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Original Article

Physiology Section

Heart Rate Variability and Cold Pressor Test before Onset of Pre-eclampsia in Pregnant Women-A Longitudinal Study

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ABSTRACT

Introduction: A major obstetric complication, that leads to severe maternal and foetal morbidity is Preeclampsia (PE). Studies evaluating the autonomic nervous activity in pregnant women with PE shows conflicting results. Previous studies are inadequate for the identification of the most useful tools to detect and monitor autonomic dysfunction prior to PE. The cold pressor test is a simple and validated test, in which the afferent sensory pathways are stimulated by the cold stimulus, resulting in an increase in Blood Pressure (BP).

Aim: To evaluate the response to cold pressor test and Heart Rate Variability (HRV) to detect increased vascular reactivity and sympathetic activity prior to the clinical manifestation of PE in pregnant women.

Materials and Methods: A longitudinal study was conducted in in the Department of Physiology at Hamdard Institute of Medical Sciences, HAHC Hospital, Jamia Hamdard, New Delhi, India. The duration of the study was 11 months, from December 2018 to November 2019. Subjects were 50 pregnant women, between age 18-40 years and of 12-14 weeks of gestation. Cold pressor test and HRV were parameters measured for the assessment of the autonomic functions during 12 and 21 weeks of gestational period. The Statistical Package for Social Sciences (SPSS) version 26.0 was used for analysis of the quantitative data. Paired t-test was done for comparison of all values.

Results: The mean age of the study participants was 29±2.7 years. There was no hyper-reaction to cold pressor test at 12 weeks of gestation, nor the subjects showed any signs of PE. At 14 weeks of gestation, 2 (4%) showed hyper-reaction to cold pressor test. At 21 weeks of gestation, 3 (6%) subjects showed hyper-reactions to cold pressor test and out of them, two developed PE. Root Mean Square Standard Deviation (RMSSD) and difference between adjacent Standard Deviation of N-N intervals (SDNN) of HRV analysis were significantly higher in the first trimester as compared to 21 weeks of pregnancy.

Conclusion: Response to cold pressor test showed increased vascular reactivity, as a sign before the development of PE. HRV analysis could not detect any significant features of increased sympathetic activity prior to the clinical manifestation of PE in pregnant women.

Keywords: Autonomic nervous system, Gestational disorders, Maternal morbidity, Non invasive assessments, Pregnancy induced hypertension

INTRODUCTION

The PE is an obstetric complication, that increases the risk of unfavourable consequences for both the mother and the foetus [1]. The severity of complications can be curtailed by early detection of PE, which can also improve clinical outcomes. PE is a complex gestational disorder, with a worldwide prevalence of 5%-8% [1,2]. Among mothers, PE increases the risk of premature death, Type 2 Diabetes Mellitus (T2DM), cardiovascular diseases, and hypothyroidism [3]. Moreover, the offspring also has an increased risk of cardiovascular and metabolic disorders later in life [4]. Previous study suggests that, there is over activity of the sympathetic nervous system in women with PE which leads to increased vascular reactivity [5]. Studies evaluating the autonomic nervous activity in pregnant women with PE show conflicting results [5-7]. These studies compared the values in pregnancy with postpartum values. Very few studies were conducted before the onset of PE in pregnant women and were inadequate for the identification of the most useful tools to detect and monitor autonomic dysfunction in pre-eclamptic women [6,7]. Majority of the previous studies, used one Autonomic Nervous System (ANS) assessment test and very few studies used two assessment tests [8-10].

The PE is defined according to the clinical criteria established by the American College of Obstetricians and Gynecologist (ACOG) as the "occurrence of Hypertension (HTN) defined as Systolic Blood Pressure (SBP) ≥140 mmHg or Diastolic Blood Pressure (DBP) ≥90 mmHg after 20 weeks of gestation in woman, who is normotensive before, and proteinuria defined as presence of 300 mg or more of protein in 24 hour urine sample or >2+on dipstick" [11]. A new onset HTN (>140/90 mmHg) after 20 weeks of pregnancy in women, who were normally normotensive was recently revised and updated by the ACOG to include other complications in case of absence of proteinuria [2]. It was found that, there is a paucity of studies, to detect the increased vascular reactivity prior to the clinical manifestation of PE. Previous studies [6-10] have not compared autonomic functions utilising both, HRV and response to cold pressor test together in pregnant women prior to the clinical manifestations of the disease. The present study therefore, evaluated the measurements of HRV and response to cold pressor test, to detect the increased sympathetic activity and vascular reactivity prior to the clinical manifestation of PE in pregnant women.

