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Identification of Mucopolysaccharide Types in Diabetic Placenta and Umbilical Cord Tissues

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Abstract: ***Background: The health of the placenta significantly influences the fetus's well - being. Placental abnormalities, especially in diabetic pregnancies, can lead to various complications affecting both the mother and the fetus. Understanding the types and distribution of mucopolysaccharides in the placenta and umbilical cord tissues of diabetic pregnancies is crucial for improving diagnostic and therapeutic strategies. Objective: The aim is to identify the types of mucopolysaccharides present in the diabetic placenta and the tissues of the umbilical cord. Materials and Methods: We conducted this non - interventional descriptive study. We analyzed a total of 36 biopsies, comprising 18 from diabetic pregnancies and 18 from healthy pregnancies. We prepared sections (3 - 5 microns) from these tissues and processed them using various staining techniques, such as hematoxylin and eosin (H&E), Alcian Blue - PAS combination, and mild methylation with Alcian Blue staining. The data were analyzed using SPSS. Results: From 216 samples, sections of the umbilical cord stained with H&E showed 72% normal histological structures. Combination techniques revealed that 34% of mucopolysaccharides were neutral and strongly acidic. Mild methylation revealed that 45% of mucopolysaccharides were strongly carboxylated and 33% were strongly sulfated. Diabetic placenta sections stained with H&E showed 33% inflammatory changes, with basement membrane thickening. Combination techniques were used to detect 45% neutral and strongly acidic mucopolysaccharides. Mild methylation revealed that 39% had no acidic mucopolysaccharides, 33% were strongly sulfated, and 17% were strongly carboxylated. Conclusion: The study highlights significant differences in the types of mucopolysaccharides between diabetic and non - diabetic placental and umbilical cord tissues. Diabetic tissues exhibited higher levels of strongly carboxylated and sulfated mucopolysaccharides, indicating potential markers for diabetes complications during pregnancy.***

Keywords: Placenta, Umbilical Cord, Mucopolysaccharides, Diabetes

1. Introduction

The well - being of the fetus is influenced by numerous factors, yet a healthy placenta remains the most crucial element in ensuring a healthy baby (1, 2). The placenta begins to form around the seventh day post - fertilization when the first layer of cytotrophoblast cells is observed. During pregnancy, a variety of complications may arise, potentially leading to fetal abnormalities or death (3). Although these issues can stem from chromosomal and genetic disorders of the fetus (e. g., Down syndrome), maternal illnesses or behaviors (e. g., pre - eclampsia, smoking), or environmental factors (e. g., radiation), placental abnormalities are the most significant (3).

Understanding the size, shape, consistency, completeness, and potential abnormalities (e. g., infarcts, hemorrhages, tumors) of the placenta is vital for the care of both the mother and the infant (3). The placenta is a complex fetal organ with multifaceted roles during fetal growth. It separates maternal and fetal circulations through distinct surfaces—syncytiotrophoblast (maternal side) and endothelium (fetal side) —making it susceptible to influences from hormones, cytokines, growth factors, and substrates present in both circulations (4).

Placental abnormalities, including increased weight and size, and abnormal placenta weight - to - fetal weight ratios, have been linked to various conditions such as maternal hypertension and diabetes, and fetal intrauterine growth restriction (5, 6). Diabetic pregnancies often feature placenta megalia, characterized by increased glycogen content and changes in cellularity and histology (7). This megalia correlates with heightened glycogen storage and cellular solute content required for maintaining osmotic balance in the presence of maternal hyperglycemia. Additionally, extracellular matrix proteins such as fibronectin are elevated in diabetic placentas (4).

Pregnancy in diabetic women is associated with increased fetal morbidity and mortality, though the precise molecular mechanisms remain unclear. Various studies suggest that alterations in extracellular matrix protein expression may be a common feature of diabetic complications, affecting not only adult tissues over prolonged periods but also the relatively short - lived placenta (8). These disturbances in extracellular matrix deposition in diabetic patients, along with overexpression of constituents like laminin B1 and fibronectin, have been observed in embryos and fetuses of diabetic individuals (4).

