

RESEARCH ARTICLE

Does the frequency domain parameters among pregnant women with gestational hypertension associated with levels of serum zinc, magnesium, and copper? A cross-sectional study

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Received: April 22, 2020; Accepted: June 01, 2020

ABSTRACT

Back ground: In addition to the imbalance in the cardiac autonomic function, deficiency of micronutrients such as zinc (Zn), magnesium, and copper (Cu) was also found to be one of the cause of hypertensive disorders of pregnancy. Although there are number of studies done to know the autonomic balance and serum trace elements among preeclamptic women, none of the studies were done among the gestational hypertensives, hence, we have chosen this study on association between the level of micronutrients and frequency domain parameters of heart rate variability (HRV) among newly diagnosed gestational hypertensives. **Aim and Objective:** This study aims to estimate the serum levels of Zn, magnesium, and Cu and its association with frequency domain parameters of HRV among non-pregnant, normotensive pregnant, and gestational hypertensives. **Materials and Methods:** The study was done in Sri Venkateswara Medical College and Hospital. Blood pressure and heart rate were recorded after 15 min rest. HRV was analyzed using Kubios HRV analyzer by recording 5 min resting electrocardiogram in supine position with PHYSIOPAC-PP4, MEDICAID system, Chandigarh, among 35 normal pregnant, 35 gestational hypertensive, and 35 non-pregnant women of 20–35 years of age group. Serum Zn, magnesium, and Cu analysis were done in the same day using standardized autoanalyzer method. **Results:** With GraphPad Prism software, statistical analysis was done. Kolmogorov–Smirnov test was done to test the normality. Data following Gaussian distribution are tabulated as mean \pm SD or it is expressed as median (interquartile range). Either one-way ANOVA, Turkey's multiple comparison, or Kruskal–Wallis and Dunn's multiple comparison test were done to compare the parameters among all the three groups. Spearman correlation was done to know the association of Zn, magnesium, and Cu with frequency domain parameters of HRV in all three groups. **Conclusion:** Frequency domain parameters of HRV show a significant higher sympathetic and lower parasympathetic activity among the study group ($P < 0.0001$). There is no significant variation in the level of micronutrients among the groups and all were within normal limit. There is no significant association between the frequency domain parameters and the level of micronutrients.

KEY WORDS: Autonomic Function; Micronutrients; Pregnant; Gestational Hypertension

Access this article online

Website: www.njppp.com

DOI: 10.5455/njppp.2020.10.04099020201062020

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INTRODUCTION

Pregnant women recording blood pressure (BP) $\geq 140/90$ mmHg for the 1st time during pregnancy after 20 weeks of gestation, without proteinuria and normalize by 12 weeks after birth of the baby which is called as gestational hypertension.^[1] In India, incidence of gestational hypertension

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was 6.9%.^[2] Worldwide, one of the most common causes of maternal death is hypertension in pregnancy.^[3] About 24.4% of the maternal death is directly related to eclampsia and preeclampsia.^[4] Invasion of trophoblastic cells abnormally into the spiral arteries during the first half of pregnancy and absence of effective remodeling in spiral arteries results in increased resistance and decreased PO₂ in placental circulation.^[5-7] As a result, there is increased oxidative stress due to the production of reactive oxygen species. Copper (Cu) and Zn-superoxide dismutase protect the trophoblastic cells from oxidative stress. Hence, reduced Cu and zinc (Zn) were associated with increased risk of hypertension.^[8,9] Lower levels of Zn were found to be associated with various pregnancy complications such as prolonged labor, pre-term and post-term births, intrauterine growth retardation, and low birth weight including gestational hypertension.^[10] Mg²⁺ deficiency was known to be involved in pathogenesis of cardiovascular disease by promoting the generation of oxygen free radicals and inflammation.^[11] Gestational hypertensives were known to have imbalance in autonomic nervous system.^[12] Heart rate variability (HRV), a non-invasive test, can be used to assess the autonomic reactivity among the pregnant women.^[13] Although the previous studies were done to know the autonomic balance and level of micronutrients among the women with preeclampsia, none were done to know the association between frequency domain parameters and micronutrients such as Zn, magnesium, and Cu among pregnant women with gestational hypertension; hence, this study was chosen.

MATERIALS AND METHODS

This cross-sectional study was carried out in Sri Venkateswara Medical College Hospital and Research Center, Ariyur, Pondicherry. The study group consists of 35 primigravida women of age 20–35 years with >20 weeks of gestation with BP \geq 140/90 measured twice, 6 h apart without proteinuria, and other symptoms of preeclampsia. Control Group 1 consisting of 35 age-matched normotensive primigravida women of >20 weeks of gestation and Group 2 consists of non-pregnant women of same age group with a history of regular menstrual cycle.