The present study hypothesised that, non invasive assessment of autonomic cardiovascular control using analysis of HRV and response to cold pressor test can be a predictive tool to identify early the women who were at increased risk of developing PE. Hence, present study was conducted, to evaluate the measurements of response to cold pressor test and HRV to detect any increased vascular reactivity and sympathetic activity, prior to the clinical manifestation of PE in pregnant women.

MATERIALS AND METHODS

A longitudinal study was conducted in the Department of Physiology at Hamdard Institute of Medical Sciences, HAHC Hospital, Jamia Hamdard, New Delhi, India. The duration of the study was 11 months, from December 2018 to November 2019. The study was approved

by Institutional Ethics Committee on 26/11/18. Informed consent was obtained from volunteered subjects. The procedure followed were in accordance with the ethical standards of the responsible committee on human experimentation.

Inclusion criteria: Pregnant women aged between 18-40 years and of 12-14 weeks of gestation were included in the study.

Exclusion criteria: Pregnant women with diabetes, chronic renal disease, neurological disorders, smokers, multiple pregnancies and cardiorespiratory illnesses were excluded from the study.

Sample size calculation: A total of 50 pregnant women were enrolled for the study by convenient sampling. Total 50 subjects were considered for both, cold pressor test and HRV analysis.

Study Procedure

Parameteric assessment of the autonomic functions was done during 12, 14 and 21 weeks of gestational period for BP response to cold pressor test and HRV was assessed at 12 and 21 weeks of gestation.

Cold pressor test: After 10 minutes of rest, the cold pressor test for the assessment of sympathetic vascular reactivity was done in sitting position by the method as described by Hines EA and Brown GE [12]. The subjects were seated comfortably and baseline BP was recorded by auscultatory method using Omron 8712 automatic BP monitor [13]. Then the subject was asked to immerse one hand up to the wrist in ice cold water (4-5°C) for one minute. The BP from the other arm, was recorded after every 30 seconds intervals for one minute. The maximum increase in SBP and DBP were noted and compared with the pretest readings. The change in the ΔSBP and DBP was calculated by subtracting pretest reading from the reading obtained after hand immersion. The subjects having ΔSBP <20 mmHg and ΔDBP <10 mmHg were labelled as normal reactors and ΔSBP ≥20 mmHg and ΔDBP ≥10 mmHg were labelled as hyper-reactors. The results were expressed as the percentages.

Heart Rate Variability (HRV): Laboratory chart data analysis software, Analog Digital (AD) instrument was used for the assessment of the autonomic functions in the subjects. The time domain and frequency domain analysis of HRV was done in uniform settings [14]. Time domain measures, included SDNN and square root of mean of the sum of the squares of differences between adjacent NN interval (RMSSD), both expressed in milliseconds (ms). Frequency domain HRV indices included Very Low Frequency (VLF) power, Low Frequency (LF) power (0.04-0.15 Hz), High Frequency (HF) power (0.15-0.4 Hz), and Total Power (TP). LF and HF were also measured in normalised units (nu). The ratio of LF to HF power, was also calculated as a measure of sympathovagal balance. The findings of HRV were interpreted as LF/HF ratio >1 as unhealthy. SDNN values below 50 ms are classified as unhealthy, 50-100 ms have compromised health, and above 100 ms are healthy [14]. The reference range for RMSSD was 13-107 ms, which reflects the integrity of vagus nerve-mediated autonomic control of the heart. Proportion of NN50 (pNN50) lower than 3% is considered indicative of high risk.

STATISTICAL ANALYSIS

The quantitative statistical data was analysed using SPSS version 26.0. Paired sample t-test was done for comparison of all values with significant p-value ≤0.05.

RESULTS

Study was conducted on 50 pregnant female subjects with average age of 29±2.7 years.

Frequency domain and time domain of Heart Rate Variability (HRV): The observed mean values of LF normal units (nu), HF (nu) and LF/HF ratio during 21 weeks of gestation, were significantly higher as compared to 12 weeks of gestation. There was significant decrease in SDNN values, RMSSD and pNN50 values during 21 weeks of gestation as compared to 12 weeks [Table/Fig-1].

