Clinicopathological Assessment of Matrix Metalloproteinase-9 (MMP-9) Expression in Preeclamptic Human Placentas

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Abstract

Objective: Insufficient trophoblastic migration and invasion of spiral arteries has been closely linked to pregnancies complications such as preeclampsia and intrauterine growth retardation (IUGR) (3). Matrix metalloproteinase (MMP) secretion by EVT and decidual cells have a potential value in controlling the extent of trophoblastic invasion. We aimed to investigate matrix metalloproteinase-9 (MMP-9) expression in extravillous trophoblasts (EVT), villous trophoblasts (VT) and decidual cells in third trimester placental tissues of preeclamptic women and to assess their relations with various clinical parameters.

Materials and Methods: A cross-sectional study was conducted on 22 preeclamptic (25^{th} to 38^{th} weeks of gestation) and 19 normotensive (27^{th} to 41^{th} weeks of gestation) pregnant women, constituting the study and control groups, respectively. Within 3 days of deliveries, uterine artery and umbilical artery Doppler velocimetric assessments were performed. Upon delivery of all cases, placental tissues were collected and evaluated with immunohistochemical method in terms of MMP-9 expression in EVT, VT and decidual cells, respectively. The degree of hypertension and proteinuria, uterine and umbilical artery Doppler velocity waveforms, gestational age at delivery, mode of delivery, gestational birthweight, Apgar scores were recorded and correlated with placental MMP-9 expression.

Results: Mean gestational age and birthweight at delivery were lower in preeclamptic group compared to controls (33.7±0.7 weeks vs 38.1±0.1 weeks and 1969±164 gr vs 3018±196 gr, respectively). In all cases, percentage of MMP-9 (+) EVT's was positively correlated with the intensity of MMP-9 expression in VT and decidual cells. Percentage of placental EVT MMP-9 expression of superimposed preeclampsia was detected to be low compared to controls (arc sine √% values: 0.72±0.1 vs 0.90±0.13, $p<0.05$). Abnormal uterine arter Doppler velocimetry findings were more prevalent in the study group (33.3% vs 7.6%, respectively). Among study group, percentage of EVT MMP-9 expression was not statistically different in terms of the degree of proteinuria ($p=0.53$) and the degree of diastolic blood pressure (< or ≥100 mmHg) ($p=0.42$), but had a negative correlation with the abnormal uterine artery Doppler waveforms (r_p:-0.39, $p=0.03$). Percentage of abnormal uterine artery Doppler findings was also negatively correlated with the intensity of MMP-9 staining in VT (r_s= -0.51, $p=0.01$) and decidual cells (r_s=-0.39, $p=0.04$); gestational birthweight (r_s=-0.37, $p=0.04$) and 5^{th} min Apgar score (r_s=-0.45, $p=0.02$).

Conclusion: In preeclampsia, placental MMP-9 expression in EVT and VT were negatively correlated with the uterine artery Doppler abnormalities, and the neonatal outcomes. MMP-9 expression of villous trophoblasts and decidual cells were negatively correlated with uteroplacental vascular resistance. A decrease in MMP-9 expression in EVT's, villous trophoblasts and host decidual cells, often preceeding clinical disease manifestations including the Doppler findings, may be associated with the uteroplacental resistance.

Keywords: matrix metalloproteinase-9, preeclampsia, uteroplacental resistance

Özet

Preklamptik İnsan Plasentalarında Matriks Metalloproteinaz-9 (MMP-9) Ekspresyonunun Klinikopatolojik Değerlendirmesi

Amaç: Yetersiz endovasküler trofoblastik migrasyon ve spiral arter invazyonu, preeklampsi ve intrauterin gelişme geriliği (IUGG) gibi gebelik komplikasyonları ile yakından ilişkilidir. Trofoblastik invazyonu sınırlamada, ekstravillöz trofoblast (EVT) ve desidüal hücrelerden salgılanan matriks metalloproteinaz (MMP) rolü bulunmaktadır. Bu çalışmada, 3. trimester preeklamptik plasenta dokularında, EVT, villöz trofoblast (VT) ve desidüal hücrelerde matriks metalloproteinaz-9 (MMP-9) ekspresyonu ve bu ekspresyonun klinik parametrelerle olan ilişkisini ortaya koymayı hedefledik.

