DOI: https://doi.org/10.3329/ewmcj.v12i1.77176

Original Article

HISTOLOGICAL CHANGE OF PLACENTA IN DIABETIC PREGNANT WOMEN IN COMPARISON TO NORMAL PREGNANT WOMEN

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Article history:
Received: November 2023
Accepted: December 2023

Abstract:

Background: *The placenta is a foeto-maternal organ and its alterations is directly related to the disease's length and severity. The human placenta experiences a number of functional and structural pathologic alterations when complicated by metabolic disorder such as diabetes mellitus.*

Methods: *This cross-sectional comparative study was conducted over a period of three-years from January 2019 to December 2021 at the Department of Anatomy, Rajshahi Medical College, Rajshahi, in collaboration with the Department of Obstetrics and Gynaecology, Rajshahi Medical College Hospital and Diabetic Hospital Rajshahi, Bangladesh. The study was carried out on 70 pregnant women, among them 35 diabetics and 35 non-diabetics. The fibrinoid necrosis, syncytial knot and immature villi of the placenta were studied in diabetic and non-diabetic pregnant women. SPSS software, version-24 was used for data analysis, with a p-value < 0.05 indicating statistically significance for all tests.*

Results: *The diabetic and non-diabetic pregnant women groups were almost identical in terms of attachment of umbilical cord to placenta with marginal attachment being more often found in either group (p = 0.829). Histological examination of placenta showed that fibrinoid necrosis was common in placenta of diabetic mothers than that of the normal mothers (p < 0.001). Syncytial knot and immature villi were higher in the former group than those in the latter group, here p values were also less than .001.*

Conclusion: *Histological findings (fibrinoid necrosis, syncytial knot and immature villi) were more in placenta of diabetic mother and they were highly significant different between the groups.*

Keywords:
Placenta, Diabetes mellitus and Histological change.

EWMCJ Vol. 12, No. 1&2, January 2024-July 2024: 27-33

Introduction

The placenta is an organ that separates the maternal and fetal circulations and plays an important metabolic role during pregnancy. The human placenta is discoid in shape and hemochorial due to chorion's direct contact with maternal blood.[1] It is also deciduate since some maternal tissue is removed after parturition.[1] It is linked to the uterine wall and connects the mother and fetus via the umbilical cord. The presence of maternal and fetal tissues in direct contact without rejection suggests that the mother's immune system accepts the fetal graft. The placenta occupies around 30% of the uterine wall.[2] It has two surfaces, one fetal and the other maternal, as well as a peripheral edge. The fetal surface is coated with smooth, sparkling amnion. The umbilical cord is linked at or around the center. Umbilical vessel branches can be seen beneath the amnion, radiating from the cord's insertion. Approximately four-fifths of the placenta is fetal origin.[2–4] The maternal surface is spongy and rough.

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Maternal blood gives the placenta its red tint. A thin greyish shaggy layer from the decidua basalis may be observed. The maternal surface is divided into 15-20 convex polygonal regions called lobes or cotyledons.[5,6] The decidual septum occupies each fissure, leaving many little grayish areas visible. They result from calcium deposition in degraded regions. The maternal part constitutes less than one-fifth of the total placenta. The decidua basalis and blood in the intervillous gaps are exclusively from the mother.[7]

Postnatal placental investigation can help understanding the fetal and maternal status during the antenatal and perinatal periods. The placenta is a complicated organ with limited metabolic and endocrine activity that are still not fully understood. It performs a variety of tasks that are critical to the fetus's growth and survival in utero.[8,9] Quantitative departures of normal parameters give a rational basis for describing placental disease.[10,11]

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The placenta of diabetic women has piqued the interest of researchers from various disciplines due to its damage or dysfunction partially responsible for the very high frequency of fetal problems in maternal diabetes. In gestational diabetes, usually there is delivery of large babies and large placentae.[10,12] While, some authors[13,14] described hypovascular villi in the diabetic placenta but Discroll[15] failed to reveal any abnormalities other than villous immaturity. Some authors claimed that there is excess syncytial knot formation[16–18] and stromal fibrosis[19]. Histological abnormalities such as the presence of nucleated fetal red blood cells, fibrinoid necrosis, villous immaturity, syncytial nodes were observed more often in the diabetic placentae compared with control placentae.[20] Considering the clinical and pathophysiological implications of placental structure in diabetes as well as the unresolved questions regarding placental changes in foetal outcome, the study was carried out to determine the histological changes of the placenta that might occur in the gestational diabetes mellitus.

