RESEARCH ARTICLE

Expression of Urotensin II of Human Placental Tissues and in Serum in Gestational Diabetic Mellitus in Iraqi Woman

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ABSTRACT

The placenta is an organ between the mother and fetus necessary for fetal growth and development. Gestational diabetes mellitus (DM) is the most frequent metabolic condition detected during pregnancy. It is characterized as hyperglycemia of various severity with onset or first detection during pregnancy that does not clearly describe any form of preexisting diabetes. Urotensin II (UII), a pluripotent vasoactive peptide, is important in developing insulin resistance.

This study aimed to determine the level of Urotensin II(UII) in placenta and in the serum of diabetic and nondiabetic women.

Methods The blood and placenta tissue collected from 50 ladies had been enrolled in this research (25 females with uncomplicated), (25 women with gestational diabetes). Immunohistochemistry (IHC) was used to look at the expression of the Urotensin II (UII) marker in placenta specimens. The IHC analysis revealed that Urotensin II expression was primarily found in placental cytotrophoblast and the syncytiotrophoblast.

Results of an immunohistochemistry investigation using the Urotensin II (UII) marker revealed a significant increase ($p \leq 0.001$) in diabetic women's placentas and serum than control groups.

Conclusion, the Urotensin II is mainly located in the cytotrophoblast and syncytiotrophoblast. That was significantly higher in the gestational DM group.

Keywords: Gestational diabetic mellitus, Placenta, Urotensin II.

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INTRODUCTION

The placenta is an organ that lies between the mother and the fetus and is important for fetal growth and development.[1] During pregnancy, the placenta performs essential functions such as the exchange of nutrients, water, respiratory gases, and waste products, as well as the synthesis of various hormones that regulate the transport of maternal nutrients to the fetus and aid maternal adaptation to different pregnancies stages, the ultrastructure of the placental exchange barrier governs these functions.[2,3] Gestational diabetes mellitus (GDM) is the most common metabolic disorder discovered during pregnancy. It is defined as hyperglycemia of variable severity with onset or first recognition during pregnancy that does not clearly characterize any type of preexisting diabetes.[4] It affects nearly 16.5% of pregnancies worldwide.[5] In a recent 2020 study in Iraq, the prevalence of GDM was found to be 13.3%.[6] The secretion of lactogen hormone from placenta alteration occurs (also known as human chorionic somatomammotropin (HCS)) and other hormones, such as progesterone, cortisol, and growth hormone, convert their effect on the maternal tissues into insulin insensitive, the pancreas is unable to provide an adequate insulin response to regulate normal insulin resistance.[7] Urotensin II (UII) is a pluripotent vasoactive peptide that is important in the development of insulin resistance.[8,9] In women with GDM, elevated Urotensin II levels are linked to insulin resistance.[9] Urotensin II is the most potent endogenous vasoconstrictor peptide discovered to date. Its Urotensin II receptor (UTR) is found in blood vessel endothelium, heart, smooth and striated muscles, thyroid, adrenal gland, and renal cortex.[10] It is the most strong vasoconstrictor peptide; however, in some arteries, it functions as a vasodilator.[11]

METHOD AND MATERIAL

The placental and blood samples was collected from 50 pregnant women were included in this study. The first group

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included 25 pregnant women with uncomplicated and the second groups included 25 pregnant women with gestational diabetes, (Department of Obstetrics and Gynecology in Al-Yarmouk Teaching Hospital), each of tissue samples were usually cut into small parts before another fixation then put the pieces of tissue into embedding cassettes.[12] The pathological examination was performed according to other methods,[13,14] after previous formaldehyde fixation of placental pieces, washing, dehydration, and embedding in paraffin block (hot paraffination), followed by cooling. The paraffin block was cut at a thickness of 5 m using a rotary microtome.[14] Then incubated with 5% hydrogen peroxide, the blocking reagent was applied to tissue sections and incubated for 10 minutes, the following incubation with the primary antibodies for 1-hour at 37°C, the tissue sections were washed twice with the phosphate-buffered saline (PBS) for 3 minutes each time. After that, the tissue slices were incubated with HRP Polymer for 30 minutes, respectively. The 3, 3′-diaminobenzidine staining in the dark was used to distinguish between positive and negative antigens. Hematoxylin was used to stain the sections. Brown deposits indicated positive staining. The sections were examined using a compound light microscope (Meijitechno, Japan) and a digital camera (Canon, Japan, 18 megapixels). The images were taken with the Live View Pro digital camera and directly uploaded to the computer in the University of Baghdad, College of Education for Pure Science (Ibn Al-Haitham), Department of Biology, advanced embryology laboratory. Using ImageJ software for add scale bars to all images.[12] As for the blood samples, venous blood samples were collected of patients. After clotted for 15 minutes at room temperature, the samples were centrifuged for 15 minutes at 2000×g to separate them. The serum samples were then stored in aliquots at -40°C before analyzing Urotensin II, commercially available human ELISA kits (abbexa® with kit number abx (570010), UK) were used to detect serum UII levels according to the manufacturer's instructions.

