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Original Research Article

Role of Metalloproteinases (PAPP-A and MMP-9) in first trimester prediction of pregnancy hypertension**Karuna Sharma^{*1}, Ritu Singh¹, Vishwajeet Rohil³, Manisha Kumar², Usha Gupta⁴,
Mujeeb Ur Rahman³ and Jayashree Bhattacharjee¹**¹Department of Biochemistry, Lady Hardinge Medical College, New Delhi, 110001, India²Department of Obstetrics and Gynecology, Lady Hardinge Medical College, New Delhi, 110001, India³Department of Clinical Biochemistry, Vallabhbbhai Patel Chest Institute, Delhi University, New Delhi, 110007 India⁴Department of Obstetrics and Gynecology, ESIC Medical College, Faribabad, Haryana, 121012 India

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New Delhi-110001, India***Article History:****Received:** 22/04/2017**Revised:** 02/06/2017**Accepted:** 02/06/2017**DOI:** <https://doi.org/10.7439/ijbar.v8i5.4127>**Abstract****Introduction:** Pregnancy hypertension is reported to affect 2-12% women in India. Currently available diagnostic criteria is applicable after 20th weeks of gestation, this delayed diagnosis is the possible cause of the associated adverse obstetrics outcomes, which can be minimized by early detection. In this study the role of MMP-9 and PAPP-A was assessed in the first trimester (11⁺⁰-13⁺⁶) for early prediction of pregnancy hypertension.**Methodology:** This study was part of an ongoing project of first trimester preeclampsia screening. In that project 2000 women were screened. Samples were taken in first trimester and women were followed till delivery. Out of 2000 women, 199 developed hypertension, 1454 remained normotensive till term and remaining 347 were excluded. In the present study 199 women who developed hypertension and 199 controls, who were normotensive till term (selected from cohort of 1454 women who were normotensive) were included.**Results:** The first trimester serum levels of PAPP-A (p<0.001) and MMP-9 (0.039) were significantly low in women who developed hypertension as compared to the women who remained normotensive till term. A significant positive correlation (p=0.03) was observed between MMP-9 and PAPP-A. The combined sensitivity, specificity and positive predictive value (PPV) of PAPP-A and MMP-9 was found to be 74.5%, 70.2% and 75% respectively.**Conclusion:** Inadequate expression of PAPP-A and MMP-9 may result in incomplete or poor trophoblast invasion and subsequent complication of pregnancy hypertension. Present study revealed that first trimester assessment of PAPP-A and MMP-9 may potentially predict risk of pregnancy hypertension.**Keywords:** Pregnancy hypertension, Pregnancy associated plasma protein –A (PAPP-A), Matrix metalloproteinase-9 (MMP-9).**1. Introduction**Pregnancy hypertension is a potential cause of fetal and maternal morbidity and mortality [1]. In India the incidence of pregnancy hypertension is 2-12%. Pregnancies are predestined to develop pregnancy hypertension during 11⁺⁰-13⁺⁶ weeks [2-5] but according to current clinical diagnostic criteria, the condition is diagnosed by onset of symptoms (blood pressure<140/90 mmHg andproteinuria<300mg/24hrs) after 20th weeks [1,6]. This delayed diagnosis is a potential reason of adverse perinatal outcomes of preeclamptic pregnancies. After onset of hypertension pregnancy hypertension progresses rapidly from mild hypertension to life threatening clinical conditions. The associated adverse outcomes of preeclamptic pregnancies can be minimized by identification of high risk women before onset of

symptoms. Precise implantation of embryo is pivotal for successful pregnancy. Inappropriate placentation may lead to pregnancy complication like IUGR (Intrauterine growth restriction) and pregnancy hypertension [7,8]. Trophoblast produces several factors which facilitates its invasion in uterine wall. MMP-9 and PAPP-A are two metalloproteinases produced by trophoblast.