Methods:

This cross-sectional study was carried out over three years, from January 2019 to December 2021, at the Department of Anatomy with partnership of Obstetrics and Gynaecology, Rajshahi Medical College Hospital and Diabetic Hospital Rajshahi, Bangladesh. The study population consisted of all diabetic and non-diabetic pregnant women aged 18 to 35 years who were admitted to hospitals during the study period. The study did not however, include any medical, systemic or obstetric illnesses during pregnancy that affected placental histology. The study sample consisted of 70 expectant mothers (35 diabetes and 35 non-diabetic) who were at term and met the qualifying requirements. The histological characteristics of the placenta (fibrinoid necrosis, syncytial knot and immature villi) were then compared in diabetes and non-diabetic pregnant women.

Collection and preparation of specimens:

After collecting placentae, they were immediately placed on a tray and the maternal and fetal surfaces were checked. The blood film on the maternal surface was gently removed by hand while leaving any solid clots embedded in the placenta's composition intact. Running tap water was used to wash the placentae and surplus water was collected with blotting paper.

Tissue for histology: The small pieces of tissue (not more than 2sq-cm) were taken from the center of the placenta for histological study. In brief each tissue sample was collected in a plastic container labeled by individual number tag, preserved and fixed by 10% formalin solution for overnight. Gradually dehydrated in ascending concentration of graded ethyl alcohol series from 70 to 100%, cleared in xylene, infiltrated in liquid paraffin then embedded with melted paraffin on a proper plane. For making paraffin blocks and sectioning according to routine paraffin section method, the steps followed were:

a) Fixation: Fixation was done for hardening and preserving the tissue specimen in a fixative.[21] It was done by 10% formol saline solution for 6-48 hours to prevent decomposition and to preserve the original architecture. 10% formol saline solution is composed of the following elements:

Formalin	10 ml
Sodium chloride	0.9 gm
Distilled water	90 ml

b) Dehydration: It was done for the removal of fixative and water from the fixed tissue and was performed by passing the tissue through upgraded ethyl alcohol as follows:

70% ethyl alcohol.	———	1 hour
80% " "	———	1 "
95% " "	———	1 "
100% " "	———	1 "
100% " "	———	1 "

c) Clearing: Clearing was done for removal of extractable or free water from the fixed tissue. As the absolute alcohol was used as final dehydrant, clearing was done by two changes of xylene for 1 hour.

d) Paraffin infiltration: It was done after tissues had been thoroughly cleared with xylene and was placed into the paraffin bath with a temperature of 58-60°C in an electric oven for 4-6 hours to infiltrate the tissue with a supporting media.

e) Paraffin embedding: Metallic molds were lubricated with liquid paraffin, then melted paraffin was poured into them. After hardening, the blocks were removed from the molds, kept in ice chamber of a refrigerator for 30 minutes before cutting the section.

f) Sectioning: Each block of tissue was fitted in a rotatory microtome, the disposable blade was placed and adjusted properly at 5° angulation. The sections were cut at 5-6 micron in thickness. A water bath regulated temperature of 45-50°C was used for floatation. Ribbons of good sections were selected and floated on warm water bath. The sections were kept on aluminized glass slides. The slides were kept in inclined position to drain off the excess water and allowed to dry at room temperature. Then they were kept in the oven at 58-60°C for melting the paraffin.

g) Staining: All slides for histological examination were stained by routine Hematoxylin & Eosin (H& E) stain. The steps followed for staining were deparaffinization, hydration, staining with hematoxylin and eosin, dehydration, clearing and mounting. From each slide, 10 fields were randomly selected. Counting of histopathological findings were done by counting 10 fields randomly in zigzag manner like counting of cotyledons.

