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Research Article

Regional Variations in Placental Protein Expression of Matrix Metalloproteinase-9 in Term Uncomplicated Pregnancy

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Abstract

Objective: The present study explored whether placental expression of matrix metalloproteinase-9 (MMP-9) would vary between regions of the same term delivered placentas that were obtained from women with uncomplicated pregnancy.

Methods: A total of 27 term delivered placentas were collected from healthy women without any medical or pregnancy related complications. Each placenta was divided into four quadrants and MMP-9 protein expression was compared between the quadrants of each placenta. Direct Sandwich Enzyme-linked immunoassay (ELISA) method was used to determine chorionic villi MMP-9 protein expression of the tissue sections from each quadrant. Commercially available assay kits from R&D Systems, Minneapolis, MN; were used that used monoclonal antibody to human MMP-9 protein as the capture antibody.

Results: MMP-9 protein expressions of 108 chorionic villi samples obtained from four different quadrants of each of 27 normal placentas was comparable between the four quadrants. Chorionic villi MMP-9 protein expression did not differ between C-section and vaginal delivery groups. The protein expression was comparable between the studied ethnic groups. Maternal age was significantly but negatively correlated with gestational age in days (r^2 = -0.201, p = 0.037).

Conclusion: The study underscores that even though each placenta may differ significantly in its structure and vasculature, MMP-9 protein expression at term is expressed equally throughout the placenta in normal healthy women.

Keywords: *MMP-9; Term Placenta; Normal Human Pregnancy; Placental Quadrants; ELISA*

Introduction

Human placenta undergoes distinctive phases of dramatic alterations in structural and functional properties throughout gestation [1,2]. These changes are orchestrated by hormones and frequently mediated by local growth factors and cytokines [3]. As the placenta undergoes changes in its shape and volume an extensive remodeling of connective tissue takes place that requires both breakdown and re-synthesis of extracellular matrix components [4]. Even though a variety of constituents participates in the degradation process, matrix metalloproteinases (MMPs) are considered to be the primary constituents in the degradation process [5]. A number of investigators have examined the placental expression of MMPs in term placentas to understand how the expressions of placental proteins differ between normal and pathological conditions [6,7]. In these investigative studies, analysis of MMP proteins was undertaken using placental tissues

from a single site. In our previous studies investigating placental protein expressions of normotensive women, we also had routinely sampled the tissue from a particular region of the placenta [8,9]. Since the placenta differ significantly in its structure and vasculature, we questioned whether the protein expression of the placenta would have been different had we sampled the tissue from another placental site. The present study was therefore undertaken to determine if the placental expression of the protein would vary based on tissue sampling sites. We focused our attention on term delivered placentas from women and investigated matrix metalloproteinase-9, a robust protein involved in human parturition.

Methods

The investigative protocol for the study was approved by the Human Subject Ethics Committee of the BronxCare Health System, New York, USA. Placentas were obtained from women following term delivery within 30 minutes of the procedure. The women were normotensive throughout pregnancy and had no signs of any pregnancy related abnormalities or any other medical complications.

Each placenta was processed as follows: First the adhering blood clots and the placental membranes were removed. The umbilical cord was also removed, leaving only a short stump at the insertion point where the umbilical cord enters the placenta. The placenta was then weighed and the weight was recorded. Keeping the orientation of the insertion point always pointing north, a photograph of the placenta was taken with a disposable measuring tape placed at the bottom of each placenta to document its size (Figure 1b). Each placenta was then flipped always from left to right to expose the chorion and was photographed once again (Figure 1c). The placenta was then visually dissected into four quadrants (Qs) (Figure 1d). The north-east section was designated Q1, south-east section as Q2, south-west section as Q3 and north-west section as Q4. A segment of tissue from each placental quadrant was then removed one section at a time and processed as stated below. Figure 1e shows the photograph of the placenta after all four sections were removed.

Tissue from each placental quadrant was first thoroughly washed to remove maternal blood, and then dissected in saline to collect free floating chorionic villi that were not anchored to the basal plate nor were emerging from the chorionic plate surface vessels (Figure 1f). Several pieces of the chorionic villi tissues from each placental quadrant were placed in several separately labelled tubes that bore the same placental ID and quadrant number. The tubes containing the tissues from each quadrant were placed in plastic bags which were then placed in ice while the tissues from the other quadrants were being processed. Finally bags of tissues from all four quadrants of the same placenta were placed in a bigger bag labeled with the placenta ID number. The plastic bags were then transported from the hospital to the laboratory on ice. In the laboratory, the tubes were arranged in cardboard freezer boxes so that tubes from each quadrant were placed in a row, and tubes from all four quadrants were placed in the same freezer box that was labeled with the same placental ID number. The freezer boxes were then stored at -80°C until assay. MMP-9 protein expression was determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems, Minneapolis, MN; that used monoclonal antibody to human MMP-9 protein as the capture antibody. A Tecan infinite 200 Pro microplate reader (Tecan Systems Inc., San Jose, CA) set at 450 nm with wavelength correction set at 540 nm was used to measure the absorbance during the ELISA assay. The manufacturer claims, that the sensitivity of the ELISA assays for MMP-9 is 31.3 pg/ml.

