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Original Article

Link of the Markers of Endothelial Dysfunction with Decreased Baroreflex Sensitivity is Crucial in the Development of Preeclampsia

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ABSTRACT

Background and Aim: Decreased Baroreflex Sensitivity (BRS) is an established marker of Cardiovascular (CV) risk. Decreased BRS has been reported in preeclampsia. In the present study, the link between endothelial dysfunction and decreased BRS in preeclampsia has been assessed. **Materials and Methods:** Pregnant women (*n*=174) having risks of preeclampsia were recruited and followed till term to note the incidence of preeclampsia. Retrospectively, they were divided into two groups: Group I (*n*=107), which did not develop preeclampsia and Group II (*n*=67), which developed preeclampsia. Blood Pressure Variability (BPV), including BRS, Heart Rate Variability (HRV), Conventional Autonomic Function Tests (CAFTs), markers of endothelial dysfunction such as Endothelin-1 (ET-1), Nitric Oxide (NO) and von Willebrand Factor and the ratio of NO to ET-1 (NO/ET-1), the inflammatory markers (interleukin-6, TNF-α, interferon-γ), and oxidative stress were measured in all the 3 trimesters. **Results:** Sympathovagal Imbalance (SVI), with decreased parasympathetic functions starting from the early part of pregnancy and increased sympathetic functions towards the later part of pregnancy, was observed in these PIH-risk women, which was more marked in Group II subjects. Decreased BRS, the marker of SVI and CV risk, was more prominent in Group II compared to Group I. Markers of endothelial dysfunction and inflammation were prominently altered in Group II subjects. Multiple regression analysis demonstrated the independent contribution of interleukin-6 (β=0.292, *p*=0.031), TNFα (β=0.308, *p*=0.025), interferon-γ (β=0.387, *p*=0.011) and NO/ET-1 (β=0.542, *p*=0.001) to the reduction in BRS. Also, markers of SVI and endothelial dysfunction were intricately linked to alterations in NO/ET-1. NO/ET-1 could predict the development of prehypertensive status in group II subjects. **Conclusion:** It was concluded that inflammation and oxidative stress contribute to vascular dysfunction, which in turn initiates SVI in the early part of pregnancy in women at risk of preeclampsia. SVI triggered by NO/ET-1 intensifies in the later part of pregnancy, which leads to the development of preeclampsia.

Keywords: Baroreflex sensitivity, Cardiovascular risks, Heart rate variability, NO/ET-1 ratio, Preeclampsia, Vascular endothelial dysfunction.

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INTRODUCTION

Gestational hypertension is the development of hypertension in a pregnant woman after the 20th week of gestation who was previously normotensive, having a Blood Pressure (BP) greater than 140/90 mmHg on two separate occasions, recorded at least 6 hr apart.[1] When associated with proteinuria, it is called preeclampsia.[1] Preeclampsia complicates 6-10% of all pregnancies, and it remains a leading cause of maternal morbidity and mortality, primarily due to the cardiometabolic complications associated with the disease.[2] Intriguingly, BP in preeclampsia usually normalises with the delivery of the placenta, suggesting that an abnormal placenta is the primary cause of preeclampsia.[3] Many putative chemicals such as Tumour Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6), the angiotensin-II type-1 receptor Autoantibody (AT1-AA), etc., secreted from an abnormal placenta have been proposed to be involved in the causation of preeclampsia,[4] though the precise biochemical mechanisms involved in the development

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of PIH have not been delineated yet. Although hypertension subsides in many preeclamptic women after delivery, it has been reported that they have a higher risk of developing hypertension later following their postpartum period, and they also have higher Cardiovascular (CV) risks compared to women who had a normotensive pregnancy.[5] It has been observed that endothelial dysfunction in pregnancy plays a major role in the pathophysiology of preeclampsia, and continuation of endothelial dysfunction postpartum could be the cause of persistent CV risk in these women in the later part of their lives.[6] Recently, we have reported the role played by Nitric Oxide (NO), a marker of endothelial dysfunction, in the development of CV risk in women with risk factors for GH.[7]

Unlike other forms of hypertension, Pregnancy-Induced Hypertension (PIH) is a state of volume contraction, and hypertension occurs in this condition despite low blood volume, which is mainly due to sympathetic overactivity.[8] We have reported that the sympathovagal imbalance assessed by Heart Rate Variability (HRV) could be a non-invasive test for the prediction of preeclampsia.[9] We have also reported the crucial role played by vagal inhibition in the genesis of hypertension in PIH, especially in the early-onset category of the disease.[10] Recently, alteration in Baroreflex Sensitivity (BRS), a precise marker of sympathovagal imbalance, has been used for the prediction of CV risks in GH that includes preeclampsia and PIH.[11,12]

NO, the endothelium-derived relaxing factor, is a predominant vasodilatory substance produced by the vascular endothelium in response to mechanical and chemical stimuli, and it has long been implicated in the pathophysiology of preeclampsia and other hypertensive disorders of pregnancy.[13,14] Endothelial dysfunction and reduced nitric oxide bioavailability have been observed to play an important role in the maternal and foetal pathophysiology, frequently leading to preterm delivery of the foetus, causing neonatal morbidity and mortality, and maternal CV morbidity and mortality in preeclampsia.[15,16] Endothelin-1 (ET-1), a potent endogenous vasoconstrictor secreted mainly from vascular endothelial cells, has been strongly implicated in the pathogenesis of PIH.[17] Though vasodilators and vasoconstrictors are known to influence the functions of the sympathetic and parasympathetic (vagal) systems,[18] to date, there are no reports of an association of NO and ET-1 with the sympathovagal imbalance in the causation of PIH. As both the reduction in NO (the vasodilator) and increase in ET-1 (the vasoconstrictor) have been observed in preeclampsia, we hypothesise that the ratio of NO to ET-1 (NO/ET-1), rather than the individual concentration of these chemicals *in* the blood, might play an important role in the alteration in sympathovagal imbalance that finally results in preeclampsia.

HRV and BRS are used as early markers of sympathovagal imbalance and autonomic dysregulation in various clinical disorders.[19,20] Recently, we have reported that a decrease in BRS is linked to inflammation and oxidative stress in PIH.[21,22] Also, retrograde inflammation and oxidative stress in various conditions are reported to be associated with increased CV risks.[23,24] Further, inflammation and oxidative stress have been reported to play a significant role in the etiopathogenesis of GH.[25,26] It has been reported that autonomic dysfunctions, as assessed by analysis of HRV, Blood Pressure Variability (BPV), and Conventional Autonomic Function Tests (CAFTs), play a significant role in the pathophysiology of various cardiometabolic disorders.[27-30] Nevertheless, to date, no study has been conducted to assess the link between alterations in the levels of markers of endothelial dysfunction and the attenuation of BRS and HRV parameters in the assessment of cardiometabolic dysfunction and the development of preeclampsia.