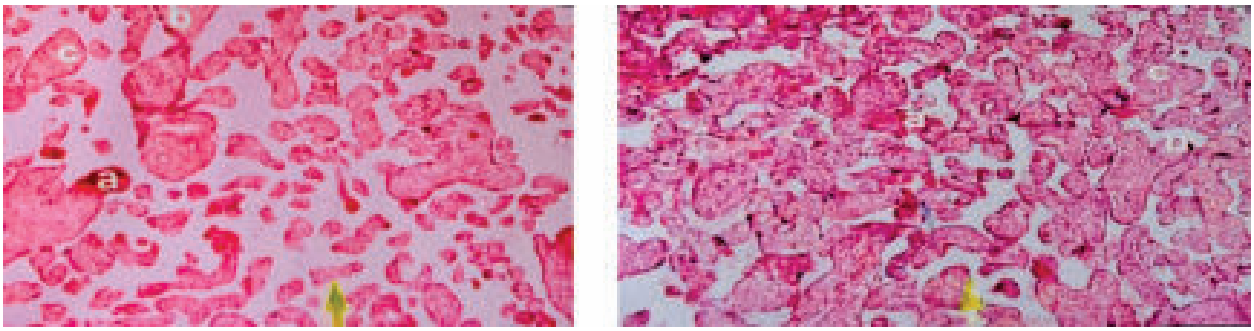

Figure 1: *Histological slide of Diabetic Placenta; (a) Fibrinoid Necrosis; (b) Syncitial Knot; (c) Immature Villous*

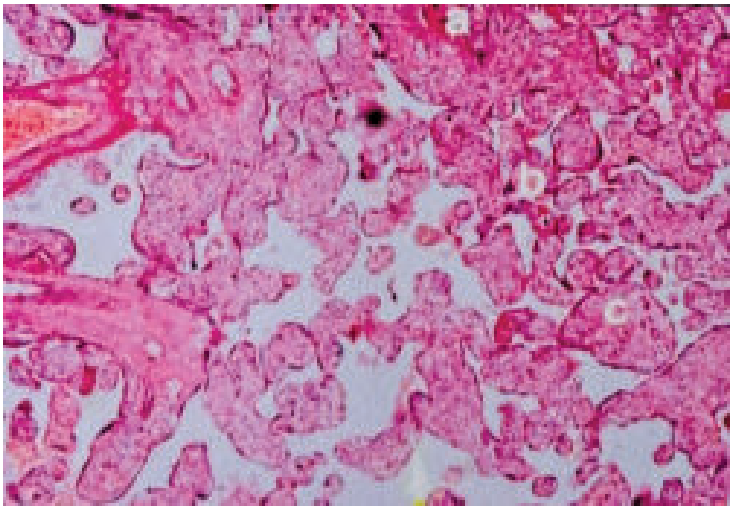

Figure 2: *Histological Slide of Normal Placenta; (a) Fibrinoid Necrosis; (b) Syncitial Knot; (c) Immature Villous*

