ORIGINAL ARTICLE

First-Trimester Inflammatory Markers for Risk Evaluation of Pregnancy Hypertension

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About the Author

About the Author I am a Ph. D. student in Biochemistry department of Lady Hardinge Medical College, New Delhi, India. I have been working in the field of "developing screening test for first-trimester prediction of Preeclampsia and other pregnancy hypertension disorders" from last five years. We are studying the role of inflammatory markers, placental markers, and endothelial dysfunction markers as predictor of pregnancy hypertension disorders. As far my knowledge, no such study (with large population) has been published in reference to Indian population. I have published two papers as co-authors in international journals.

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Abstract

Introduction Hypertension in pregnancy is one of the potential causes of maternal and fetal morbidity and mortality. It complicates 7–10% of pregnancies. As of today, prediction of pregnancy hypertension is not possible.

Aim and Objectives Evaluation of pregnancy associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin, tumor necrosis factor-α (TNF-α) and interferon gamma (INF-γ) in establishing a biomarker or combination of biomarkers for the early identification of pregnancy hypertension.

Methodology This prospective study was carried out in two phases. Phase I was a cohort study in which 2000 pregnant women were enrolled in their first trimester ($11 + 0$ to $13 + 6$ weeks of gestation) and followed till delivery. Women who developed hypertension were compared with normotensive cohort (women who remained normotensive till term). Phase II was a case–control study. The women

who were diagnosed with hypertension in phase I were cases and their controls were matched for gestational age and sample storage time from normotensive cohort population. Two additional proinflammatory markers TNF-α and INF-γ were evaluated in this case–control population.

Results Out of 2000 women, 199 women developed hypertension and 1454 women remained normotensive throughout their pregnancy. Among 199 hypertensive women, 151 (9.13%) cases had gestational hypertension, 45 (2.72%) had preeclampsia (PE) and 3 (0.18%) had eclampsia (E). First trimester mean arterial pressure (MAP) ($p < 0.001$) and body mass index (BMI) ($p < 0.001$) were found significantly higher in hypertensive women when compared with normotensive women. Maternal serum levels of PAPP-A ($p < 0.001$) were significantly low in hypertensive women as compared to normotensive women, while free β-hCG ($p = 0.59$) was high, but the difference was not statistically significant. TNF-α ($p < 0.001$) and INF-γ ($p = 0.014$) both were high in hypertensive women. When all biomarkers were combined we found the positive predictive value (PPV) of 51.6% an negative predictive value (NPV) of 71.4%.

Conclusion Increased levels of proinflammatory cytokines suggest the role of underlying inflammation in pathogenesis of pregnancy hypertension, and low PAPP-A may be attributed to impaired implantation. Combining biomarkers may improve the prediction of pregnancy hypertension in the early stages of gestation. NPV of 71.4% depicts that if woman has all biomarkers in normal ranges during first trimester, she will have 71.4% chances of remaining normotensive during pregnancy.

Keywords Pregnancy hypertension · PAPP-A · Free β-hCG · TNF-α · INF-γ

Introduction

Pregnancy hypertension is one of the major causes of maternal and fetal morbidity and mortality. It affects 7–10% of pregnancies [1]. It is multiorgan and heterogeneous disorder. This disease is initially characterized by high blood pressure (systolic >140 and diastolic >90) after 20th weeks. Proteinuria (0.3 gm/24 h) appears along with or followed by hypertension in severe cases [2].

Global incidence of pregnancy hypertension is reported to be 3–18% [3]. If untreated, pregnancy hypertension can progress to PE and E, which are life-threatening maternal neurovascular disorders. The adverse outcomes of the pregnancies exposed to pregnancy hypertension can be minimized by early detection of the disorder. The pathogenesis of disorders starts much earlier than the appearance of the symptoms. There is evidence that a high proportion of pregnancies are destined to develop pregnancy hypertension at 11–13 weeks of gestation [4–7]. The role of maternal immunity [8], impaired implantation [9], increased coagulation activity [10], oxidative stress and endothelial damage has been reported in pathophysiology of pregnancy hypertension [11, 12].