It has been observed from our previous studies that in the Indian population, the incidence of hypertension in women having high risks of developing preeclampsia is about 40%.[9-12] It is not known why 60% of women with risks for preeclampsia do not develop preeclampsia, whereas 40% of them develop the disease. Therefore, in the present study we have assessed the cardiometabolic and endothelial dysfunctions and entire gamut of autonomic dysfunctions in preeclampsia and attempted to determine the link of markers of endothelial dysfunction with CV risk in preeclampsia assessed by BRS, and its role in the genesis of preeclampsia in women having risks of developing preeclampsia.

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MATERIALS AND METHODS

Study design

After obtaining approval of the study protocol from the Institute Ethics Committee of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, India, 310 pregnant women with risk factors for preeclampsia were screened in the outpatient department of the Obstetrics and Gynaecology of JIPMER hospital. Following the inclusion and exclusion criteria, 238 subjects were recruited in their 1st trimester. With the dropout of 64 subjects, a total of 174 subjects who attended antenatal check-ups in all 3 trimesters and had participated in all the investigations were followed up till term. The study parameters were recorded thrice, at the 12th week, 24th week and 36th week of gestation, and they were followed up till term to note the development of hypertension, if any.

Inclusion criteria

Inclusion criteria included normotensive pregnant women having any of the established risk factors for preeclampsia,[1] such as family history of preeclampsia or GH, preeclampsia or GH in previous pregnancy, extremes of reproductive age, Body Mass Index (BMI) <35, Diastolic Blood Pressure (DBP) >80 mmHg at the first visit, first pregnancy, multiple pregnancy, etc.

Exclusion criteria

Exclusion criteria included subjects with unknown last menstrual date, women not willing to deliver at JIPMER Hospital, women known to have hypertension, cardiac arrhythmias, and endocrine disorders including diabetes mellitus, before pregnancy and those who had received oral contraceptive pills within 6 months before pregnancy.

Sample size calculation

The major outcome parameter was assessment of the association of BRS with the parameters of endothelial dysfunction. But there was no previous report of assessment of BRS and endothelial dysfunction markers in preeclampsia. Therefore, as per the previous report of BRS in pregnancy-induced hypertension[12] and keeping the power of the study at 80% and the confidence interval at 95%, the sample size was calculated to be a minimum of 65.

Brief procedures

All the subjects were asked to report to the Autonomic Function Testing laboratory and Cardiovascular Research laboratory of the Physiology Department between 8 AM and 10 AM, following a light breakfast. Written informed consent was obtained from all the subjects prior to initiation of the study. They were clearly informed about the entire procedure of HRV, BRS and CAFT recordings. The temperature of the lab was maintained at 25 °C throughout the recordings. Their Body Weight (BW) in kg and

Table 1: Age, body weight, BMI, heart rate, blood pressure parameters and RPP in subjects of study group I (pregnant women with risk factors for PIH but did not develop PIH) and study group II (pregnant women having risk factors for PIH and developed PIH) at all 3 trimesters.

Parameters	Study Group I (*n*=107)	Study Group II (*n*=67)	*p* value
First Trimester			
Age (Yrs)	26.36±4.83	26.63±4.72	0.7178
Body weight (Kg)	59.92±5.63	60.23±6.87	0.7461
BMI (Kg/m^2)	25.15±3.10	25.87±3.93	0.1812
BHR (per min)	81.41±7.62	90.83±6.72	<0.0001
SBP (mmHg)	108.60±10.36	123.88±6.85	<0.0001
DBP (mmHg)	78.99±7.42	80.90±8.47	0.3232
MAP (mmHg)	88.86±8.12	94.73±7.82	<0.0001
RPP (mmHg/min)	88.50±10.13	112.52±11.86	<0.0001
Second Trimester			
Body weight (Kg)	64.50±5.90	65.61±6.93	0.2608
BMI (Kg/m^2)	28.42±3.23	28.90±4.15	0.3947
BHR (per min)	90.69±8.77	97.78±6.47	<0.0001
SBP (mmHg)	113.29±10.92	131.92±11.62	<0.0001
DBP (mmHg)	81.90±7.73	85.24±6.02	0.0030
MAP (mmHg)	92.36±7.91	100.80±9.78	<0.0001
RPP (mmHg/min)	103.38±10.76	129.09±11.12	<0.0001
Third Trimester			
Body weight (Kg)	68.92±5.88	74.63±6.33	<0.0001
BMI (Kg/m^2)	30.36±3.31	32.89±3.55	<0.0001
BHR (per min)	103.27±8.69	112.27±6.04	<0.0001
SBP (mmHg)	117.43±12.15	139.90±8.36	<0.0001
DBP (mmHg)	83.14±8.36	92.21±6.12	<0.0001
MAP (mmHg)	94.57±8.13	108.01±9.38	<0.0001
RPP (mmHg/min)	121.79±11.72	157.02±9.23	<0.0001

The data presented are mean±SD. *p*-value <0.05 was considered statistically significant. BMI, Body Mass Index; BHR, Basal Heart Rate; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; MAP, Mean Arterial Pressure; RPP, Rate-Pressure Product.

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height in cm were measured, and Body Mass Index (BMI) was calculated.

HRV recording

Following 10 min of supine rest in the laboratory, Basal Heart Rate (BHR), Systolic Blood Pressure (SBP), and Diastolic Blood Pressure (DBP) were recorded. For recording of short-term HRV, recommendation of the Task Force on HRV and as described earlier was followed.[12,27] For this purpose, ECG electrodes were connected, and Lead II ECG was acquired at a rate of 1000 sample/s during supine rest using a BIOPAC MP 100 data acquisition system (BIOPAC Inc., USA). The data were transferred from BIOPAC to a Windows-based PC with AcqKnowledge software version 3.8.2. Ectopics and artefacts were removed from the recorded ECG. RR tachogram was extracted from the edited 256 s ECG using the R wave detector in the AcqKnowledge software and saved in ASC-II format, which was later used offline for short-term HRV analysis. HRV analysis software (version 1.1, Biomedical Signal Analysis Group, University of Kuopio, Finland), which analyses the frequency spectrum components using Fast Fourier transformation and time-domain components in the RR trend, was used. The frequency-domain indices of HRV included Total Power (TP), Low Frequency power expressed in normalised units (LFnu), High Frequency power expressed in normalised units (HFnu), and ratio of Low-Frequency to High-Frequency power (LF-HF ratio); and the time-domain components included mean and Standard Deviation of RR intervals (SDNN), Square Root of the Mean of the Sum of the

Table 2: Heart Rate Variability (HRV) parameters in subjects of study group I (pregnant women with risk factors for PIH but did not develop PIH) and study group II (pregnant women having risk factors for PIH and developed PIH) in all 3 trimesters.

