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Sequential profile of endothelial functions and arterial stiffness in preeclampsia during the course of pregnancy

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ABSTRACT

Objective: To evaluate the temporal profile of arterial functions during the course of pregnancy and also to determine the predictive accuracy of vascular function indices in development of preeclampsia (PE).
Study design: Longitudinal study, two hundred and eight women participated in the study and vascular functions were assessed at 11–13, 20–22 and 30–32 weeks of gestation.
Main outcome measures: Flow mediated dilatation (FMD), augmentation index (AIx), pulse wave velocity (PWV).
Results: Out of 208 women, 13 women developed PE while 70 remained healthy pregnant (HP). In HP women, normalized FMD decreased gradually from 11 to 13 weeks to 30–32 weeks of gestation ($p < 0.05$). While in PE, Normalized FMD decreased from 11 to 13 to 20–22 weeks of gestation ($p < 0.05$) and was significantly lower in PE than HP group at 20–22 weeks of gestation ($p < 0.05$). AIx showed a mid trimester drop in HP group ($p < 0.05$) while demonstrated a rising trend in PE. Both AIx and PWV were significantly higher in PE than HP group during the course of pregnancy ($p < 0.05$). AIx demonstrated good sensitivity and specificity at both 11–13 and 20–22 weeks of gestation. Carotid femoral PWV showed an area under curve (AUC) of 78.18% and 69.75% at 11–13 and 20–22 weeks of gestation respectively. Carotid radial PWV showed good accuracy at 20–22 weeks (AUC-77.58%) of gestation.
Conclusions: Compromised arterial functions precede the onset of PE. AIx and carotid femoral PWV constitute potential predictive marker in early pregnancy for later development of PE.

1. Introduction

Preeclampsia (PE) is a major complication of pregnancy and a significant risk to the health of fetus and women in both developed and developing countries [1,2]. The worldwide incidence of PE ranges from 3 to 5% [3] and in India is around 8–10% [4] and being a developing country, the impact of this condition is of particular significance in relevance to clinical as well as financial burden. Despite the fact, it's a serious condition affecting both mother as well as fetus, the pathophysiology of PE is still not clearly understood. However, reports suggest that PE is a vascular disorder and sub-clinical vascular dysfunction may pre-exist in women that makes her vulnerable for the development of PE [5,6] Arterial functions can be assessed noninvasively by flow mediated dilatation (FMD), pulse wave velocity (PWV) and augmentation index (AIx). FMD is a gold standard measure of endothelial functions and assesses nitric oxide dependent vasodilatation while PWV and AIx are principle measures of arterial stiffness which assess structural and functional component of arterial stiffness [7] Reduced endothelial function and increased arterial stiffness in PE observed in previous cross sectional reports may manifest in high blood pressure and proteinuria in this disease [8,9].

Though, PE manifests late in gestation, it is accepted that pathophysiological processes begin early in first trimester. However, majority of existing literature in PE is after its clinical onset and of cross-sectional design which makes it difficult to define a clear patho-physiological mechanism and the specific mediators of disease. We could not find any study which assesses endothelial functions and arterial stiffness, together and in temporal manner starting from 1st trimester of pregnancy, at multiple time points during pregnancy. In order to get better understanding of patho-physiological mechanism and a comprehensive picture in PE, it is critical to study multiple but related features of the arterial functions (endothelial functions and arterial

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stiffness) simultaneously beginning with 1st trimester. Information obtained will not only help in understanding the time course of pathological processes in PE but also will assist in identifying a set of indicators that may help in prediction of PE. Its early prediction may further decrease the onset of PE by frequent monitoring of the high-risk women for PE to the hospital. Therefore, we aimed to determine the sequential changes in endothelial functions and arterial stiffness (FMD, AIx and PWV) in women who subsequently develop PE during pregnancy and also to determine the predictive accuracy of arterial function indices in development of PE.

2. Materials and methods

Pregnant women between the age of 18–35 years were enrolled in their first trimester from Dept. of Obstetrics & Gynecology of All India Institute of Medical Sciences, New Delhi, India, from January 2014 to July 2015 and followed up till delivery. Pregnant women with multiple pregnancy, morbid obesity, diabetic vasculopathy, any known vascular disorder (systemic or local), known case of renal disease, valvular heart disease and autoimmune disorder were excluded from the study. The study was approved by the institutional ethics committee (reference number – IESC/T/429/1.11.2013). Written informed consent was obtained from all pregnant women. Endothelial functions and arterial stiffness were assessed at three time points i.e., visit 1 at first trimester (11–13 weeks), visit 2 at second trimester (20–22 weeks), and visit 3 at third trimester (30–32 weeks) during the course of pregnancy in Department of Physiology, All India Institute of Medical Sciences, New Delhi.

2.1. Diagnosis of preeclampsia (PE)

Diagnosis of PE was made after 20 weeks of gestation with new onset hypertension with two readings of systolic blood pressure (BP) ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg, separated by a 4 to 6 h period and proteinuria (≥300 mg per 24 h) in a previously normotensive women [10].

2.2. Assessment of endothelial functions and arterial stiffness

All measurements were performed after a 20 min rest period in temperature-controlled room with participants in supine position. All women were asked to refrain from tea and coffee 24 h before the testing. Women were also instructed not to take any meal within 2 h of the tests. Brachial artery blood pressure (BP) was recorded twice consecutively in supine position, with a 1 min interval between each measurement, using a sphygmomanometer. The average of the two measurements of BP was used as brachial systolic BP and diastolic BP.

