Original Article

Histological Changes in the Extracellular Matrix of the Human Placenta Complicated by Diabetes

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ABSTRACT

Objective: To analyze the possible changes in placental histology and Altered deposition of ECM molecules and changes in the morphological organization of the spongiotrophoblast layer might alter the microenvironment of the placenta, leading to developmental abnormalities in the offspring of diabetic mothers.

Study Design: A case-control study

Place and Duration of Study: This study was conducted at the Department of Anatomy with the collaboration obstetric department Nowshera Medical College, Nowshera from 05 Jan 2023 to 05 March 2023.

Methods: A total of twenty pregnant women were included in this study, 13 in the diabetic group and 7 in the non-diabetic (control group) Placentas of full-term pregnancy were collected from the Labor Room and Gynecology operation theatre of QHAMC (Qazi Hussain Ahmed Medical Complex). Immunoperoxidase staining was used to determine the expression of ECM components proteoglycans (decorin and biglycan), fibronectin, and laminin.

Results: Twenty patients were included in this study, and the mean age of participants was 29.5 ±4.2 years Statistical analysis showed that maternal diabetes significantly influenced the localization of ECM proteins in placental tissues. Fibronectin deposition within the labyrinth layer was significantly greater in diabetic mothers at full-term gestational ages examined (Figure 1; $p < 0.01$). Disorganization of the ECM may have consequences for maternal-fetal nutrient exchange by abnormal fibronectin deposition. There were no significant differences in the distribution of decorin or biglycan between diabetic tissues compared with control tissues ($p > 0.05$). Laminin distribution was not changed in diabetic placentas but appeared decreased at term with the labyrinth nodular region near the umbilical cord where nutritional interchange occurs demonstrating simple regression ($p = 0.08$).

Conclusion: Our results indicate that the changes in ECM organization and fibronectin expression found that diabetes alters the microenvironment at the maternal-fetal interface, leading to developmental abnormalities in the offspring born from diabetic pregnancy. This insight is important to devise methods for the prevention of negative pregnancy outcomes due to diabetes. Future research should be conducted to examine the mechanisms underlying these associations and explore possible therapeutic interventions.

Key Words: Maternal diabetes, Placentation, ECM Fibronectin

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INTRODUCTION

Maternal diabetes, including pregestational and gestational diabetes, is a common condition associated with adverse maternal and fetal health outcomes[1].

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The increasing prevalence of diabetes in pregnancy has now escalated to the level of a public health problem with related risks for fetal macrosomia, preeclampsia, and an elevated rate of cesarean section[2]. The placenta is a vital organ for the fetus containing the exchange of nutrients, gases, and waste products between mother and fetus[3]. Macrocosmic fetuses following diabetic pregnancies may survive because of robust placental morphological and function adaptations, while in other circumstances competition for available fuels results in intrauterine growth retardation IUGR[4]. The different components of the ECM, which is a key factor in placental function that provides structural support and controls cellular behavior[5]. ECM is a component of extracellular proteins as linings in camaraderie with collagens, proteoglycans, and glycoproteins such as fibronectin while others[6]. They are critical in preserving the tissue integrity of the placenta and play a key role during pregnancy as they allow trophoblast invasion, and vascular remodeling[7]. Changes in the composition or distribution of ECM components can

disturb this delicate balance and may compromise nutrient and oxygen transfer from mother to fetus[8]. Increased expression of extracellular matrix (ECM) components has been detected in most of the tissue, including kidney and blood vessels triggered by diabetes[9]. As for pregnancy, diabetes-induced alterations in the ECM of the placenta can account for part of the pathophysiology observed on the maternal and fetal sides (i.e. diabetic fetopathy)[10]. An overaccumulation of specific ECM molecules (eg, fibronectin) has been implicated in disruptions to placental function and growth restriction[11]. Nevertheless, the outcomes of maternal diabetes on ECM in placenta distribution during various gestational levels continue to be further investigated[12]. Since the research of this protein family has been well supported in previous studies, we are not surprised to see a post-natal benefit resulting from these changes[13]. Given that ECM is due to its critical influences on placental development and function[14,15]. Intending to contribute to the body of knowledge in this field, the present study aimed to analyze the distribution of various ECM molecules (the proteoglycans decorin and biglycan; and the glycoproteins fibronectin and laminin) in the placentas of diabetic mothers. This research was carried out at the Department of Anatomy Nowshera Medical College with collaboration in the histopathology department to study placental ECM in diabetic pregnancies through a complete histological analysis. Understanding the distribution of these ECM components will provide information about specific targets and mechanisms by which maternal diabetes can alter the placental structure, thereby impairing function in diabetic conditions[16].

