American Journal of Clinical Anatomy & Physiology

Research Article

Morphometric and Structural Changes of the Placenta of Gestational Diabetics

Shawky M tayel, Mohamed G A Zaki and El Sayed AM Metwally*

Anatomy Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt

***Address for Correspondence:** Elsayed Aly Mohammed Metwally, Assistant Professor, Anatomy Department, Faculty of Medicine, Alexandria University, Tel: +20-100-567-1448; E-mail: sayedmetwally2020@yahoo.com

Submitted: 10 February 2021; **Approved:** 19 February 2021; **Published:** 29 March 2021

Citation this article: Tayel SM, A Zaki MG, AM Metwally ES. Morphometric and Structural Changes of the Placenta of Gestational Diabetics. American J Clin Anat Physiol. 2021 Mar 29;3(1): 005-009.

ABSTRACT

Background: The incidence of diabetes mellitus as one of the non-communicable disease is expected to increase rapidly due to rapid socio-demographic changes. One of the important causes of increased fetal complications is impaired placental function in diabetic women.

Objective: The aim of present research was to identify structural changes in the placenta of gestational diabetics.

Materials and methods: Placentas of 30 women; 15 controls and 15 with GDM were studied with free consent. The whole thickness of the placenta in all specimens, were taken near the attachment of the umbilical cord. Paraffin-embedded blocks of formaldehyde fixed tissue were made and sections (0.5 µm thickness) were cut out of each block and stained with H&E and other sections were stained with Masson Trichrome stain to be examined by light microscopy (Optica-B-150).

Results: The number of placental microvilli of GDM was less compared to the control placenta and this difference was of statistical significance. Moreover, the width of the intervillous spaces, the thickness of the blood vessels wall and fibrosis showed a statistically significant increase in cases of GDM than the control.

Conclusion: The present research showed that increased maternal glycemic levels is accompanied with a decrease in the number of microvilli and increased blood vessels thickness, also the intervillous spaces width was higher with marked areas of fibrosis. This necessity the strict control of blood glucose level in pregnancies in GDM and diabetes mellitus as well.

Keywords: Placenta; Gestation; Diabetes mellitus; Microvilli

INTRODUCTION

The placenta plays essential roles during pregnancy and is fundamental for growth and development of the fetus [1]. Placenta performs multiple important functions during pregnancy, namely the gas exchange, nutrition, and waste removal together with hormonal synthesis which control the maternal transport of nutrients to the fetus and maternal adaptation to various stages of pregnancy. The placental functions are determined by the Blood Placental Barrier (BPB) structure which is an exchange surface area between the mother and fetus [1].

The BPB separates the fetal and maternal blood and allows the exchange between the fetus and mother without blood mixing [2]. With the progress of pregnancy, it decreases in thickness to compensate for the increased fetal needs [3]. A proper coordination of trophoblastic proliferation, differentiation and invasion is required for development of placenta [2].

The prevalence of DM as a major noncommunicable disease in Egypt is rapidly growing probably due to the rapid sociodemographic changes. Egypt was identified to be the ninth leading country worldwide in terms of the number of patients with DM with a prevalence rate of 15.9% [4].

Gestational Diabetes Mellitus (GDM), defined as diabetes diagnosed during pregnancy that is not clearly overt diabetes [5]. Hyperglycemia has an inductive effect in trophoblast apoptosis [6]. One of the main causes of the increased frequency of fetal complications is impaired placental function in diabetic pregnancies [7]. This necessitates the study of the structural placental changes in gestational diabetes.

