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Research Article

Investigation of the Sox-9 and Caspase-6 Immune Activity in Placentas of Pregnant Women with GDM

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Abstract

Aim: In this study, we investigated the immunohistochemical staining of Sox-9 and Caspase-6 expression in placentas of pregnant woman with gestational diabetes mellitus (GDM).

Material and Methods: Placentas of 20 healthy and 20 women with GDM were processed for routine histological tissue processing. The biochemical and clinical parameters of patients were recorded. Placentas were stained with hematoxylin-eosin and Sox-9 and Caspase-6 immunostaining.

Results: In control group, Sox-9 expression was negative in decidual and connective cells and endothelial cells. In GDM group, Sox-9 expression was increased especially in the decidual cells. For the Caspase-6 expression, Caspase-6 reaction was mainly in maternal region in control group. In GDM group, Caspase-6 reaction was increased in decidual cells, in endothelial cells and in the syncytial nodes.

Conclusion: Expression of Sox-9 transformed the decidua cells and lead to apoptotic pathway via Caspase-6 expression

Keywords: Sox-9, caspase-6, GDM, placenta

INTRODUCTION

Many complications can develop during pregnancy and these are called maternal morbidity. The most common diseases among pregnancy complications are gestational hypertension (GHT), preeclampsia, eclampsia, superimposed preeclampsia, gestational diabetes mellitus (GDM), postpartum hemorrhage and infections (1,2). Regular pregnancy follow-ups are important for the early diagnosis of these diseases, but there is no definitive and clear screening and test according to studies. Detailed medical and obstetric history is still the most commonly used method for diagnosis. Most pregnancy complications may be resolved after delivery however their long-term effect may be continued future (3,4).

Gestational diabetes mellitus (GDM) is carbohydrate intolerance that occurs during pregnancy or is first noticed during pregnancy (5). It affects approximately 1 out of every 10 women worldwide. It may have various negative consequences such as macrosomia, fetal hypoglycemia, neonatal intensive care support and fetal mortality. There are also long-term effects. Conditions such as various cardiovascular diseases, insulin resistance, and diabetes may occur (5,6). The placenta is an important organ for fetal growth and development. It provides adaptation to the changes that occur in the uterus during normal and pathological pregnancies. Compared to normal pregnancies, increased vascularization is observed in placentas with GDM. This may seem counterintuitive

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because of increased maternal nutrition, but improved fetal aerobic metabolism stimulated by insulin reflects the increased oxygen demand of the fetus. The mechanisms underlying enhanced angiogenesis have not been fully elucidated (7,8).

The aim of this study is to investigate Sox-9 and Caspase-6 expression of in placentas of women with GDM by histochemical and immunohistochemical methods.

MATERIAL AND METHOD

Ethical approval was taken from Dicle University Medical School, Non-interventional Clinical Trials Ethical Committee (2020/68). In our study, 20 healthy women and 20 women with GDM were included. Placentas were obtained from Gynecology and Obstetrics Clinics. All patients were informed about the data and experimental protocol. For each patient, biochemical and clinical parameters were noted after their approval. for each patient were recorded. Patient informed consent form was read to all patients and they signed the forms.

Histological Tissue Processing

Placental samples were dissected and stored for histological dye experiments. The placental tissues were taken into formalin solution, dehydrated in increasing alcohol series, soaked in xylol solution and incubated in paraffin wax at 58°C. samples were put into paraffin blocks and 4 µm sections were cut and stored for hematoxylin eosin staining (9).

Immunohistochemical Examination

Placental sections were cleared in xylol solution, dehydrated in alcohol and cleared in distilled water. Epitope retrieval was inducted by EDTA (ethyl diamine tetra acetic acid) solution (pH:8.0) for 15 minutes in a microwave oven at 90°C. After sections were cooled down, they were rinsed in phosphate buffered saline (PBS) three times for 5 minutes. 3% hydrogen peroxide (H2O2) was dropped on slides to block endogen peroxidase activity. After washing in PBS, sections were incubated with rabbit polyclonal Sox-9 and Caspase-6 (AFG Scientific, US, 1/100) overnight at + 4°C. Sections were dipped into PBS and biotinylated antibody solution (ThermoFischer, US) was dropped onto slides for 14 minutes. Sections were reacted with streptavidin peroxidase solution was (ThermoFischer, US) for 15 minutes. After PBS washing, diaminobenzidine (DAB) chromogen was used to observe color change for maximum 10 minutes. Reaction were stopped with PBS solution and sections were stained with hematoxylin dye. Slides were analyzed under light microscope (10).