Maternal intolerance for the growing fetus [8] and impaired implantation [9] are considered as the central causes of the pathogenesis of pregnancy hypertension. It has been reported that the proinflammatory cytokines produced by placenta trigger the production of vasoconstrictors. The endothelial damage is attributed to these vasoconstrictors.

In our study, we are focussing on the evaluation of PAPP-A, free β-hCG, TNF-α and INF-γ during the first trimester for the prediction of pregnancy hypertension. Women identified at high risk can be given better antenatal care or advised to follow-up in tertiary care hospitals for improved perinatal and maternal outcomes.

Materials and Methods

Study Populations

This prospective study was conducted in two phases. Phase I was cohort study, and phase II was case–control study. In phase I, women attending antenatal outpatients department (at 11 + 0 to 13 + 6 weeks of gestation) of Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi, from September 2013 to November 2015 were enrolled. Women with singleton pregnancy and who have no medical history of cardiac, hepatic, renal disorders and endocrine disorders were included in the study. Written consent was taken from women who were willing to participate in the study which was approved by ethical committee of Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi.

All women underwent ultrasonography for the confirmation of gestational age, missed abortion and any other fetal anomaly. There were 2000 women enrolled in the study during the first trimester ($11 + {}^{0} - 13 + {}^{6}$) and followed till delivery. During that visit, their age, parity, weight, height and blood pressure were recorded. Serum was separated by centrifuging 5 ml of blood sample at 5300 rpm for 3 min. Aliquots were taken for PAPP-A and free β-hCG, and rest of the sample was stored at −80 °C. In phase I, demographic characters, serum levels of PAPP-A and free β-hCG were evaluated. Women who developed hypertension were compared with normotensive cohort. In phase II, two additional investigations, i.e., TNF-α and INF-γ in hypertensive women and their gestational age and sample storage time matched controls from normotensive cohort.

Fig. 1 Flow chart showing total population stratification
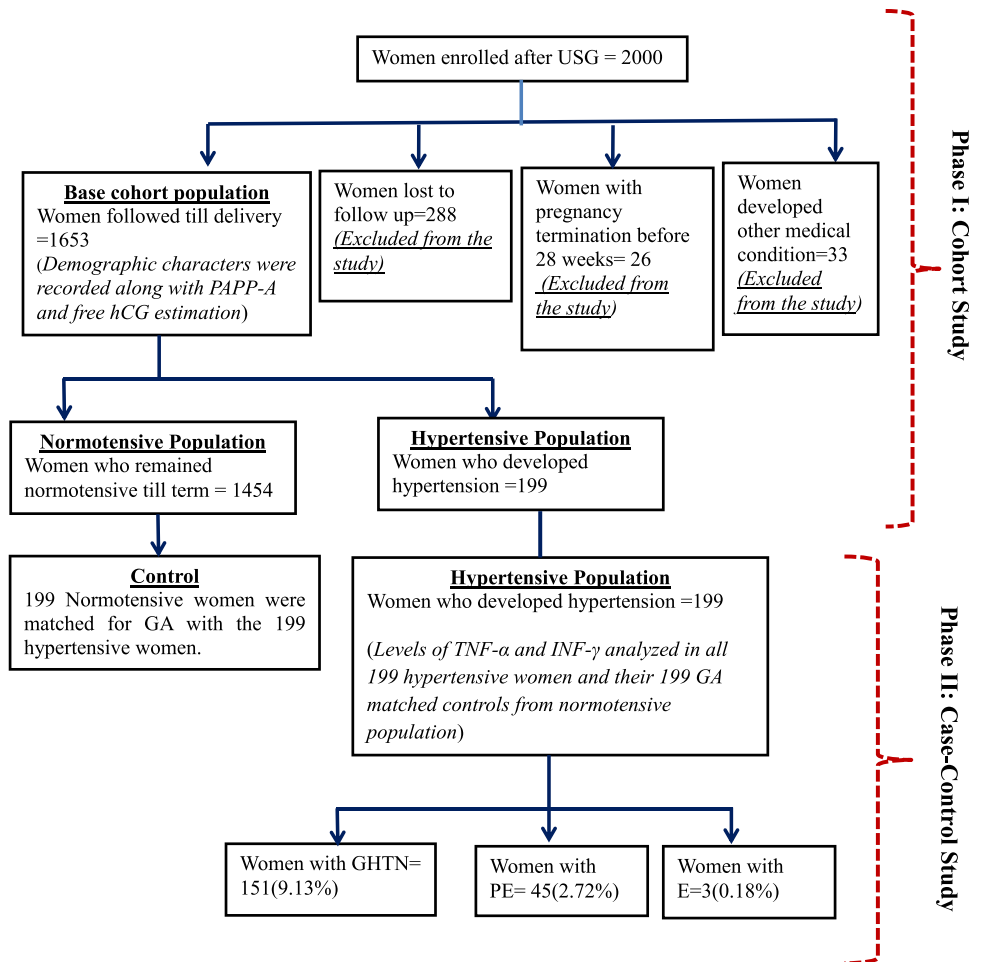
Methods