2.2.1. Endothelial functions

Endothelial functions were assessed by flow mediated dilatation (FMD) using vascular ultrasound (Vivid e, GE Healthcare, Germany). B-mode images of right brachial artery were recorded with a 12 MHz linear array probe. A blood pressure cuff placed below the right elbow and ultrasound probe was placed above the cuff. Lead II ECG was simultaneously recorded to correlate the brachial artery diameter with cardiac cycle. After collection of the baseline data, arterial blood flow was occluded by rapid inflation of a blood pressure cuff to 50 mmHg above systolic blood pressure. Occlusion was maintained for a period of 5 min. After release of occlusion by rapid deflation of the blood pressure cuff, artery diameter was measured for 3 min at every 10 sec intervals. The diameter of brachial artery was measured at three points on each image from the intima-media interface of one side to the intima-media interface of the other side using electronic calipers. Based on the ECG cycle, an average of three arterial diameters at end diastole (peak of R-wave of ECG) was analyzed per image. All diameter measurements were performed before the pregnancy outcome. Flow mediated dilatation % (FMD%) was calculated as the percentage increase in diameter from baseline according to the equation: $FMD\% = D_P - D_B/D_B$, where D_P is maximal post occlusion diameter and D_B is baseline diameter. It is known that smaller vessels dilate more than the larger ones and FMD is dependent on the baseline artery diameter [16] therefore, FMD was normalized for baseline artery diameter at each trimester: Normalized FMD (%/cm) = $FMD\%/D_B$.

2.2.2. Arterial stiffness

Arterial stiffness was quantified by augmentation index (AIx) and pulse wave velocity (PWV) by applanation tonometry using SphgmoCor® CVMS CPVH device (Atcor Medicals, Australia). The SphgmoCor® system uses a validated transfer function to derive central aortic pressure waveform from recorded radial arterial waveforms. Waveforms were captured when a high amplitude reproducible signal was obtained for at least 10 consecutive beats. The pulse wave analysis (PWA) was used to determine AIx. AIx value was normalized to the heart rate 75 beats/min (AIx@75) to nullify the effect of heart rate as a confounding factor.

In the present study, the PWV was calculated at three arterial segments- carotid radial, carotid distal and carotid femoral. Carotid radial PWV is a measure of peripheral stiffness while carotid femoral is a measure of central arterial stiffness. Carotid distal PWV is a measure of both central (includes part of aorta) as well as peripheral arterial stiffness. The carotid-radial PWV was recorded first, for which the distance from the suprasternal notch to the point of best palpable carotid artery pulsation (D_c) and the distance from the suprasternal notch to the radial artery (D_r) were measured. Pressure signal was obtained by placing the tonometer probe at the carotid and radial artery sequentially. ECG was simultaneously recorded to allow for an R-gated reference point for timing of pressure signal. Automated, foot to foot method was used to calculate the time elapsed between onset of pulse wave at carotid (T_c) and radial sites (T_r) by the software. The value was averaged over 10 cardiac cycles to obtain the mean time difference. PWV was reported as the distance between the two arterial sites divided by the mean time taken to travel the distance: carotid radial $PWV = D_r - D_c/T_r - T_c$. For measuring carotid distal and carotid femoral PWV, the same procedure was repeated with the dorsalis pedis and femoral artery as the distal site respectively. Data for AIx and PWV were not available in some patients due to recording artefacts, therefore respective n number for each parameter has been mentioned in table.

2.3. Statistical analysis

Data of normally distributed parameters are expressed as the mean ± standard deviation (SD) and non-parametric data are expressed as median with inter-quartile range (IQR). Maternal demographic characteristics between PE and normal pregnancy group were compared using Mann-Whitney test and unpaired *t*-test. Analysis of changes for a parameter in the three visits in the healthy pregnant group and PE group was done using repeated measures ANOVA (Analysis of Variance) with Tukey's multiple comparison test for parametric data and Friedman test with Dunn's post hoc test for non-parametric data. Analysis of differences in PE and normal pregnancy with three visits was done using unpaired *t*-test for parametric data and Mann-Whitney test for non-parametric data. Receiver operating characteristics (ROC) curves were analyzed to determine sensitivity and specificity. All statistical analyses were done using GraphPad prism version 5.00 for Windows (GraphPad Software, Inc., USA). Multiple regression analysis was performed for adjustment of blood pressure using R-software.

3. Results

A total of 256 pregnant women were recruited in their first trimester of pregnancy (< 14 weeks of gestation) and followed up till delivery.