PAPP-A is produced by syncytiotrophoblast and increases insulin-like growth factor availability by cleaving the complex of IGF (Insulin-Like Growth Factors) and IGFBP (Insulin-Like Growth Factor binding protein)[9]. IGF system facilitates trophoblast invasion and amino acid transport through placenta [10]. Lower concentration of the PAPP-A is associated with a higher incidence of the preeclampsia [11]. The case-control studies did not report any significant differences in obstetrical outcomes associated with PAPP-A level but recent cohort studies describe increased adverse obstetrical outcomes associated with PAPP-A level below the fifth or tenth [12]. Low levels PAPP-A has been associated with pregnancies that subsequently developed hypertension [12].

MMP-9 secreted by cytotrophoblast promotes trophoblast penetration in the maternal endometrium and myometrium by proteolysis of ECM (Extra cellular matrix) [15,16]. In reference to pregnancy hypertension, a contradictory data of MMP-9 is available. Poon *et al.* (2009) reported increased MMP-9 level at 11+0 – 13+6 weeks gestation in pregnancies which later developed hypertension [17]. The studies by Karampas *et al.* (2014) and Kolben *et al.* (1996) suggests no significant association

of first trimester MMP-9 levels with development of preeclampsia which is pregnancy hypertension disorder [18,19].

In the present study, role of PAPP-A and MMP-9 in the first trimester prediction of pregnancy hypertension will be evaluated. This study may possibly enhance the current knowledge of early prediction of pregnancy hypertension which may help to reduce the hypertension associated adverse complications and outcomes.

2. Material and Methods

This was a prospective case control study and was part of an ongoing project of preeclampsia screening. In that project 2000 women having singleton pregnancy, gestational age between 11+⁰- 13+⁶weeks and with no history of endocrine, cardiac, renal and hepatic disorders were enrolled from antenatal OPD of Lady Hardinge Medical College and SSK Hospital New Delhi, India [20]. The blood samples were collected after confirmation of gestational age by ultrasonography (11+⁰- 13+⁶ weeks) and all women were followed till delivery. Out of 2000 women, 199 women developed hypertension, 1454 remained normotensive till term and remaining 347 were excluded (for lost to follow up and development of medical conditions other than hypertension) (Figure 1). The study protocol was approved by institutional ethical committee of Lady Hardinge Medical College and SSK Hospital, New Delhi, India. All women were enrolled after taking their written and informed consent.

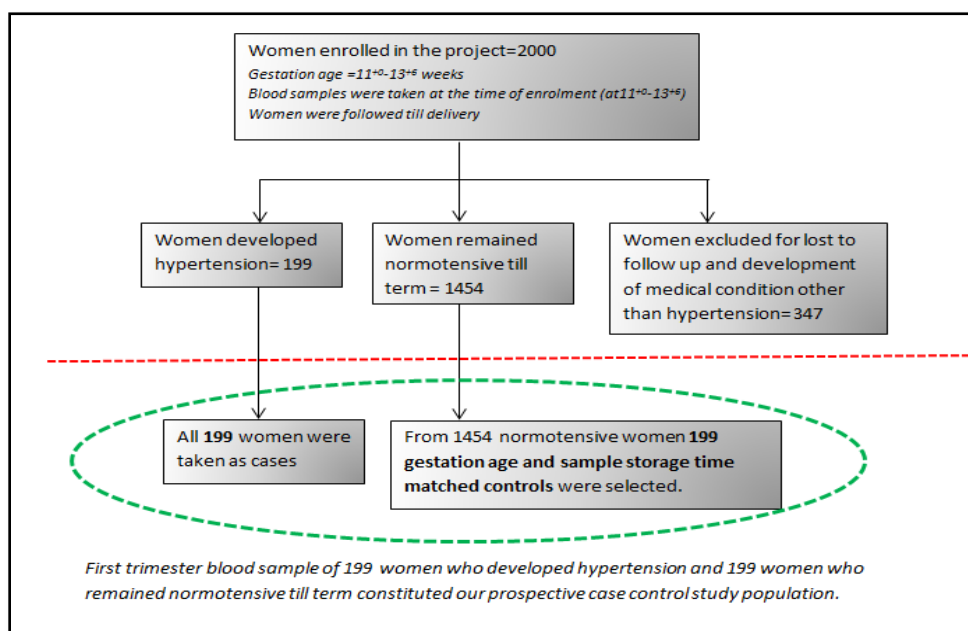


Figure 1: Patient’s recruitment pattern: Study population is part of another research study being conducted at Lady Hardinge medical college, New Delhi, India, for the First trimester screening of Pregnancy Hypertension [20].