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Materyal ve Metot: 22 preeklamptik olguyu (25-38. gebelik haftası) içeren çalışma grubu ve 19 normotensif olgunun (27-41. gebelik haftası) bulunduğu kontrol grubuyla kesitsel bir çalışma yapıldı. Doğum zamanı öncesi 3 gün içinde, uterin arter Doppler ve umbilikl arter Doppler akım hızı ölçümleri yapıldı. Olguların doğum sonrası, plasental dokular toplanarak, immünohistokimyasal yöntemle, EVT, VT ve desidüal hücrelerde MMP-9 ekspresyonu değerlendirildi. Hipertansiyon ve proteinüri derecesi, uterin ve umbilikal arter Doppler akım hızı dalgaformları, doğum haftası, doğum şekli, yenidoğan ağırlığı, Apgar skorları saptanarak, plasental MMP-9 ekspresyonu ile korelasyonu araştırıldı.

Sonuçlar: Ortalama doğum haftası ve yenidoğan ağırlığı, preeklamptik grupta, kontrol grubuna göre daha düşüktü (sırasıyla, 33.7±0.7 hafta vs 38.1±0.1 hafta ve 1969±164 gr vs 3018±196 gr). Bütün olgularda, EVT MMP-9 ekspresyonu, VT ve desidüal hücrelerdeki ekspresyon ile pozitif korelasyon gösterdi. Plasental EVT MMP-9 ekspresyon yüzdesi süperempoze preeklampsi olgularında kontrol grubuna göre daha düşüktü (arc sine √% değerleri: 0.72±0.1 vs 0.90±0.13, $p<0.05$). Anormal uterin arter Doppler akım hızı bulguları, çalışma grubunda daha belirgindi (sırasıyla %33.3 ve %7.6). Çalışma grubunda, EVT MMP-9 ekspresyonu, proteinüri derecesi ($p=0.53$) ve diyastolik kan basıncı değerleri (< veya ≥100 mmHg) ($p=0.42$) açısından bir farklılık gözlenmezken; anormal uterin arter dalgaformu ile negatif bir korelasyon içinde saptandı (rp:-0.39, $p=0.03$). Anormal uterin arter Doppler bulgu yüzdesi, VT (r_s= -0.51,$p=0.01$) ve desidüal hücre MMP-9 immün boyanma yoğunluğu (r_s=-0.39, $p=0.04$) ile yenidoğan kilosu (r_s=-0.37, $p=0.04$) ve 5. dk Apgar skoru ile (r_s=-0.45,$p=0.02$) negatif bir korelasyon gösterdi.

Tartışma: Preklampside, EVT ve villöz trofoblastlara ait plasental MMP-9 ekspresyonu uterin arter Doppler anormallikleri ve neonatal sonuç ile negatif bir ilişki gösterdi. Bununla birlikte, villöz trofoblastlar ve desidüal hücrelerde MMP-9 ekspresyonu ile uteroplasental vasküler direnç arasında negatif bir korelasyon mevcuttu. Doppler bulguları gibi klinik hastalığın bulgularının ortaya çıkmasından önce EVT, villöz trofoblast ve desidüal hücrelerde azalan MMP-9 ekspresyonu, uteroplasental direnç ile yakından ilişkilidir.

Anahtar sözcükler: matriks metalloproteinaz-9, preeklampsi, uteroplasental direnç

Introduction

In pregnancies complicated with preeclampsia, trophoblastic invasion is almost completely restricted to decidual segment of the spiral arteries with little or no evidence of invasion beyond decidual-myometrial junction (1,2). Insufficient trophoblastic migration and invasion of spiral arteries has been closely linked to pregnancies complications such as preeclampsia and intrauterine growth retardation (IUGR) (3). Matrix metalloproteinase (MMP) secretion by EVT and decidual cells have a potential value in controlling the extent of trophoblastic invasion (4). *In vivo* or *in vitro* studies of the placenta and the decidua have shown that EVT cell proliferation, migration and invasiveness are stringently regulated in the placental microenvironment (5,6). Those studies suggested the role of a variety of molecules (TGFβ1-2, VEGF, plasminogen activator, cytokines, leptin, integrins, proto-oncogenes, growth factors etc.) produced at the feto-maternal interface *in situ* on implementing migratory and invasive functions of EVT cells and ensuing the maintenance of high capacitance placental vessels (5-10). Derangements in the functionality of secretion of proteolytic enzymes or plasminogen activator from EVT cells as well as decidual cells in a susceptible mother may lead to cascade of events culminating in full-blown disease (5,8). Cross-talks between EVT, VT and decidual cells in cellular levels have been extensively studied in preeclampsia. However, there is a paucity of studies showing extracellular matrix degrading enzymes expression in those cells and uterine/umbilical artery Doppler findings as well as neonatal outcome parameters.