Trimester	HRV Indices	Parameter	Study Group I (*n*=107)	Study Group II (*n*=67)	*p*-value
First Trimester	Frequency Domain Indices	TP (ms^2)	239.01±97.83	207.22±82.88	0.0285
		LFnu	50.44±17.30	53.85±18.90	0.2239
		HFnu	49.56±17.85	46.15±17.01	0.2136
		LF:HF ratio	1.01±0.47	1.16±0.51	0.0491
	Time Domain Indices	RMSSD	31.12±9.25	25.84±8.10	<0.0001
		SDNN	30.90±5.17	24.10±3.5	<0.0001
		NN50	37.84±6.61	33.90±7.41	0.0003
		pNN50	10.38±1.06	8.40±1.17	<0.0001
Second Trimester	Frequency Domain Indices	TP (ms^2)	199.45±77.36	154.58±55.12	<0.0001
		LFnu	52.34±15.36	57.92±13.45	0.0155
		HFnu	47.66±14.30	42.08±12.85	0.0101
		LF:HF ratio	1.10±0.51	1.38±0.70	0.0027
	Time Domain Indices	RMSSD	27.32±7.45	20.38±5.32	<0.0001
		SDNN	28.94±6.78	18.98±5.65	<0.0001
		NN50	34.80±9.34	21.87±7.40	<0.0001
		pNN50	8.77±1.72	5.21±1.05	<0.0001
Third Trimester	Frequency Domain Indices	TP (ms^2)	162.20±77.40	84.63±13.29	<0.0001
		LFnu	61.83±10.57	67.77±9.86	0.0003
		HFnu	38.17±10.57	32.23±9.86	0.0003
		LF:HF ratio	1.62±0.75	2.10±0.84	0.0001
	Time Domain Indices	RMSSD	22.50±4.43	12.80±3.37	<0.0001
		SDNN	21.90±3.89	14.68±3.70	<0.0001
		NN50	28.81±9.32	12.10±2.68	<0.0001
		pNN50	7.35±1.16	2.20±0.55	<0.0001

The data presented are mean±SD. *p*-values <0.05 were considered statistically significant. TP, Total Power of HRV; LFnu, Normalised Low-Frequency (LF) Power; HFnu, Normalised High-Frequency (HF) Power; RMSSD, the Square Root of the Mean of the Sum of the Squares of the Differences between Adjacent NN Intervals; SDNN, Standard Deviation of Normal-to-Normal Intervals; NN50, the Number of Interval Differences of Successive NN Intervals greater than 50; pNN50, the Proportion derived by Dividing NN50 by the Total Number of NN Intervals.

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Table 3: Conventional Autonomic Function Testing (CAFT) parameters and Blood Pressure Variability (BPV) parameters in subjects of study group I (pregnant women with risk factors for PIH but did not develop PIH) and study group II (pregnant women having risk factors for PIH and developed PIH), at all 3 trimesters.

Trimester	Parameters	Study Group I (*n*=107)	Study Group II (*n*=67)	*p*-value
First Trimester				
	CAFT Parameters			
	30:15 ratio	1.47±0.07	1.40±0.08	<0.0001
	E:I ratio	1.30±0.08	1.28±0.06	0.0803
	ΔDBP_{IHG}	17.32±3.18	20.68±3.71	<0.0001
	BPV Parameters			
	SV (mL)	73.90±10.65	79.56±9.86	0.0006
	LVET (ms)	300.86±35.42	310.55±22.61	0.0473
	CO (L/min)	6.05±0.90	7.25±0.97	<0.0001
	TPR (mmHg·min/L)	1.05±0.30	1.17±0.32	0.0133
	BRS (ms/mmHg)	18.20±2.30	16.38±3.64	<0.0001
Second Trimester				
	CAFT Parameters			
	30:15 ratio	1.42±0.07	1.33±0.09	<0.0001
	E:I ratio	1.24±0.09	1.17±0.09	<0.0001
	ΔDBP_{IHG}	21.64±4.10	24.60±3.82	<0.0001
	BPV Parameters			
	SV (mL)	74.84±8.48	82.43±10.15	<0.0001
	LVET (ms)	309.22±25.17	327.90±26.54	<0.0001
	CO (L/min)	6.78±0.77	8.05±0.94	<0.0001
	TPR (mmHg·min/L)	1.10±0.31	1.25±0.36	0.0040
	BRS (ms/mmHg)	17.35±3.12	14.04±2.16	<0.0001
Third Trimester				
	CAFT Parameters			
	30:15 ratio	1.35±0.08	1.21±0.05	<0.0001
	E:I ratio	1.18±0.05	1.05±0.04	<0.0001
	ΔDBP_{IHG}	24.12±3.28	29.75±4.17	<0.0001
	BPV Parameters			
	SV (mL)	77.80±9.78	90.38±9.62	<0.0001
	LVET (ms)	332.33±30.12	387.86±38.52	<0.0001
	CO (L/min)	8.03±0.92	10.12±1.02	<0.0001
	TPR (mmHg·min/L)	1.12±0.29	1.46±0.40	<0.0001
	BRS (ms/mmHg)	15.20±2.75	9.32±1.76	<0.0001

The data presented are mean±SD. *p*-value <0.05 were considered statistically significant. 30:15 ratio, the ratio of maximum RR interval at the 30th beat to the minimum RR interval at the 15th beat following standing; E:I ratio, the ratio of average RR interval during expiration to that of during inspiration in six cycles of deep breathing; ΔDBP_{IHG}, the maximum rise in DBP above baseline following 30% of maximum voluntary contraction by isometric hand grip method; SV, stroke volume; LVET, left ventricular ejection time; CO, cardiac output; TPR, total peripheral resistance; BRS, baroreceptor reflex sensitivity.