The purpose of this study is to enhance our understanding of the histological and histochemical changes, particularly mucopolysaccharides, in diabetic placentas using chemical control techniques such as Alcian Blue staining with mild methylation. This research aims to identify specific types of mucopolysaccharides in the umbilical cord and placenta tissues, contributing to better diagnostic and therapeutic strategies for managing pregnancies complicated by diabetes.

2. Materials and Methods

Study Design
This is a non - interventional descriptive study.

Study Duration
The study was conducted from November 2023, to April 2024.

Study Population
The study included a total of 36 biopsies: 18 biopsies from diabetic pregnant women and 18 biopsies from healthy pregnant women.

Study Sample
Sections of 3 - 5 microns were prepared from the biopsy samples on a random basis.

Tissue Processing

Selection
At the end of fixation, tissues were examined, and specimens were selected.

Selection of Specimen
Specimens (3 - 5 microns) were selected after fixation in 10% formal saline for 20 days and processed manually.

Dehydration
Dehydration was performed using ethyl alcohol at different concentrations.

Dehydration Stage	Start	End	Period
70% Alcohol	09:30	02:30	05:00
90% Alcohol	02:30	09:00	18:30
ABS1	09:00	12:00	03:00
ABS2	12:00	02:00	02:00
ABS3	02:00	04:00	02:00

Clearing
Clearing was done using chloroform and impregnation.

Clearing Stage	Start	End	Period
Chloroform	05:00	11:30	18:30
Impregnation	11:30	03:30	04:00

Embedding
Embedding was done using clean wax in a dispenser machine.

Sectioning
Sections of 3 - 5 microns in thickness were cut using a microtome. The sections were mounted on frosted slides using 20% alcohol to spread the sections in a water bath. Ribbons of sections were separated by a needle, and adhesive media were used on the frosted slides to mount the sections. After sectioning, slides were dried and placed in an oven for dewaxation.

Staining Methods

Organ	H&E	Combination	Alcian Blue Methylation (37°C)	Total
Placenta	18	18	18	54
Umbilical Cord	18	18	18	54
Total	36	36	36	108

Hematoxylin and Eosin (H&E)
- Take sections to water.
- Stain nuclei with Mayer's Hematoxylin for 5 - 8 minutes.
- Wash and blue in tap water for 10 minutes.
- Counterstain with eosin for 1 - 3 minutes.
- Dehydrate, clear, and mount (9)

Combination Alcian Blue - PAS
- Take sections to water.
- Stain with Alcian Blue (pH 2.5) for 20 minutes.
- Wash in tap water.
- Oxidize in 10% periodic acid for 5 minutes.
- Wash in water for 5 minutes and rinse in distilled water.
- Place in Schiff's reagent for 10 - 20 minutes.
- Wash in water for 10 minutes.
- Stain nuclei with Mayer's Hematoxylin for 5 - 8 minutes.
- Blue in tap water for 10 minutes.
- Dehydrate, clear, and mount (9).

Alcian Blue
- Take sections to water.
- Stain with Alcian Blue for 10 - 30 minutes.
- Wash in water for 5 minutes.
- Counterstain with 1% neutral red.
- Wash in water.
- Dehydrate, clear, and mount. (9)

Blocking Techniques

Mild Methylation Technique
- Take sections to water.
- Incubate in hydrochloric acid in methanol at 37°C for 4 hours.
- Wash.
- Incubate in distilled water at 37°C for 4 hours.

Data Analysis
Data obtained from this research were analyzed using SPSS software. Descriptive statistics, including mean, median, and standard deviation, were used to summarize the data. Inferential statistical tests such as t - tests and chi - square tests were applied to compare the differences between diabetic and healthy groups, with a significance level set at $p < 0.05$.

Ethical Considerations
All participants provided written informed consent before enrollment, and confidentiality of their personal information was strictly maintained throughout the study.