The activity of matrix degrading and secretory capacity of the EVT cells is higher in first trimester placentas compared to term placentas. This finding has clearly shown that MMP's activity and the invasive potential of EVT cells is more pronounced in the initial placentation than later periods of gestation (9). Furthermore, MMP-9 expression is a prerequisite for matrigel invasion by human cytotrophoblast cells (7).

This cross-sectional study was aimed firstly, to anticipate the expression of MMP-9 (gelatinase-B), a member of zinc and calcium dependent MMP's in placentae of normotensive and hypertensive cases and secondly, to relate its expression among EVT, VT and decidual cells with uterine and umbilical artery Doppler waveforms and some clinical parameters such as degree of proteinuria, blood pressure, gestational birth weight , 1 and 5 min Apgar scores.

Materials and Methods

A total number of 22 preeclamptic women (severe preeclampsia, n=8; superimposed preeclampsia, n=8 eclampsia, n=6) and 19 normotensive pregnant women without any medical illnesses, admitted to University of Osmangazi Medical School, Department of Obstetrics and Gynecology, between January 2000 and March 2003 constituted the study and the control group, respectively. During this time period, control groups were matched in regard to maternal age and previous obstetrics histories. The study was approved by the Ethical Committee of Medical Faculty and confirmed written consent forms were obtained from all the participants. Hypertensive cases were classified as severe preeclampsia or superimposed preeclampsia following the criteria defined by the report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (11).

Women were diagnosed with preeclampsia if they met both of the following criteria: (1) A blood pressure of ≥140/90 mmHg after 20th weeks of gestation and, (2) proteinuria defined as 24-hour urine specimen with a total protein excretion of ≥300 mg. If a 24-hour urine specimen was unavailable, a random urine dipstick value of 1 + (30 mg/dl) at the admission was accepted. Eclampsia was defined as the presence of those above findings and tonic-clonic convulsions in a patient known to be free of any previous cerebral disturbances. Severe preeclampsia was defined as the presence of one or more of the following features: systolic blood pressure ≥160 mmHg; diastolic blood pressure ≥110 mmHg; persistent proteinuria 2+ or more; neurologic symptoms such as visual disturbances, headache and seizures; oliguria; elevated serum creatinine; presence of HELLP (hemolysis, elevated liver enzymes, thrombocytopenia) syndrome or pulmonary edema. Superimposed preeclampsia was defined as worsening of hypertension or new-onset proteinuria in a pregnant women known to be hypertensive before 20 weeks' gestation. Cases with multiple pregnancy, diabetes mellitus, chronic renal disease, platelet disorders, fetal infections, fetal anomalies, rupture of membranes and autoimmune diseases were excluded from the study.

Within 3 days of delivery, majority of the study (15/22) and control group (13/19) participants were undergone uterine and umbilical artery Doppler investigation. All the study cases were not on antihypertensive medication during Doppler velocimetry. Patients were examined in a semirecumbent position and insonated by means of office ultrasonography (Toshiba SSA Sonoline 250, Tokyo, Japan) with 5 mHz transabdominal transducer. The uterine artery Doppler recording was performed at the appearent cross-over point of uterine and external iliac artery. Three consecutive pulsatility index (PI) values of each side (placental and non-placental) were obtained and averaged accordingly. Presence of unilateral or bilateral early diastolic notch and/or the mean PI and RI's ≥95% percentile of the reference value for the index gestational weeks value were considered as abnormal uterine artery Doppler findings (12). In addition, umbilical artery RI values were also recorded. Managing physicians were not blinded to the results of Doppler findings. Placental tissues were collected from all cases following the delivery and evaluated via means of a unique immunohistochemical method by two independent co-author pathologists who were blinded to the clinical findings (13).

