. This is supported by the study conducted by Turki Gasim (2012), at King Fahad hospital, Dammam University,

Saudi Arabia who concluded that there is significantly higher incidence of fetal macrosomia and higher mean birth weight in gestational diabetes women[21] .

The current study showed low Apgar score in the gestational diabetic group in comparison to the control group, this is supported by the study of Segregur J et al (2009), in Croatia, that showed similar lower Apgar score and more frequent neonatal complications in the GDM group[22]. This study concluded that GDM have effect on structure and function of the placenta, and eventually fetal outcome. In diabetic patients treated with insulin, the placenta showed more changes than those controlled with diet; this may be related to the severity of the disease process itself or may be related to the insulin.

References

1. Clare L Tower. Gestational diabetes. In: David M Luesley, Mark D Kilby (eds.) Obstetrics and Gynecology, An Evidence-based Text for MRCOG. Third edition. CRC Press Taylor & Francis Group. 2016 P.191-2.
2. Ahmed SA, Shalayel MH. Role of cortisol in the deterioration of glucose tolerance in Sudanese pregnant women. East Afr Med J 1999; 76: 465-7.
3. Buchanan TA, Xiang AH. Gestational diabetes mellitus. J Clin Invest 2005; 115: 485-91.
4. Catalano PM. Obesity, insulin resistance, and pregnancy outcome. Reproduction 2010; 140: 365-71.
5. Briana DD, Malamitsi-Puchner A. Reviews: adipocytokines in normal and complicated pregnancies. Reprod Sci 2009; 16: 921-37.
6. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci USA 1994; 91: 4854-8.
7. Hotamisligil GS, Peraldi P, Budavari A, et al. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α and obesity-induced insulin resistance. Science 1996; 271: 665-8.
8. Al-Noaemi M, Shalayel MHF. Adiponectin. Sudan J Med Sci 2009; 4: 297-305.
9. Berthold Huppertz, John CP Kingdom. The Placenta and Fetal Membranes. In: D. Keith Edmonds (ed.). Dewhurst's Textbook of Obstetrics and Gynaecology. Eighth edition. Wiley-Blackwell, 2012. P.16-7.
10. F Gary Cunningham, Kenneth J Leveno, Steven L Bloom, eds. Placental Abnormalities. Williams Obstetrics. 24th edition, McGraw-Hill Education, 2014. P.116-26
11. Benirschke K, Kaufmann P, Baergen R. Pathology of the Human Placenta. 5th edition. New York: Springer, 2006.
12. Kaufmann P, Huppertz B, Frank HG. The fibrinoids of the human placenta: origin, composition and functional relevance. Ann Anat 1996;178:485-501.
13. Huppertz B, Kadyrov M, Kingdom JC. Apoptosis and its role in the trophoblast. Am J Obstet Gynecol 2006;195:29-39.
14. Arshad R, Karim N, Hasan JA. Effects of insulin on placental, fetal and maternal outcomes in gestational diabetes mellitus. Pak J Med Sci 2014;30(2):240-4.
15. Jarmuzek P, Wielgos M, Bomba-Opon DA. Placental pathologic changes in gestational diabetes mellitus. Neuroendocrinology Letters 2015; 36(2):101-5.
16. Ashfaq M, Janjua MZ, Channa MA. Effect of gestational diabetes and maternal on gross morphology of placenta. J Ayub Med Coll Abbottabad 2005; 17(1):44-7.
17. Saha S, Biswas S, Mitra D, Adhikari A, Saha C. Histologic and morphometric study of human placenta in gestational diabetes mellitus. Ital J Anat Embryol 2014; 119(1):1-9.
18. Lateef Al-Mamori RH. Morphopathology of human placenta in diabetic pregnancy. J Biol Agricul Healthcare 2014;4(3):99-104.
19. Verma R, Mishra S, Kaul JM. Cellular changes in the placenta in pregnancies complicated with diabetes. Int J Morphol 2010;28(1):259-64.
20. Augustine G, Pulikkathodi M, Renjith S, et al. A study of placental histological changes in gestational diabetes mellitus on account of fetal hypoxia. Journal of Medical Science and Public Health 2016; 5(12):2457-60.
21. Gasim T. Gestational Diabetes Mellitus: Maternal and Perinatal Outcomes in 220 Saudi. Oman Medical Journal 2012;27(2): 140-4.
22. Segregur J, Buković D, Milinović D, et al. Fetal macrosomia in pregnant women with gestational diabetes.2009;33(4):1121-7.

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