Statistical Analysis

The data were recorded as median (minimum – maximum). Statistical analysis was done using the IBM SPSS 25.0 software (IBM, Armonk, New York, US).

RESULTS

Biochemical Parameters

Age, gravida, parity, systolic BP, diastolic BP, hemoglobin, platelet, glucose, urea, creatinine, ALT, AST-urine protein was recorded in healthy and GDM women. Data were shown in Table I. Glucose level were higher in GDM group than in healthy group. Graphical illustration of Table I was shown in Figure 1.

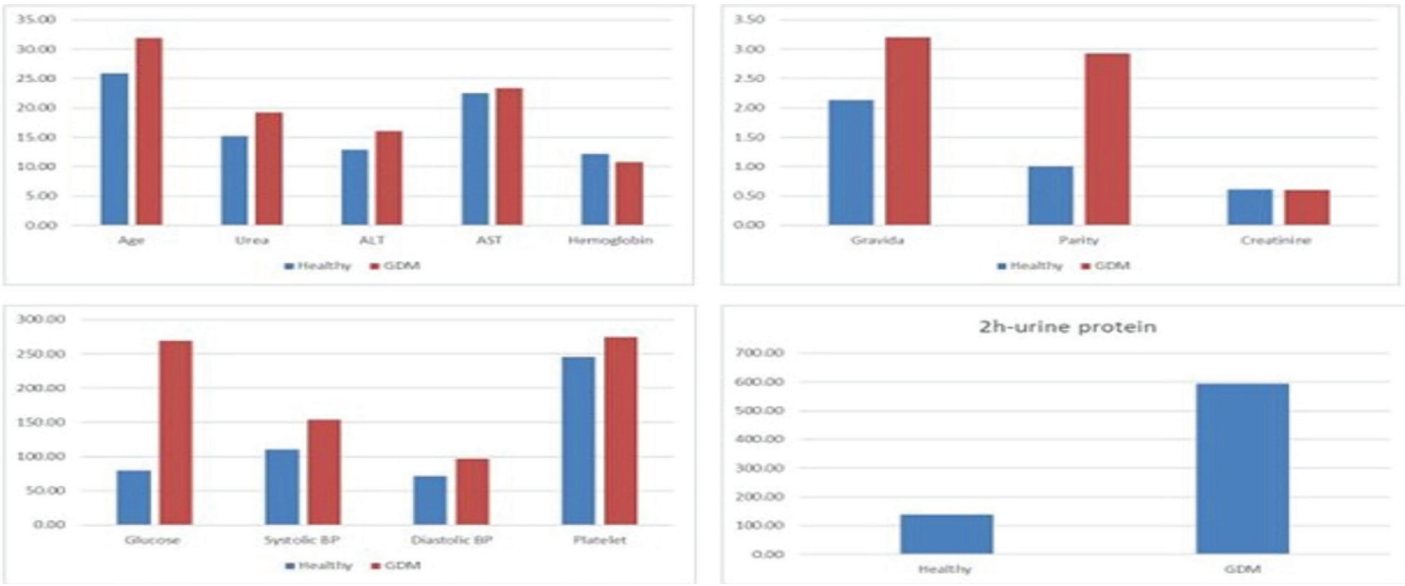

Figure 1. Graphical illustration of biochemical and demographical parameters

Histopathological Staining

Although mild dilatations were observed in some vessels in the GDM control group section, it was observed that the Sox-9 reaction was negative in endothelial cells and negative in connective tissue cells in general, while positive Sox-9 expression was detected in moderate decidual cells. It was also observed that Sox-9 was negative in the syncytial regions. Again, no Sox-9 reaction was observed in intervillous areas (Figure 2a). In the GDM preparation taken from the maternal region, that the reaction increased especially in the decidual cells, the nucleus showed

negativity in some places, but the cytoplasmic activity was positive in the direction of Sox-9 in the aggregated areas. Again, although there was shrinkage in nuclei in some areas, the expression was found to be positive for Sox-9 (Figure 2b).

