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Reduced Cardiac Sympathetic Activity and Subclinical Vitamin B12 Status among Indian Mothers During Early Pregnancy

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Abstract

Objectives: Prevalence of subclinical vitamin B12 deficiency is high during early pregnancy especially among Indian women. Early pregnancy is also associated with modulations in autonomic nervous activity to adapt to haemodynamic changes. Current study aimed to compare the autonomic nervous activity through heart rate variability (HRV) indices during early pregnancy in women with low and high vitamin B12 status.

Methods: Forty-two healthy pregnant Indian women in their early pregnancy (<14 weeks) were evaluated. Blood samples were analysed for plasma vitamin B12, haemogram and ECG. ECG was subjected to heart rate variability analysis. Subjects were divided into four groups based on quartile values of vitamin B12 levels.

Results: There was a significant difference in Log LF (low frequency) HRV between the study groups (p=0.03). Log LF HRV was lower in lowest vitamin B12 group (Quartile 1 of Vitamin B12 levels <151.7 pmol/l) compared to group with highest vitamin B12 levels (Quartile 4 of Vitamin B12 levels >248.7 pmol/L) (p=0.05). There was a significant positive correlation between plasma vitamin B12 levels and Log LF (r=0.36, p=0.02).

Conclusion: This study showed reduced sympathetic activity among subjects with low vitamin B12 status compared to subjects with high vitamin B12 status. Thus, emphasising the use of vitamin B12 levels during routine clinical evaluation and utility of simple measures like HRV as a screening tool during pregnancy along with postural blood pressure evaluation to rule out orthostatic intolerance. Further, longitudinal studies are required to evaluate vitamin B12 status and its long-term impact on autonomic nervous system during pregnancy.

Keywords: Vitamin B12; Early pregnancy; Heart rate variability; Autonomic nervous system

Significance

What is known: Autonomic modulations especially reflex sympathetic activation plays a critical role in adapting to haemodynamic changes during early pregnancy. Vitamin B12 is associated with decreased sympathetic activity and orthostatic intolerance especially among the elderly.

What it will add: Recurrent syncopal and presyncopal attacks is a significant problem during pregnancy. However, the mechanisms remain unclear. In this study there was a reduction in cardiac sympathetic activity in women with low vitamin B12 status during early pregnancy suggesting a blunted compensatory response. Since prevalence of subclinical vitamin B12 deficiency is high during pregnancy, this could potentially increase their susceptibility to presyncopal attacks during early pregnancy.

Introduction

Prevalence of vitamin B12 deficiency is high (ranging from 50-75%) during pregnancy among Indian women [1-3]. However, there is no defined cut-off to identify vitamin B12 deficiency during pregnancy. Majority of the studies during pregnancy have linked low vitamin B12 status with adverse birth outcomes for instance intrauterine growth retardation (IUGR) [4]. Also, much of the focus has been on clinical vitamin B12 deficiency (pernicious anemia) during pregnancy demonstrating neurological deficits and delayed developmental milestones during infancy and early childhood [5,6]. Among the triad of complications linked to clinical vitamin B12 deficiency in non-pregnant patients (anaemia, neuropathy and cognitive defects) neuropathy has been demonstrated to precede other deficits [7]. In particular autonomic neuropathy is said to be affected much earlier than other defects [8].

One of the most commonly used technique to evaluate autonomic nervous system is heart rate variability (HRV). Heart rate variability is a simple non-invasive measure that detects subtle changes in

autonomic nervous system [9]. Decreased HRV indices have been associated with increased cardiovascular morbidity and mortality [10]. Our earlier studies have demonstrated decreased heart rate variability in children born to women with low vitamin B12 levels [11] thus increasing the possibility of future cardiovascular risk during adulthood [12]. There is lack of data exploring the autonomic neural activity among subclinical vitamin B12 deficient pregnant Indian mothers. Subclinical vitamin B12 deficiency during pregnancy is important to evaluate in an Indian context as majority of Indians adhere to vegetarianism and even when they follow non-vegetarian diet, the reported quantity of vitamin B12 rich food consumption is less [13,14]. Autonomic nervous system plays a critical role in maternal adaptation to hemodynamic changes which occurs during early pregnancy particularly during first half of the pregnancy [15]. Consequence of altered autonomic nervous activity during pregnancy could potentially bring functional changes not only in the developing fetus but could also impact infants following birth. There is also a greater possibility of increased risk including obstetrical complications like orthostatic hypotension, gestational diabetes and gestational hypertension [16-18]. Therefore, the current study aimed to study the autonomic nervous activity (HRV indices) in mothers with low vitamin B12 status and compare them with high vitamin B12 status during early pregnancy.