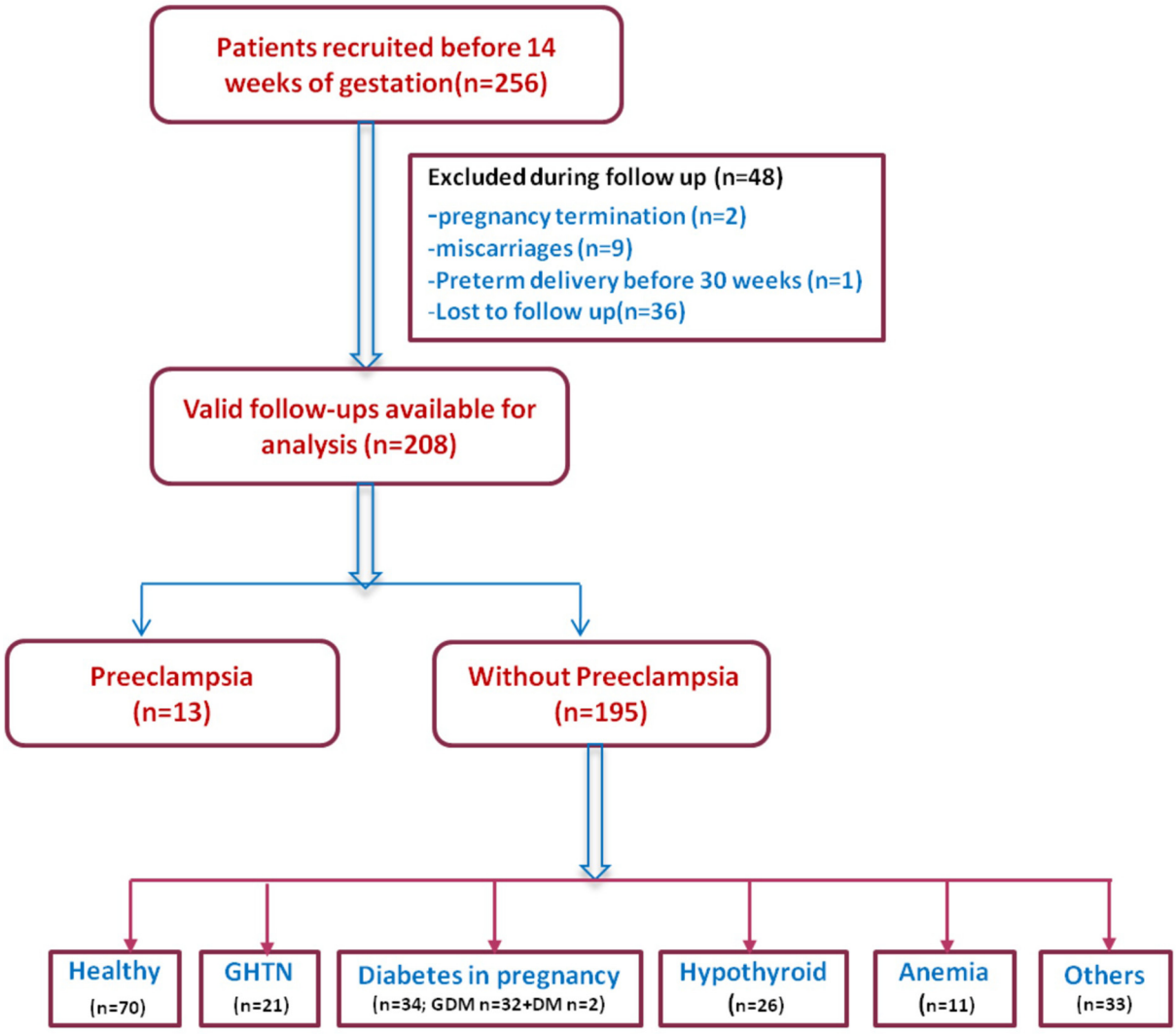

Fig. 1. Study flow chart, Abbreviations: GHTN = gestational hypertension, GDM = gestational diabetes mellitus, DM = diabetes mellitus.

Table 1
Trimester specific changes in demographic data of healthy pregnancy (HP) and preeclampsia (PE).

Indices	Healthy pregnant (HP)			Preeclampsia (PE)			P value PE vs. HP
	11–13 weeks n = 70	20–22 weeks n = 70	30–32 weeks n = 70	11–13 weeks n = 13	20–22 weeks n = 13	30–32 weeks n = 12	
Weight (kg)	54.03 ± 8.32	57.61 ± 8.88 ^{a*}	61.53 ± 9.15^{b*,c*}	57.31 ± 11.15	60.54 ± 9.95^{A*}	64.67 ± 10.81^{B*,C*}	ns
SBP (mmHg)	110.00 (106.00–116.00)	104.00 (98.00–112.00)^{a*}	104.00 (98.00–110.50)^{c*}	118.00 (111.0–124.0)	112.00 (106.0–128.0)	112.00 (108.0–127.0)	**0.003X*, 0.008Y*, 0.01Z***
DBP (mmHg)	64.00 (60.00–70.50)	60.00 (58.00–64.50)^{a*}	66.00 (60.00–70.00)^{b*}	74.00 (68.00–81.00)	70.00 (61.00–81.00)	77.00 (74.00–97.00)	**0.001X*, 0.0004Y*, < 0.0001Z***
MBP (mmHg)	79.00 (75.00–86.00)	74.00 (71.00–78.20)^{a*}	79.00 (74.00–83.00)^{b*}	90.00 (83.50–97.50)	87.00 (77.0–100.0)	94.50 (86.25–109.30)	**0.0003X*, < 0.0001Y*, 0.008Z***
PP (mmHg)	45.00 (40.00–50.50)	44.00 (38.00–50.00)	38.00 (34.00–46.00)^{b*,c*}	40.00 (36.00–48.00)	40.00 (33.0–49.0)	35.00 (32.00–40.00)	ns
Heart Rate (beats/min)	85.00 (75.70–90.20)	86.50 (78.00–95.00)^{a*}	94.50 (88.00–101.00)^{b*,c*}	85.50 (77.25–96.50)	87.50 (77.75–94.75)	89.50 (78.75–104.5)	ns

Values are represented as median (IQR) and mean ± SD. Repeated measures ANOVA has been applied on data available for all three visits- HP (n = 70) and PE (n = 12), ns = non-significant, SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure, PP = pulse pressure.
a* represents $p < 0.05$ between 11 and 13 weeks and 20–22 weeks in HP group, b* represents $p < 0.05$ between 20 and 22 weeks and 30–32 weeks in HP group, c* represents $p < 0.05$ between 11 and 13 weeks and 30–32 weeks in HP group, A* represents $p < 0.05$ between 11 and 13 weeks and 20–22 weeks in PE group, B* represents $p < 0.05$ between 20 and 22 weeks and 30–32 weeks in PE group, C* represents $p < 0.05$ between 11 and 13 weeks and 30–32 weeks in PE group, X* represents $p < 0.05$ between healthy pregnant and PE group at 11–13 weeks, Y* represents $p < 0.05$ between healthy pregnant and PE group at 20–22 weeks, Z* represents $p < 0.05$ between healthy pregnant and PE group at 30–32 weeks.