RESULTS

An immunohistochemical study revealed an alteration in the pattern of receptor scattering between the two studied groups. (Table 1) showed the expression and quantification of the Urotensin II marker in the gestational DM and control groups placentae. The expression of the Urotensin II marker differed between the two groups in two significant ways. First, in the control group, Urotensin II marker expression was associated primarily with negative, medium, and strong staining [2(8.00%), 13(52.00%), and 10(40.00%)]. However, in the diabetes groups, there was a plentiful Urotensin II marker signal with medium [1(4.00%)] and strong staining [24(96.00%)]. Second, less than 10% immunostaining quantification was considered negative patterns, and it was founded in the control group [2(8.00%)] only and appeared the significant difference ($p \leq 0.001$) between two groups. While medium staining was observed in the two studied groups, the control group recorded the higher number percentage [13 (52.00%)] compared with the gestational diabetes mellitus group [1(4.00%)]. Therefore, there was significantly high variation ($p \leq 0.001$) between the groups studied. Finally, the strong staining in the gestational diabetes mellitus group showed approximately [24(96.00%)] more positive signals than in the control group [10 (40.00%)]. Statistically, an extremely significant ($p \leq 0.001$) was recorded between control and gestational diabetes mellitus in strong staining scoring as shown in (Table 1) and (Figures 1)

Table 2 and Figure 2 showed elevated Urotensin II serum in the gestational diabetes mellitus group 45.63 ± 3.82 pg/dL compared with the control group recorded (16.47 ± 1.28) pg/dL from Urotensin II serum. Statically, there was a difference highly significant at probability level ($p \leq 0.001$) between groups (control and gestational diabetes mellitus).

Table 1: Occurrence of cases with positive reaction in control and gestational diabetes mellitus groups using digital quantification in the central section of the placenta.

Scoring	*Control N = 25 n (%) (Rang)*	*GDM N = 25 n (%) (Rang)*	*p-value*
Negative (<10%)	2(8.00) (7.80-8.20)	0(0.00)	0.001**
Medium (10-50%)	13(52.00) (10.50-45.40)	1(4.00) (45.80)	0.000**
Strong (>50%)	10(40.00) (58.10-77.1)	24(96.00) (51.70-81.40)	0.000**

(2-tailed) independent t-test; * highly significant at $p \leq 0.001$.

