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ORIGINAL RESEARCH

Immunolocalization of MMP-2 and MMP-9 in Placenta of Humans with Gestational Diabetes Mellitus

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Abstract

Background: *The aim of this study was to compare of term placentas, which has gestational diabetes mellitus and normal pregnant women in terms of immunolocalization of MMP-2 and MMP-9.*
Methods: *Fifteen gestational diabetes mellitus patients and fifteen women without any systemic disease of the placenta were collected. Fetal and maternal faces of the placentas, as well as peripheral and central parts were examined for immunohistochemical investigation of MMP-2 and MMP-9.Groups were evaluated through use of the SPSS 15.0 package program. P values ≤0.05 were considered statistically significant.*
Results: *MMP-2 reaction was weakly expressed in maternal and fetal surface, and no significant difference between the two groups was observed. When MMP-9 expression was compared between the groups, an increase in decidual cells of diabetic group and a decrease in syncytial nodes were noticed. In fetal surface, a decrease in expression level was detected in chorionic villus syncytiotrophoblasts, chorionic villus stroma, stem villus syncytiotrophoblasts, stem villous stroma and chorionic plate.*
Conclusion: *Regarding our findings, MMP-9 plays a more active role than MMP-2 in gestational diabetic placentas was concluded.*
Key Words: *Full-term human placenta, gestational diabetes mellitus, MMP-2, MMP-9*

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Introduction

Gestational diabetes mellitus represents a failure to maintain normal glucose tolerance during extreme metabolic stress of pregnancy. This disease, defined as any degrees of glucose intolerance first appearing or recognized during pregnancy, is potentially hazardous to both the mother and the fetus (1). It is estimated that GDM affects 1-14% of all pregnancies (2).
Human placenta is a complex system of fetal and maternal blood circulation system, which is adjacent to each other. The placental fetal vessels are contained in chorionic villi composed of an outer trophoblast layer and a mesenchymal core. Villi are washed with maternal blood. Trophoblast and the endothelium regulate the transfer of nutrients and oxygen to maternal and fetal circulation[3].
The development and functioning of the human fetoplacental vascular system are vulnerable to the maternal diabetic milieu. These vessels are in direct continuum with the fetal vascular system and are therefore also vulnerable to fetal endocrine derangements. Deteriorated conditions in the critical phase of the development of the placenta may disrupt the vasculogenesis, angiogenesis and maturation of vascular system (3). Placental vascularization is processes, which have a lot of factor, resulted by organized tracking of so many steps each other. These factors take part not only for the formation of vascular structures, but also in trophoblastic invasion and maturation. Placentation is developed by the interaction and eventually invasion of trophoblast with endometrium (4).
The placentas of diabetic pregnants have shown villous lesions on light microscopy, such as excess syncytial knot formation, increased thickness of vasculosyncytial membrane, villous fibrinoid necrosis, villous immaturity and abnormalities of villous vascularisation but others have failed to reveal any of these abnormalities (5,6).
Placental MMPs take part in physiological processes of human gestation and birth and the majority of them are produced by trophoblastic cells. The changes of MMPs may result in placental abnormalities and premature rupture of membranes. It is reported that, extracellular matrix disruption or accumulation because of the imbalance of MMP and its inhibitors cause pathologic complications such as neuropathy, retinopathy and degenerative aortic disease (7).
Matrix metalloproteinases are a family of zinc-dependent enzymes capable of degrading extracellular matrix (ECM) components and thus contribute to connective tissue breakdown. MMP-2 and MMP-9, also known as gelatinase A and gelatinase B, respectively, mostly degrade collagen type IV, which is an important constituent of basement membranes (8). Mainly of type IV collagen, as well as gelatinases which are a member of MMP family, smashing proteins in extracellular matrix (ECM) such as type I, V, VII, IX collagen, fibronectin, lamin, elastin and vitronectin, are the most studied MMPs in placental invasion (9). İn addition, the increase of activity of MMP-2 and MMP-9 in both fetal and maternal side of the placenta had been shown in diabetic rats (10).
In this study, our aim is MMP-2 and MMP-9 expression was under gestational diabetic placenta to reveal whether the difference.