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Table 4: Inflammatory and Oxidative Stress (OS) markers and vascular endothelial dysfunction markers in subjects of study group I (pregnant women with risk factors for PIH but did not develop PIH) and study group II (pregnant women having risk factors for PIH and developed PIH) at all 3 trimesters.

Trimester	Parameters	Study Group I (*n*=107)	Study Group II (*n*=67)	*p* Value
First Trimester				
	Inflammatory & OS Markers			
	hs-CRP (µmol/L)	6.42±1.67	6.90±2.72	<0.0001
	IL-6 (pg/mL)	55.96±8.84	66.94±8.84	<0.0001
	TNF-α (pg/mL)	109.97±9.92	167.62±15.74	<0.0001
	IFN-γ (pg/mL)	92.28±10.29	155.11±13.90	<0.0001
	TBARS (µmol/L)	2.57±0.36	3.42±0.65	<0.0001
	Vascular Endothelial Dysfunction Markers			
	Endothelin-1 (µg/L)	7.97±0.38	8.25±0.71	0.0009
	vWF (mU/mL)	50.64±6.54	51.03±10.48	0.7627
	NO derivatives (µmol/L)	26.27±4.36	25.84±3.70	0.4876
	NO/Endothelin-1 ratio	3.96±0.68	3.13±0.55	<0.0001
Second Trimester				
	Inflammatory & OS Markers			
	hs-CRP (µmol/L)	7.54±0.58	8.17±0.74	<0.0001
	IL-6 (pg/mL)	72.92±18.12	87.93±21.02	<0.0001
	TNF-α (pg/mL)	153.65±15.53	218.33±16.84	<0.0001
	IFN-γ (pg/mL)	123.81±10.90	202.32±18.74	<0.0001
	TBARS (µmol/L)	2.90±0.47	4.84±1.36	<0.0001
	Vascular Endothelial Dysfunction Markers			
	Endothelin-1 (µg/L)	8.40±0.52	8.75±0.51	<0.0001
	vWF (mU/mL)	52.59±6.05	56.29±11.01	0.0134
	NO derivatives (µmol/L)	27.61±5.43	23.92±4.83	<0.0001
	NO/Endothelin-1 ratio	3.28±0.52	2.73±0.40	<0.0001
Third Trimester				
	Inflammatory & OS Markers			
	hs-CRP (µmol/L)	8.86±0.77	9.84±1.21	<0.0001
	IL-6 (pg/mL)	90.20±8.57	132.66±15.20	<0.0001
	TNF-α (pg/mL)	179.77±15.57	270.72±21.88	<0.0001
	IFN-γ (pg/mL)	133.36±11.81	269.09±18.38	<0.0001
	TBARS (µmol/L)	3.88±0.53	6.46±1.19	<0.0001
	Vascular Endothelial Dysfunction Markers			
	Endothelin-1 (µg/L)	8.77±0.54	10.57±0.81	<0.0001
	vWF (mU/mL)	56.51±6.25	68.07±9.61	<0.0001
	NO derivatives (µmol/L)	29.40±4.50	20.48±4.10	<0.0001

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Table 4: (Continued)

Trimester	Parameters	Study Group I (*n*=107)	Study Group II (*n*=67)	*p* Value
	NO/Endothelin-1 ratio	3.35±0.65	1.93±0.32	<0.0001

The data presented are mean±SD. *p* value <0.05 were considered statistically significant. hs-CRP, High-Sensitive C-Reactive Protein; IL-6, Interleukin 6; TNF-α, Tumour Necrosis Factor α; IFN-γ, Interferon gamma; TBARS, Thiobarbituric Acid Reactive Substance; vWF, von Willebrand factor; NO, nitric oxide.

Squares of differences between adjacent RR interval (RMSSD), adjacent RR interval differing more than 50 ms (NN50), and NN50 counts divided by all RR intervals (pNN50).

Conventional autonomic function tests

Three conventional AFTs were performed following the standard procedures.[28]

HR and BP response to standing

For HR and BP response to standing, BP and ECG were recorded in the supine position. The subject was instructed to attain the standing posture in 3 sec. The ECG was continuously recorded during the procedure. BP was recorded every 40 sec by an automatic monitor (Omron, SEM-1, Kyoto, Japan) till 5th min. The 30:15 ratio (ratio of maximum RR interval at the 30th beat to minimum RR interval at the 15th beat after standing) was calculated.

HR response to deep breathing

For HR response to deep breathing, the subject was in a sitting posture and HR and respiration monitoring were done from electrocardiographic recording and stethographic respiratory tracings recorded on the multichannel polygraph (Nihon-Kohden, Tokyo, Japan), respectively. A baseline recording of ECG and respiration was taken for 30 sec. The subject was asked to take slow and deep inspiration followed by slow and deep expiration such that each breathing cycle lasted for 10 sec, consisting of 6breathing cycles/min. E/I ratio (ratio of average RR interval during expiration to average RR interval during inspiration in 6 cycles of deep breathing) was calculated from electrocardiographic tracing.

BP response to isometric handgrip

For BP response to isometric handgrip, baseline BP was recorded. The subject was asked to press a handgrip dynamometer at 30% of maximum voluntary contraction for 2 min. BP was recorded at the 1st and 2nd min of contraction. ΔDBP_{IHG} (maximum increase in DBP above baseline) was noted.

Recording of BPV parameters

The CV parameters and BRS were measured by continuous BPV method using Finapres (Finometer version 1.22a, Finapres Medical Systems, Amsterdam, The Netherlands); a non-invasive continuous haemodynamic CV monitor based on the principle of measurement of finger arterial pressure with the volume clamp technique of Penaz and the Physical criteria of Wesseling.[29] In this method, the brachial artery pressure measured was the reconstructed pressure from the finger pressure, estimated through generalised waveform inverse modelling and generalised level correction. The subjects were asked to lie down, and the brachial cuff of finapres was tied around the mid-arm about 2 cm above the cubital fossa, and the finger cuff of small, medium, or large size was tied around the middle phalanx of the middle finger depending on the finger width. For the height correction, two sensors were placed, one at the heart level and another at the finger level. The recordings were obtained following connection of the cuffs' cables to the Finometer, after 10 min of supine rest. The "return-to-flow calibration and the physical" was done for the level correction between the brachial and finger pressure during the initial 5 min of the recordings. Following this, the continuous BP recording was done for a period of 10 min. The reconstructed brachial pressure was acquired through a PC-based data acquisition system (Finapres Medical Systems BV, Amsterdam, The Netherlands). The parameters recorded from the reconstructed brachial pressure tachogram were HR, systolic BP, diastolic BP, Mean Arterial Pressure (MAP), Rate-Pressure Product (RPP), interbeat interval, Left Ventricular Ejection Time (LVET), Stroke Volume (SV), cardiac output, Total Peripheral Resistance (TPRS), and BRS.