Parameters	12 weeks gestation Mean±SD	21 weeks gestation Mean±SD	p-value
LF (nu)	21.46±5.49	23.08±5.92	<0.0001*
HF (nu)	63.75±4.88	67.51±6.9	<0.0001*
LF/HF ratio	0.31±0.09	0.35±0.14	<0.0001*
SDNN (ms)	677.45±153.3	520.25±295.54	0.0257**
RMSSD (ms)	676.8±153.27	520.62±296	0.0354**
pNN50 (%)	0.91±0.82	0.89±0.08	0.0098***

[Table/Fig-1]: HRV parameters between 12 and 21 weeks of gestation and p-values. Paired sample t-test was done for comparison of all values with significant p-value ≤0.05. *significant, **very significant and ***extremely significant; nu: Normal unit; ms: Milliseconds

Cold pressor test: There was no hyper-reaction to cold pressor test at 12 weeks of gestation, nor the subjects showed signs of PE or Pregnancy Induced Hypertension (PIH). At 14 weeks of gestation, two subjects showed hyper-reaction to cold pressor test. At 21 weeks of gestation, three subjects showed hyper-reactions to cold pressor test and out of these, two developed PE or PIH [Table/Fig-2].

Response to cold pressor test	Parameters	Gestational age		
		12 weeks (mean±SD)	14 weeks (mean±SD)	21 weeks (mean±SD)
Resting pulse and BP	Pulse/minute	81.5±18.48	101.8±12.99	96.08±11.74
	SBP (mmHg)	96±8.48	108.8±12.53	104.76±8.63
	DBP (mmHg)	61.5±6.85	70.09±7.73	65.82±7.42
Maximum increase during one minute of immersion of hand in cold water	Pulse/minute	82±18.77	103.66±12.94	97.96±10.88
	SBP (mmHg)	114.75±6.39	126.41±9.33	121.06±8.63
	DBP (mmHg)	69.75±3.30	77.08±7.26	72.6±8.14
Change in pulse and BP	Pulse/minute	5.5±2.12	4.75±3.09	8.75±4.12
	SBP (mmHg)	13.5±3.53	18.16±9.84	17.15±5.84
	DBP (mmHg)	7.8±4.64	8.57±4.64	8.67±4.86
Normal reactors (%)		50 (100%)	48 (96%)	47 (94%)
Hyper-reactors (%)		0	2 (4%)	3 (6%)
PIH/PE (%)		0	0	2 (66.6% of hyperactive subjects)

[Table/Fig-2]: Response to cold pressor test during 12, 14 and 21 weeks of gestation.

There was significant increase in SBP during the cold pressor test. When compared with healthy pregnant women, the change in BP was more evident in women developing PE. (SBP: 20.8±3.4 vs 14.15±5.84 mmHg) [Table/Fig-3].

Cold pressor test parameters	Women developing PE (n=2)	Healthy pregnant women (n=48)	p-value
Increase in SBP	20.8±3.4	14.15±5.84	0.0621*
Increase in DBP	11.8±3.8	8.67±4.86	0.231
Increase in heart rate	7±2.2	9.3±5.8	0.0326*

[Table/Fig-3]: Change in Blood Pressure (BP) during cold pressor test. *significant; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; N=50

DISCUSSION

The present study compared the response to cold pressor test between 12,14 and 21 weeks of gestation and found that, there was no hyper-reaction to cold pressor test at 12 weeks of gestation, nor the subjects showed signs of PE or PIH. At 14 and 21 weeks of gestation, 4% and 6% of the total subjects showed hyper-reaction to cold pressor test respectively. A total of 2 (4%) out of 50 pregnant women developed PE. Previous study has found that, the central sympathetic output was significantly increased in women with PIH, as compared to normal pregnant women [15]. Studies have also reported decreased vagal tone, along with increased sympathetic activity to be associated with PIH/PE [16-18]. Previous attempts for early prediction of PIH/PE in clinical practice by using a variety of biological, biochemical and biophysical markers have been inadequate [19,20]. However, in the present study, there was significant increase in BP during the cold pressor test in women

developing PE between 14 to 21 weeks of gestation. Study by Woisetschlager C et al., found significant increase in SBP and DBP during the cold pressor test [19]. The change was significant in women, who developed PE between 16 to 20 weeks of gestation. It was interpreted as increased vascular activity detected prior to clinical manifestation of PE.

The present study has found that, the pregnant women, who developed PE or PIH showed, increased BP reactivity to cold pressor test as early as 14 weeks of gestation. Therefore, in the present study, increased vascular activity prior to clinical manifestation of PE was detected during the early 14 weeks of gestation. Cold pressor test was done to assess the percentage of hyper-reactors, who develop PE in later pregnancy. The present study found 4% of the total pregnant women subjects developed PE. The finding is in concordance with the finding of previous study that, PE affected 2%-8% of the total pregnancy [19,20].