Materials and Methods

Placental samples collection

The Institutional Ethics Committee of University of Dicle approved the study according to the principles of the Declaration of Helsinki, and all placental samples were collected after the mothers had provided written informed consent. 15 pregnant women (elective caesarean) diagnosed as GDM which participated into study and 15 pregnant women (12 elective and 3 emergency caesarean) constitute control group, which were clinically normal pregnancies, informed after exposition consent form is taken. The specimens were obtained from 3rd trimester of pregnancy with post-

conception 36-38 weeks delivery. Pregnant women in the study group were aged between 24 and 37 years. After the whole placentas were carefully and quickly washed in physiologic saline solution, placental tissue samples, were obtained from peripheral and central part of maternal and fetal faces.

Preparation of placental samples for immunohistochemical examination

In this study, 2 different proteins (Table.1) were investigated. Four-six μm sections of paraffin-embedded placental tissue were dewaxed, rehydrated, and rinsed in PBS pH 7.4. For antigen retrieval, the slides were heated in microwave by using etilendiamintetraasetikasit (EDTA) (pH: 8.0) solutions. Endogenous peroxidase was blocked by soaking the sections in 3% v/v hydrogen peroxide/methanol for 20 min at room temperature followed by washing them with distilled water. They were thereafter rinsed in PBS pH 7.4. Non-specific protein binding was minimized by covering the slides with a serum-free protein blocking reagent (Invitrogen) for 10 min at room temperature. Sections were then incubated with the primary antibodies overnight at 4°C. The sources, dilutions, and time of incubation of these antibodies are shown in Table 1. The slides were subsequently rinsed in PBS pH 7.4 (3 x 3 min) followed by incubation with biotinylatedIgG (Invitrogen) for 20 min at room temperature. Visualization of the bound antibodies was carried out with DAB (Invitrogen) for 8-11 min at room temperature. All incubations were performed in a humidified chamber. Sections were counterstained in Harri's haematoxylin, dehydrated, and mounted with DPX. Staining results were evaluated using a semi-quantitative score, with 0 indicating that the sample was not immunoreactive and 1, 2, and 3 indicate weak, moderate, and intense immunoreactivity, respectively (11). Immunostained slides were blindly evaluated under light microscope and photographed with it (Eclipse 80i, Nikon, Japan).

Statistical analysis

The data from this study were evaluated through use of the SPSS 15.0 package program. Statistical analysis of the data was performed using Kruskal–Wallis one-way analysis of variance and Mann–Whitney U tests. Pearson Chi-Square test was used in categorical data as a test dependency. P-values <0.05 were considered statistically significant throughout the study.