3. Results

This study focused on identifying the different types of mucin present in the placenta and umbilical cord tissue of pregnant women. We prepared 216 samples from the placenta and umbilical cord using a combination of hemoglobin and eosin, as well as alcian blue and blocking techniques. We prepared sections from the umbilical cord and stained them with hematoxylin and eosin stain, revealing 72% of normal histological structures (refer to figure 1). When we used a combination staining technique, we observed neutral mucopoly saccharides and strongly acid mucopoly saccharides (34%), as shown in figure 4 - 2. Sections stained with mild methylation at 37 oC and 7 oC, on the other hand, showed that 33% were strongly sulfated and 45% were strongly carboxylated (see figure 4 - 3).

We cut up a diabetic placenta and stained sections with hematoxylin to look at their histological structure. We saw changes in the thickness of the basement membrane that were caused by inflammation (see figures 4–4).

We found that the sections stained with a mix of techniques had the same amount of neutral muco polysaccharides and strongly acid mucopoly saccharides (45%; see Alcian blue with mild methylation). We also found three types of acid mucopoly saccharides: no acid muco polysaccharides, 33% strongly sulfated acid mucopoly saccharides, and 17% strongly carboxylated acid mucopoly saccharides (see figures 4 - 6).

We made sections from the umbilical cords of people with and without diabetes and stained them with hematoxylin and eosin. The diabetic umbilical cord had 72% normal histological structure, and the undiabetic umbilical cord had 61% normal histological structure (see figures 4–7). Arides, saccharides, and 17% were strongly carboxylated acid mucopoly saccharides (saccharides), as shown in figures 4 - 6. We made sections from umbilical cords of people with and without diabetes and stained them with hematoxylin and eosin. The diabetic umbilical cord had 72% normal histological structure and the undiabetic umbilical cord had 61% normal histological structure (see figures 4–7).

The alcian blue mild - methylation blocking technique produced the most mucin. In the diabetic umbilical cord, 45% was strongly caroxylated mucopoly saccharides, and 38% was weakly carboxylated acid mucopoly saccharides (see figure 4 - 8). We also found the most normal histological structure in sections from diabetic and non - diabetic placentas stained with hematoxylin and eosin. In the non - diabetic placenta, 83% of the normal histological structure was present, and 50% of it had normal histological structure (see figure 4 - 9).

Using a mild methylation method to stain with alcian blue, the placenta from the woman who did not have diabetes had the most strongly sulfated acid mucopoly saccharide (44%), while the placenta from the woman who did have diabetes had the most mucin (39%), which was a neutral mucopoly saccharide (see figures 4–10).

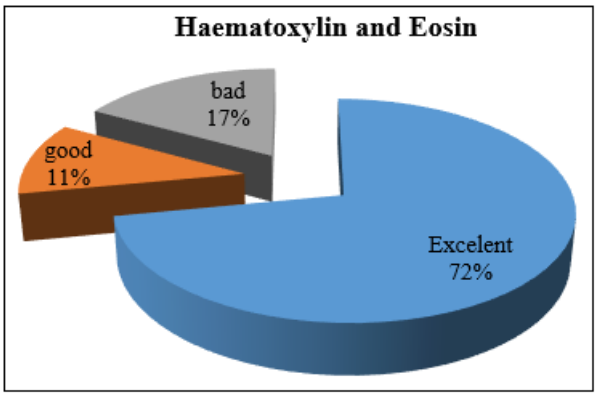


Figure 1: Frequency of results in diabetic umbilical cord with haematoxylin and eosin.

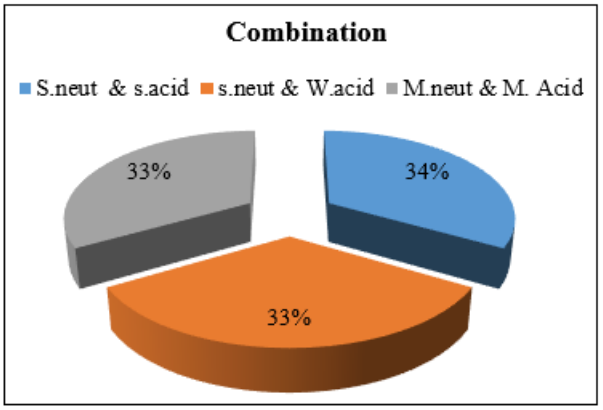


Figure 2: Types of mucopoly saccharides in diabetic umbilical cord using the combination P. A. S. reaction and Alcian blue technique