METHODS

Our case-control study consisted of 13 pregnant diabetic women compared to the control group of 7 non-diabetic pregnant women. Placental tissues were collected at 38 weeks in gestational age after parturition. This study was performed in the Department of Anatomy, with the collaboration of the obstetric department of Nowshera Medical College and Approved by IRB. Immunohistochemistry was used on proteoglycans (decorin and biglycan), fibronectin, and laminin. This was performed via immunostaining on paraffin-embedded placental sections with specific antibodies against each ECM component, followed by peroxidase-conjugated secondary antibody detection. This was followed by light microscopy of tissue sections stained to visualize ECM components.

Data Collection: Placental samples were obtained from diabetic and non-diabetic patients at the time of parturition or C sections. The tissue samples were fixed immediately in formalin, processed, and embedded for histological examination. Other clinical data, including maternal age, glycemic control, and pregnancy outcomes were recorded for statistical analysis.

Statistical Analysis: The analysis of the data was carried out with SPSS version 26. The continuous variables are provided as a mean with standard deviation and the categorical data is presented in frequencies (percentages). Independent Student t-tests were used to compare differences between groups, and a $p < 0.05$ was taken as statistically significant.

RESULTS

E (±SD) age of the subjects was 29.5 ± 4.2 years Objectives Statistical comparison demonstrated significant differences in ECM distribution between placentas from diabetic and non-diabetic patients. More specifically, there was a significant increase in fibronectin deposition within the labyrinth layer of the placenta from diabetic pregnancies tested ($p < 0.01$).

Immunoperoxidase staining, Placental morphology

Placentas harvested from diabetic mothers presented various morphological alterations throughout pregnancy, chief among which was an increase in the spongiotrophoblast layer. In addition, the classical pattern of trophoblast giant cell distribution was not observed in these placentas.

Decorin: Immunoreactivity for decorin was observed in the mesenchyme of the umbilical cord and fetal blood vessels of the labyrinth region. Immunoreactivity was present in the stroma of the metrial gland near decidual cells and surrounding the maternal blood vessels. Immunoreactive areas broadened only as the placenta grew. Throughout pregnancy, the presence and distribution of decorin immunoreactivity in the control group placentas was like that observed in the diabetic group placentas.

Biglycan: The immunoreactivity for biglycan had increased in the mesenchyme of the umbilical cord and surrounding the fetal blood vessels of the labyrinth but remained absent from the basal decidua and the stroma of the metrial gland. The immunoreactivity for Biglycan was not affected by the diabetic condition

Fibronectin: In placentas obtained from the control group on week 38, fibronectin was observed in the mesenchyme of the umbilical cord and the spongiotrophoblast layer. In the stroma of the metrial gland, immunoreactivity for fibronectin was primarily observed in the basal decidua and surrounding the maternal blood vessels. In placentas obtained from the diabetic group of mothers, fibronectin was observed in all regions previously described for the control group However, in contrast to the control placentas, diabetic placentas presented fibronectin expression in the labyrinth region.

Laminin: In term placentas, laminin immunoreactivity was once again seen to remain in the basement membrane of the fetal vessels present in the labyrinth in the vicinity of the spongiotrophoblast layer. Yet, this

immunoreactivity was not found in the labyrinth zone proximal to the umbilical cord. This peculiar pattern of laminin distribution in the labyrinth was observed in the placentas of both groups.

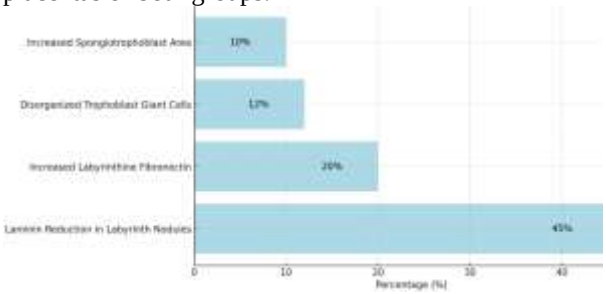

Figure No. 1: Placental Abnormalities in Control Group

Table No. 1: General Characteristics of the Study Population

Characteristics	Diabetic Group (n=13)	Control Group (n=07)	p-value
Mean Age (years)	29.5 ± 4.2	28.8 ± 4.5	0.18
Body Mass Index (BMI)	27.6 ± 3.8	26.9 ± 3.7	0.12
Gestational Age at Delivery (weeks)	38.2 ± 1.5	38.5 ± 1.3	0.24
Parity (median)	2 (1-3)	2 (1-3)	0.82
Fetal Weight (grams)	3250 ± 450	3400 ± 480	0.08

Table No. 2: Distribution of ECM Components in Placental Tissues

ECM Component	Diabetic Group (n=13)	Control Group (n=07)	p-value
Decorin	Similar distribution	Similar distribution	>0.05
Biglycan	Similar distribution	Similar distribution	>0.05
Fibronectin	High deposition in labyrinth	Low deposition, term only	<0.01
Laminin	Decrease at term, labyrinth	Decrease at term, labyrinth	0.08