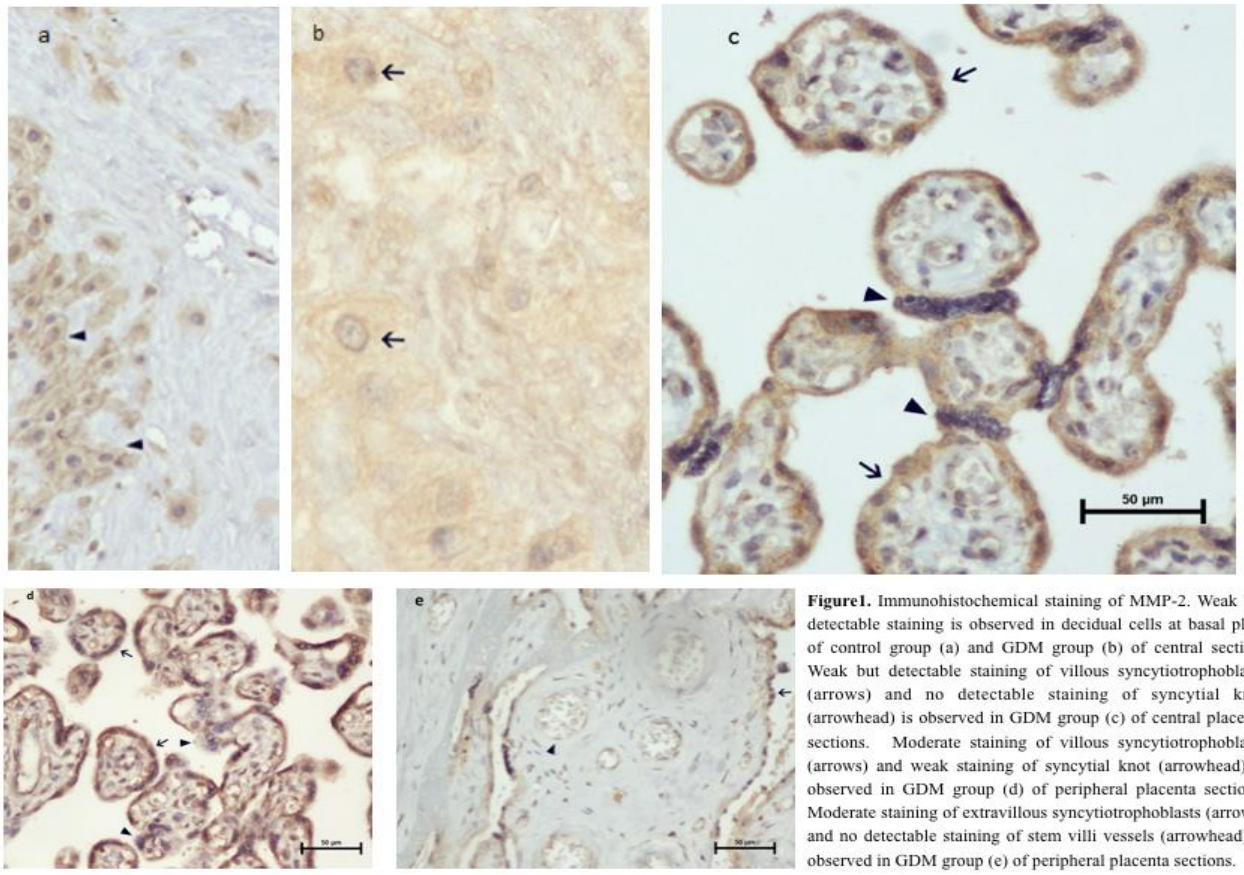

Figure1. Immunohistochemical staining of MMP-2. Weak but detectable staining is observed in decidual cells at basal plate of control group (a) and GDM group (b) of central section. Weak but detectable staining of villous syncytiotrophoblasts (arrows) and no detectable staining of syncytial knot (arrowhead) is observed in GDM group (c) of central placenta sections. Moderate staining of villous syncytiotrophoblasts (arrows) and weak staining of syncytial knot (arrowhead) is observed in GDM group (d) of peripheral placenta sections. Moderate staining of extravillous syncytiotrophoblasts (arrows) and no detectable staining of stem villi vessels (arrowhead) is observed in GDM group (e) of peripheral placenta sections.

Results

MMP-2 Findings

There were generally poor levels of expression in the maternal part of the central sections that were evaluated in terms of MMP-2 immun reaction, also mid-level immune reaction were present in some regions.

No differences were observed when the two groups were compared. There was a weak-moderate expression in the fetal areas. Inreased stem villus syncytiotrophoblasts, decreased expression of syncytial nodes were identified in cases of GDM.

There were weak immune reactions at both of the fetal and maternal side of MMP-2 peripheral sections and there was no difference in expression between the two groups.