MATERIAL AND METHODS

Placentas of 30 women from Elshatby Gynecology and Obstetrics department, Faculty of Medicine, Alexandria University; 15 controls aged 26-28 years with maternal weight 64.29 ± 0.58 kg and 15 with GDM aged 27-33 years with maternal weight 66.37 ± 1.09 kg were examined with the written agreement of the Ethics Committee, Faculty of Medicine, University of Alexandria. The research was performed after free consent. Placental specimens, including the entire thickness of the placenta, were taken from the middle of the placenta near the cord attachment point. Paraffin-embedded blocks of 10% formaldehyde fixed tissue were prepared and sections (3-5 µm thickness) were cut out of each block and processed for hematoxylin and eosin (H & E) [8] and Gomori's trichrome staining [9]. Digital images from the sections were collected by digital cameras linked to the microscope. (Olympus BX41). The magnification of the images were (100X) and (400 X).

Every woman with a history of hypertension, smoking or pre-gestational diabetes has been excluded from the study.

After at least 8 hours of fasting at 25–28 weeks of gestation, fasting plasma sugar was estimated. GDM diagnosis was made on the basis of the American Diabetes Association (2017) [10]. Both control and GDM women were delivered at Elshatby Gynecology and Obstetrics Department, Faculty of Medicine, Alexandria University; Caesarian section was performed for all GDM women in gestational weeks 37 - 38. Vaginal delivery was performed for 11 control women and Caesarian section for 4 women at the gestational age 36 - 39 weeks.

Placental samples were randomly selected for blind morphometric assessment with an image analyzer [11].

Histomorphometric Studies

The data were obtained using the image analyzer computer system (Leica Qwin 500, Leica, Cambridge, England). Three histological sections per slide with a total of nine per placenta were used in the morphometric study. The examination was done on Gomori trichrome stained sections. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. For each study group, the number of microvilli, thickness of the wall of blood vessels (μm), width of intervillous spaces (μm) and areas of fibrosis (μm) were measured. The brown coloration of the immunoreaction was covered automatically by a blue mask (binary image). The area of this binary image was then calculated [12].

STATISTICAL ANALYSIS

The results were analyzed statistically using SPSS software version 22 as mean± standard deviation and the 2 groups were compared using Paired-Samples T test and the level of significance was accepted as $p < 0.05$ [13].

RESULTS

The blood glucose levels of the control women ranged from 95 to 120 mg / dL during pregnancy, while those of the GDM women ranged from 120 to 165 mg / dL. After diet management and insulin therapy, the fasting blood glucose levels of the GDM women remained in a sufficient range from 100 to 135 mg / dL till delivery. The maternal weight at gestation ranged from 65 - 67.5 Kg for the controls and 67 - 71 kg for GDM.

As regards the fetal birth weight; it ranged from 2.80 to 3.15 Kg in the control group and from 3.21to 3.40 Kg in GDM women. The placental weight after umbilical cord removal in control women ranged from 450 to 480 gm and from 470 to 500 gm in GDM women.

I: Variables of the studied cases

(Table 1) show that there was no statistically significant difference in the maternal age between GDM and control women. Moreover, the maternal weight showed a significant increase in cases of GDM than the control. On the other hand, there was no statistically significant difference in the gestational age between the study groups. Regarding the neonatal and the placental weights, they were significantly increased in women with GDM than the control (Figure 1).

II. Histological examination

H&E stain: Histological examination of the placenta of control women stained by H&E revealed a number of tightly packed microvilli. Among these microvilli, there were intervillous spaces that were narrow and filled with blood cells. Syncytial knots that are aggregations of the syncytiotrophoblast nuclei; the outer covering of the microvilli; were observed occupying different areas at the poles of the microvilli. The villi showed small thin walled blood vessels containing blood cells (Figure 2).

On examination of sections of the placenta of GDM stained with H&E under light microscope, few microvilli with syncytial knots were found. The number of microvilli was less than that in the control placenta. Among the microvilli, there were large intervillous spaces filled with blood cells. Several microvilli showed well-seen areas of fibrosis. Some microvilli also showed thick walled dilated blood vessels filled with blood cells. In addition, areas of perivillous fibrinoid degeneration were also evident (Figure 3).