Materials and Methods

Study population

Out of 50 pregnant women <14 weeks registered for the antenatal screening at an OBG outpatient department in a tertiary government hospital, 42 mothers were recruited for the study after obtaining written informed consent. Mothers were screened for following inclusion and exclusion criteria's: women with multiple pregnancies, those with clinical diagnosis of chronic illness such as diabetes mellitus, hypertension, heart disease, thyroid disease, those who tested positive for hepatitis B surface antigen (HbSAg), HIV or syphilis venereal disease research laboratory test infections were excluded from the study. None of the subjects reported any symptoms suggestive of peripheral or autonomic neuropathy like giddiness on standing, urinary urgency, tingling sensation of limbs or limb weakness or gastrointestinal symptoms like burning sensation in epigastric region, diarrhoea and constipation. All mothers were clinically evaluated to rule out neuropathy. Blood pressure measurements were performed to rule out orthostatic intolerance. All the study parameters were collected in accordance with the Helsinki Declaration and The Institutional Ethics Committee (IEC) of St John's Medical College approved the research protocol (IEC study Ref No 168/2013).

Sociodemographic and anthropometric data

Sociodemographic status was evaluated using education levels, family income, occupation and dietary information were obtained through interview. Gestational age in days was calculated from the reported first day of the last menstrual period (LMP) and was confirmed through ultrasound. All subjects underwent anthropometric assessment including weight recorded to the nearest 0.1 kg, using a digital scale (Alfoset, Model HW- 100KA1, Mumbai, India) and height recorded to the nearest 0.1 centimeter, using a vertically mobile scale (Holtain, Crymych, UK). Body mass index was calculated as weight/height2. Skinfolds were taken (Holtain callipers, Crymych, UK) as the

mean of three measurements at four sites (biceps, triceps, suprailliac and subscapular). Percent fat and fat free mass was derived using Durnin and Womersley equation.

Biochemical data

Approximately 4 ml of blood (non-fasting state) was drawn from subjects by venepuncture. Hemoglobin (Hb) and complete blood count were analysed on whole blood samples in an automated Coulter counter (ABX Pentra C+, Horiba medicals, CA, US). The serum was separated for vitamin B12 analysis. Vitamin B12 was measured by the electrochemiluminescence method (Elecsys 2010, Roche Diagnostics Mannheim, USA). The inter-assay and intra-assay CVs for vitamin B12 was 7.81% and 12.5% respectively.

Heart rate variability assessment

Preparation of the subject: After explaining the process of ECG recording, subjects were asked to rest and relax in supine posture for 30 minutes. ECG recording was performed in a quiet room with soothing lighting and with comfortable temperature being maintained. Any electronic gadgets or metal or magnetic objects which could interfere with the ECG recording were removed. Skin was kept dry and rubbed with alcohol pad before the application of electrodes.

Electrode placement: Pre-gelled disposable silver electrodes were used (Ambu blue sensor, Copenhagen, Denmark). These electrodes were placed firmly on to the skin and made sure that a good contact between the skin and the electrode was maintained. Five electrodes were placed on the abdomen - four electrodes at four quadrants of the abdomen and one at the right upper abdomen which acts as reference electrode.

ECG module and collection of ECG signals: Subjects were instrumented for recording of maternal ECG (Monica DK, UK). The data acquisition system included a threshold peak detection system, from which RR intervals were determined. The RR intervals were plotted as a tachogram (RR values Vs interval number). The data were further processed for analysis of heart rate variability (AD Instruments, Australia). Heart rate variability analysis was performed using the frequency domain method. Power spectral density (PSD) was calculated using mathematical algorithm Fast Fourier transform (FFT). The PSD was analyzed by calculating powers and peak frequencies for different frequency bands. No more than 3 data points were edited in a given segment [19-21]. Power was calculated in three bands. The 0.04-0.15 Hz band of RR power (referred to as the low-frequency band) reflects, at least in part, sympathetic nerve activity to the heart and partly parasympathetic activity, while the 0.15-0.4 Hz band (high-frequency band) reflects parasympathetic nerve activity to the heart. Absolute total power (TP) represents the total variance of the power spectral density i.e., -0-0.4 Hz band ($msec^2$). In addition to the absolute power, data for heart rate variability are also presented as normalized units, as recommended [22], where the power in the low and high frequency bands is expressed as a percentage of the total power minus the power of the very-low-frequency band (0.0-0.04 Hz).