Serum levels of PAPP-A and free β-hCG were analyzed on immulite-1000 (Siemens) which is chemiluminescence-based auto analyzer. TNF-α and INF-γ levels were analyzed by ELISA (enzyme-linked immunosorbent assay) kits (Diaclone). BMI was calculated by taking a person's weight in kilograms and dividing it by the square of their height (Weight (kg)/Height (m)^2). MAP was calculated as diastolic $+1/3$ pulse pressure (difference in systolic and diastolic blood pressure).

Statistical Analysis

The present study constitutes a cohort population and case–control population. The quantitative variables were expressed as Mean ± SD and compared between the two groups using unpaired t test. The qualitative variables were expressed as frequencies and percentages and compared across two groups using Chi-square/Fisher's exact tests. Multivariate analysis using logistic regression was performed to determine the best set of predictors for an outcome. The "Forward: LR" model selection criteria was

used to obtain the best model. ROC was created to find out the cutoff values and calculating sensitivity, specificity, PPV and NPV.

Result

In this study, we prospectively examined 2000 singleton pregnancies. Out of 2000, we had excluded 347 women (288: lost to follow-up, 26: pregnancy termination before 28 weeks, 33: medical conditions other than hypertension). There were 199 women who developed hypertension in which 151 (9.13%) were gestational hypertension (GHTN), 45 (2.72%) were PE, 3 (0.18%) were E (Fig. 1).

Results of Phase I

The demographic data obtained from the patients are documented in Table 1. Among maternal factors maternal age ($p < 0.0001$), MAP ($p < 0.0001$) and BMI ($p < 0.0001$) were significantly high in women who developed hypertension as compared to the women who remained normotensive till term. Baby birth weight

Table 1 Comparison of demographic characters in cohort population

Variables	Normotensive cohort ($n = 1454$)	Hypertensive ($n = 199$)	p value
Age (Years)	24.34 ± 3.57	25.89 ± 4.26	<0.0001
Parity			
Primipara	645 (44.368%)	84 (42.21%)	0.22
Multipara	809 (55.63%)	115 (57.78%)	
BMI (kg/m^2)	20.76 ± 3.39	23.88 ± 4.76	<0.0001
MAP (mmHg)	79.54 ± 9.19	88.74 ± 12.49	<0.0001
GA at delivery (Days)	277.60 ± 9.43	265.50 ± 18.85	<0.0001
Baby birth weight (kgs)	2.93 ± 0.33	2.85 ± 0.45	0.0015

Table 2 Comparison of biochemical markers in cohort population

Variables	Normal cohort ($n = 1454$)	Hypertensive ($n = 199$)	p value
PAPP-A (mlU/ml)	5.53 ± 3.03	3.99 ± 2.96	<0.0001
Free β-hCG (ng/ml)	49.78 ± 35.38	48.45 ± 32.70	0.59