Placental tissues were collected from all cases following the delivery. Specimens were sliced and fixed in 10% neutral buffered formalin solution for 12-24 hours. The aeras including basal plate and anchoring villi were marked out on the slide stained with hematoxylin and eosin. Thereafter, appropriate tissue sections 3-4 µm in thickness were prepared and stained with double staining method (Histostain-DS Kit, Broad spectrum, Zymed Laboratories Inc. CA, USA). Paraffin sections are deparaffinized with xylene and rehydrated in a graded series of ethanol. After washing in distilled water, slides were incubated at 121°C for 20 min for antigen retrieval procedure, and than tissue sections were submerged in peroxidase quenching solution and washed with phosphate buffered saline (PBS). After incubation with serum blocking solution; in brief, the procedure was as follows: monoclonal mouse antibody directed against cytokeratin (Pan Ab-1, AE1-AE3, Neomarkers, Fremont, CA, USA) as a first primary antibody was incubated for 45 min and then,biotinylated second antibody, streptavidin-alkaline phosphatase (AP) and substrate–chromogen mixture for AP and double staining enhancer reagents were incubated respectively. After incubation with primary antibody, between each step the sections were rinsed well (2 min, 3 times) in PBS containing 0.05% Tween-20. As a second stage tissues again were incubated with serum blocking solution, and without rinsing tissues were incubated (30 min) with a second primary monoclonal antibody for MMP-9 (Neomarkers, Fremont, CA, USA) and following with biotinylated second antibody. The sections were well rinsed (2 min, 3 times) with PBS containing 0.05% Tween-20 between these two step. After incubation streptavidin-peroxidase, sections were rinsed well with PBS (2 min, 3 times). Substrate-chromogen (AEC) mixture was incubated and tissue rinsed well with tap water containing 0.05% Tween-20 to stop reaction. Tissues were mounted in an aqeous medium. Cytokeratin expression was evaluated as a dark purple and MMP-9 expression as a intense red staining. Both markers showed cytoplasmic stainings. Slides were evaluated under x20. magnification, covering 8-10 microscope examination field. Percentage of MMP-9 positive stained EVT cells were converted into normal distribution via arc-sine$\sqrt{}$% conversion (14). The intensity of MMP-9 staining in EVT, VT and decidual cells were scored as negative, mild, moderate or intense. Uterine and umbilical arteries Doppler waveforms, degree of proteinuria (24-hr urinary protein loss of ≥ or < 2 gr/day) or the degree of hypertension (diastolic blood pressure ≥ or <110 mmHg), gestational birthweight were determined among each group. Any correlation, if any, was assessed with the placental MMP-9 expression.

Data were analysed by using SPPS (SPSS 10.0 Inc., Chicago, Illinois, USA) statistical package programme. Statistical analysis included χ^2 test with Yates correction, Fisher exact test, Kolmogorov-Smirnov test for multiple comparisons and Spearman correlation analysis for categoric variables. For continous variables, Student's *t* test and ANOVA with posthoc Tukey HSD were performed where appropriate, and the values were expressed as mean ±standard error of mean (SEM). P values <0.05 were considered statistically significant.

Results

Mean age, number of parity and gravidity did not differ among two groups, as shown in Table 1. As clearly depicted in Table 1, gestational age at delivery of the study group was lower than that of control normotensive pregnant women (33.7 ±0.8 weeks vs 38.1 ±0.7 weeks, p<0.001) Uterine ar-

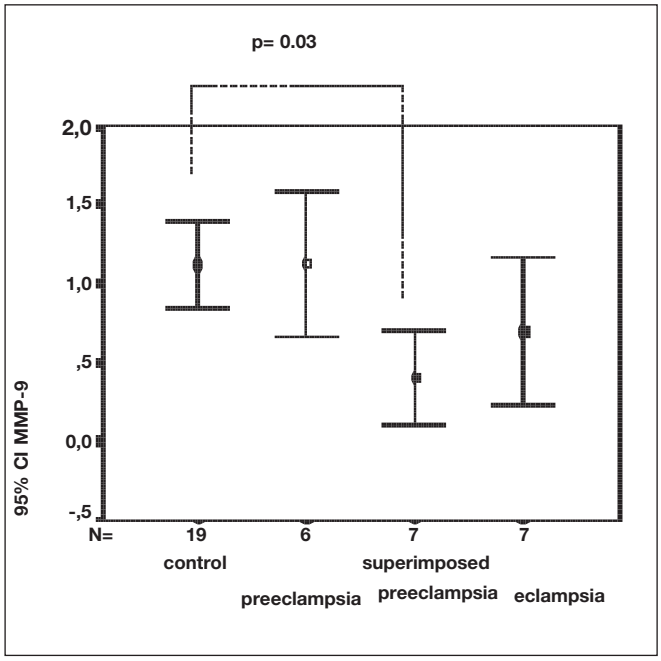

Figure 1. Percentage distributions of EVT cells MMP-9 expression (arc-sine % values) among normotensive controls, cases with preeclampsia, superimposed preeclampsia and eclampsia, respectively.