The IRB approved protocol also allowed the collection of clinical information pertaining to the women from whom placental tissues were obtained. These included: maternal age, race/ethnicity (self-reported), gestational age (as determined by ultrasound or by initial date of the last menstrual period), last maternal systolic and diastolic blood pressure prior to term delivery, medicine(s) administered during pregnancy and newborn's weight and sex. Tissues and clinical information gathered during sample collection were de-identified before exiting the delivery suites.

Statistical analysis

The statistical software package SPSS, version 26 (IBM Corporation, Armonk, NY) was used for statistical analyses. One way ANOVA and Pearson's correlation were used for analysis and the level of statistical significance was set at $P < 0.05$.

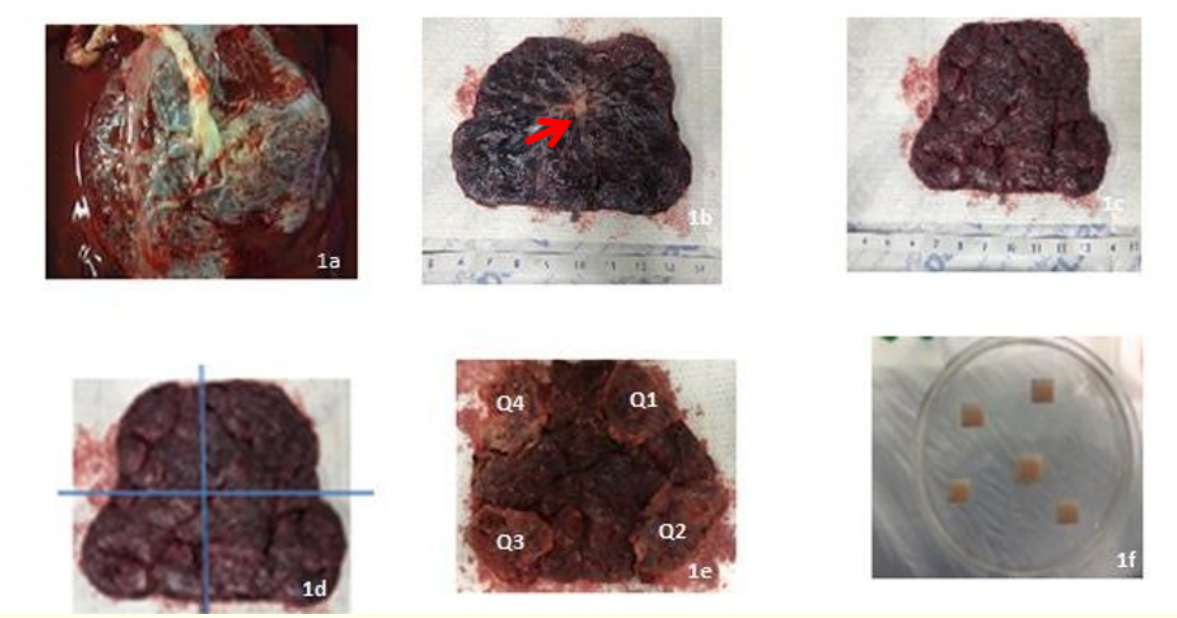

Figure 1: *1a: Normal term delivered placenta with membranes and umbilical cord just after delivery; 1b: Fetal side of the placenta with the red arrow showing the insertion point of the umbilical cord into the placenta; 1c: Maternal side of the placenta; 1d: Placenta visually divided into four quadrants; 1e: sampling of placental tissue from each placental quadrants; 1f: Sections showing isolated chorionic villi that were free of blood and emerging blood vessels.*

Results and Discussion

The present study explored whether placental expression of MMP would vary between regions of the same placenta. In the study, we focused our attention on term delivered placentas from women with uncomplicated pregnancy. A number of MMPs are secreted by human trophoblasts [10,11], however, MMP-2 and MMP-9 are the two MMPs that have been studied more frequently [12-14]. Of these two MMPs, MMP-9 protein expression is reported to be higher than MMP-2 in the third trimester of human pregnancy [15]. Moreover, placental MMP-9 is also known to be involved in the parturition process and is reported to be induced during active labor in humans [16]. Hence, to investigate the regional distribution of placental protein in term delivered placentas, MMP-9 protein was selected as the candidate protein for this investigative study.