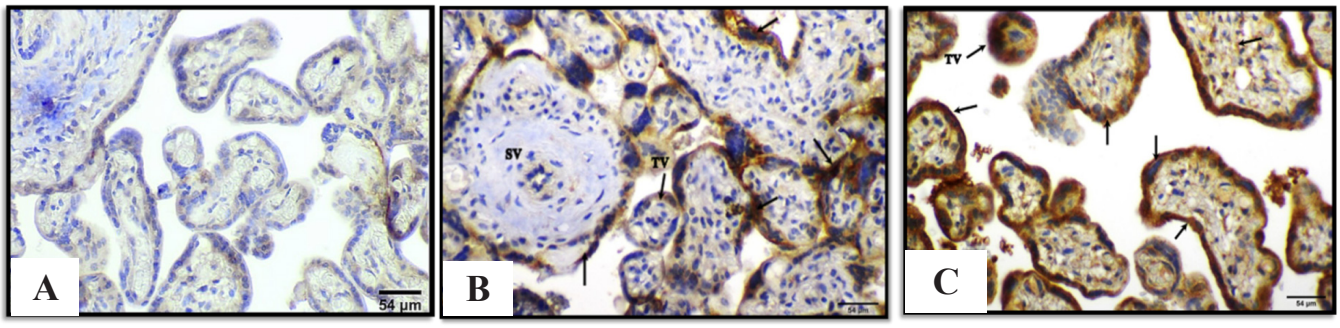

Figure 1: Expressions of Urotensin II in placental tissue by immunochemistry. Primarily found in the cytoplasm of placenta trophoblastic cells and syncytio-trophoblast cells (brown deposit). A, control group (negative reaction) b, gestational DM group (medium reaction) c, gestational DM group (strong reaction)

Table 2: Immunohistochemical quantity for Urotensin II serum (pg/dL) in the control and gestational diabetes mellitus groups.

Serum	*Controls (Mean ± SEM)(pg/dL)*	*GDM (Mean ±SEM)(pg/dL)*	*p-value*
Level of Urotensin II	16.47 ± 1.28	45.63 ± 3.82	0.000**

(2-tailed) Independent t-test, * **highly significant at p ≤0.001.

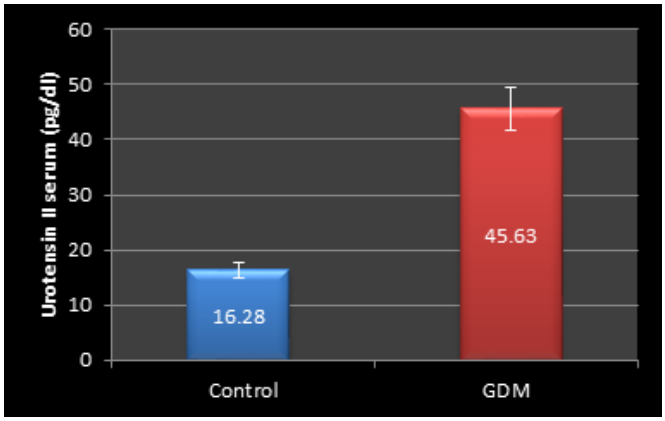

Figure 2: serum level of Urotensin II (pg/dL) in control and GDM groups

DISCUSSION

The pathophysiology of pregnancy complications is much debated; however, that is commonly thought to be the form of placental malfunction. This is caused by the arteriolar vasoconstriction with abnormal cytotrophoblast cell invasion of the spiral arteries.[15] According to research, Urotensin II can constrict blood vessels, cause atherosclerosis, promote cell proliferation and regulate the secretion of soluble vascular endothelial growth factor receptor 1 by tissues of the placenta, which may be linked to the pathogenesis of pregnancy complications.[15,16] Urotensin II is the most powerful endogenous vasoconstrictor identified to date.[16] Which is found in the small intestine as well as in the prostate, kidney, and placenta.[16,17] This is a multifunctional peptide that is essential for glucose metabolism as well as insulin resistance development.[17,18] The current findings revealed that the reaction was primarily confined to cytotrophoblast, particularly in the peri-nuclear region, and to syncytiotrophoblast, also, Urotensin II expression in the placenta was found to be concentrated in the trophoblast, with strong cytoplasmic staining but no staining of nuclei. The gestational DM group had the most cases, followed by the control group. The findings were consistent with those of,[19] demonstrating that Urotensin II expression occurs in the cytoplasm of placental trophoblastic cells. Furthermore,[20] found that the expression of Urotensin II was mainly found in the cytotrophoblast and syncytiotrophoblast of the placenta.

When compared to the control group, the mean level of Urotensin II in blood was significantly greater in the gestational diabetes mellitus groups. This results agree with,[9] who found that circulating Urotensin II levels are higher in gestational DM subjects than in controls. Another study found an increase in Urotensin II in diabetic patients.[11] But in another study, Urotensin II levels were observed to be increased in gestational DM patients in a small-sample study.[8] The increased circulating Urotensin II in the patients with pregnancy complications may be due to the placentae secretes Urotensin II.[19] It could be caused by a lack of conversion of spiral artery, which results in lower blood flow in uteroplacental, resulting in ischemia-reperfusion or hypoxia.

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