Statistical Analysis

The normality of the data was examined using Kolmogorov–Smirnov test. The data are expressed as mean (standard deviation). As

the HRV indices were not normally distributed, log transformed data were used for further analysis and the antilog values are represented. As there are no defined cutoffs for vitamin B12 deficiency among pregnant women, subjects were divided into four groups based on the quartile values of vitamin B12 levels. Subjects were categorized as Group 1 (Quartile 1 of Vitamin B12 levels <151.7 pmol/l), Group 2 (Quartile 2 of Vitamin B12 levels between 151.7 pmol/L and 196.8 pmol/L), Group 3 (Quartile 3 of Vitamin B12 levels between 196.8 pmol/L and 248.7 pmol/L) and Group 4 (Quartile 4 of Vitamin B12 levels >248.7 pmol/L). The HRV indices were compared between the four vitamin B12 groups using one-way analysis of variance and post hoc pair wise comparisons were performed using Tukey adjustment. Correlation of vitamin B12 status and HRV indices was also examined using Pearson's correlation coefficient. Results were considered significant if $P < 0.05$. All statistical analyses were performed using SPSS (v20, SPSS, Chicago, IL, USA).

Results

Maternal characteristics including anthropometry and biochemical measures: Average age of the mothers was 25 ± 3 years and 60% of the mothers were primiparous. 27 mothers reported their education level as below higher secondary school and 17 mothers were employed. 85% of the mothers consumed non-vegetarian diet. Table 1 represents the anthropometric and biochemical characteristics of study subjects. These parameters were comparable between the groups. There was no significant change in systolic and diastolic blood pressure from supine to standing posture among pregnant mothers.

	Entire group (n=42)	Group 1 (n=12)	Group 2 (n=9)	Group 3 (n=11)	Group 4 (n=10)
Basic Characteristics					
Age (year)	24.9 ± 3.4	24.2 ± 3.1	25.3 ± 4.1	24.4 ± 3.0	25.9 ± 3.8
Gestational age (day)	79.3 ± 20.7	79.9 ± 14.1	88.9 ± 19.2	71.1 ± 27.2	78.9 ± 19.8
Systolic BP (mmHg)	109.1 ± 9.2	106.6 ± 7.0	106.5 ± 5.0	114.1 ± 9.8	108.0 ± 13.9
Diastolic BP (mm Hg)	70.4 ± 7.4	71.6 ± 10.2	68.5 ± 3.2	72.3 ± 6.1	67.3 ± 7.8
Anthropometric Characteristics					
Height (cm)	154.0 ± 11.3	155.7 ± 5.4	151.4± 23.0	152.5 ± 4.3	156.8 ± 5.8
Weight (kg)	48.9 ± 8.0	49.3 ± 5.5	49.1 ± 10.2	46.5 ± 10.2	50.3 ± 6.5
BMI (kg/m2)	20.4 ± 3.7	20.1 ± 2.8	20.6 ± 4.0	19.9 ± 3.9	20.8 ± 2.3
% Fat	29.2 ± 5.5	29.3 ± 6.2	28.9 ± 4.1	27.6 ± 7.0	29.8 ± 3.7
Fat Free Mass (kg)	34.2 ± 6.5	32.1 ± 7.9	36.6 ± 8.3	33.2 ± 4.8	35.8 ± 5.1
Biochemical parameters					
Vitamin B12 (pmol/L)	204.4 ± 77.6	121.4 ± 21.9	177.4 ± 14.1	217.4 ± 17.0	314.1 ± 53.5*
Haemoglobin (g%)	11.9 ± 1.3	11.7 ± 1.7	11.8 ± 1.0	12.0 ± 1.4	12.1 ± 1.2
Mean Corpuscular Volume (fl)	79.0 ± 8.0	79.4 ± 9.7	80.3 ± 7.1	75.6 ± 9.1	80.8 ± 4.7
Data are represented as Mean ± SD, *p <0.05 by ANOVA for comparison between four groups					

Table 1: Descriptive characteristics of study population.