tery Doppler velocimetric measurements could be obtained in 13 (68.4%) and 15 (68.1%) cases of the control and study groups, respectively. In rest of the cases, an urgent delivery was undertaken, compromising the managing physician to perform and evaluate the Doppler waveform analysis. More cases in the study group notably revealed abnormal uterine artery findings (7.6% vs 33.3%, p<0.01). However, umbilical artery RI did not differ significantly between the control and study groups, although there was a trend toward an increased vascular resistance in the study group (0.37 ±0.3 vs 0.58±0.4, respectively). Gestational birthweight (1969.9±164 gr vs 3018.9±196 gr), 1 and 5 mn Apgar scores (3.3±0.7 vs 8.1±0.5 and 5.3±0.7 vs 9.3±0.4, respectively) were detected to be lower in the study group. Meanwhile, percentage of cesarian section due fetal distress was more prevalent in the study group (21% vs 68.1%, p<0.01).

The percentage of EVT cells expressing MMP-9 presented with arc sine√% values was lower in the superimposed preeclampsia group compared to control group (0.42±0.11 vs 0.90±0,13 respectively, p=0.03), as shown in Figure 1, 2a, 2b and 3a. Severe preeclampsia (0.70±0.14), eclampsia (0.91±0.18) and control group (0.90±0.13) did not differ in terms of percentage EVT MMP-9 expression.

Furthermore, the intensity of MMP-9 expression of EVT, VT and decidual cells did not differ significantly among two groups (p>0.05), as shown in Table 2. In the half of the cases of the study group (11/22 cases), MMP-9 expression in EVTs was mild in intensity (Figure 3b).

In terms of EVT cell cytokeratin expression, in the control group, numbers of mild and moderately cytokeratin stained cases were 4 and 15, respectively. In the study group, these figures were 4 and 17 and, one case stained strongly. No statistically significant difference was depicted from those of controls (D_{max}=0.001, z=0.1, p>0.05).

In both group, no significant change was detected among in terms of percentage of EVT MMP-9 expression, among cases delivered primarily with cesarian section and those delivered vaginally (p>0.05). Furthermore, in study group, percentage of EVT MMP-9 expression was not statistically different in terms of proteinuria < or ≥2 gr/24-hour urine