Estimation of biochemical parameters

5 mL of fasting blood sample was collected through venipuncture, and serum was separated and stored at −80 °C for analysis. The oxidative stress parameter, Thiobarbituric acid reactive substances (TBARS), was measured using a colourimetric assay kit (Elabscience, USA), and inflammatory markers such as high-sensitivity C-Reactive Protein (hsCRP) (Calbiotech, USA), Tumour Necrosis Factor α (TNF-α), Interferon gamma (IFN-γ), and Interleukin-6 (IL-6) (Diaclone, France) were measured using an ELISA kit according to the manufacturer's instructions. Nitric oxide derivatives (nitrate and nitrite), Endothelin 1 (ET-1) and von Willebrand factor (vWF) were estimated using a chemical method (Elabscience, USA).

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Statistical analysis of data

SPSS version 13 (SPSS Software Inc., Chicago, IL, USA) was used for statistical analysis. All data were expressed as mean±SD for parametric data and median (IQR) for non-parametric data. Normality of data was tested by the Kolmogorov-Smirnov test. For parametric data, the level of significance between study group I and study group II was tested by Student's unpaired *t* test and for nonparametric data, the Wilcoxon signed-rank test was used. The association of BRS with various parameters was assessed by Spearman correlation analysis. The independent contribution of various parameters to BRS and NO/ET-1 was assessed by multiple regression analysis. Multiple logistic regression was done to assess the prediction of prehypertension status by NO/ET-1in study group II. The P values less than0.05 were considered statistically significant.

RESULTS

There was no significant difference in age between the two groups. Body weight and BMI showed no significant change in the 1st and 2nd trimesters, but both were significantly increased ($p<0.0001$) in the 3rd trimester in study group II subjects. Basal cardiovascular parameters like BHR, SBP, MAP and RPP were significantly higher ($p<0.0001$) in study group II compared to study group I women in all the 3 trimesters of gestation, whereas DBP was significantly higher only in the 2nd trimester ($p=0.0030$) and 3rd trimester ($p<0.0001$) (Table 1).

Table 5: Correlation of BRS with important cardiometabolic parameters in all 3 trimesters in the study group I and study group II.

Trimester	Parameter	Study Group I (r)	Study Group I (p)	Study Group II (r)	Study Group II (p)
First Trimester	hs-CRP	0.036	0.352	0.052	0.320
	IL-6	0.090	0.293	-0.142	0.205
	TNF-α	0.040	0.332	-0.110	0.256
	IFN-γ	0.082	0.308	-0.180	0.099
	TBARS	0.030	0.380	-0.140	0.208
	Endothelin-1	-0.102	0.256	-0.190	0.091
	NO derivatives	0.150	0.137	0.115	0.242
	NO/Endothelin-1 ratio	0.115	0.173	0.260	0.045
Second Trimester	hs-CRP	0.098	0.287	-0.112	0.195
	IL-6	-0.117	0.170	-0.164	0.193
	TNF-α	-0.134	0.142	-0.186	0.095
	IFN-γ	-0.102	0.174	-0.154	0.167
	TBARS	0.081	0.308	-0.151	0.170
	vWF	-0.048	0.341	-0.117	0.251
	Endothelin-1	-0.160	0.128	-0.278	0.037
	NO derivatives	0.172	0.110	0.237	0.058
	NO/Endothelin-1 ratio	0.107	0.167	0.338	0.008
Third Trimester	hs-CRP	-0.182	0.091	-0.265	0.041
	IL-6	-0.180	0.092	-0.305	0.012
	TNF-α	-0.198	0.072	-0.327	0.009
	IFN-γ	-0.191	0.085	-0.386	0.005
	TBARS	-0.155	0.112	-0.262	0.041
	vWF	-0.095	0.288	-0.196	0.091
	Endothelin-1	-0.195	0.081	-0.358	0.007
	NO derivatives	0.240	0.058	0.437	0.001
	NO/Endothelin-1 ratio	0.246	0.055	0.485	0.000

The p-values less than 0.05 were considered significant; hs-CRP, high-sensitivity C-Reactive Protein; IL-6, Interleukin 6; TNF-α, Tumour Necrosis Factor α; IFN-γ, Interferon gamma; TBARS, Thiobarbituric Acid Reactive Substances; NO, Nitric Oxide.

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Table 6: Multiple regression analysis to assess independent contribution of various important cardiometabolic parameters to BRS (as dependent variable) in third trimester in the study group II.

Independent Variables	Standardised Regression Coefficient (β)	Confidence Interval - Lower Limit	Confidence Interval - Upper Limit	p-value
hs-CRP	-0.122	-0.015	0.005	0.104
IL-6	-0.292	-0.003	0.045	0.031
TNF-α	-0.308	-0.032	0.156	0.025
IFN-γ	-0.387	-0.002	0.088	0.011
TBARS	-0.244	-0.001	0.072	0.062
Endothelin-1	-0.395	-0.033	0.134	0.009
NO	0.416	0.002	0.085	0.007

p values <0.05 were considered significant. BRS, baroreflex sensitivity; hs-CRP, high-sensitivity C-Reactive Protein; IL-6, Interleukin 6; TNF-α, Tumour Necrosis Factor α; IFN-γ, Interferon gamma; TBARS, Thiobarbituric Acid Reactive Substances; NO, Nitric Oxide.

Table 7: Multiple regression analysis to assess the independent contribution of various important cardiometabolic parameters to BRS (as the dependent variable) in the 3rd trimester in study group II.

Independent Variables	Standardised Regression Coefficient (Beta)	Lower Limit	Upper Limit	*p* Value
hsCRP	-0.124	-0.017	0.005	0.107
IL6	-0.295	-0.002	0.048	0.030
TNF-α	-0.312	-0.033	0.158	0.024
IFN-γ	-0.395	-0.006	0.080	0.010
TBARS	-0.250	-0.004	0.078	0.060
NO/Endothelin-1	0.532	0.005	0.092	<0.001

p values <0.05 were considered significant. BRS, baroreflex sensitivity; hs-CRP, high-sensitivity C-Reactive Protein; IL-6, Interleukin-6; TNF-α, Tumour Necrosis Factor α; IFN-γ, Interferon gamma; TBARS, Thiobarbituric Acid Reactive Substances; NO/ET-1, ratio of nitric oxide to Endothelin-1.