Vitamin B12 and HRV indices: Table 2 summarizes the HRV data across the study groups. One-way ANOVA showed a significant difference in Log LF HRV between the study groups (p=0.03).

On performing Tukey Post Hoc test, Log LF HRV was lower in group 1 (lowest vitamin B12 levels) compared to group 4 (highest vitamin B12 levels) (p=0.05). HRV indices namely Log HF and Log TP were not statistically different between the study groups.

The correlation of vitamin B12 levels with Log LF and Log TP was 0.36 (p=0.02) and 0.28 (p=0.07) respectively, while the correlation with Log HF was 0.27 (p=0.08) (Figure 1).

HRV indices	Entire group (n=42)	Group 1 (n=12)	Group 2 (n=9)	Group 3 (n=11)	Group 4 (n=10)
Anti-Log Very Low Frequency (msec^2)	691.8 (346.7-1380.4)	467.7 (186.2-1174.9)	794.3 (501.2-1258.9)	631.0 (316.2-1258.9)	955.0 (398.1- 1584.9)
Anti-Log Low Frequency (msec^2)	616.6 (309.0- 1230.3)	478.6 (239.9-955.0)	645.7 (323.6-1288.2)	501.2 (251.2-1000.0)	1047.1 (524.8- 2089.3)[*]
Anti-Log High Frequency (msec^2)	831.8 (263.0- 2630.3)	741.3 (295.1- 1862.1)	562.3 (223.1-1412.5)	707.9 (281.8-1778.3)	1479.1 (467.7-4677.4)
Anti-Log Total Power (msec^2)	2238.7 (1122.0-4466.8)	1698.2 (851.1-3388.4)	2187.8 (1096.5-4365.2)	1905.5 (281.8-1778.3)	3548.1 (467.7-4677.4)
Low Frequency nu	41.5 ± 13.4	36.8 ± 10.9	49.6 ± 13.3	40.1 ± 11.8	41.3 ± 16.3
High Frequency nu	54.9 ± 12.2	56.9 ± 12.4	48.6 ± 11.9	56.4 ± 11.1	56.4 ± 13.4
Low Frequency/ High Frequency ratio	0.9 ± 0.5	0.7 ± 0.4	1.2 ± 0.6	0.8 ± 0.4	0.9 ± 0.6
nu- normalised units, Data are represented as Mean ± SD					
[*]$p < 0.05$ by ANOVA for comparison between 4 groups, Post-hoc Tukey's test significant between Groups 1 and 4					

Table 2: Representation of HRV Indices of the study subjects across four study groups.
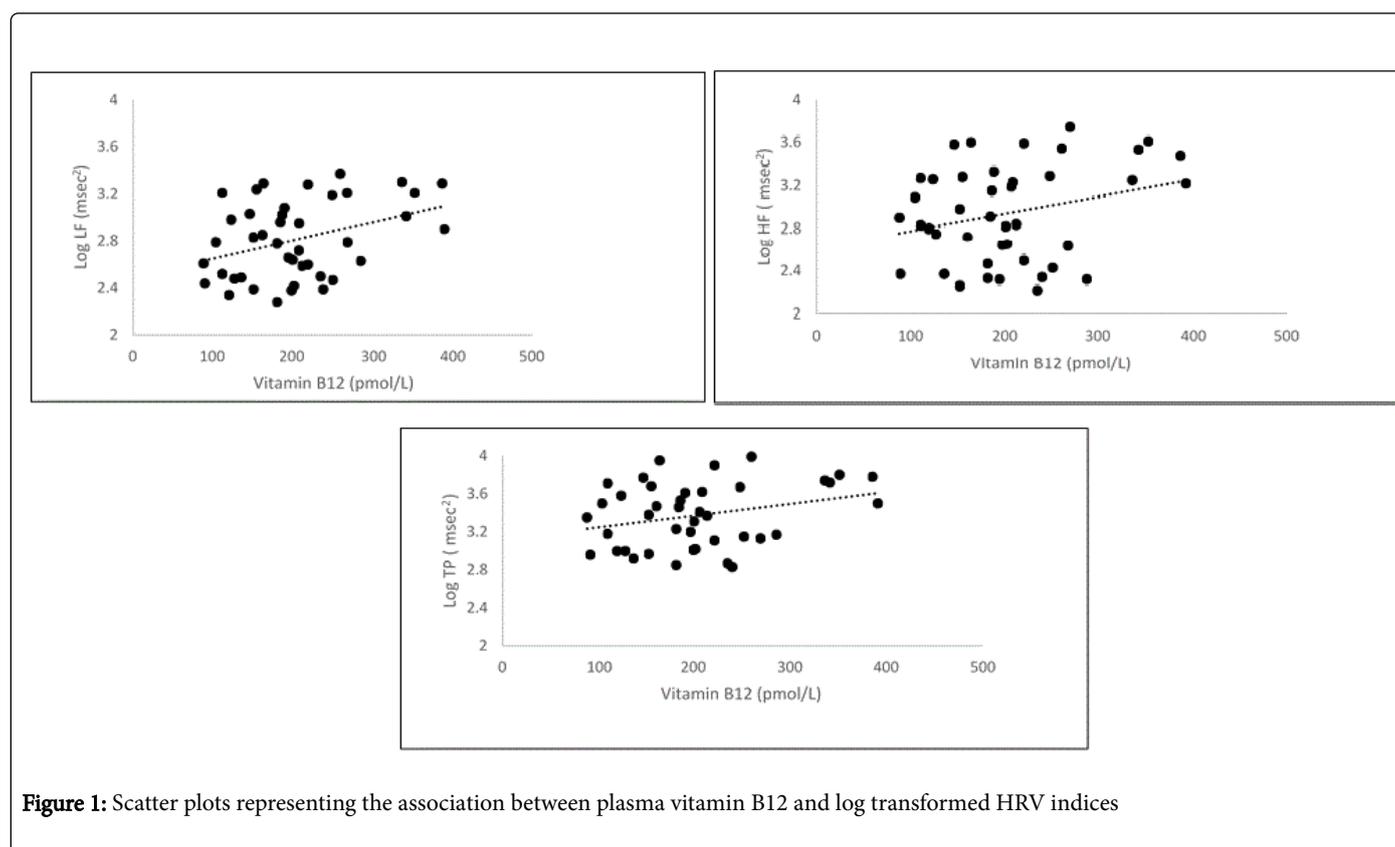