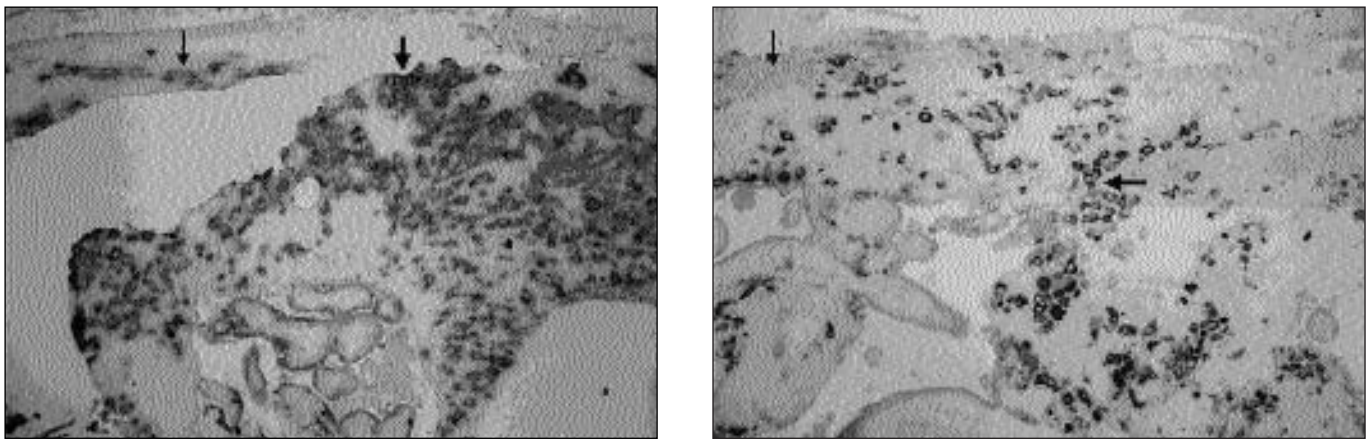

Figure 2a. Matrix metalloproteinase-9 (MMP-9) expression (dark red) in a high percentages of keratin positive EVT's (thick arrow) in an anchoring villus and basal plate in the control group . Small number of EVT's were stained keratin positive only (dark purple, thin arrow). Villous trophoblasts (VT) also showed MMP-9 and keratin positivity with no decidual MMP-9 and keratin staining (Double immunostaining, x10) ; **2b:** Matrix metalloproteinase-9 (MMP-9) expression in a high percentages of EVT's (thick arrow) with a mild decidual MMP-9 cell expression (thin arrow) in a preeclamptic case. VT's showed mild keratin expression (Double immunostaining, x10).

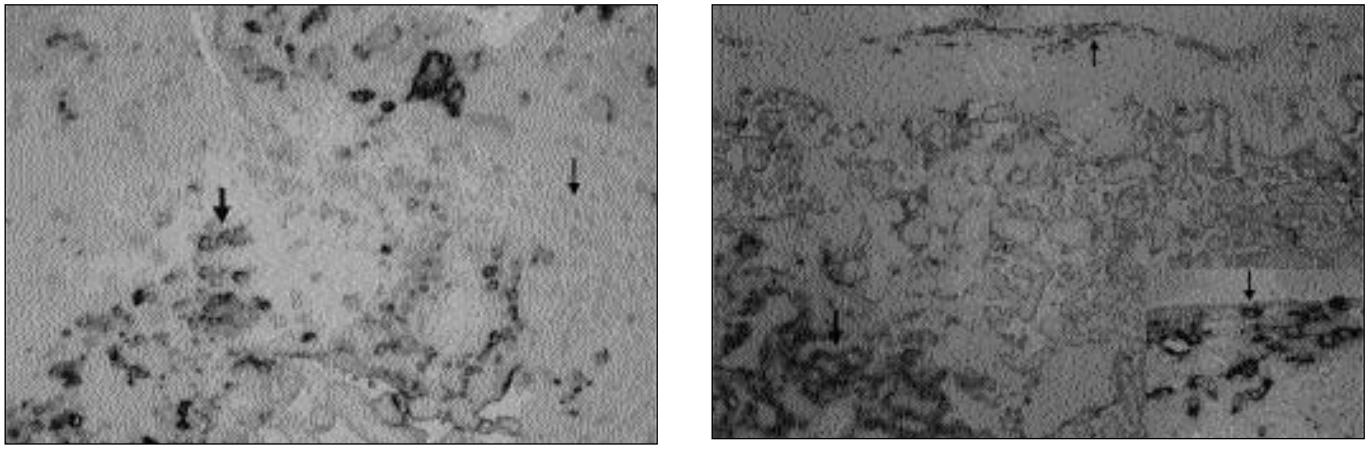

Figure 3a. Matrix metalloproteinase-9 (MMP-9) expression (dark red) in a high percentages of keratin positive EVT's (thick arrow) in an anchoring villus and basal plate in the control group. Small number of EVT's were stained keratin positive only (dark purple, thin arrow). Villous trophoblasts (VT) also showed MMP-9 and keratin positivity with no decidual MMP-9 and keratin staining (Double immunostaining, x10); **3b:** Matrix metalloproteinase-9 (MMP-9) expression in a high percentages of EVT's (thick arrow) with a mild decidual MMP-9 cell expression (thin arrow) in a preeclamptic case. VT's showed mild keratin expression (Double immunostaining, x10).

(0.8±0.13 vs 0.6±0.12, p=0.53) and the degree of diastolic blood pressure < or ≥100 mmHg (0.56±0.13 vs 0.82±0.4, p=0.42). Abnormal uterine artery Doppler waveform patterns were also negatively correlated with the percentage of EVT MMP-9 expression (rs=-0.39, p=0.03); with gestational birthweight (r_s=-0.37, p=0.04) and 5 min Apgar score (r_s=-0.45, p=0.02). Same negative correlation was present with degree of MMP-9 staining among decidual and VT's (r_s=-0.39, p=0.04 and r_s=-0.51, p<0.01). No significant correlation, in either way, was detected among umbilical artery RI and placental MMP-9 expression.