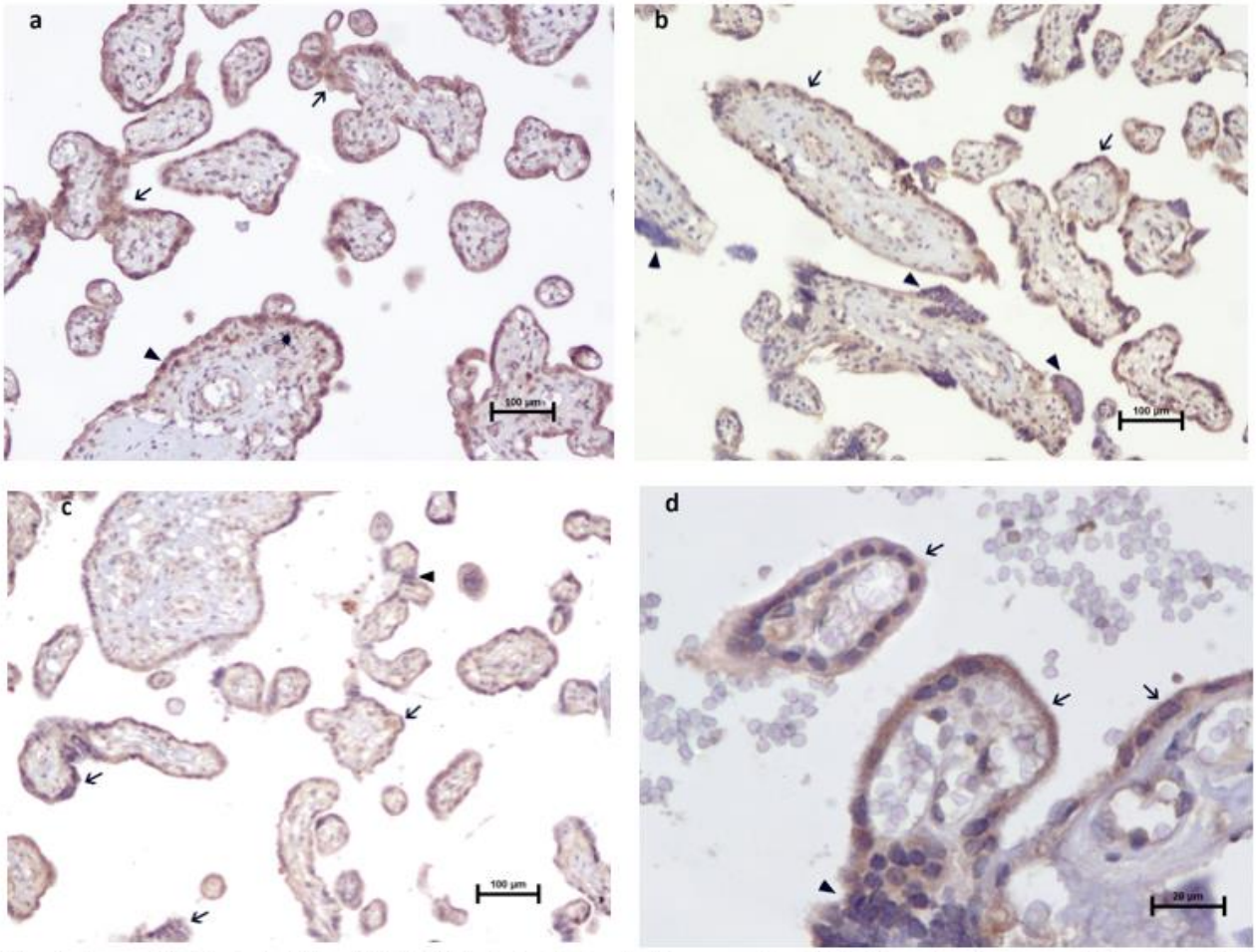

Figure2. Immunohistochemical staining of MMP-9. Moderate staining of villous syncytiotrophoblasts (arrowhead) and syncytial knot (arrows) is observed in control group (a) of central placenta sections. Moderate staining of villous syncytiotrophoblasts (arrows) and no detectable staining of syncytial knot (arrowhead) is observed in GDM group (b) of central placenta sections. Weak but detectable staining of villous syncytiotrophoblasts (arrowhead) and syncytial knot (arrows) is observed in control group (c) of peripheral sections. Moderate staining of villous syncytiotrophoblasts (arrows) and weak but detectable staining of syncytial knot (arrowhead) is observed in GDM group (d) of peripheral placenta sections.