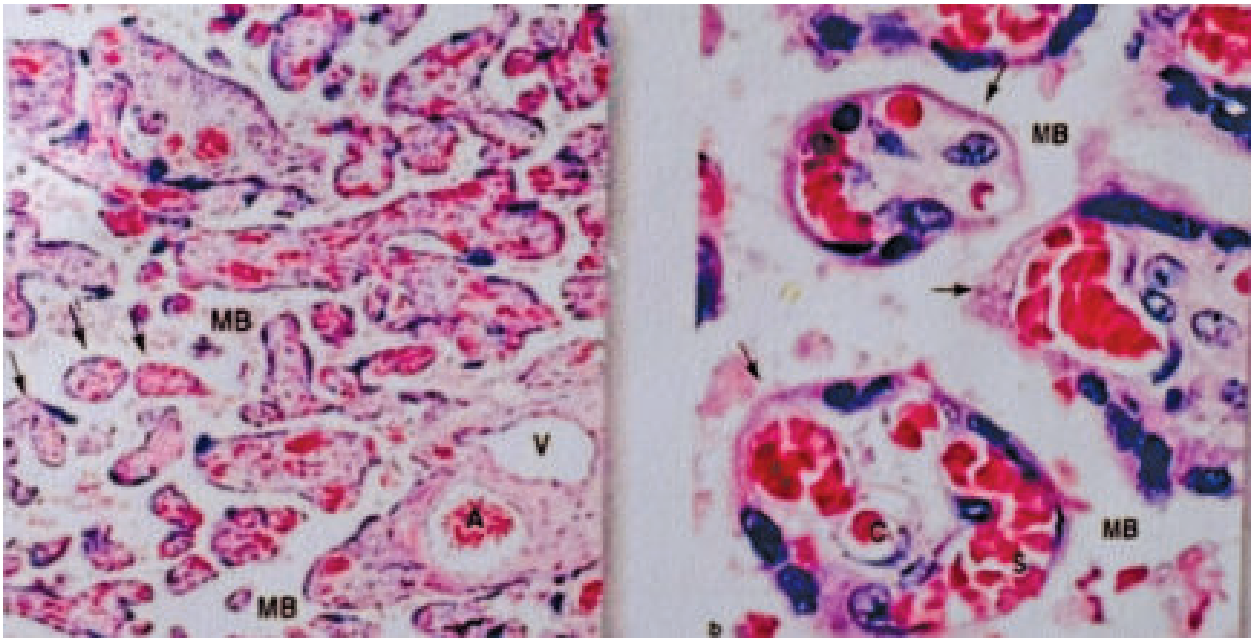

Figure 3: *Histological findings of Placenta*

Results:

Majority of the diabetic mothers (54.3%) and all of the non-diabetic mothers (100.0%) had fasting blood sugar level < 6.5 mmol/L. Majority of the fetus of diabetic mothers (65.7%) and non-diabetic mothers (60.0%) were delivered at 38 weeks gestational age.

The mean number of cotyledons between diabetic and normal placentae were 18 and 19 respectively (p = 0.891). Marginal attachment of the cord was more or less common in both the groups followed by eccentric lateral and eccentric medial with no significant intergroup difference (p = 0.829).

Histological examination of the placenta showed that fibrinoid necrosis was fairly common in placentae of diabetic mothers than that in the placenta of normal pregnant women (p < 0.001).This is highly significant. Syncytial knot and immature villi were staggeringly higher in the former group than those in the latter group (p < 0.001 and p < 0.001 respectively).This is also highly significant.

Table-I

Comparison of FBS and gestational age between diabetic and non-diabetic group (n=35 in each group).

Variables	Group		p-value
	DM(n = 35)	Normal(n = 35)	
FBS*			
< 6.5	19(54.3)	35(100.0)	
6.5 – 7.0	10(28.6)	0(0.0)	< 0.001
> 7.0	6(17.1)	0(0.0)	
Gestational age* (weeks)			
37	3(8.6)	4(11.4)	0.867
38	23(65.7)	21(60.0)	
39	9(25.7)	10(28.6)	

*Chi-squared Test (c^2) was done to analyze the data; figures in the parentheses denote corresponding percentage.

Table-II

Comparison of no. of cotyledon and attachment of cord between groups (n=35 in each group).

Morphology of placenta	Group		p-value	Significant (S) / Non-Significant (NS)
	DM (n = 35)	Normal (n = 35)		
No. of cotyledon#	18 ± 3	19 ± 10	0.891	NS
Attachment of cord*				
Marginal	15(42.9)	17(48.6)		
Battle door	0(0.0)	1(2.9)		
Central	7(20.0)	6(17.1)	0.829	NS
Eccentric Lateral	8(22.9)	6(17.1)		
Eccentric Medial	5(14.3)	5(14.3)		

*Chi-squared Test (c^2) was done to analyze the data; figures in the parentheses denote corresponding percentage.
#Data were analyzed using Unpaired t-Test and were presented as mean ± SD.

Table-III

Comparison of histology of placenta between groups (n=35 in each group).

Histology of placenta	Group		p-value	Significant (S) / Non-Significant (NS)
	DM (n = 35)	Normal (n = 35)		
Fibrinoid necrosis	25.5 ± 13.0	12.0 ± 8.3	< 0.001	S
Syncytial knot	359.1 ± 120.6	51.8 ± 18.2	< 0.001	S
Immature villi	22.9 ± 10.8	3.7 ± 2.0	< 0.001	S

#Data were analyzed using Unpaired t-Test and were presented as mean ± SD.