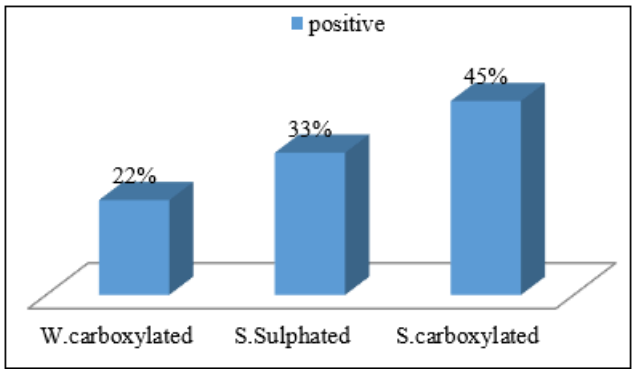


Figure 3: Types of mucopoly saccharides in diabetic umbilical cord with Alcian blue stain by blocking technique.

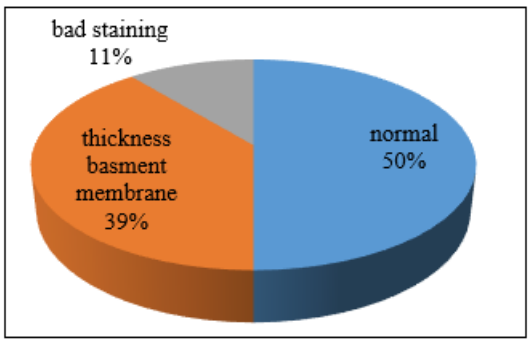
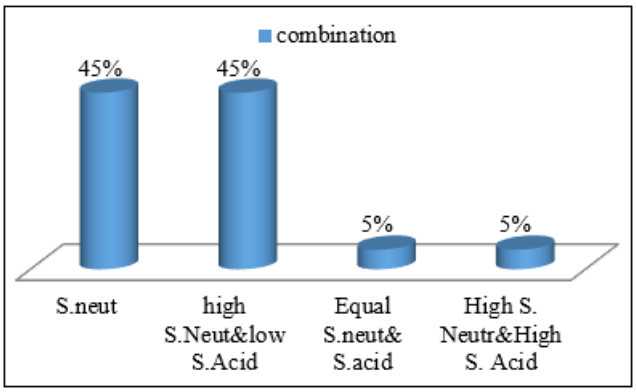


Figure 4: Frequency of result in diabetic placenta with haematoxylin and eosin.



Figures 5: Types of mucopoly saccharides in diabetic placenta with combination P. A. S. reaction and Alcian blue technique.

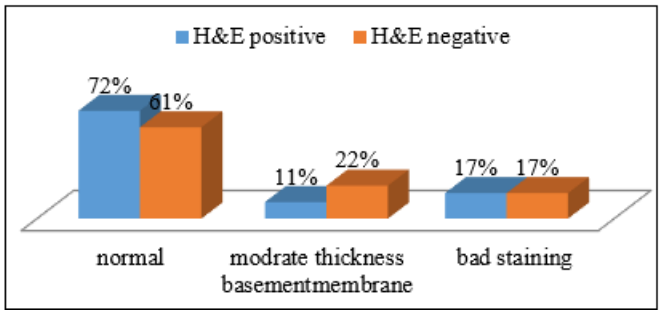


Figure 7: Comparison between diabetic and non - diabetic umbilical cords stained with haematoxylin and eosin stain

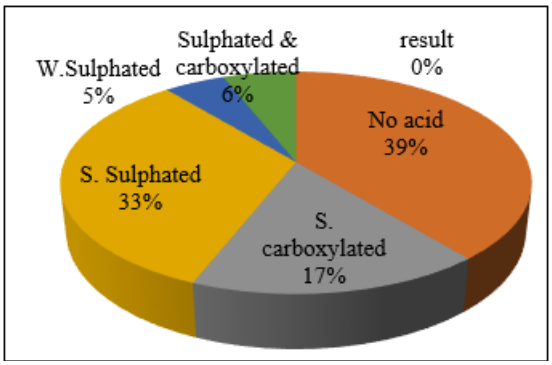


Figure 6: Types of mucopoly saccharides in diabetic placenta stained with Alcian blue using a blocking technique.