Exclusion Criteria

Women with a history of chronic hypertension, gestational diabetes mellitus, renal disease, thyroid disorders, known cardiac patients, chronic liver disease, multiple pregnancy, history of previous abortion or still birth, or those who on chronic medication were excluded from the study. The study was conducted for 6 months from November 2018 to April 2019, the subjects were selected for the study based on the inclusion and exclusion criteria by subjecting them to history taking, general physical examination, vitals, and systemic examination. The study group consists of 14 women

in the second trimester and 21 women in the third trimester of pregnancy. Among the normal pregnant women (control Group 1), 18 were in the second trimester and 17 were in the third trimester of pregnancy. After getting Institutional Ethical Committee clearance, informed written consent was obtained from all the participants. In non-pregnant control group, HRV was recorded during their follicular phase (6th–7th days of menstruation) of menstrual cycle.^[14]

Methodology

After 15 min of rest, basal BP and heart rate were measured. Instructions were given to all the subjects before the test and their doubts were clarified. Subjects were asked to breathe normally, not to talk, move, or sleep during the recording.^[14] Five minutes computerized electrocardiogram was recorded using PHYSIOPAC-PP4, MEDICAID system, Chandigarh, in the Department of Physiology using limb leads in supine position in a quiet room with temperature of 22°C–24°C. Mercury sphygmomanometer was used for recording BP. Kubios HRV analyzer was used to analyze HRV. Frequency domain parameters such as low frequency (LF) nu, high frequency (HF) nu, LF/HF ratio, and total power were obtained. Normalization represents the balance in sympathetic and parasympathetic divisions.^[15] Total power representing the cardiovagal modulation. Recordings were done in the morning between 9.00 am and 11.00 am in the autonomic function test laboratory. The participants were advised not to take coffee or tea 12 h before the test and to avoid breakfast 2 h before the test.^[14] Blood was collected and analyzed for Zn, magnesium, and Cu on the same day.

Sample Collection

A 3 ml of venous blood was collected in Vacutainer. After clotting, the blood samples were centrifuged at 3000 rpm for 5 min. The sera were collected into sterile containers for assay. All the micronutrients are estimated using autoanalyzer.^[15]

Magnesium – Magnesium reacts with xylydyl blue to form a colored compound in alkaline solution. The color intensity is proportional to the concentration of magnesium in the sample.

Zn – In alkaline medium, Zn reacts with nitro-PAPS to form a purple-colored complex. Color intensity is proportional to the concentration of Zn in the collected sample.

Cu – In an acidic media, Cu released from ceruloplasmin, reacts with Di-Br-PAESA to form a colored complex. Intensity of color is directly proportional to the amount of Cu present.

Statistical Analysis

Data are tabulated as mean \pm SD for the values follow Gaussian distribution; otherwise, it is expressed as median

(interquartile range). The tests were done using GraphPad Prism software. Kolmogorov–Smirnov test was done to test the normality. Depending on the frequency distribution, either one-way ANOVA, Turkey's multiple comparison was done or Kruskal–Wallis and Dunn's multiple comparison test was done to compare the parameters among all the three groups. Spearman correlation was done to know the association of Zn, magnesium, and Cu with frequency domain parameters of HRV in all three groups.

RESULTS

In Table 1, Kruskal–Wallis test was done to compare among the three groups. Dunn's multiple comparison test was done to compare between the two groups. $*P < 0.05$ is considered as statistically significant. Age between the groups was not significant; hence, three groups are comparable. Systolic BP and heart rate are significantly different between the groups. Diastolic BP is significantly different in three groups. Between the groups comparison shows significant difference in gestational hypertensive versus normotensive pregnant group, gestational hypertensive versus non-pregnant group but not between pregnant versus non-pregnant group.

In Table 2, three groups comparison was done using Kruskal–Wallis test. Between the groups comparison was done using Dunn's multiple comparison test. $*P < 0.05$ is considered as statistically significant. LF nu, HF nu, and LF/HF values

show a significant difference on comparison among all three groups. Between the groups comparison for HRV parameters showed a significant result between gestational hypertensive versus normotensive pregnant and gestational hypertensive versus non-pregnant but not between normotensive pregnant and non-pregnant groups. Total power did not show a significant difference among all the three groups.

In Table 3, one-way ANOVA was done to compare among all the three groups and Turkey's multiple comparison was done to compare between the groups. Magnesium and Cu values are mentioned as median (interquartile range) as it does not follow Gaussian distribution. Kruskal–Wallis test was done to compare among all the three groups and Dunn's multiple comparison was done to compare between the groups. $*P < 0.05$ is considered as statistically significant. None of the parameters showed statistical significance.

Table 4 did not show any significant association between frequency domain parameters of HRV and Zn, magnesium, and Cu among gestational hypertensive, normotensive, and non-pregnant women.