Table 8: Multiple regression analysis of NO/ET-1 (as the dependent variable) with various parameters (as independent variables) in the 3rd trimester in study group II

Independent Variables	Standardised Regression Coefficient (β)	95% CI Lower Limit	95% CI Upper Limit	*p* Value
MAP	-0.265	-0.320	-0.019	0.022
LF-HF ratio	-0.242	-2.588	-0.143	0.039
E:I ratio	0.305	0.217	0.452	0.008
ΔDBP_{IHG}	-0.334	0.316	0.947	0.005
IL-6	-0.296	0.051	-0.356	0.009
TNF-α	-0.280	-0.390	-0.012	0.016
IFN-γ	-0.255	-0.295	-1.39	0.035
TBARS	-0.221	0.000	-0.010	0.054

MAP, Mean Arterial Pressure; TP, Total Power of HRV; LF-HF ratio, ratio of LF power to HF power of HRV; E: I ratio, the ratio of average RR interval during expiration to that during inspiration in six cycles of deep breathing; $\Delta DBP_{IHG,}$ the maximum rise in DBP above baseline following 30% of maximum voluntary contraction by isometric hand grip method; IL-6, Interleukin 6; TNF-α, Tumour Necrosis Factor α; IFN-γ, Interferon gamma; TBARS, Thiobarbituric Acid Reactive Substance.

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Among the frequency domain indices of HRV, TP was significantly lower in study II compared to study group I in all the 3 trimesters. Though LFnu and HFnu were not significantly altered in the 1st trimester, increases in LFnu and decreases in HFnu were more prominent in the 2nd and 3rd trimesters in study group II compared to study group I. LF-HF ratio was significantly increased in all 3 trimesters. There was a significant decrease (p<0.0001) in time domain parameters such as RMSSD, SDNN and pNN50 in all 3 trimesters, and NN50 was significantly decreased in the 1st trimester (p=0.0003), 2nd trimester (p<0.0001) and 3rd trimester (p<0.0001) (Table 2).

Among the CAFT parameters, the 30:15 ratio was significantly reduced (p<0.0001) and ΔDBP_{IHG} was significantly increased (p<0.0001) in all the 3 trimesters in study group II compared to study group I subjects. E:I ratio showed no significant change in the 1st trimester, whereas in the 2nd and 3rd trimesters, the E:I ratio was significantly reduced (p<0.0001) in study group II compared to study group I subjects (Table 3).

In BPV parameters, in the 1st trimester, there was a significant increase in SV (p=0.0006), LVET (p=0.0473), CO (p<0.0001) and TPRS (p=0.0133) and a significant decrease in BRS (p<0.0001) in study group II compared to study group I. Also, SV, LVET and CO were significantly increased (p<0.0001), and BRS was decreased (p<0.0001) in 2nd and 3rd trimester in study group II in 2nd and 3rd trimesters of gestation (Table 3). TPRS was significantly increased in the 2nd trimester (p=0.0040) and in the 3rd trimester (p<0.0001) (Table 3).

TBARS, the oxidative stress marker, and inflammatory markers like hs-CRP, IL-6, TNF-α and IFN-γ were significantly increased (p<0.0001) in study group II compared to study group I in all the 3 trimesters. Among the endothelial dysfunction markers, endothelin-1 was significantly increased in the 1st trimester (p= 0.0009), 2nd trimester (p<0.0001) and 3rd trimester (p<0.0001) (Table 4).

vWF was statistically significant in the 2nd trimester (p=0.0134) and 3rdtrimester (p<0.0001), and NO derivatives were significantly decreased (p<0.0001) in the 2nd and 3rd trimesters in study group II compared to study group I (Table 4). NO/Endothelin-1 ratio was significantly reduced (p<0.0001) in all 3 trimesters (Table 4).

In study group II, BRS showed significant correlation only with the NO/Endothelin-1 ratio (p=0.045) in the 1st trimester. In 2nd trimester, BRS was significantly correlated with Endothelin-1 (p=0.037) and NO/Endothelin-1 ratio (p=0.008), and it was significantly correlated with hs-CRP (p=0.041), IL-6 (p=0.012), TNF-α (p=0.009), IFN-γ (p=0.005), TBARS (p= 0.041), Endothelin-1 (p=0.007), NO derivatives (p=0.001) and NO/Endothelin-1 ratio (p=0.000) in 3rd trimester, but no correlation was found with vWF in 2nd and 3rd trimester (Table 5).

In multiple regression analysis, markers like IL-6 (p=0.031), TNF-α (p=0.025), IFN-γ (p=0.011), Endothelin-1 (p=0.009) and NO derivatives (p=0.007) independently contributed to BRS in study group II subjects in the 3rd trimester (Table 6). NO/Endothelin-1 ratio significantly contributed to BRS in study group II subjects in the 3rd trimester (Table 7). MAP (p=0.022), LF-HF ratio (p=0.039), E:I ratio (p=0.008), ΔDBP_{IHG} (p=0.005), IL-6 (p= 0.009), TNF- α (p=0.016) and IFN-γ (p=0.035) independently contributed to NO/ET-1 in the 3rd trimester in study group II by multiple regression analysis (Table 8).

Bivariate logistic regression analysis showed that NO/Endothelin-1ratio could significantly predict prehypertension status in study group II subjects in the 2nd trimester (p=0.025), as well as in the 3rd trimester (p=0.005) (Table 9).

DISCUSSION

In the present study, there was a significant alteration in blood levels of the markers of vascular endothelial dysfunction in all the 3 trimesters of pregnancy in women who developed PIH compared to the women who did not develop PIH (Table 4). The ET-1 was significantly increased, and the NO/ET-1 ratio was significantly reduced in study group II compared to study group I in the 1st trimester. These alterations in study group II were more pronounced in the 2nd trimester, in addition to a significant reduction in NO and a rise in vWF, and in the 3rd trimester, the alterations in these parameters were observed to be maximal. These findings suggest that endothelial dysfunction had started much earlier in pregnancy, before the development of hypertension that manifested in the later part of pregnancy. Thus, it appears that molecular mechanisms of endothelial dysfunction precede the clinical expression of the disease in PIH. All the markers of endothelial dysfunction except vWF, in the 3rd trimester in group II, were significantly correlated with a decrease in BRS (Table 5), the most important indicator of dysregulation of BP. BRS is a marker of sympathovagal control of BP, and decreased BRS is invariably associated with increased BP,[30] which is an established indicator of CV risk.[7] Further, there was a significant independent association of NO and ET-1 with BRS (Table 6), indicating that endothelial dysfunction contributes to an increase in BP and increased CV risks in these high-risk pregnant women. An increase in endothelin-1 and a decrease in NO have been reported to be associated with PIH.[31,32] But, to date, there is no report of the assessment of the ratio of NO to ET-1 in PIH patients.