Out of 256 women, 48 were excluded from the study due to incomplete follow up (n = 36), miscarriages (n = 9), pregnancy termination due to chromosomal anomaly (n = 2), spontaneous preterm delivery before 30 weeks of gestation (n = 1). Remaining 208 women completed all the three visits and out of which, 13 women developed preeclampsia (PE) and 70 remained healthy. Therefore, the incidence of PE in the present study was 6.25%. Rest of the women were gestational hypertension (n = 21), gestational diabetes mellitus (n = 34) and hypothyroid (n = 26) and anemia (n = 26) and others complications (n = 33) (Fig. 1). In the present study, data of 70 healthy pregnant (HP) women and 13 women with PE were analyzed while remaining women with other complications were excluded from the analysis. One woman with PE developed intrauterine death at 28 weeks of gestation before the third visit therefore data of 30–32 weeks of gestation for that woman is not available. Out of 13 preeclamptic women, 5 women developed severe and early onset preeclampsia while 8 women developed mild and late onset preeclampsia. The mean gestational age of development of PE was 33^{+5} weeks (33 weeks and 5 days) thus in the present study, arterial functions were assessed before the clinical onset of disease at all trimesters.

Both the groups comprised non-smokers and were comparable in terms of age {HP; (25.87 ± 3.1 years) and PE; (27.15 ± 4.5 years), p = 0.35} and BMI {HP; 22.59 (20.63–24.57) kg/m^2 and PE; 22.70 (21.00–28.52) kg/m^2, p = 0.17} at first trimester of pregnancy. The brachial blood pressure was significantly higher in women with PE than HP women (p < 0.05) even at first trimester of pregnancy and remained high throughout the gestation (Table 1).

3.1. Flow mediated dilatation (FMD)

On sequential analysis in HP group, there was no change in FMD% over the course of pregnancy while baseline brachial artery diameter increased significantly from 11 to 13 weeks to 20–22 weeks and 20–22 weeks to 30–32 weeks of gestation. FMD normalized to baseline artery diameter (normalized FMD) decreased gradually through 11–13 weeks till 30–32 weeks of gestation (p < 0.05). In PE group, FMD % showed a steep fall from 11 to 13 weeks to 20–22 weeks of gestation (p < 0.05) and remained similar till 30–32 weeks of gestation. Baseline artery diameter increased gradually in PE group also similar to HP group. Normalized FMD also decreased significantly at 20–22 weeks from 11 to 13 weeks of gestation in PE (Fig. 2).

On intergroup comparison, both FMD and normalized FMD were comparable in HP and PE group at 11–13 weeks of gestation. At 20–22 weeks of gestation, both FMD% and normalized FMD (%/cm) were significantly lower in PE as compared to HP women. This significance persisted even after adjustment for SBP, DBP and MBP (p = 0.02, R^2 = 0.29). At 30–32 weeks, FMD% (0 = 0.09) and normalized FMD (p = 0.07) were lower in PE than HP group although it was non-significant. Baseline brachial artery diameter increased significantly with advancing gestation in both PE as well as in HP group (p < 0.05) (supplementary table).

3.2. Augmentation Index

On sequential analysis, augmentation index at heart rate 75 beats/min (AIx@75) showed a mid trimester drop in HP group while demonstrated a rising trend in PE group during the course of pregnancy. The mid trimester drop observed in HP group disappeared after adjustment for blood pressure between 11 and 13 weeks and 20–22 weeks (R^2 = 0.09, p = 0.89) and also between 20 and 22 weeks and 30–32 weeks of gestation (R^2 = 0.11, p = 0.24) (Fig. 3).

AIx@75 was significantly higher in PE as compared to HP group throughout the pregnancy however after correction for blood pressure, the difference was significant only at 20–22 weeks of gestation (p = 0.0005, R^2 = 0.35) before the development of PE.

3.3. Pulse wave velocity

PWV was calculated at three arterial segments- carotid radial (measure of peripheral arterial stiffness), carotid distal (measure of both central and peripheral arterial stiffness) and carotid femoral (measure of central arterial stiffness). There was no significant difference in carotid radial (cr) PWV and carotid femoral (cf) PWV during the course of pregnancy in both HP and PE group. While, carotid distal (cd) PWV decreased significantly from 11 to 13 weeks to 20–22 weeks of gestation in HP group (Table 2) however, this significance was lost after correction for blood pressure (p = 0.09, R^2 = 0.11). While in PE group, cd-PWV was comparable among three trimesters of pregnancy (Fig. 3).

On inter-group comparison, cr-PWV and cf-PWV were significantly higher in PE as compared to HP group throughout the pregnancy while cd-PWV was higher in women with PE at 11–13 weeks and 30–32 weeks of gestation (Fig. 3). After adjustment for blood pressure, cr-PWV was significantly higher at 20–22 weeks (p = 0.002, R^2 = 0.33) and 30–32 weeks (p = 0.002, R^2 = 0.49) of gestation. Cd-PWV (p = 0.01,