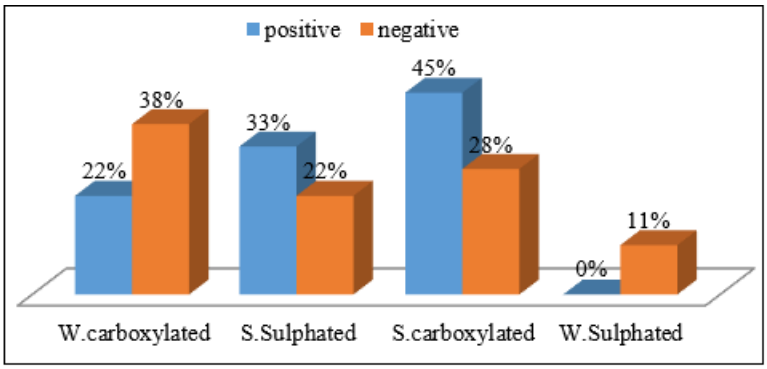


Figure 8: Comparison of diabetic and non - diabetic umbilical cords stained with alcian blue stain and mild methylation technique.

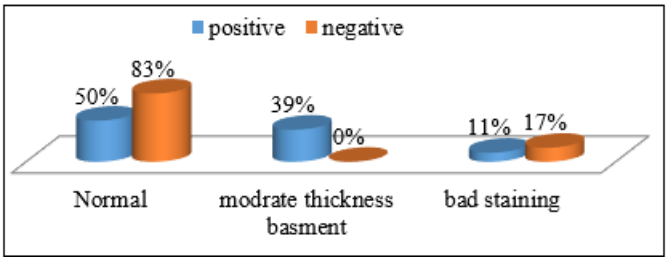


Figure 9: Comparison between diabetic and undiabetic placentas stained with hematoxylin and eosin stain.

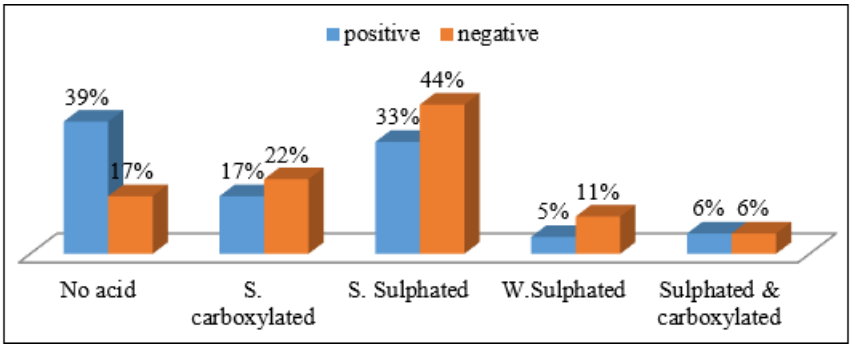


Figure 10: A comparison between diabetic and undiabetic placentas stained with Alcian Blue using the mild methylation technique.

4. Discussion

Diabetes mellitus, indicated by abnormal glucose tolerance with onset or first recognition during pregnancy, is associated with an increased risk of maternal complications. This study aimed to analyze the placental histopathological changes in pregnancies complicated by diabetes.

Lavonia et al. (2012) studied the placental histological changes in pregnancies with diabetes at the University of Medicine and Pharmacy of Craiova (10). Their study showed important changes in the cells' histopathology, such as immature villi, swollen villi, a thickening of the basement membrane, chorangiosis (clotted capillaries), fibrinoid deposits inside and outside of villi, and glycogen buildup (10). These findings reflect a severe degree of placental damage associated with maternal diabetes, suggesting a detrimental impact on placental structure and function (10).

Contrary to these findings, our study did not observe such extensive histopathological changes. One possible explanation for this discrepancy is the difference in population and severity of diabetes among the study subjects. Most women in our study had effectively managed gestational diabetes mellitus (GDM) with treatment, resulting in minimal histological damage to the placenta. Our subjects' lower diabetes severity likely contributed to the less pronounced histopathological alterations observed in our study (11).