MMP-9 Findings

In terms of expression of MMP-9, while slightly more intensive immune reaction than MMP-2 had been seen, usually there was a predominance of mid-level expression. When the central sections were examined, decreased expression was detected at maternal face, syncytial nodes and bridges. There was no difference between groups in amniotic epithelium and strong expressions were observed. There was usually mid-level expression at fetal face and no difference of expression between the groups.

In the peripheral sections, considering the maternal side, increase of expression in decidua cells and decrease of expression in syncytial nodes were detected. At the fetal side, there were decrease of expression in chorionic villus syncytiotrophoblasts, chorionic villus stroma, stem villus syncytiotrophoblasts, stem villus stroma and chorionic plate.

Table1: Primary antibodies, incubation period and their commercial sources.

Primary antibodies					
Against	Source	Origin	Dilution	Incubation time	Antigen retrieval
MMP-2	Santa Cruz	Mouse	1/200	Overnight	EDTA pH: 8.0, heating in microwave
MMP-9	Santa Cruz	Mouse	1/200	Overnight	EDTA pH: 8.0, heating in microwave

Discussion

Matrix metalloproteinases are a homologous family of enzymes, which have the capability of shredding the components of extracellular matrix and basal membrane and contain zinc in their active site. These enzymes play an important role in physiological states such as tissue remodelling, morphogenesis, wound healing and normal developmental process as well as they take place in pathological processes such as tumor cell invasion, angiogenesis and metastasis. Increased number of syncytial nodes, fibrinoid necrosis, villous edema and capillary proliferation were identified under the light microscopic examination of the terminal villi derived from placentas of GDM cases (6). In addition to similar findings in the present study, we detected syncytial bridges and focal necrosis in some areas. Structural placental abnormalities, malformations of the embryo and embryo-placental metabolic disorders which are formed as a result of Diabetes Mellitus, are associated with pathologies. This ECM changes in embryonic tissues were defined in gestational diabetes. Changing MMPs in the components of extracellular matrix are related with the disorder of regulation of their activities (10). Hyperglycemic condition provided, by creating cell cultures, which has observed increased expression of gelatinases.

In a study showing the immune localizations of gelatinases in human term placentas; mid-level expression in basal plate decidua cells and extratrophoblasts were recorded while expression was weak in MMP-2 amniotic epithelium, chorionic plate, extratrophoblasts and stem villi. Results of MMP-9 and MMP-2 expression were similar. Differently from MMP-2, they identified strong expression in extratrophoblasts while there were weak expressions in amniotic epithelium, amniotic mesenchyme, chorionic plate mesenchyme, anchor villi and vessel walls of chorionic villi (12). When we compared this study with our control group, we detected usually parallel results in terms of MMP-2 but while the presence of strong expression in MMP-9 amniotic epithelium took our attention, we usually determined a mid-level expression in other regions.

In a study (8) , while the amount of active form of MMP-2 was high in decidua, chorionic leve and umbilical cord by zymographic measurements, it was found as low in amnion and chorionic villi. In the Pro MMP-9 measurement, the most prominent band was found on the Nitabuch line. It was followed by decidua which has almost the same integrated optical density, chorionic plate, chorionic leve and basal plate. It had been seen weakly in placental villus and amnion and could not be determined in umbilical cord.

In another study, when they compared MMP-9 activity of placentas of pre-existing diabetes and gestational diabetes with placentas of normal pregnant cases, increases of placental MMP-9 activities in pre-diabetic cases and decreases of MMP-9 activities in gestational diabetic cases had been identified by zymographic measurements. We saw in our study that, MMP-9 expression decreased in cells and areas of some sights in gestational diabetes (13).

Stanovic et al. had not seen any statistical difference between MMP-2 and MMP-9 in their MMP-2, MMP-9 and TIMP measurements by ELISA method in serums of pregnant groups including 20 control cases and 16 cases of Gestational Diabetes Mellitus who are in 26^{th} and 28^{th} gestational weeks (14).