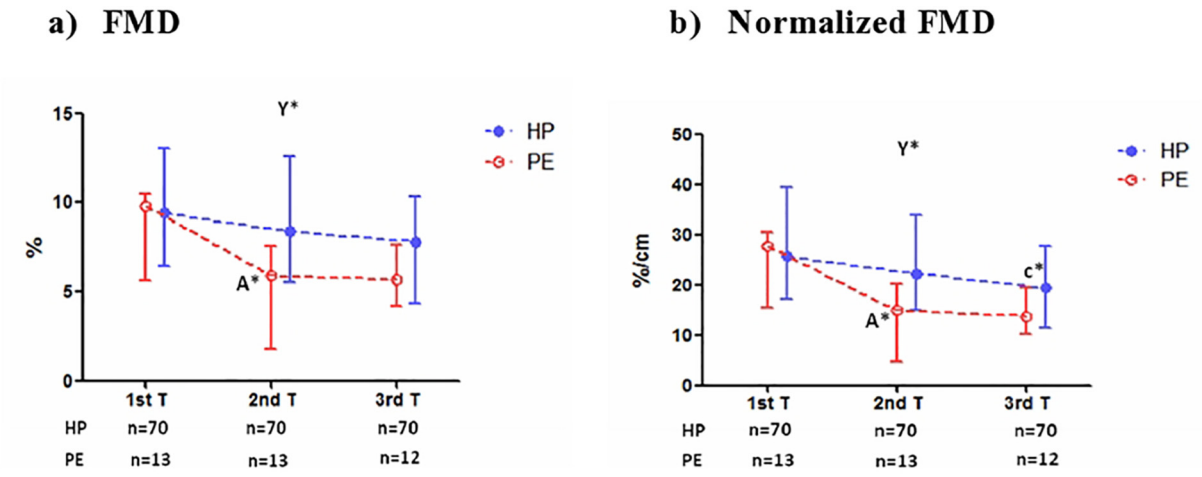

Fig. 2. The figure show sequential changes in (a) FMD% and (b) Normalized FMD (%/cm) during the course of pregnancy in preeclampsia (PE) and healthy pregnancy (HP) group. Repeated measures ANOVA have been applied on data available for all three visits –HP; FMD% (n = 70), Normalized FMD (n = 70), and PE; FMD% (n = 12), Normalized FMD (n = 12). Values are represented as median (IQR). FMD: flow mediated dilatation, T = trimester, c* represents p < 0.05 between 1st trimester and 3rd trimester in HP group, A* represents p < 0.05 between 1st trimester and 2nd trimester in PE group, Y* represents p < 0.05 between HP and PE group at 2nd trimester.