In this study, chorionic villi protein expression of MMP-9 was compared between four different quadrants of each placenta. A total of 108 chorionic villi samples from 27 placentas were analyzed by Direct Sandwich ELISA method. The intra-assay and inter-assay variations for the assays were between 7 - 10%.

The clinical characteristics of the women from whom placentas were taken are presented in table 1. All women were normotensive. The average maternal age was 28.3 ± 6.5 years. The average gestational age was $39^{5/7} \pm 1^{0/7}$ weeks. Self-reported race/ethnicity showed 67% of the women were of Hispanic origin, 11% were of African American origin and 22% were of other ethnic groups. A total of 17

women delivered by C-section and the remaining 10 women delivered vaginally. Information on the gender of newborns showed that 13 were females and 14 were males. The last recorded systolic and diastolic blood pressures of the women prior to their delivery were collected, and the average systolic and diastolic blood pressure of the women from whom placental samples were collected were 117 ± 13 and 71 ± 10 mm Hg, respectively.

	N = 27
Maternal characteristics	**Mean + SD**
Age (yrs)	28.3 ± 6.5
Gestational Age (wks)	$39^{5/7} \pm 1^{0/7}$
Systolic blood pressure (mmHg)	117 ± 13
Diastolic blood pressure (mmHg)	70 ± 10
Placental Weight (gms)	431.6 ± 88.6
Ethnic groups	
Hispanic	67%
African American	11%
Others	22%
Mode of Delivery	
C-Section	17
Vaginal	10
Newborn's weight (gms)	3330.0 ± 424.1
Newborn's gender	
Female	13
Male	14

Table 1: *Maternal clinical characteristic.*

The results of MMP-9 protein expressions of 108 chorionic villi samples obtained from four different quadrants of each of 27 normal placentas were analyzed by one way ANOVA. The data as presented in table 2 shows that MMP-9 protein expression was comparable between the four quadrants. The scatter plot of MMP-9 protein expression in the four quadrants accentuates, in further details, the comparability of the protein in the four quadrants (Figure 2). Placental distribution of MMPs has been studied before. Investigators have reported on the distribution pattern of several MMPs in first, second and third trimester placentas [15]. The primary focus of their study was, however, in determining whether the distribution pattern of MMPs depended on the stage of pregnancy and on the degree of differentiation of trophoblast cells. In another study, the distribution pattern of MMP-9 protein was compared between decidual cells and adjacent interstitial trophoblasts cells, in tissue sections obtained from preeclamptic and control women [17]. The focus of our study is however entirely different. We investigated whether MMP-9 protein expression was different between different regions of the same placenta.

Placental	N	MMP-9 Protein (ng/100 mg tissue) (Mean ± SD)	Sig.
Quadrant 1	27	23.45 ± 18.54	0.715
Quadrant 1	27	21.04 ± 12.73	
Quadrant 1	27	20.22 ± 11.61	
Quadrant 1	27	24.37 ± 16.58	

Table 2: *Chorionic villi MMP-9 protein expression in placental quadrants. By one-way analysis of variance.*

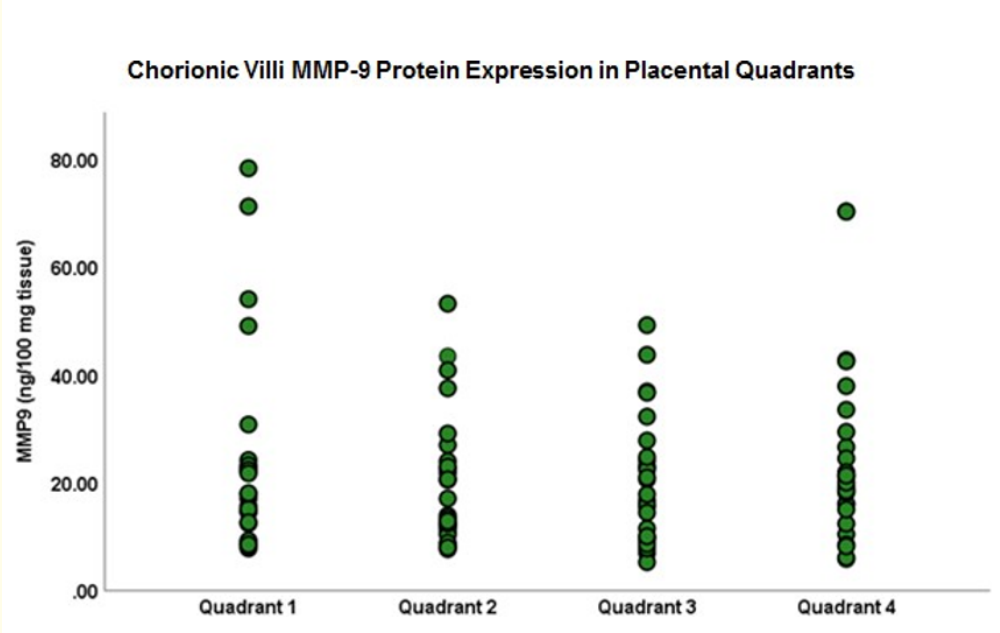

Figure 2: *Scatter plot of chorionic villi Matrix Metalloproteinase-9 (MMP-9) protein expression by placental quadrants.*