Table 3 Biochemical parameters in case–control population

Serum markers	Control ($n = 199$)	Hypertensive ($n = 199$)	p value
TNF-α (pg/ml)	64.97 ± 17.48	80.40 ± 13.05	<0.0001
INF-γ (pg/ml)	85.38 ± 22.85	94.11 ± 16.92	0.014

Table 4 Sensitivity, specificity, PPV and NPV

Biomarkers	Cutoff values
BMI	>22 kg/m^2
MAP	>82 mmHg
PAPP-A	<3.3 mlU/ml
TNF- α	>76 pg/ml
INF-γ	>84.5 pg/ml

($p = 0.001$) was significantly low in hypertensive women as compared to normotensive. Gestational age at the time of delivery ($p < 0.0001$) was significantly low in hypertensive women as compared to normotensive women. The biochemical data of cohort population is given in Table 2. PAPP-A ($p < 0.0001$) was found significantly low in women who developed hypertension. But there was no significant difference found in serum levels of free β-hCG ($p = 0.59$) (Table 2).

Results of phase II

There were 199 women who developed hypertension. Serum level of two additional markers TNF-α and INF-γ was assessed in all hypertensive women and their gestational age and sample storage time matched controls (selected from normotensive cohort of 1454 women). The serum levels of TNF-α ($p < 0.0001$) and INF-γ ($p = 0.014$) were significantly high in cases as compared to controls (Table 3).

Biomarker's Cutoff Values, Sensitivity, Specificity, PPV and NPV

ROC curve was plotted to find out the cutoff values of all biomarkers. The cutoff values are given in Table 4. At these cutoff values, sensitivity, specificity, PPV and NPV values were determined (Table 5). Among demographic markers, MAP was giving moderate sensitivity and specificity (71.6 and 51.6%). Individual sensitivity and specificity of BMI were relatively poor (Table 5). When BMI and MAP were combined the sensitivity was slightly enhanced to 78.6%; however, specificity was reduced to 41%. Serum biomarkers were shown to have better sensitivity and specificity as compared to demographic markers (Table 5). TNF-α has maximum sensitivity (89%) and specificity (86%) among all serum biomarkers. When all the biomarkers were combined the sensitivity was increased up to 96.5% with specificity of 66.8% (Table 5). In the present study, the NPV was found to be 71.4% and PPV was 51.7%. From our finding, it can be stated that if a woman has biomarkers (studied) in normal ranges during the first trimester, then she has 71.4% chances of remaining normotensive during pregnancy.

Prediction of Pregnancy Hypertension

The cutoff values of all biomarkers were determined by ROC. The impact of demographic and biochemical markers on the prediction of pregnancy hypertension was assessed by multivariate logistic regression. The best model was selected using "Forward-LR" model selection criteria. The best model was conquered in four steps where explanatory power (R^2) was increased from 2.7 to 6.3%.

Table 5 Sensitivity, specificity, PPV and NPV

Biomarkers	Sensitivity	Specificity	PPV	NPV
BMI	55.8	58.3	66	47.2
MAP	71.6	56.1	70.3	57.1
BMI + MAP	78.7	41	57.8	65.3
PAPP-A	72.7	65.9	60	77.5
TNF-α	89	86	90.6	83.1
INF-γ	73	51.2	68.6	56.4
PAPP-A + TNF-α + INF-γ	93.6	19.2	54.3	75
BMI + MAP + PAPP-A + TNF-α + INF-γ	97.5	66.8	51.7	71.4

Table 6 Best set of predictors of pregnancy hypertension

Variables	B	S.E.	p value	Odds ratio	95% C.I for odds ratio	
					Lower	Upper
Step 4						
BMI	0.206	0.086	0.017	1.228	1.037	1.455
MAP	0.078	0.031	0.013	1.081	1.017	1.149
PAPP-A	−0.443	0.112	0.000	0.642	0.516	0.800
TNF-α	0.121	0.027	0.000	1.128	1.071	1.189
Constant	−17.374	4.215	0.000	0.000		

BMI ($p = 0.017$), MAP ($p = 0.013$), TNF-α ($p < 0.0001$) and PAPP-A ($p < 0.0001$) were found to be significant predictors (Table 6).