Although placental weights (490±12.3 gr vs 340±22.4 gr, p<0.03) did differ significantly among two groups, placental weight did not appear to correlate with the extent of trophoblast MMP-9 expression and Doppler findings in each group.

Discussion

Although the trophoblastic invasion occurs mostly during the first trimester of gestation, through this study, it was possible to demonstrate that term villous and extravillous trophoblasts can express MMP's to a variable degree. In addition, we also accomplished to demonstrate diminished trophoblastic MMP expression in third trimester placental tissues.

The invasion of extravillous trophoblasts (EVT) into the maternal endometrium is one of the key events in human implantation (15). Histopathologic studies of placental bed biopsy specimen obtained from cases with preeclampsia revealed that trophoblastic invasion of uterine spiral arteries is diminished (16). Degradation of extracellular matrix (ECM) proteins and their migratory capacity in ECM require the expression of MMP's on EVT and decidual cells (4,5). These enzymes play an important role in the degradation of basement membrane and ECM, facilitating the remodeling of maternal uterine vasculature. In this setting, these proteolytic enzymes may also contribute to the maintenance of blood fluidity in the villous space. Reduced EVT MMP-9 expression in preeclampsia early in gestation leads to the aggregations of platelet debris, fibrin and macrophages within the spiral arteries, leading to impaired placental perfusion and incresased uteroplacental resistance (5). MMP-9 and other protease expression and secretion from EVT cells contribute to physiologically protecting perivillous deposition of fibrin and clots, an important histopathologic finding in preeclamptic placentas (15). Our study failed to demonstrate any difference in terms of degree of MMP-9 expression among villous trophoblastic cells of preeclamptic and normotensive cases. This may be related to the small number of cases evaluated in the present study.

Based on *in vitro* studies, villous trophoblasts have been found to express MMP's and urokinase receptors, thereby preventing fibrin deposition within the intervillous space (17). Although villous MMP expression is high during third trimester trophoblasts, EVT MPP-9 expression diminishes as the gestational age progresses (18).

In vitro and *in vivo* studies have found that MMP secretion from EVT are tightly regulated by the MMP tissue inhibitor (TIMP) expressed and secreted from the decidual cells, confronting the further invasion of EV trophoblast cells (18,19). Furthermore, Campbell et al.(20) performed an co-culture experiment using cytotrophoblasts from normal pregnancies, together with decidual cells from both normal and preeclamptic pregnancies. They concluded that preeclamptic decidual cells showed reduced MMP-1 secretion, suggesting a possible role for these maternal cells in controlling the extent

of endovascular invasion. However, Vettraino et al. (21) found that the placental specimens recovered from pregnancies complicated by severe preeclampsia did not differ in collagenase-I and stromelysin-I immunostaining from those placentae recovered from uncomplicated pregnancies. Gallery et al (22), through their study about the secretion of MMP by cultured human decidual endothelial cells from normal and preeclamptic pregnancies, has shown that decidual cells from preeclamptic cases expressed reduced MMP-1, inhibiting the endovascular invasion by cytotrophoblasts. This findings demonstrates that protease expression in the EVT trophoblasts and decidual cells may play important role in the setting of endovascular trophoblast invasion (23).

As seen in Table 1, mean gestational age of placental sampling was lower in the preeclamptic group, as well as the EVT MMP-9 expression, compared to term normotensive controls. But, as one would expect, MMP-9 expression should be higher in the preterm delivered cases to term placental tissues of the control cases. In contrast, even in preterm placentae, preeclamptic changes resulted in diminished MMP-9 expression compared to those of uncomplicated term pregancies. Hence, at the end of this study, we thought that gestational age difference between study and control group did not influenced the results.

There are several reports in the literature that pointed that immunoreactive MMP-9 protein was significantly increased during and after labour compared with before labor (24,25). Those labor-associated changes were also examined in this present study. With regard to EVT MMP-9 expression, we failed to detect any difference among cases delivered primarily with cesarian section and those delivered vaginally or by cesarian section following a short period of labor stimulation.