In 2008, Abromovici and Svejcar looked at the mucopolysaccharide content in both normal and diabetic placentas, focusing on the labyrinth zone (12). They found that the two zones had different amounts of total mucopolysaccharides, with hyaluronic acid and neutral polysaccharides deposited outside of cells. Diabetic placentas showed an increase in all types of mucopolysaccharides, suggesting a correlation between the accumulation of mucopolysaccharides and the degree of maternal hyperglycemia (12).

Due to differences in methodology and the degree of diabetes control among the study populations, our study did not find such extensive mucopolysaccharide accumulation. The accumulation of mucopolysaccharides in the diabetic placenta might be a consequence of a shift in carbohydrate metabolism induced by hyperglycemia, as suggested by Abromovici and Svejcar (12). However, in our study, the effective control of diabetes in the subjects likely mitigated these metabolic shifts, resulting in less pronounced histochemical changes (13).

Furthermore, the methods we used were not as complex as those used by Abromovici and Svejcar. This could be why the results we got were different when it comes to finding mucopolysaccharides (13). Our findings underscore the importance of diabetes management during pregnancy to minimize histopathological and histochemical alterations in the placenta (13).

5. Conclusion

while previous studies have documented significant histopathological and histochemical changes in the placentas of diabetic pregnancies, our findings suggest that effective diabetes management can mitigate these alterations. The differences between our findings and those of other studies highlight the impact of diabetes severity and control on placental health, emphasizing the need for careful monitoring and management of diabetes in pregnant women.

References

[1] Van den Broek N, White SA, Goodall M. The impact of placental pathology on maternal and fetal health: A review. Placenta.2005; 26 (4).
[2] Kliman HJ. From Trophoblast to Human Placenta. Advances in Experimental Medicine and Biology.1997; 447: 3 - 22.
[3] Yetter JF. Examination of the Placenta. American Family Physician.1998; 57 (5): 1045 - 1054.
[4] Mylonas I, Gingelmaier A. Diagnosis of placental insufficiency. Journal of Perinatal Medicine.2007; 35 (1): 17 - 21.
[5] Molteni RA, Stys SJ, Battaglia FC. Relationship of fetal and placental weight in human beings. The Journal of Reproductive Medicine.1978; 21 (5): 327 - 334.
[6] Heinonen S, Taipale P, Saarikoski S. Weights of placentae from small - for - gestational age infants revisited. Placenta.2001; 22 (5): 399 - 404.
[7] Desoye G, Shafrir E. Placental metabolism and its regulation in health and diabetes. Molecular Aspects of Medicine.1994; 15 (6): 505 - 682.
[8] Knock G. The role of diabetes in placental development and function. Placenta.2002; 23 (4): 330 - 337.
[9] Lavonia GH, Plesea IE, Gheorman V. Studying the placental histological changes in pregnancies with diabetes in the context of the real (epidemic) of diabetes

and lack of pathognomonic information on histological damage of the placenta in diabetes. University of Medicine and Pharmacy of Craiova.2012.
[10] Lavonia GH, Plesea IE, Gheorman V. Villous immaturity, villous edema, basement membrane thickening, chorangiosis, intra and extra villous fibrinoid, and glycogen deposits in diabetic placentas. University of Medicine and Pharmacy of Craiova.2012.
[11] Abromovici AL, Svejcar JM. Histochemical analysis of mucopolysaccharides in diabetic and normal placentas: Differences in total content and extracellular deposition of hyaluronic acid and neutral polysaccharides. Journal of Histochemistry & Cytochemistry.2008; 56 (5): 457 - 465.
[12] Abromovici AL, Svejcar JM. Correlation between mucopolysaccharide accumulation in the placenta and maternal hyperglycemia: A shift in carbohydrate metabolism induced by hyperglycemia. Placenta.2008; 29 (4): 312 - 319.
[13] Abromovici AL, Svejcar JM. The impact of diabetes on placental histological and histochemical characteristics. Placenta Research Journal.2008; 30 (6): 428 - 434.