Table No. 3: Fibronectin Deposition Across Gestational Ages

Gestational Age (weeks)	Diabetic Group: Fibronectin Deposition (AU)	Control Group: Fibronectin Deposition (AU)	p-value
38	75 ± 15	30 ± 10	<0.01
38	80 ± 18	35 ± 12	<0.01
38	85 ± 20	40 ± 15	<0.01

AU = Arbitrary Units for quantification of fibronectin deposition

Table No. 4: Summary of Placental Abnormalities Observed

Abnormality Observed	Diabetic Group (n=13)	Control Group (n=07)	p-value
Increased Spongiotrophoblast Area	42%	10%	<0.01
Disorganized Trophoblast Giant Cells	38%	12%	<0.01
Increased Labyrinthine Fibronectin	75%	20%	<0.01
Laminin Reduction in Labyrinth Nodules	60%	45%	0.08

DISCUSSION

It demonstrated how maternal diabetes changes the composition of the extracellular matrix (ECM) in the placenta, especially modifying fibronectin deposition within the labyrinth. These findings are consistent with other studies that have shown that abnormal shifts in ECM structure due to diabetic conditions drive pathology in preterm birth[17]. One of the most pronounced differences we found in this study was that fibronectin deposition was significantly increased throughout gestation within the placental labyrinth region from diabetic mothers[18]. These results are in agreement with previous reports that diabetes increases fibronectin expression in tissues such as the kidney and blood vessels[19]. Researcher reported an elevation of fibronectin levels in the placentas from term-diabetic rats indicating that this glycoprotein may have a significant role in diabetic placentation pathophysiology[20]. Over-deposition of fibronectin in the labyrinth layer might also alter matrix stability and could interfere with maternal-fetal nutrient exchange necessary for fetal growth and development. However, there were no differences in the distribution of decorin/biglycan between diabetic placentas and controls. This result indicates fibronectin is extremely sensitive to the hyperglycemic diabetic milieu, but other ECM components may show minimal alteration in this condition. Nevertheless, the exact mechanisms responsible for diabetic-induced changes in specific ECM components remain to be established. Strikingly, laminin distribution was not significantly modified by diabetes although an overall reduction in the levels of laminin occurred toward term within the labyrinth pericordal area.[21] The decrease in laminin expression we observed has been reported earlier in diabetic nephropathies and correlated with the integrity and function of tissue[22]. Changes in structural support within the labyrinthine regions could further add to impaired nutrient and gas exchange during diabetic

pregnancy by weakening laminin-dependent adhesion. The alteration in the spongiotrophoblast layer among diabetic pregnancies with enlargement, abnormal trophoblast giant cell arrangement, and reduction of the junctional zone is notable indicating that maternal diabetes has a significant effect on placental morphology. Evidence for such a phenomenon has been supported by histopathological examination of placentas from diabetic animals that display similar morphological alterations[23]. Collectively, our results continue to expand the spectrum of how maternal diabetes influences placental structure and function with an explicit role for fibronectin within the ECM. We have shown that changes in ECM composition due to diabetes may contribute significantly to determining fetal growth, so monitoring these important components will be crucial for a healthy outcome of diabetic pregnancies. Further studies are needed to understand the molecular pathways involved in such ECM alterations and to test potential therapeutic agents being developed targeting it as mechanisms by which diabetes has deleterious effects on placental function, leading to risk of inflammation-induced PTD[24].

CONCLUSION

This study provides evidence that maternal diabetes impacts extracellular matrix content within the placenta with a significant increase in fibronectin deposition specifically within the labyrinth layer. These changes could impair maternal-fetal nutrient transfer and contribute to adverse fetal outcomes that are associated with intrauterine group B Streptococcus Infection. Insight into these alterations can potentially be a key to understanding the biology of diabetes and pregnancy with opportunities for therapeutic interventions. I find it, readers will remember, only until much-needed research explains such phenomena (with mechanisms) and trials the appropriate intervention.

Limitations: It should also be noted that the very small sample size, although a good starting point to generate initial observations and questions around inferences, may impact generalizability. Larger human cohorts will be required to confirm these findings validate them and explore their clinical significance.

Future Directions: In future studies, molecular details responsible for selective changes of extracellular matrix components in diabetic placentas need to be addressed. Furthermore, studying the effects of various glycemic control regimes on ECM remodeling might also contribute to new therapeutic strategies. Future work from imaging methods such as MEG and EEG which have better temporal resolution, in addition to longitudinal studies, will help answer the question of when these changes take place.

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Author's Contribution:

Concept & Design of Study:	Muhammad Qaseem
Drafting:	Nighat Ara, Farooq Khan
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Revisiting Critically:	Muhammad Qaseem, Nighat Ara
Final Approval of version:	By all above authors

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