Discussion and Conclusions

In this study, there was no significant difference between placentae of diabetic mothers and normal pregnant women in terms of gestational age. Findings were in good agreement with Meng et al.[22] and Tandon et al.[23]. But these values were not consistent with the study done by Whittington et al.[24] where there was significant difference between placentae of diabetic mothers and normal pregnant women in terms of gestational age.

However, in the present study, groups did not differ in terms of mean number of cotyledons. But this finding was not consistent with the study done by Bhanu et al.[25] where cotyledons was significantly higher and revealed an overabundance in pregnant mothers with diabetes or GDM.

In the current study, attachment of umbilical cord to placenta did not differ between the two groups and marginal attachment of the placenta being more or less common in either group which was 42.9% in diabetic group and 48.6% in control group. But in another study that was done by Bhanu et al.[25] showed that most of GDM placenta had eccentric type found to be 43.75% and the next observed type was marginal with 33.33% which were different from our findings.

GDM alters the formation, structure and function of the placenta. According to the review, these changes are related to the foetus oxygen shortage, changes in the transplacental transport of nutrients and other modifications that cause fetal enlargement by increasing their availability, among other repercussions for the developing fetus. Among the morphological abnormalities, it was discovered that the placental weight/fetal weight ratio is higher in GDM and is mostly linked to histological changes.[26]

In our study, histological examination of the placenta showed that fibrinoid necrosis was fairly common in placentae of diabetic mothers than that in the placenta of normal pregnant women. Syncytial knots and immature villi were much higher in the former group than those in the latter group. These findings were in accordance with the studies done by Rudge and

colleagues[27], Evers et al.[20], Daskalakis et al.[28] and Madazli et al.[29].

Rudge and colleagues[27] reported that placentas from mild gestational hyperglycemia (MGH) group presented 17 types of histopathological change and higher rates of syncytial knots and endarteritis. GDM placentas presented only nine types of histopathological changes, high rates of dysmaturity, low rates of calcification and no syncytial knots. Overt DM placentas showed 22 types of histopathological change, 21 of which were present in the preterm period. There were histopathological similarities between MGH and DM placentas. The distance between maternal and fetal circulation is increased because of an increase in the chorionic villi on the surface as well as greater thickness of the syncytiotrophoblast basal membrane due to an increased type IV collagen deposition. The stroma between the villi is edematous which modifies the metabolic and endocrinal function of these placentas. Moreover, the capillary surface is enlarged due to the phenomena of vascular neoformation and a greater penetration of these vessels within the villi. Low oxygen partial pressure (PO_2) was detected which would produce a compensatory hyperplasia of terminal chorionic villi.[26] When pregnancy is complicated by diabetes, the human placenta undergoes a number of functional and structural pathologic changes, such as increased placental weight and increased incidence of placental lesions including villous maturational defects and fibrinoid necrosis.[30] From the above discussion it is evident that standardization of terminology used to define placental lesions is an essential prerequisite to study histological changes in placentas of diabetic mothers.

There were some limitations of the study. Although the present study had an intention to study the complete histological features but due to unavailability of histopathologists experienced in studying the histological features of placenta, only syncytial knots, fibrinoid necrosis and immature vilies were feasible to be studied. As the Anatomy Department has no histopathologic study facilities of its own, we had to depend on the Histopathology Department of the Medical College. So, processing of the specimen was hampered to some extent ion some cases.

Acknowledgements:
All doctors and stuffs of Department of Anatomy, Rajshahi Medical College, Rajshahi and my family members were acknowledged for their co-operation to conduct the study.

Authors' contributions: MIK and SF: Concept and design, data acquisition, interpretation, drafting, final approval and agree to be accountable for all aspects of the work.

Declarations:
Funding: The authors received no financial support for the research, authorship and/or publication of this article.

Conflict of interest: Authors declared no conflict of interest.

Ethical approval: Ethical approval of the study was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi. Informed consent was taken from all participants. All the study methodology was carried out following the relevant ethical guidelines and regulations.

Consent for publication: Taken.

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Peer-Review History:

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