In the present study, NO/ET-1 was significantly less in study group II compared to study group I in all 3 trimesters (Table 4), and NO/ET-1 had a maximal level of significant correlation with BRS in study group II in all the trimesters. Further, NO/ET-1 had a significant correlation with BRS in study group II in the 1st and 2nd trimesters, when the individual correlation of NO

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or ET-1 with BRS was not significant (Table 5). Moreover, the independent contribution of NO/ET-1 to BRS had the highest level of significance (Table 7) compared to the level of individual contribution of NO or ET-1 to BRS in the 3rd trimester in study group II (Table 6). To avoid multicollinearity, the NO/ET-1 ratio was not used in the same regression model along with NO and ET-1. All these findings clearly indicate that the NO/ET-1 ratio is a better marker than NO or ET-1 alone in the prediction of decreased BRS in PIH. Also, a rise in mean arterial pressure (MAP) had an independent association with NO/ET-1 in the 3rd trimester (Table 8) in women who developed PIH. Moreover, NO/ET-1 could predict the development of prehypertensive status in the 2nd and 3rd trimesters in PIH women (Table 9). It appears that the NO/ET-1 ratio has a crucial role to play in the development of PIH. Though ET-1 was significantly increased in the 1st trimester in group II, the rise in BP was not considerable, when the decrease in NO became significant from the 2nd trimester onward, the increase in BP became more substantial. Thus, it seems that the decrease in NO, and therefore the decrease in the NO/ET-1 ratio, alters the balance between vasoconstriction and vasodilation that tilts the net effects towards vascular contraction, resulting in an increase in BP.

Although the exact underlying mechanisms of endothelial dysfunction in PIH are still not known, the most well-accepted hypothesis is that placental ischaemia/hypoxia results from inadequate uteroplacental vascular remodelling, which leads to a decrease in placental blood flow and the ischaemic placenta releases cytokines such as Tumour Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6), the angiotensin II type 1 receptor Autoantibody (AT1-AA), and Interferon-γ (IFN-γ).[32,33] These immunological-inflammatory cytokines released from the abnormal placenta cause maternal endothelial dysfunction characterised by elevated circulating Endothelin (ET-1), vWF, and enhanced vascular sensitivity to angiotensin II.[34,35] These factors act in concert to decrease renal function and cause hypertension during pregnancy. In the present study, the circulating levels of inflammatory markers such as hsCRP, IL-6, TNF-α and IFN-γ were significantly high in study group II subjects from the 1st trimester of pregnancy, indicating that abnormalities in the placenta were present since the early part of pregnancy, which were intensified in the 3rd trimester of pregnancy. Further, all these cytokines were significantly correlated with BRS (Table 5), and all except hsCRP had an independent association with BRS (Table 6) in the 3rd trimester. Furthermore, IL-6, TNF-α and IFN-γ had a significant contribution to the NO/ET-1 ratio in PIH women in the 3rd trimester. These findings suggest that these inflammatory markers could be the molecular intermediaries between the abnormal placenta and the endothelial dysfunction-mediated rise in BP in PIH.

Table 9: Bivariate logistic regression analysis for prediction of prehypertension status (as dependent variable) by NO/ET-1 (as independent variable) in the subjects of study group II in all 3 trimesters.

First Trimester	Second Trimester	Third Trimester
OR (95% CI), *p*-value	**OR (95% CI), *p*-value**	**OR (95% CI), *p*-value**
1.95 (1.16-6.48), 0.062	2.84 (1.45-7.32), 0.025	4.45 (1.06-9.75), 0.005

CI: 95% confidence interval; OR, odds ratio after adjusting for age and body mass index; NO/ET-1, ratio of nitric oxide to endothelin-1.

In the present study, TBARS, the Reactive Oxygen Species (ROS) molecules, were significantly high in all the 3 trimesters of pregnancy in group II subjects (Table 4), indicating the presence of oxidative stress in women who developed PIH throughout their pregnancy. Many studies have indicated that preeclamptic women have elevated oxidative stress within the placental unit.[36,37] Placental oxidative stress and release of oxidants from the placenta in PIH have been proposed to mediate endothelial cell dysfunction and contribute to the pathophysiology of hypertension, as there is evidence of increased pro-oxidant activity formation along with decreased antioxidant protection in preeclampsia.[36] In the present study, TBARS was significantly correlated with BRS (Table 5) and had an independent contribution to BRS (Tables 6 and 7) and NO/ET-1 (Table 8), suggesting that oxidative stress could be a possible mechanism in the endothelial dysfunction-mediated rise in BP in PIH.

There is report of markers of endothelial dysfunction, especially ET-1causing enhancement in vascular sensitivity to angiotensin II in PIH, a potent vasoconstrictor that increases BP.[34] Also, it is known that both ET-1 and angiotensin II stimulate sympathetic activity.[38,39] PIH has been reported as a state of sympathetic overactivity.[8] However, the role of these markers of inflammation and endothelial dysfunction in the causation of autonomic dysregulation in PIH has not been adequately studied. Therefore, in the present study, we have assessed the entire battery of autonomic function tests, including HRV, BPV and CAFT. Among the HRV parameters, LFnu, the marker of cardiac sympathetic drive, was significantly increased, and the indices of cardiac vagal drive (HFnu, RMSSD, SDNN, NN50 and pNN50) were significantly reduced in group II subjects compared to group I subjects (Table 2), indicating increased sympathetic and decreased parasympathetic activities since the 1st trimester of pregnancy in women who developed PIH later in pregnancy. Further, TP of HRV, the marker of overall cardiac vagal modulation, was reduced and LF-HF ratio, the marker of sympathovagal imbalance, was increased in these subjects, indicating that these subjects are at increased CV risk, as reduction in TP is associated with adverse cardiac events.[40] Moreover, the LF-HF ratio was independently correlated with NO/ET-1, indicating that NO and ET-1 have greater influence