The present study, compared the time and frequency domain analysis of HRV between 12 weeks and 21 weeks of gestation. The ACOG states that, heart rate fluctuation is very much varied throughout the gestation [21]. Therefore, HRV was analysed during end of first trimester and during 21 weeks of second trimester, when PE changes are expected to begin. It has been recommended that, short term recordings of five minutes performed in the present study under uniform and stable conditions would be processed by frequency domain methods [14]. Frequency domain analysis of HRV showed significant increase of LF waves during 21 weeks of pregnancy compared to first trimester. The LF/HF ratio during 21 weeks of gestation was found to be less than one, which is considered as good cardiovascular health [14]. LF modulation (0.04-0.15Hz) of R-R Interval changes corresponds to the sympathetic and parasympathetic activities together. HF modulation (0.15-0.4 Hz) of R-R interval changes is primarily regulated through innervation of the heart through the parasympathetic (vagal) nerve [14]. The time domain parameters i.e., RMSSD and SDNN were significantly higher in the first trimester, as compared to 21 weeks of pregnancy. The time-domain Standard Deviation (SD) of all N-N intervals (SDNN) reflect the total variability and along with the RMSSD between adjacent N-N intervals reflects parasympathetic activity [14].

In the present study, there was a significant decrease in SDNN, RMSSD values during 21 weeks of gestation, as compared to 12 weeks suggesting reduced parasympathetic activity. It is known that, SDNN is a measure of combined sympathetic and parasympathetic activity, and RMSSD represents parasympathetic activity, which reflects the integrity of vagus nerve mediated autonomic control of the heart [14]. Previous study has observed that, increased sympathetic activity and may already be present before the clinical presentation of PE, eventhough, the signs and symptoms of PE appear late in pregnancy [21]. The results of the present study are similar to the prospective cohort study, which evaluated the predictive value of spectral analysis of heart rate and BP for hypertensive diseases of pregnancy at 28 weeks of pregnancy. The study by Flood P et al., concluded that, although, useful for PIH, it was not able to detect women who developed PE afterward in pregnancy [22].

The HRV analysis in the present study, could not detect features of increased sympathetic activity during 12 weeks of gestation. Women, who developed increased sympathetic activity leading to PE after 12 weeks of pregnancy could not be detected by analysis of HRV. This could be attributed to the fact that, BP regulation by ANS is disturbed at several levels between the hypothalamus and the periphery. Therefore, the results are limited [23]. Previous reports of HRV in PE patients are conflicting. Some studies have found elevated sympathetic activity as a manifestation of ANS dysfunction and reduced parasympathetic activity [21,23] while other have showed, no significant difference between the PE group and the control group [24]. Comparison of previous studies with the present study on HRV during pregnancy has been shown in [Table/Fig-4] [25-28].

Previous studies	Place and year of the study	Sample size	HRV parameters in pregnant women
Kimmel MC et al., 2021 [25]	Sweden, 2014-2018	120	Significant ↓HF power, ↓LF power, ↓RMSSD, and ↓SDNN ↑HR in third trimester
Solanki JD et al., 2020 [26]	Gujarat, India, 2016	89	Significant ↓SDNN, ↓RMSSD ↑LF/HF in third trimester
Moors S et al., 2020 [27]	Netherlands, 2018	33	Significant ↓HRV, ↑LF/HF-ratio, ↑LF(n.u.), ↓HF(n.u.) in women with PE in third trimester
Gandhi PH et al., 2014 [28]	Gujarat, India, 2013	60	Significant ↓ Very Low Frequency (VLF) and HF normalised unit (nu) LF (nu) and LF/HF significantly ↑ Significant ↓SDNN, RMSSD, NN50 count, pNN50 in third trimester
Present study	New Delhi, India, 2019.	50	Significant ↓in SDNN, RMSSD, ↑LF/HF values during 21 weeks of gestation as compared to 12 weeks

[Table/Fig-4]: Studies on HRV during pregnancy [25-28].

Limitation(s)

Long term follow-up of subjects could not be done. Delivery outcomes and foetal status of the detected PE subjects could not be assessed. Future studies can include appropriate comparison groups with longer follow-ups.

CONCLUSION(S)

Response to cold pressor test showed increased vascular reactivity, as a sign before the development of PE. HRV analysis could not detect features of significantly increased sympathetic activity prior to the clinical manifestation of PE in pregnant women. HRV analysis could not detect women, who developed increased sympathetic activity and PE after 12 weeks of pregnancy. Therefore, cold pressor test in early pregnancy may be a useful indicator for development of PIH or PE later in pregnancy.

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