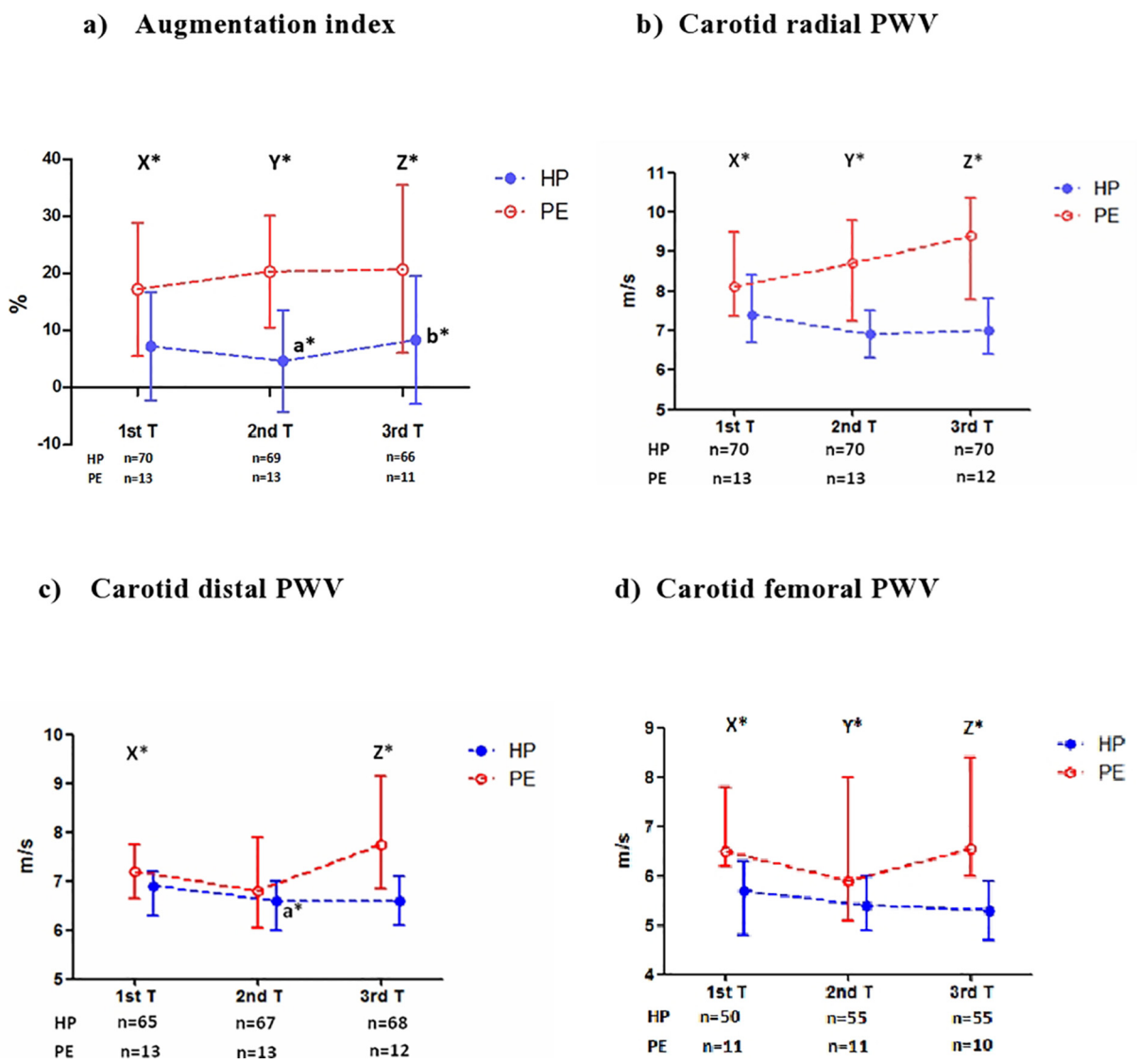

Fig. 3. The figure shows sequential changes in (a). augmentation index @ heart rate 75 (AIx@75), (b). carotid radial PWV, (c). carotid distal PWV and (d) carotid femoral PWV in preeclampsia (PE) and healthy pregnant (HP) group during the course of pregnancy. Values are represented as mean ± SD for (AIx@75) and all three PWV are expressed as median (IQR) in both the groups. Repeated measures ANOVA have been applied on data available for all three visits -Healthy pregnant: AIx@75 (n = 66), cr-PWV (n = 70), cd-PWV (n = 65), cf-PWV (n = 50) and preeclampsia: AIx@75 (n = 11), cr-PWV(n = 12), cd-PWV(n = 12), cf-PWV(n = 10), T = trimester, PWV = pulse wave velocity. a* represents $p < 0.05$ between 1st trimester and 2nd trimester in HP group. b* represents $p < 0.05$ between 2nd trimester and 3rd trimester in HP group. X* represents $p < 0.05$ between HP and PE group at 1st trimester. Y* represents $p < 0.05$ between HP and PE group at 2nd trimester. Z* represents $p < 0.05$ between HP and PE group at 3rd trimester.

$R^2 = 0.49$) and cf-PWV ($p = 0.02$, $R^2 = 0.44$) were significantly higher in PE group than HP group only at 30–32 weeks of gestation after correction for blood pressure (supplementary table).

3.4. ROC curve analysis

PE develops after 20 weeks of gestation, therefore its early prediction is utmost important in developing country like India to minimize the risk of maternal and fetal complications. ROC curves were analyzed at 11–13 weeks and 20–22 weeks of gestation before the development of PE. The area under ROC curves showed that the sensitivity and specificity were low for FMD at both 11–13 weeks, 20–22 weeks of gestation (Table 2, Fig. 4a) however, AIx demonstrated an area under curve > 75% at all time points (Table 2, Fig. 4b). Out of the three PWV, cf-PWV gave an AUC of 78.18% and 69.75% at 11–13 weeks and 20–22 weeks of gestation respectively (Table 2, Fig. 4e). cr-PWV was able to predict PE at 20–22 weeks (AUC-77.58%) of gestation (Table 2, Fig. 4c) and cd-PWV showed an AUC of 70.24 and 62.28 at 11–13 weeks and 20–22 weeks of gestation respectively (Table 2, Fig. 4d).

4. Discussion

In the present study, endothelial functions and arterial stiffness have been assessed together and sequentially starting from 11 to 13 weeks at multiple time points over the course of pregnancy in preeclampsia (PE). We observed that endothelial functions [assessed by flow mediated dilatation (FMD)] were lower and functional arterial stiffness [assessed by augmentation index (AIx)] was higher in PE than healthy pregnancy at second trimester of pregnancy. While structural arterial stiffness [assessed by pulse wave velocity (PWV)] was higher in PE than healthy pregnant women at third trimester of pregnancy. Results also showed that AIx, carotid-femoral (cf) PWV can be used for prediction of PE at 11–13 weeks as markers to triage women and timely refer them to

Table 2
Overview of the sensitivity and specificity of arterial functions in prediction of preeclampsia.

Parameter	Gestational age of assessment	AUC (95% CI)	Cut off	Sensitivity (%)	Specificity (%)	Likelihood ratio
FMD (%)	11–13 weeks (HP; n = 70, PE; n = 13)	53.96(39.66–68.26)	< 8.6%	46.15(19.22–74.87)	55.71(43.34–67.59)	1.04
	20–22 weeks (HP; n = 70, PE; n = 13)	71.65(57.36–85.93)	< 7.05%	76.92(46.19–94.96)	60.00(47.59–71.53)	1.92
AIx@75 (%)	11–13 weeks (HP; n = 70, PE; n = 13)	**77.97(62.56–93.37)**	**> 11.5%**	**76.92(46.19–94.96)**	**67.14(54.88–77.90)**	**2.34**
	20–22 weeks (HP; n = 69, PE; n = 13)	**89.07(80.60–90.09)**	**> 11.5%**	**84.62(54.55–98.08)**	**79.71(68.31–88.44)**	**4.17**
cr-PWV (m/s)	11–13 weeks (HP; n = 70, PE; n = 13)	70.49(55.19–85.80)	> 7.60 m/s	61.54(31.58–86.14)	60.00(47.59–71.53)	1.54
	20–22 weeks (HP; n = 70, PE; n = 13)	**77.58(62.32–92.85)**	**> 7.65 m/s**	**76.92(46.19–94.96)**	**82.86(71.97–90.82)**	**4.49**
cd-PWV (m/s)	11–13 weeks (HP; n = 65, PE; n = 13)	70.24(54.44–86.03)	> 7.05 m/s	69.23(38.57–90.91)	67.29(54.95–78.77)	2.14
	20–22 weeks (HP; n = 67, PE; n = 13)	62.28(41.97–82.60)	> 6.75 m/s	61.54(31.58–86.14)	59.70(47.00–71.51)	1.53
cf-PWV (m/s)	11–13 weeks (HP; n = 50, PE; n = 11)	**78.18(62.32–94.04)**	**> 6.10 m/s**	**90.91(58.72–99.77)**	**72.00(57.51–83.77)**	**3.25**
	20–22 weeks (HP; n = 55, PE; n = 11)	69.75(55.78–88.72)	> 5.75 m/s	72.73(39.03–93.98)	69.09(55.19–80.86)	2.35

Bold values indicate the parameters which can be use for prediction of preeclampsia at different trimesters of pregnancy. Abbreviations: FMD = flow mediated dilatation, AIx@75 = augmentation index @ heart rate 75, cr-PWV = carotid radial pulse wave velocity, cd-PWV = carotid distal pulse wave velocity, cf-PWV = carotid femoral pulse wave velocity.

higher centers for prevention of the disease. Additionally, AIx and carotid-radial (cr) PWV can be used as predictive marker at 20–22 weeks of gestation.