In the present study 199 women who developed hypertension and 199 gestational age matched controls who were normotensive till term (Selected from cohort of 1454 women who were normotensive till term) were included.

Serum levels of MMP-9 were determined by quantitative ELISA (enzyme linked immunosorbent assay) technique using Ray Bio Human MMP-9 immunoassay (Ray Biotech, Inc. USA). PAPP-A serum concentration was measured by autoanalyser immulite-1000 (Siemens, Berlin).

Statistical analysis was performed using SPSS 20 (IBM Corporation, NY, USA). The data was represented as Multiple of median (MoM). The means of two groups were compared using unpaired t-test. The correlation of serum concentration of MMP-9 and PAPP-A determined using Pearson’s correlation test.

3. Results

The first trimester serum levels of PAPP-A ($p < 0.001$) and MMP-9 ($p = 0.03$) were significantly lower in women who developed pregnancy hypertension as compared to women who remained normotensive till term (Table-1). The sensitivity, specificity and PPV of PAPP-A was 72.7%, 66% and 60% respectively. MMP-9 show sensitivity, specificity and PPV to be 65%, 60.4% and 52% respectively. When both the biomarkers were combined the sensitivity, specificity and PPV was increased slightly up to 74.5%, 70% and 75% (Table-2). In our study we found a moderate positive correlation between MMP-9 and PAPP-A ($r = 0.4$; $p = 0.03$) Figure-2.

Table 1: First trimester serum levels of PAPP-A and MMP-9: Cases Vs. Controls

Biomarkers	Controls (n=199)		Cases (n=199)		p-value
	Mean	±SD	Mean	±SD	
PAPP-A MoM	1.45	0.72	0.90	0.72	<0.0001
MMP-9 MoM	1.01	0.23	0.94	0.19	0.039

Values are given as Mean±SD (Standard Deviation); $p < 0.05$ is statistically significant

Table 2: Sensitivity, Specificity, PPV and NPV of PAPP-A and MMP-9

Biomarkers	Cut-off Value	Sensitivity	Specificity	PPV	NPV
PAPP-A MoM	0.8	72.7%	65.9%	60%	77%
MMP-9 MoM	0.9	65.4%	60.4%	52%	72.5%
Combined (PAPP-A and MMP-9)	--	74.5%	70.2%	75%	78.7%