In this study, we have further analyzed whether the mode of delivery had any effect on chorionic villi MMP-9 protein expression of term delivered placentas. The results reveal that chorionic villi MMP-9 protein expression was comparable between C-section and vaginal delivery groups (Table 3). In a study that has compared placental MMP-9 protein expression between spontaneous vaginal delivery and C-sectioning group in preterm pregnancy, a significantly lower MMP-9 protein expression was reported in the preterm group [18].

	Mode of Delivery					
	C-Section			Vaginal		
Placenta	**N**	**MMP-9 (ng/100 mg tissue) Mean ± SD**	**Sig.**	**N**	**MMP-9 (ng/100 mg tissue) Mean ± SD**	**Sig.**
Quadrant 1	17	20.60 ± 17.21	0.876	10	28.29 ± 20.62	0.833
Quadrant 2	17	18.05 ± 10.74		10	26.11 ± 14.76	
Quadrant 3	17	17.78 ± 9.18		10	24.38 ± 14.46	
Quadrant 4	17	20.08 ± 10.11		10	31.67 ± 22.76	

Table 3: *Effects of method of delivery on chorionic villi MMP-9 protein expression.*
By one-way analysis of variance.

Chorionic villi MMP-9 protein expression in this study was also not found to differ between Hispanic, African American and other ethnic groups. Additionally, the gender of the newborn the woman was carrying had no impact on chorionic villi MMP-9 protein expression as well.

Pearson's correlation was carried out to determine the correlation between the studied variables and the results are shown in table 4. MMP-9 was not found to be correlated to any of the studied variables. However, the results showed that maternal age was significantly but negatively correlated with gestational age in days (r^2 = -0.201, p = 0.037, Table 4). Our findings are in agreement with a study that had examined the effect of maternal age on gestational age- specific perinatal mortality; and has reported that older mothers deliver preterm, with no adverse neonatal outcome [19].

		MMP-9	GAD	Age	Placental Weight	New Born Weight
MMP-9	Pearson's Correlation	1	-0.025	-0.081	0.056	0.041
	Sig. (2-Tailed)		0.797	0.406	0.565	0.676
	N		108	108	108	108
GAD	Pearson's Correlation	-0.025	1	-0.201	0.031	0.033
	Sig. (2-Tailed)	0.797		0.037	0.749	0.734
	N	108		108	108	108
Age	Pearson's Correlation	-0.081	-0.201	1	0.087	0.046
	Sig. (2-Tailed)	0.406	0.037		0.368	0.638
	N	108	108		108	108
Placental Weight	Pearson's Correlation	0.056	0.031	0.087	1	0.798
	Sig. (2-Tailed)	0.565	0.749	0.368		0.0001
	N	108	108	108		108
New Born Weight	Pearson's Correlation	0.041	0.033	0.046	0.798	1
	Sig. (2-Tailed)	0.676	0.734	0.638	0.0001	
	N	108	108	108	108	

Table 4: *Correlation between studied variables.*

MMP-9: Matrix Metalloproteinase-9 Protein; GAD: Gestational Age in Days.

The placenta dictates the ability of the fetus to grow and thrive in utero. Since careful examination of the placenta could provide information on in utero environment of the fetus before delivery, placental weight and its relationship to infant size at birth have been studied for more than a century. Investigators have reported that newborn and placental weights are positively correlated [20,21]. In this study, Pearson's correlation data also revealed a significant positive correlation between placental and newborn weights (r^2 = +0.798, p = 0.0001, Table 4). The mean placental weight of a woman carrying a male (n = 14) or female fetus (n = 13) was found to be comparable (424g vs 439g, respectively). The mean newborn weight of males (3389g, n = 14) and females (3274g, n = 13) was comparable as well.

Conclusion

The study primarily validates that even though the placenta differ significantly in its structure and vasculature, MMP-9 protein is equally expressed in normal human term delivered placenta, irrespective of the tissue sampling sites. The other points that need to be made are that methods of delivery, either spontaneously vaginal or C-sectioning does not influence placental MMP-9 protein expression. Our findings show that in normal pregnancy, placental and newborn weights are significantly correlated. Additionally, though a typical normal pregnancy may last 40 weeks our data highlight that older mothers may have shorter gestational age. Hence, prolongation of pregnancy in older mothers may only be recommended with caution because it could adversely affect the fetus.

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Conflict of Interest

The authors declare no conflicts of interest with respect to the research, authorship and/or publication of this article.

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