In the present study, women who developed PE later in pregnancy showed a decrease in FMD% with advancing gestation similar to previous reports [11,12] and fall was 3.21% from 11 to 13 weeks to 20–22 weeks of gestation indicating endothelial dysfunction in PE. In healthy pregnancy, FMD did not show any change during the course of pregnancy consistent with previous reports [13,14] however, a fall of 1.03% was observed between 11 and 13 weeks and 20–22 weeks of gestation. Incongruently, a meta-analysis of 14 studies [15] showed that FMD% increases by 1.89% between 15 and 21 weeks during healthy pregnancy. Best of our knowledge, none of previously reported studies have used normalized FMD as a measure of endothelial function in Asian women. Normalized FMD decreased significantly from 11 to 13 weeks to 30–32 weeks of gestation healthy pregnancy, while from 11 to 13 to 20–22 weeks in PE. In line with earlier report, both FMD [11,12] and normalized FMD were lower in PE than healthy pregnancy at 20–22 weeks and 30–32 weeks of gestation suggesting that nitric-oxide mediated vasodilatation is hampered before the development of PE. A couple of studies are available with a single time point measurement in different trimesters of pregnancy and reported a lower FMD in PE than healthy pregnant women [16–20].

PE is associated with the exaggerated inflammatory response with advancing gestation [13,21] which may manifest in oxidative stress and decreased NO bioavailability in the systemic as well as in placental vasculature, therefore may result in endothelial dysfunction. Evidence signify that abnormal maternal inflammation is associated with faulty trophoblastic invasion and spiral artery remodeling leading to increased vascular resistance and blood pressure [22]. Additionally, compromised endothelial function in kidney may result in loss of integrity of glomerular endothelium leading to the proteinuria in PE [23].

Although we found that FMD% was significantly different between healthy pregnancy and PE even before the clinical onset of disease however area under curve (AUC) for FMD was < 75% at both 11–13 weeks and 20–22 weeks of gestation. Therefore, FMD can't be considered an adequate tool to predict PE at any age of gestation [24] in agreement with previous reports [25,26]. However, Brandao et al. 2014 [27] have assessed FMD at 24–27^{+6} weeks of gestation in 20 PE and 72 normotensive controls and reported FMD to be an independent predictor of PE.

Arterial stiffness is an important predictive marker for various cardiovascular diseases and determined by the smooth muscle tone, transmural distending pressure and structural components (collagen and elastin) of the arterial wall [28,29]. AIx and PWV are easy to measure and reproducible method for evaluation of arterial stiffness. In the present study, AIx (systemic arterial stiffness) showed a mid-trimester drop in healthy pregnant women similar to earlier reports [9,14] which could be because of fall in blood pressure (BP) in second trimester of pregnancy as no fall was observed in AIx after correction for BP. Within the PE group, a continuous rise was observed in AIx during the course of pregnancy consistent to previous reports [30,31] and women with PE also demonstrated a significantly higher AIx than healthy pregnancy at 20–22 weeks which is consistent with the findings from few cross-sectional studies [32,33] and a longitudinal report [30]. Higher AIx in PE represents the increase vascular tone (vasoconstriction), earlier wave reflection and decreased elasticity of arteries and arterioles.

In concordance with the earlier reports by Khalil et al., in 2010 (AUC-87%) and 2009 (AUC-94%) [34,35] at 11–13 weeks, the sensitivity and specificity was found to be high both at 11–13 and 20–22 weeks before the development PE for AIx in our study. Therefore, AIx can be used as a marker of prediction of PE in early pregnancy.

To the best of our knowledge, this is the first study where PWV has been assessed at three arterial segments together and sequentially during pregnancy. PWV showed comparable results in both PE and healthy pregnancy during pregnancy consistent with previous reports [30,36–39]. PWV was higher in PE than healthy pregnancy throughout the pregnancy however after correction for BP, the difference between the groups was significant only at 30–32 weeks of gestation for all three arterial segments. Carotid radial-PWV was higher in PE than healthy pregnancy at 20–22 weeks of gestation also after correction of BP. Interestingly, with a cut of value of > 6.10 m/s for carotid femoral-PWV, the sensitivity and specificity for prediction of PE was 91% and 72% respectively at 11–13 weeks of gestation which suggest that cf-PWV is an adequate parameter for prediction of PE even at 11–13 weeks. Unfortunately, we could not find any study determining sensitivity and specificity of cf-PWV at 11–13 weeks. Additionally, cr-PWV (AUC-