Discussion

In pregnancy hypertension, vasoconstriction and reduced peripheral vascular amenability result in hypertension [13]. As per current clinical practices, pregnancy hypertension is diagnosed after 20th weeks of gestation by raised maternal blood pressure and proteinuria. Several proofs substantiate the role of increased first- and second-trimesters blood pressure in women who develop PE [14–16]. Our study confirms findings that increased first-trimester BMI [17, 18] and MAP [17] can predict whether women will develop pregnancy hypertension.

We found that PAPP-A is low in first trimester of women who develop pregnancy hypertension in second half of pregnancy. Our study is in accordance with the previous findings which show low PAPP-A as predictor of pregnancy hypertension [17, 19, 20]. PAPP-A a syncytiotrophoblast secreted metalloproteinase that enhances the mitogenic function of the insulin-like growth factor by cleaving the complex of IGF (insulin-like growth factor)s in and IGFBP (insulin-like growth factor binding protein)

[21], because IGF system is believed to play a significant role in the trophoblast invasion, so the low concentration of the PAPP-A might be associated with a higher risk of the developing pregnancy hypertension.

The tolerance of maternal immune system is vital for success of healthy pregnancy. Alterations in the maternal immune system in the form of amended Th1-cytokine and CD4 cell expression are known to occur in pregnancy hypertension [22, 23]. Sharma et al. [23] and Mihu et al. [24] have demonstrated elevated values of TNF-α in PE however, Roudsari et al. [25] inferred that the increase in the levels of TNF-α in preeclamptic patients is not statistically significant. It has been reported that increased levels of TNF-α in vascular smooth muscle lead to endothelial cell dysfunction, generalized vascular changes and hypertension [10]. In our study, we found an enhanced inflammatory response, i.e., significantly higher levels for TNF-α, and INF-γ in maternal circulation in hypertensive women when compared with normotensive women. Our findings show that first-trimester TNF-α is significantly associated with prediction of pregnancy hypertension. TNF-α is known to induce structural and functional alterations in endothelial cells, which induces the production, enhances the formation of endothelin-1 and reduces vasodilatation [25] which leads to endothelial damage.

Increased levels of the INF-γ are associated with PE [26]. The human placental tissue expression of INF-γ was intense in the first trimester while imperceptible at term [27]. We found increased levels of INF-γ in cases as compared to controls in first trimester. Our findings suggest that INF-γ when analyzed in first trimester can be useful to predict pregnancy hypertension. Decidual IFN-γ is secreted by uNK (uterine natural killer cells) during first trimester of pregnancy and suggested to be involved in extravillous trophoblast invasion inhibition [28]. In a review, it was suggested that IL-12 inhibits trophoblast proteases in IFN-γ-dependent pathway and also upregulates the tissue invasion inhibitors [8].

Conclusion

The data obtained from our study suggests, the prediction of women predisposed to pregnancy hypertension is possible in the first trimester. Our finding shows increased levels of proinflammatory cytokines and lowering of PAPP-A in hypertensive women as compared to normotensive women. Raised proinflammatory cytokines are suggestive of underlying inflammations in pregnancy hypertension pathogenesis, while low PAPP-A is attributed to impaired trophoblastic implantation. Statistical analysis of logistic regression indicates that biochemical markers are significant predictors associated with pregnancy

hypertension. The NPV is >70% which indicates that women with all biomarkers (Studied) in normal ranges in first trimester have >70% chances of remaining normotensive throughout pregnancy. Prediction of pregnancy hypertension before onset can potentially reduce associated fetal and maternal morbidity and mortality in a developing nation like India where deliveries are still conducted at home without any medical supervision or primary health care centres. The role of all these biomarkers as potential predictors of pregnancy hypertension needs to be further evaluated by large-scale epidemiological studies.

Compliance with Ethical Standards

Conflict of interest None.

Ethical statement All procedures performed were with the standard of institutional research ethical committee and the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent was obtained from all individuals who participated in the study.

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