In the section taken from the maternal region, although Caspase-6 reaction was negative in cells in some areas, positivity was observed in individual decidual cells, it was seen that the reaction was negative in the maternal region in general. Similarly, negativity for Caspase-6 was detected in endothelial cells (Figure 2c). In the GDM group, the caspase reaction showed widespread and intense positivity especially in decidual cells, the Caspase-3 reaction increased in the same way in endothelial cells, apoptotic cells were abundant, and Caspase-6 was positive in some areas due to vessel dilatation, especially in the vascular endothelium. It was determined that the Caspase-6 reaction increased in the syncytial nodes in these regions (Figure 2d).

Table 1. Clinical and biochemical parameters of healthy and GDM patients

Parameter	Healthy (N=20)	GDM (N=20)
Age	26 (20-35)	28 (24-42)
Gravida	2 (0-5)	3 (1-7)
Parity	0 (0-5)	3 (1-8)
Systolic blood pressure	110 (93-135)	148 (125-220)
Diastolic blood pressure	69 (64-82)	96 (87-109)
Hemoglobin	12 (10-14.5)	10.4 (9.5-13)
Platelet	231 (123-447)	269 (148-398)
Glucose	78 (68-105)	269.39 (105-608)
Urea	15 (12-20)	16 (13.5-42.58)
Creatinine	0.61 (0.54-0.71)	0.58 (0.53-0.84)
ALT	12 (8-23)	13 (7-44)
AST	18 (13-48)	22 (14-43)
2h-urine protein	142 (104-178)	534 (300-980)