Masson Trichrome stain: Sections of control and diabetic placentas were stained with Masson Trichrome stain and examined under light microscope. Control placenta showed minimal/negligible deposition of collagen type I in the microvilli. On the other hand, the microvilli of the diabetic placentas showed widespread multiple areas of fibrosis and deposition of collagen type I with significantly higher fibrosis index than the control (fibrosis index of control = 2% while in GDM = 34%) (Figure 4).

Table 1: Distribution of different parameters among studied groups.

Variables	Control(n=15)	GDM(n=15)	t-test	P value
Maternal age	30.79 ± 0.64	31.11 ± 0.71	0.83	0.211
Maternal weight	64.29 ± 0.58	66.37 ± 1.09	3.97	0.001*
Gestational age	36.74 ± 0.57	36.39 ± 0.43	0.91	0.049
Neonatal weight	2.79 ± 0.27	3.31 ± 0.89	4.01	0.001*
Placental weight	451.74 ± 6.05	479.91 ± 8.97	5.81	0.001*

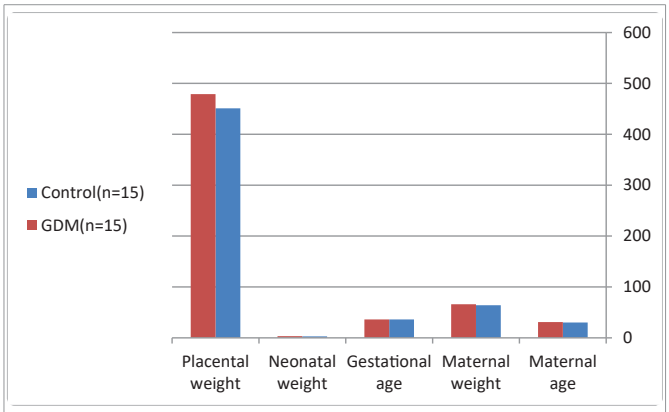

Figure 1: A bar chart showing the different variables among control and GDM groups.

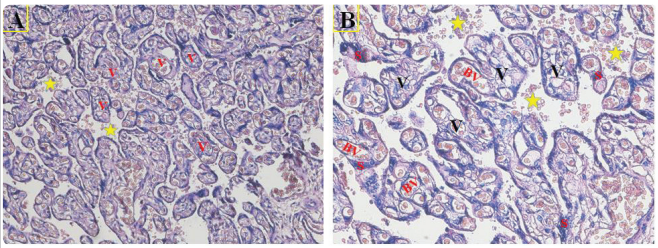

Figure 2(A,B): A photomicrograph of the control placenta showing densely packed numerous microvilli (V) with narrow intervillous spaces (yellow star) filled with blood cells. The microvilli have thin walled blood vessels (BV) containing blood cells. Aggregations of the nuclei of the syncytiotrophoblast; Syncytial knots (S) were observed occupying different areas at the poles of the microvilli (A) H&E X200 (B) H&E X400.

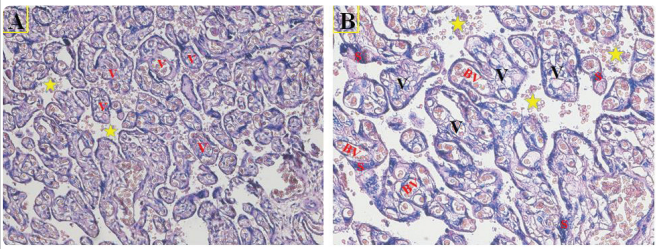

Figure 3(A,B): A photomicrograph of placenta of GDM showing smaller numbers of microvilli (V) with wide intervillous spaces (yellow star) containing blood cells. The microvilli have thick walled (black arrow) blood vessels (BV) containing blood cells. The microvilli show multiple areas of fibrosis (F) and areas of perivillous fibrinoid degeneration (FD). (A) H&E X200 (B) H&E X400.