Figure 1: Scatter plots representing the association between plasma vitamin B12 and log transformed HRV indices

Discussion

Data from the present study demonstrated a significant correlation between low frequency (LF), HRV, an indicator of cardiac sympathetic activity (as suggested by majority of available literature) and serum vitamin B12 levels among pregnant mothers during early pregnancy.

LF HRV was significantly reduced in mothers with lowest vitamin B12 status compared to mothers with highest vitamin B12 status.

In a recent review, broad ranges of cut off for identifying vitamin B12 deficiency in the general population ranging from 100 pmol/L to 350 pmol/L was defined [23]. Findings from this study suggests that vitamin B12 has a significant impact on HRV. When the study

population were categorized based on their vitamin B12 quartiles association between vitamin B12 and functional outcomes was better. This suggests that usage of continuum of vitamin B12 status rather than by any defined cut offs is recommended.

Studies during pregnancy have focussed upon late pregnancy with special emphasis on increased sympathetic activity (as measured by HRV) and its relations with obstetrical complications [24,25]. During early pregnancy major hemodynamic changes including blood volume expansion and reductions in systemic vascular resistance have been reported. These changes are said to begin during early phase of the pregnancy, reaching their peak during the second trimester and then remain relatively constant until delivery [26,27]. Recently, a study using microneurographic technique demonstrated increase in vasomotor sympathetic activity in normotensive early pregnant mothers. The proposed mechanism for sympathetic activation could be a reflex response or a compensatory mechanism to reduced diastolic pressure and total peripheral resistance [27]. Animal studies during early pregnancy have demonstrated that levels of angiotensin II could increase sympathetic activity due to stimulation of receptors in the juxtaglomelular cells of the kidney leading to increase in rennin release thus increasing blood volume [28-30]. Elevated sympathetic activity during early pregnancy seems to be a normal response given the above evidences. However, reduction in cardiac sympathetic activity as demonstrated by reduced low frequency component of HRV among pregnant mothers with low vitamin B12 status from the current study suggests that there could be blunted compensatory responses and reduced vascular resistance. This could potentially increase their susceptibility to presyncopal attacks during early pregnancy if associated with vitamin B12 deficiency. Syncope and recurrent presyncope is common during course of pregnancy. A study reported that 4.6% of pregnant women experience syncope, 28.2% report at least one episode of presyncope and 10.3% reporting recurrent presyncopal episodes sufficient to cause a change in daily activity or lifestyle [31]. However, there is still lack of our understanding of mechanisms causing the same.