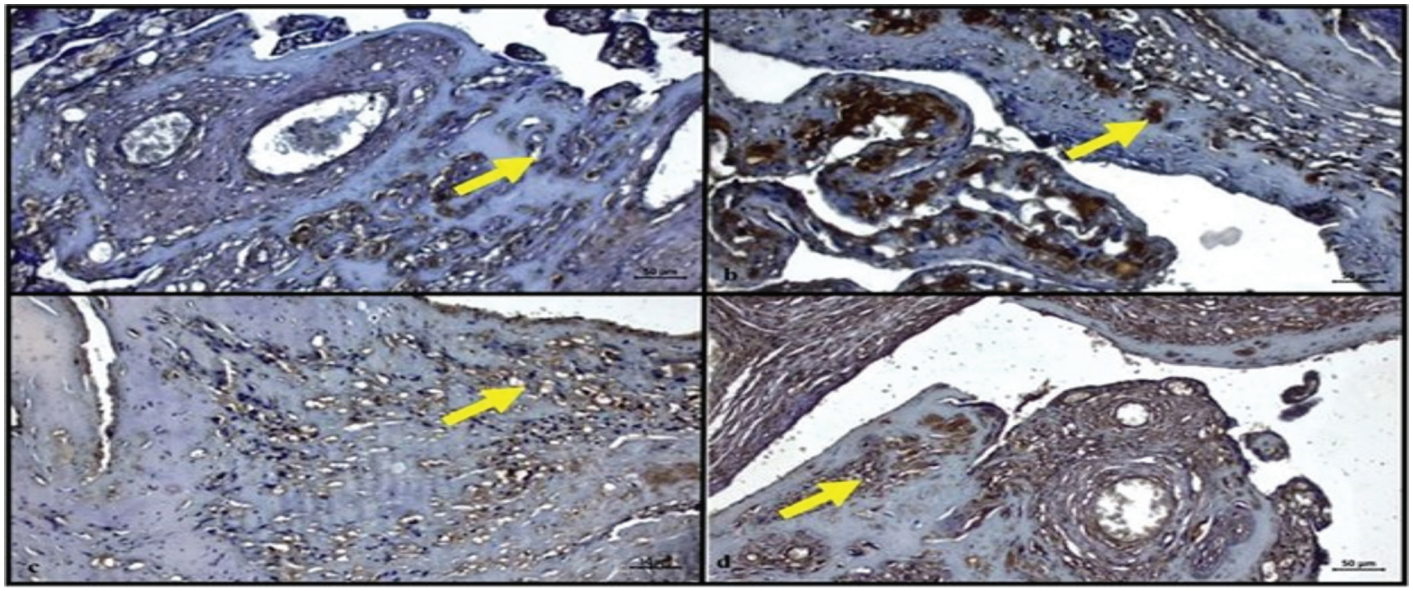

Figure 2. Healthy and GDM placentas with immune staining. Decidual cells (arrows)

DISCUSSION

During pregnancy, complications can lead the placental abnormalities such as preeclampsia, gestational diabetes mellitus (GDM), placenta previa and accrete. GDM can affect placental histology in short or long term. Aldahmash et al studied Saudi women with GDM. 84 placentas were examined and results showed that syncytial knots, calcification, villous agglutination, decidual vasculopathy, and retroplacental hemorrhage were common findings in maternal side. Villous fibrinoid necrosis, chorangiosis, fibromuscular sclerosis, and villous edema were common findings in fetal side (11). Another study by Rudge et al studied mild GMD, GDM and overt GDM patients and analyzed their histopathological lesions. Results showed villous edema and fibrosis, congestion, interstitial hemorrhage, focal hyaline degeneration (12).

SRY-box transcription factor 9 or Sox-9 is a transcription factor that is required for testicular development, organogenesis of liver and pancreas, cytoskeleton and chondrocytes. Mutations in Sox-9 gene can lead to autosomal sex reversal, skeletal formation and testis development (13,14). Sekido et al studied two genes in Sertoli cell by investigating SRY expression. They found that upregulation of Sox-9 gene in supporting cells determine their fate as Sertoli cells, which shows importance of Sox-9 gene in testis (15). Zhao et al studied endothelial to mesenchymal transition in murine endovascular progenitors. They found that endothelial to mesenchymal transition was dependent on relative expression of Sox-9 along with Notch signaling, affecting their plasticity which may be a therapy tool for fibrotic diseases (16). Xian et al studied showed that stimulation of Sox-9 can induce cellular differentiation gene and this can be a mechanism in transformation of extra villous trophoblast to endovascular trophoblasts during placentation (17). In

our study, in control group, Sox-9 reaction was negative in decidual and endothelial cells (Figure 2a). In GDM group, Sox-9 expression was increased in the decidual cells. Shrinkage nuclei showed positive Sox-9 expression (Figure 2b)

Caspases are cysteine proteases that are involved in cell death, immune responses. Caspase-6 is ana executioner caspase. Its role in apoptosis is well known however other roles remain unclear. Development of placenta is dependent on implantation to the uterus and invasion of decidual plate by trophoblast cells (18,19). Cheng et al investigated inflammatory pathway in preeclamptic patients via caspase-1 expression. They found that cell death pathway in increased via elevated active caspase-1 expression (20). Mu et al studied apoptosis in placenta of transgenic mice. They found that apoptosis was observed predominantly in syncytiotrophoblast cells via Tunnel assay. They also performed immunohistochemistry to analyze caspase-3 expression and found that active caspase-3 expression was observed in cells undergoing apoptosis (21). In control group, Caspase-6 reaction was negative in some cells but mainly negative in the maternal decidual cells and endothelial cells (Figure 2c). In the GDM group, Caspase-6 expression was showed widespread and intense positivity especially in decidual cells and endothelial cells (Figure 2d).

CONCLUSION

In conclusion, during GDM development, decidual cells were affected due to trophoblastic invasion and inflammation. Expression of Sox-9 signal in syncytial region and decidua cells could induce transformation and apoptotic process with Caspase-6 expression.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Ethical approval was taken from Dicle University Medical School, Non-interventional Clinical Trials Ethical Committee (2020/68).*

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