Another important finding of the present study is that percentage of EVT MMP-9 staining was more prevalent in superimposed preeclampsia compared to controls, as shown in Figure 1. In the present study, being allocated as preeclampsia group, only cases with severe preeclampsia, eclampsia and superimposed preeclampsia were enrolled in the study, and compared with the normotensive pregnant women. Mild or severe gestational hypertension were not included to the study. Hence, MMP-9 expression of various cells in the placenta could not be assessed among different forms of hypertensive diseases of pregnancy.

Rather than having patients from 25-38 weeks GA and with not only mild preeclampsia but with severe preeclampsia and "superimposed preeclampsia", the hypothesis could be tested more effectively by studying a group of term patients with "mild preeclampsia". The control group would then be comprised of term patients who were normotensive. This was an important question that remained to be answered following this study. We could not include mild preeclampsia cases due to inadequate number reached at the end of the study. This may be, somewhat, related to admission of more severe preeclamptic cases to our unit during the study period instead of referring mild preeeclamptic cases that were easily be managed in our state or social security hospitals in our city. Another limitation of this sudy is the fact that the expression of MMP-9 from third trimester placentas (trophoblasts) did not appear to offer a valid representation of a first trimester process (abnormal trophoblastic migration and invasion of arteries). It would be more relevant to study the placenta in early pregnancy rather than in the third trimester.

Motile and invasive extravillous cytotrophoblasts are found as cytokeratin positive cells in the decidua, the intima of the uterine spiral arteries and the proximal third of the myometrium. We failed to detect any difference in terms of the intensity of trophoblastic cytokeratin expression among pregnant women with preeclampsia and normotensive pregnant women. However, an association of preeclampsia and excessive proliferation and shedding of immature trophoblasts into maternal circulation has been suggested, upregulating the cytokeratin expression in the preeclamptic placentae (26,27).

To the best of our knowledge, there are inadequate data in the literature, relating the uteroplacental resistance detected with Doppler studies with placental MMP-9 expression. Based on the result of the current study, elevated uterine artery resistance was negatively correlated with the intensity of MMP-9 expression in extravillous, villous and decidual cells as well as neonatal outcomes such as birthweight and Apgar scores. Similar results obtained from the studies of Olofsson (28) and Lin et al. (29), pointing out that patients with placental bed biopsies with impaired trophoblastic migration and invasion delivered more prematurely and had a higher rate of small-for-gestational age infants. Nevertheless, these studies were based on the histopathologic confirmation of impaired trophoblastic invasion, instead of immunohistochemical identification of protease expression in EVT cells or decidual tissues.

Among preeclamptic group, percentage of MMP-9 stained EVT cells did not differ, regardless of both the degree of proteinuria and diastolic blood pressure. Taken into consideration the negative correlation between uteroplacental resistance and the percentage of MMP-9 stained EVT cells, the latter finding is in accordance with the results of the previous studies, underlying the fact that abnormal uterine artery Doppler studies (increased PI, RI, and the presence of diastolic notch) is not mediated by high blood pressure, but rather are the consequence of abnormally elevated uteroplacental resistance determined by the inadequate and shallow endovascular trophoblast invasion into the myometrium (30). Kolben et al. (31) has also demonstrated that in patients with abnormal uterine artery Doppler findings were more likely to have reduced MMP-9 and TIMP-1 concentrations in the plasma and placental extracts compared to those with normal Doppler velocimetry. In that study, only preeclamptic women showed reduced placental MMP-9 expression.

Another limitation of our study was the fact that more than 30% of cases among each group failed to undergo uterine and umbilical artery Doppler studies. In this regard, the missing data together with small number cases enrolled in each group may be problematic in assessing the correlation of Doppler results with various clinico-pathologic parameters. Moreover, one should consider the fact that the given medications to the study group may have an influence on this staining experiment.

In conclusion, the present data suggest that in preeclampsia, placental MMP-9 expression is responsible from the elevated uteroplacental resistance and its expression in EVT cells is lower in preeclampsia. Although no significant changes were observed among those cells in cases with preeclampsia of different degree, interactions between trophoblast cells (extravillous/villous) and decidua cells have important implications on the placental function. This issue needs to be elucidated by future studies, on a large data set, in an attempt to clarify the intraplacental cell cross-talks in preeclampsia.

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