Saglam et al. detected the relationship between MMP-9 expression and Preterm Premature Rupture of Membranes (PPROM) and 4 times increase of MMP-9 in group of PPROM in cervical region compared to the control group by measurements of specimens from MMP-9 cervical, midzone, periplacental regions with ELISA method in their study; in which 8 pregnant cases between 26^{th} and 37^{th} weeks without evidence of infection and without active birth and 8 control pregnant cases of the same week interval, whose birth were realized by caesarean due to several obstetric indications, were included. However, there was no difference between the groups in other regions (15).

Gyorgy et al. (16) immunostained MMP-2 and MMP-9 of matrix metalloproteinases and inhibitors in trophoblastic diseases and control group of placentas in 1^{st} Trimester. In this study, generally

weak expressions were indicated in placentas of 11 cases of control group. They detected weak expression in extravilloustrophoblasts and strong expression in close to half of the other part.
In Ping Xu et al.'s (17) study in 2002, they examined term expressions of MMP-2 and MMP-9 in human placentas and fetal membranes. When local expression in MMP-2 amniotic mesenchyme was observed, they recorded strong staining in chorionic levetrophoblasts, decidua parietalis and syncytiotrophoblasts of placenta. In our study, we determined local weak expressions in amniotic mesenchyme. The expression levels of MMP-9 and MMP-2 in synctiotrophoblasts of the placenta were parallel to our study.
In Shokry et al.'s study in 2002, in which MMP-2 and MMP-9 expressions were examined in preeclamptic and normal human placentas, there were strong expressions in MMP-9 trophoblastic cells and decidual cells in 40 placentas of control group, while there was weak expressions in stromal cells (18). In terms of MMP-2, while mid-level expressions were observed in decidual cells and trophoblastic cells, strong expressions has been seen in stromal cells. Our study was parallel to MMP-9 findings of this study and we saw that, MMP-2 expressions were weak in trophoblastic cells, decidual cells and stromal cells.
In Huppertz et al.'s study in 1998 (4), they expressed that, the immunoreactivity for MMP-2 was observed in all extravilloustrophoblasts of paraffin sections of term placentas and in the matrix around them, also more significantly in decidual cells. In the part of the study about MMP-9, the situation at the end of the pregnancy was not so clear. While there were intracellular and extracellular immunoreactivities in all invasive phenotypic extratrophoblasts of cryostatic sections, they reported that, immunoreactivity was completely lost in extratrophoblasts of paraffin sections.
In Pustrovrh et al.'s (19) study in 2005, showing MMP-2 and MMP-9 expressions in the middle of pregnancy in 6 cases of control group and 6 experimentally generated diabetic pregnant rats, increase of immunoreactivities were shown both at fetal and maternal face of trophoblasts of the labyrinth zone and the connection zone according to the control group in immunostaining.
In Mauro et al.'s (20) study in 2010, MMP-2 and MMP-9 expressions were observed intensively in umbilical cord epithelium cells and Wharton's jelly fibroblasts.ELISA method in serum of patients in one study that compared spontaneous early pregnancy failure levels of MMP-9 was found to be higher than the ongoing pregnancy (21).
Productions of MMP-9 from trophoblasts, decidua and macrophages increases at birth and helps the rupture of fetal membranes. MMP-9 takes place in situations about remodelling of decidua and fetal membranes in term placenta. The elevated activity of MMPs is due to the elevation of tissue damage. Increased MMP-9 activity in extremely violent pre-known diabetic pregnant is possibly related with the fetal and neonatal inconveniences, created by structural and functional abnormalities caused by diabetes. Decrease of MMP-9 in Gestational Diabetes Mellitus may cause accumulation of interstitial matrix and furthermore may be relevant to placental abnormalities. Indeed, relation of increase and decrease of MMPs with tissue abnormalities in different pathologies have been propose (22).
Based on these informations, more important role playing of MMP-9 than MMP-2 in trophoblastic invasion was emphasized.
In our study, immunohistochemical methods were used at tissue level and when comparison was made between groups in tissue, important differences were observed between two groups. This situation can be clarified by studies at the level of both serum and tissue.

Conflicts of interest

There is no conflict of interest

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