III. Morphometric measurements

Table 2 shows that the number of microvilli of placenta of GDM was less compared to the control placenta and the difference was statistically significant. Moreover, the width of the intervillous spaces, the thickness of the wall of the blood vessels and fibrosis showed a significant increase in cases of GDM than the control (Figure 5).

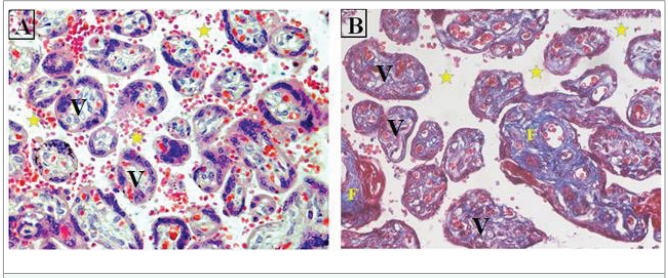

Figure 4(A,B): A photomicrograph of the control placenta (A) showing negligible or minimal fibrosis (blue colour) and GDM placenta (B) where the microvilli (V) show widespread areas of fibrosis (F) (blue colour). Yellow star = intervillous spaces. (A,B) Masson Trichrome stainX400.

Table 2: Distribution of different parameters of image analyzer among studied groups.

Parameter	Control placenta	cGDM placenta	t-test	P value
Number of microvilli	7.1 ± 3.5	5.9 ± 2.8	0.83	0.001*
Thickness of the wall of blood vessels(μm)	387.6 ± 3	556.6 ± 2.1	3.97	0.001*
Width of intervillous spaces (μm)	8890.0 ± 2.1	18840.0 ± 1.1	0.91	0.001*
Areas of fibrosis (μm)	8012.8 ± 1	9017.3 ± 1.2	4.01	0.001*

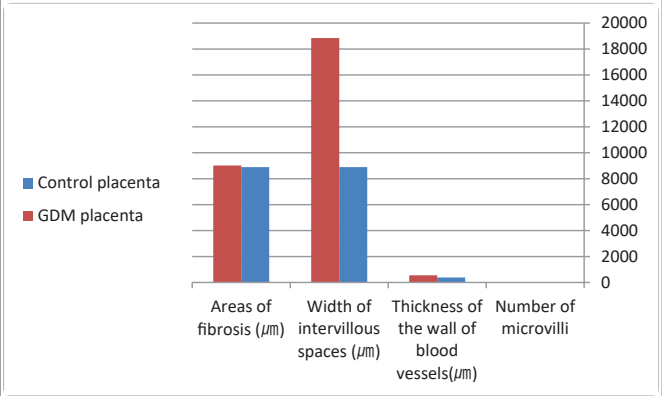

Figure 5: A bar chart showing the different morphometric parameters among control and GDM groups.

DISCUSSION

The human placenta is at the feto-maternal interface. The placenta is widely exposed to numerous adverse intrauterine conditions. Glucose is the primary placental energy substrate. Glucose exchange between mother and fetus is vital for fetal survival and is observed throughout pregnancy. The gestational changes in maternal glucose metabolism and increased blood sugar level reflect the maternal metabolic adaptations to fulfill the nutrition needs of the developing fetus and this is exacerbated in GDM [10].

In the present work, the maternal weight, neonatal weight and the placental weight of GDM women showed a significant increase than the control. On the other hand, the maternal age and gestational age has no statistical significant difference between both groups. This could be explained in that pregnancy is characterized by a physiological state of insulin resistance, which is further magnified in GDM. Furthermore, GDM pregnant women have many features of the metabolic syndrome and have a high risk of developing type 2 diabetes [14].

Maternal insulin sensitivity is inversely related to neonatal birth weight [15] this is in agreement with the present study. On the other hand, studies stated that insulin resistance and a maternal birth weight are not linked during pregnancy, and there are conflicting results regarding the relationship between GDM and maternal birth weight [16].