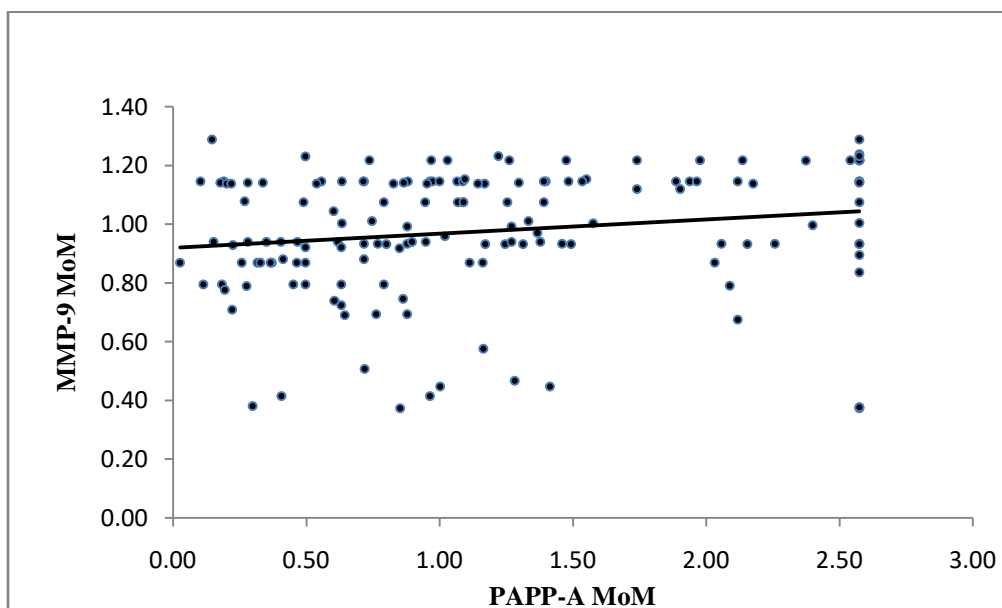


Figure 2: Correlation of First Trimester Serum Level of MMP-9 and PAPP-A; A moderate positive correlation with $r = 0.4$ was found between MMP-9 and PAPP-A in the first trimester of women who later developed hypertension.

4. Discussion

Complete and precise trophoblast invasion is vital for healthy and successful pregnancy. Trophoblast invasion is facilitated by several factors produced by trophoblast. MMP-9 and PAPP-A are metalloproteinases produced by trophoblast believed to facilitate trophoblast migration and proliferation through uterine wall. Trophoblast has two layers cytotrophoblast which produces MMP-9 and syncytiotrophoblast which produces PAPP-A [9,15,16].

The first trimester serum level of PAPP-A was lower in women who later developed pregnancy hypertension. Present study affirms the previous findings that indicates association of lowering of PAPP-A during first trimester with development of pregnancy hypertension [4,11,21]. PAPP-A is a metalloproteinase which enhances the mitogenic function of the IGFs by cleaving the complex of IGFs-IGFBPs which helps in trophoblast invasion [9,10]. The lowering concentration of the PAPP-A in first trimester may have inhibitory effect on trophoblast invasion in IGF-IGFBP dependent manner. MMP-9 is one of those metalloproteinases which are most actively involved in ECM degradation and rearrangement at the placentation site [15,22]. First trimester serum level of MMP-9 ($p=0.03$) in pregnancies which progressed to pregnancy hypertension was lower than the pregnancies which remained normotensive till delivery. Our finding is different from the findings of Poon *et al* (2009) who reported association of raised MMP-9 in first trimester with development of pregnancy hypertension and Kolben *et al* (1996) who have reported no significant association of MMP-9 levels with pregnancy hypertension [17,19]. Karampas *et al* (2014) also reported no association between first trimester MMP-9 and development of pregnancy hypertension [18]. Lowering of MMP-9 during first trimester may influence trophoblast invasion in maternal endometrium by reducing the ECM degradation. Our finding indicates the trophoblast invasion promoting role of PAPP-A and MMP-9. The correlation between PAPP-A and MMP-9 during first trimester in women who developed pregnancy hypertension could be suggestive of their similar impact on trophoblast invasion or involvement of common expression regulatory pathways. The correlation was found to be moderate therefore further evaluation is required. In our prospective case-controls study we found that sensitivity and specificity of PAPP-A was 72.7% and 66% respectively which was slightly increased up to 74.5% on combining MMP-9. PAPP-A alone was found to predict 60% high risk women for development of pregnancy hypertension, however the predictive value was enhanced up to 75% on addition of MMP-9. In a prospective cohort study Odibo *et al* (2011) reported that sensitivity and specificity of PAPP-A was 58% and 68% respectively [23]. The difference in the sensitivity

and specificity could be due to regional variation of population and study type. In our previous prospective cohort study we reported that the individual sensitivity and specificity of PAPP-A was 68.2% and 72.2% respectively [12]. Our findings are comparable with the previous findings [12,23].

5. Conclusion

Serum level assessments of PAPP-A and MMP-9 in first trimester can predict 75% women who are at risk of developing pregnancy hypertension later. PAPP-A and MMP-9 are important factors for trophoblast implantation. Lowering of these two markers in first trimester may have resulted in incomplete or impaired trophoblast invasion, which is a vital for successful pregnancy. The cause of lowering of PAPP-A and MMP-9 could be a quest of further research in reference of pregnancy hypertension prediction.

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