DISCUSSION

Our results in Table 1 show that the BP and heart rate are significantly greater ($P < 0.0001$) in the study group. Table 2 shows that LF nu and LF/HF ratio were significantly greater

Table 1: Comparison of age, BP, and heart rate parameters among gestational hypertensive, normotensive pregnant, and non-pregnant women

Parameters	Gestational hypertensives	Normotensive pregnant	Non-pregnant	<i>P</i> -value
Age (years)	28 (26–30)	26 (24–28)	28 (24–31)	0.0512
SBP (mm of Hg)	146 (144–152)	112 (110–122)	106 (100–110)	<0.0001*
DBP (mm of Hg)	100 (98–102)	80 (76–82)	78 (72–80)	<0.0001*
HR (beats/min)	85 (81–90)	80 (76–86)	75 (70–81)	<0.0001*

All the values are expressed as median (interquartile range) as the sample is not normally distributed. BP: Blood pressure. SBP: Systolic blood pressure, DBP: Diastolic blood pressure

Table 2: Comparison of HRV parameters among gestational hypertensive, normotensive pregnant, and non-pregnant women

Parameters	Gestational hypertensives	Normotensive pregnant	Non-pregnant	<i>P</i> -value
LF nu	62 (58–68)	52.60 (46.20–55.20)	49.10 (44.10–53.10)	<0.0001*
HF nu	38 (32–42)	47.40 (44.80–53.70)	50.90 (46.90–55.90)	<0.0001*
LF/HF	1.632 (1.381–2.125)	1.110 (0.8600–1.232)	0.9650 (0.7890–1.132)	<0.0001*
Total power	841 (654–920)	852 (623–1370)	952 (557–1282)	0.5160

All the values are expressed as median (interquartile range). HRV: Heart rate variability

Table 3: Comparison of Zn, magnesium, and Cu among gestational hypertensive, pregnant, and non-pregnant women

Micronutrients	Gestational hypertensive	Normotensive pregnant	Non-pregnant women	<i>P</i> -value
Zn (µg/dl)	59.57±9.951	62.49±6.771	60.94±9.113	0.3791
Magnesium (mg/dl)	1.80 (1.7–2.0)	1.9 (1.5–2.1)	1.8 (1.6–2.0)	0.9601
Cu (µg/dl)	110 (86–144)	122 (105–134)	121 (100–148)	0.5314

Zinc values are mentioned as Mean±SD as it follows Gaussian distribution. Other values are expressed as median (interquartile range), Cu: Copper, Zn: Zinc

Table 4: Spearman correlation between Zn, magnesium, and Cu rate and frequency domain parameters among gestational hypertensive, normotensive, and non-pregnant women

Parameters	Zn (µg/dl)						Magnesium (mg/dl)						Cu (µg/dl)					
	Gestational hypertensive		Normotensive pregnant		Non-pregnant women		Gestational hypertensive		Normotensive pregnant		Non-pregnant women		Gestational hypertensive		Normotensive pregnant		Non-pregnant women	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
LF nu	-0.04	0.83	0.14	0.42	-0.07	0.70	-0.03	0.84	-0.00	0.96	-0.05	0.76	0.30	0.08	-0.09	0.57	0.28	0.10
HF nu	0.04	0.82	-0.14	0.42	0.07	0.68	0.04	0.84	0.00	0.96	0.06	0.74	-0.30	0.08	0.09	0.58	-0.28	0.10
LF/HF	-0.03	0.85	0.14	0.41	-0.07	0.69	-0.03	0.87	-0.00	0.97	-0.05	0.78	0.31	0.07	-0.09	0.58	0.28	0.09
Total power	0.00	0.98	-0.04	0.80	-0.12	0.48	-0.13	0.47	-0.18	0.29	0.12	0.48	0.05	0.76	0.18	0.28	-0.04	0.79

Cu: Copper; Zn: Zinc

($P < 0.0001$) in the study group. Table 3 did not show any significant difference in Zn ($P = 0.3791$), magnesium ($P = 0.9601$), and Cu ($P = 0.5314$) among the study and control groups. Normotensives were known to have dominant sympathetic activity as the pregnancy advances and decrease in parasympathetic influence over heart rate. Hence, in hypertensives, the sympathetic activity is further exaggerated.^[16]

This is similar to our results with significantly higher sympathetic activity among the women with gestational hypertension.^[17] Our results are further supported by various authors.^[12,18,19] Reduced micronutrients were found to increase the oxidative stress-induced endothelial dysfunction and peripheral vascular resistance.^[20] In contrast, Fan *et al.* have reported that high serum Cu level was found to be associated with preeclampsia.^[21] Low serum magnesium level was found to be associated with the development of hypertension.^[22] All the three groups were found to have normal levels of serum Zn, magnesium, and Cu and there is no significant difference between them in our study. Similarly, Tabandeh *et al.* did not find any significant difference in serum Zn and magnesium between preeclamptic and normotensive pregnancies.^[23]

Limitations

Atomic absorption spectrometry was not used to estimate the serum Zn, magnesium, and Cu in our study due to lack of financial support.

CONCLUSION

Thus, we conclude that though the sympathetic activity is significantly higher in gestational hypertensives, there is no significant association with serum levels of Zn, magnesium, and Cu. Thus, from our study, we conclude that pathogenesis of pregnancy-induced hypertension is multifactorial and there might be various other mechanisms associated with the development of gestational hypertension apart from the level of micronutrients.

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How to cite this article: Mohan P, Udayakumar KP, Hiramath PB. Does the frequency domain parameters among pregnant women with gestational hypertension associated with levels of serum zinc, magnesium, and copper? A cross-sectional study. *Natl J Physiol Pharm Pharmacol* 2020;10(09):744-748.

Source of Support: Nil, **Conflicts of Interest:** None declared.