Histological examination of placenta of control women stained by H&E exhibited numerous densely packed microvilli with narrow intervillous spaces filled with blood cells. The villi showed small thin walled blood vessels while placenta of GDM showed smaller numbers of microvilli with wide intervillous spaces containing blood cells. The microvilli had thick walled blood vessels and showed multiple areas of fibrosis and areas of perivillous fibrinoid degeneration.

Meng, et al. [17] attributed the alternations in the morphology and ultrastructure of GDM placentas to the compensatory mechanism to maintain homeostasis at the maternal-fetal interface in GDM and assumed that those changes in the placenta and materno-fetal interface are related to the pregnancy complications and even to fetal diseases.

In this study, placental morphometric characteristics were significantly increased in GDM as compared to the control placenta. The number of microvilli of placenta of GDM was less compared to the control placenta and the difference was statistically significant. Moreover, the width of intervillous spaces, the thickness of the wall of the blood vessels and fibrosis showed a significant increase in cases of GDM than the control. This could be explained by the chronic hypoxia in the fetuses of diabetics that induces angiogenesis and cellular aggregation and fibrosis later on.

Feto-placental angiogenesis in well-controlled diabetes has been reported to be increased and to occur whatever the control of maternal diabetic profile [18].

Calderon, et al. [11] stated that the size and number of placental microvilli as well as total villous area in placenta of diabetics were similar to those in the control group, a finding which is not in agreement with the present study, but this could be explained by the other confounding factors in the control group of their study including smoking, arterial hypertension which induce a state of chronic hypoxia in the control placenta.

CONCLUSION

In conclusion, our results show that increased maternal glycemic levels is associated with decreased number of microvilli and inceased thickness of wall of blood vessels, also the width of intervillous spaces was higher with marked areas of fibrosis. This is relevant in clinical practice, as it necessities the strict control of blood sugar in pregnancies GDM as well as diabetes mellitus.

DATA AVAILABILITY STATEMENT

The data supporting these findings are from previously reported studies and datasets, which have been cited. And this data are included within the article.

REFERENCES

1. Abdelghany AH, TM Eissa, S Idris. Study of the ultrastructure of the placenta in gestational diabetes mellitus. International Journal of Anatomical Variations. 2018. 11(1):004-010. https://tinyurl.com/2zeeskk3

2. Aires MB, Dos Santos AC. Effects of maternal diabetes on trophoblast cells. World J Diabetes. 2015 Mar 15;6(2):338-44. doi: 10.4239/wjd.v6.i2.338. PMID: 25789116; PMCID: PMC4360428.

3. al-Okail MS, al-Attas OS. Histological changes in placental syncytiotrophoblasts of poorly controlled gestational diabetic patients. Endocr J. 1994 Aug;41(4):355-60. doi: 10.1507/endocrj.41.355. PMID: 8528350.

4. El Sagheer. Prevalence and risk factors for gestational diabetes mellitus according to the diabetes in pregnancy Study Group India in comparison to international association of the diabetes and pregnancy study groups in El-Minya, Egypt. The Egyptian Journal of Internal Medicine, 2018;30(3):9. https://tinyurl.com/35z5n4zw

5. Bentley-Lewis R, Dawson DL, Wenger JB, Thadhani RI, Roberts DJ. Placental histomorphometry in gestational diabetes mellitus: the relationship between subsequent type 2 diabetes mellitus and race/ethnicity. Am J Clin Pathol. 2014 Apr;141(4):587-92. doi: 10.1309/AJCPX81AUNFPOTLL. PMID: 24619761; PMCID: PMC4040002.

6. Basnet KM, Bentley-Lewis R, Wexler DJ, Kilic F, Roberts DJ. Prevalence of intervillous thrombi is increased in placentas from pregnancies complicated by diabetes. Pediatr Dev Pathol. 2016 Nov/Dec;19(6):502-505. doi: 10.2350/15-11-1734-OA.1. Epub 2015 Dec 15. PMID: 26669929.