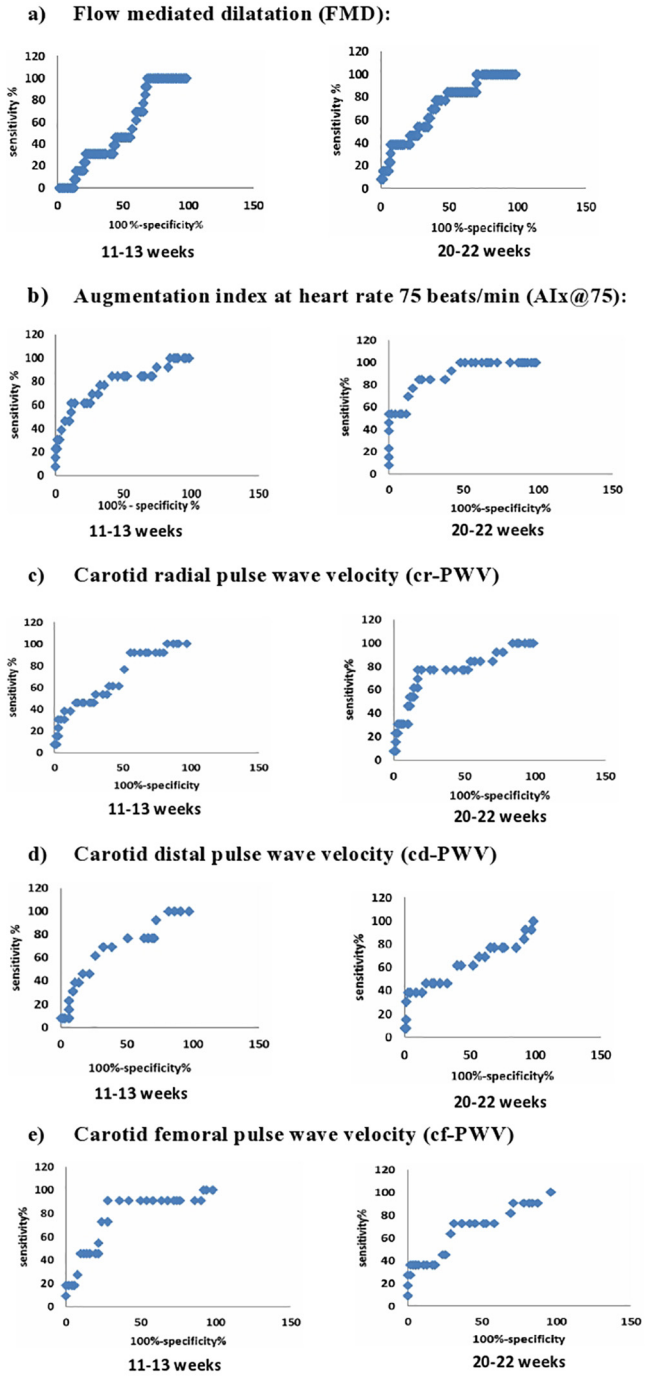

Fig. 4. The figure shows receiver operator characteristic (ROC) curves for (a). FMD% (b). AIx@75c). cr-PWV (d). cd-PWV (e). cf-PWV at 1st trimester (11–13 weeks) and 2nd trimester (20–22 weeks) of pregnancy.

77.58%) can be used as predictor of disease at 20–22 weeks of gestation whereas cf-PWV (AUC-69.75%) should not be used as predictive marker at 20–22 weeks. Katsipi et al., in 2014 [40] indicated aortic PWV (AUC-92%) as a useful tool for prediction of PE at 24–26 weeks of gestation.

Inflammation, oxidative stress and endothelial dysfunction, all can contribute to the increased arterial stiffness in PE [41]. Observations of present study suggest that reduced endothelial functions could possibly be contributing to both functional (second trimester) and structural (third trimester) arterial stiffness in women with PE. Vascular dysfunctions may manifest in platelet activation, vasodilator to vasoconstrictor imbalance, increased endothelial cell permeability and possibly contribute to clinical signs i.e. hypertension and proteinuria of PE.

Endothelial function and arterial stiffness were assessed simultaneously, in a temporal manner and at multiple time points, which is the biggest strength of this study. Additionally, arterial functions have been assessed via noninvasive methods. Present study has an important clinical implication, as the sensitivity and specificity have been determined for different parameters for early prediction of PE.

5. Limitation

The major limitation of the present was the small number of women who developed preeclampsia later during the course of pregnancy. However, study gives an insight into the patho-physiological sequence of events of development of PE.

6. Conclusion

We conclude that compromised vascular functions precede the onset of PE later in pregnancy. AIx and cf-PWV constitute potential predictive marker in early pregnancy for later development of PE. Results of present study are helpful in improving the adverse maternal and fetal outcome in high risk women for PE. However, validation of the results on a larger scale and a molecular level will further enhance in understanding the disease mechanism and will assist in prevention of disease costs.

Contributors

Priyanka Garg: "I declare that I participated in the research design, volunteer recruitment, data collection, data analysis, review of literature and writing of the manuscript and that I have seen and approved the final version. I have no conflict of interest".

Ashok Kumar Jaryal: "I declare that I participated in the research design, data analysis and writing of the manuscript and that I have seen and approved the final version. I have no conflict of interest".

Garima Kachhawa: "I declare that I facilitated access to antenatal clinics to allow volunteer recruitment. I have participated in the research design, data analysis and writing of the manuscript and have seen and approved the final version. I have no conflict of interest".

Alka Kriplani: "I declare that I participated in the research design, data analysis and writing of the manuscript and that I have seen and approved the final version. I have no conflict of interest".

Kishore Kumar Deepak: "I declare that I participated in the research design, data analysis and writing of the manuscript and that I have seen and approved the final version. I have no conflict of interest".

Details of ethics approval

Institute ethics committee, All India Institute of Medical Sciences, New Delhi, India, date of approval – 09.12.13 and reference number – IESC/T/429/1.11.2013.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.09.013.

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