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on sympathovagal imbalance in PIH. Among the CAFTs, there was a significant reduction in parameters of parasympathetic reactivities (30:15 ratio and E:I ratio) and a significant elevation in the parameter of sympathetic reactivity (ΔDBP_{IHG}) (Table 3) in these PIH women. Thus, these findings suggest that in PIH, there is an increase in sympathetic activities and reactivities and there is decrease in vagal activities and reactivities. Further, the E:I ratio, the essential marker of vagal reactivity and ΔDBP_{IHG}, the important marker of sympathetic reactivity, were independently associated with NO/ET-1, which indicates that NO and ET-1 have a profound influence on alterations in autonomic functions in PIH. There are reports of ET-1 and NO affecting sympathetic activity.[38,41] Autonomic functions in the present study were also assessed by BPV analysis. The Total Peripheral Resistance (TPR) was significantly increased in all the 3 trimesters in study group II compared to study group I, indicating that sympathetic tone was significantly high throughout the pregnancy in women who developed PIH, as TPR is a physiological measure of sympathetic discharge in the peripheral circulation. BRS assessed by BPV analysis is an important parameter of sympathovagal function, and decreased BRS reflects sympathovagal imbalance.[30] Independent contribution of NO/ET-1 to BRS in group II subjects (Table 7) indicates a close association of alterations in NO and ET-1 with autonomic modulation in PIH. Thus, it appears that a decrease in NO and an increase in ET-1 contribute to increased sympathetic and decreased parasympathetic activities in PIH, which in turn leads to hypertension.

Findings of the present study indicate that sympathovagal imbalance, with increased sympathetic and decreased parasympathetic activities, is present from the 1st trimester of pregnancy in women having risk factors for developing PIH. The sympathovagal imbalance with sympathetic hyperactivity intensifies in the 3rd trimester of pregnancy causing persistent vasoconstriction and further increasing BP in women who develop PIH. The inflammatory and oxidative stress markers released from an abnormal placenta induce endothelial dysfunctions. Chemicals released from vascular endothelium, especially decreased production of NO and increased secretion of ET-1, trigger sympathovagal imbalance to decrease BRS, an important marker of BP dysregulation and CV risk. The ratio of NO to ET-1 (NO/ET-1) appears to be a more powerful indicator of sympathovagal imbalance and decreased BRS in women who develop PIH. NO/ET-1 is found to be significantly decreased, along with a decrease in BRS from the 1st trimester of pregnancy in these high-risk pregnant women. NO/ET-1 could predict the development of prehypertensive status in group II subjects. It was concluded that inflammation and oxidative stress contribute to vascular dysfunction, which in turn initiates Sympathovagal Imbalance (SVI) in the early part of pregnancy in women at risk of PIH. SVI triggered by a decrease in NO/ET-1 intensifies in the later part of pregnancy, leading to the development of PIH. Future studies should be conducted to assess if NO/ET-1 can be used as a marker of vascular endothelial dysfunction for prediction of PIH in women with risks for PIH. Also, future studies should address if practice of yoga by women having risk factors for PIH from the 1st trimester of pregnancy can prevent the development of PIH, as yoga is known to improve endothelial function and bring balance in sympathovagal functions.

There are many merits in the study. Women with risk factors for PIH were investigated in all the 3 trimesters of gestation, and they were followed up till term to note the incidence of hypertension. All the autonomic function tests to assess both sympathovagal activity at rest (HRV and BPV) and reactivity to stressors (CAFTs) were performed to determine the robustness of sympathetic and parasympathetic drives and tones of the subjects. Thus, assessment of autonomic dysfunction was comprehensive. The alteration of the ratio of NO to ET-1 (NO/ET-1) was studied in addition to the individual alterations of NO and ET-1. Regression analysis was done to assess the individual contribution of factors influencing BRS and prehypertension status.

Limitations of the study

Our study has a few limitations. The sample size of study group II (women who developed PIH) was modest. There were no second control group of pregnant women who do not have risk factors for PIH but still develop PIH, to compare the difference in alterations in cardiometabolic profile, and endothelial and autonomic dysfunctions with women having risks of developing PIH. Immunological markers of PIH such as angiotensin-II type-1 receptor autoantibody (AT1-AA) that influences autonomic functions have not been estimated. The biochemical markers of sympathovagal imbalance, such as urinary estimation of metanephrine, normetanephrine, and vanillylmandelic acid (VMA), have not been estimated. Oxidative stress markers in the placental tissue have not been estimated.

CONCLUSION

Sympathovagal imbalance with increased sympathetic and decreased parasympathetic activities occurs from the 1st trimester of pregnancy in women having risk factors of developing PIH, which intensifies in the 3rd trimester of pregnancy with sympathetic overactivity. It appears that the inflammatory and oxidative stress markers inhibit the production of NO and stimulate the secretion of ET-1from vascular endothelium. Alteration in the levels of NO and ET-1, especially the ratio of NO to ET-1 (NO/ET-1) seems to be more powerful in inducing sympathovagal imbalance and decreasing BRS in women who develop PIH. NO/ET-1 could predict the development of prehypertensive status in PIH women. Thus, inflammation and oxidative stress in PIH contribute to vascular dysfunction, which in turn initiates SVI in the early part of pregnancy in women at risk of PIH. SVI triggered by NO/ET-1 is accentuated in the 3rd

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trimester of pregnancy and could be the physiological basis of PIH.

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ABBREVIATIONS

BRS: Baroreflex Sensitivity; **PIH:** Pregnancy-Induced Hypertension; **BPV:** Blood Pressure Variability; **HRV:** Heart Rate Variability; **CAFT:** Conventional Autonomic Function Tests; **NO/ET-1:** Ratio of Nitric Oxide to Endothelin-1; **SVI:** Sympathovagal Imbalance; **GH:** Gestational Hypertension; **AT1-AA:** Angiotensin-II Type-1 Receptor Autoantibody; **TP:** Total Power; **LFnu:** Low-Frequency Power Expressed in Normalized Units; **HFnu:** High-Frequency Power Expressed in Normalized Units; **LF-HF Ratio:** Ratio of Low-Frequency to High-Frequency Power; **SDNN:** Standard Deviation of NN Intervals; **RMSSD:** Root Mean Square of Successive Differences; **NN50:** Number of Adjacent NN Intervals Differing by More Than 50 ms; **pNN50:** Percentage of NN50 Counts Relative to All NN Intervals; **MAP:** Mean Arterial Pressure; **RPP:** Rate-Pressure Product; **LVET:** Left Ventricular Ejection Time; **SV:** Stroke Volume; **CO:** Cardiac Output; **TPR:** Total Peripheral Resistance; **TBARS:** Thiobarbituric Acid Reactive Substances.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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