7. Daskalakis G, Marinopoulos S, Krielesi V, Papapanagiotou A, Papantoniou N, Mesogitis S, Antsaklis A. Placental pathology in women with gestational diabetes. Acta Obstet Gynecol Scand. 2008;87(4):403-7. doi: 10.1080/00016340801908783. PMID: 18382864.

8. Tripp EJ, MacKay EH. Silver staining of bone prior to decalcification for quantitative determination of osteoid in sections. Stain Technol. 1972 May;47(3):129-36. doi: 10.3109/10520297209116467. PMID: 4111887.

9. Urlaub KM, Lynn JV, Carey EG, Nelson NS, Polyatskaya Y, Donneys A, Mazzoli AC, Buchman SR. Histologic improvements in irradiated bone through pharmaceutical intervention in mandibular distraction osteogenesis. J Oral Maxillofac Surg. 2018 Dec;76(12):2660-2668. doi: 10.1016/j.joms.2018.05.013. Epub 2018 May 19. PMID: 29883588.

10. Jayabalan N, Nair S, Nuzhat Z, Rice GE, Zuñiga FA, Sobrevia L, Leiva A, Sanhueza C, Gutiérrez JA, Lappas M, Freeman DJ, Salomon C. Cross talk between adipose tissue and placenta in obese and gestational diabetes mellitus pregnancies via exosomes. Front Endocrinol (Lausanne). 2017 Sep 27;8:239. doi: 10.3389/fendo.2017.00239. PMID: 29021781; PMCID: PMC5623931.

11. Calderon IM, Damasceno DC, Amorin RL, Costa RA, Brasil MA, Rudge MV. Morphometric study of placental villi and vessels in women with mild hyperglycemia or gestational or overt diabetes. Diabetes Res Clin Pract. 2007 Oct;78(1):65-71. doi: 10.1016/j.diabres.2007.01.023. Epub 2007 Mar 13. PMID: 17360067.

12. Shawky LM, Morsi AA, El Bana E, Hanafy SM. The biological impacts of sitagliptin on the pancreas of a rat model of type 2 diabetes mellitus: drug interactions with metformin. Biology (Basel). 2019 Dec 25;9(1):6. doi: 10.3390/biology9010006. PMID: 31881657; PMCID: PMC7167819.

13. Kirkpatrick LA. A simple guide to IBM SPSS statistics : for version 20.0. 2013. Wadsworth Cengage Learning. https://tinyurl.com/3pwjbfmu

14. Elsennawy TMA. Effect of gestational diabetes on gross morphology, histology and histochemistry of human placenta. Endocrinol Metab Syndr. 2016;5(5):1-13. DOI:10.4172/2161-1017.1000227

15. Jarmuzek P, Wielgos M, Bomba-Opon D. Placental pathologic changes in gestational diabetes mellitus. Neuro Endocrinol Lett. 2015;36(2):101-5. PMID: 26071574.

16. Seghieri G, Anichini R, De Bellis A, Alviggi L, Franconi F, Breschi MC. Relationship between gestational diabetes mellitus and low maternal birth weight. Diabetes Care. 2002 Oct;25(10):1761-5. doi: 10.2337/diacare.25.10.1761. PMID: 12351474.

17. Meng Q, Shao L, Luo X, Mu Y, Xu W, Gao C, Gao L, Liu J, Cui Y. Ultrastructure of placenta of gravidas with gestational diabetes mellitus. Obstet Gynecol Int. 2015;2015:283124. doi: 10.1155/2015/283124. Epub 2015 Aug 24. PMID: 26379710; PMCID: PMC4561319.

18. Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. Int J Mol Sci. 2014 Sep 12;15(9):16153-85. doi: 10.3390/ijms150916153. PMID: 25222554; PMCID: PMC4200776.