Linking reduced low frequency component of heart rate variability to reduced vitamin B12 status is supported by data from our previous work among cohorts of elderly, young adults and children [32,33,11]. Reduced low frequency was reversed with vitamin B12 supplementation among elderly [32]. Evidence of sympathetic involvement during long term vitamin B12 deficiency is supported by studies demonstrating reduced serum noradrenaline concentrations, decreased sympathetic skin responses and reduced local sweat responses to acetylcholine [34,35]. Reduced sympathetic activity among pregnant mothers with low vitamin B12 status compared to mothers with high vitamin B12 status from the current study indicate that early autonomic changes could occur in the absence of clinical signs and symptoms of autonomic dysfunction. As pregnancy progresses reduction in vitamin B12 status is a possibility due to increased demand by the growing fetus and haemodilution [36]. Continuous monitoring of vitamin B12 levels throughout pregnancy could add value particularly in susceptible population like for instance among vegetarians. Lack of adequate vitamin B12 levels during pregnancy may not only affect the neural development in fetus by restricting myelination, dendritic arborisation and synaptic connectivity [37], but could also affect the maternal health both short and long term.

In summary, data from the present study explored the association between vitamin B12 status and autonomic nervous activity during early pregnancy among Indian mothers within physiological framework. Data suggested that there was reduced cardiac sympathetic activity among pregnant mothers with low vitamin B12 status. Vitamin B12 estimation is not a regular screening tool during pregnancy. Findings from current study not only emphasises the use of vitamin B12 levels during pregnancy but also usage of simple physiological indices like HRV during routine clinical practice. Further, longitudinal studies among pregnant mothers are required to evaluate the role of vitamin B12 status and its long-term impact on autonomic nervous system.

Acknowledgements

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Pathak P, Kapil U, Yajnik CS, Kapoor SK, Dwivedi SN, et al. (2007) Iron, folate, and vitamin B12 stores among pregnant women in a rural area of Haryana State, India. Food Nutr Bull 28: 435-438.

2. Samuel TM, Duggan C, Thomas T, Bosch R, Rajendran R, et al. (2013) Vitamin B (12) intake and status in early pregnancy among urban South Indian women. Ann Nutr Metab 62: 113-122.

3. Finkelstein J, Kurpad AV, Thomas T, Bose B, Samuel T, et al. (2014) Vitamin B12 status in pregnant women and their children in India. The FASEB Journal 28: 135-136.

4. Muthayya S, Kurpad AV, Duggan CP, Bosch RJ, Dwarkanath P, et al. (2006) Low maternal vitamin B12 status is associated with intrauterine growth retardation in urban South Indians. Eur J Clin Nutr 60: 791-801.

5. Banka S, Roberts R, Plews D, Newman WG (2010) Early diagnosis and treatment of cobalamin deficiency of infancy owing to occult maternal pernicious anemia. J Pediatr Hematol Oncol 32: 319-322.

6. Casella EB, Valente M, de Navarro JM, Kok F (2005) Vitamin B12 deficiency in infancy as a cause of developmental regression. Brain Dev 27: 592-594.

7. Carmel R, Green R, Rosenblatt DS, Watkins D (2003) Update on cobalamin, folate, and homocysteine. Hematology Am Soc Hematol Educ Program 1: 62-81.

8. Vinik AI, Mehrabyan A (2003) Diagnosis and management of diabetic autonomic neuropathy. Compr Ther 29: 130-145.

9. Kamath MV, Fallen EL (1993) Power spectral analysis of heart rate variability: a noninvasive signature of cardiac autonomic function. Crit Rev Biomed Eng 21: 245-311.

10. Greiser KH, Kluttig A, Schumann B, Swenne CA, Kors JA, et al. (2009) Cardiovascular diseases, risk factors and short-term heart rate variability in an elderly general population: the CARLA study 2002–2006. Euro J Epid 24: 123-142.

11. Sucharita S, Dwarkanath P, Thomas T, Srinivasan K, Kurpad AV, et al. (2012) Low maternal vitamin B12 status during pregnancy is associated with reduced heart rate variability indices in young children. Matern Child Nutr 10: 226-233.

12. Rakow A, Katz-Salamon M, Ericson M, Edner A, Vanpee M (2013) Decreased heart rate variability in children born with low birth weight. Pediatric Research 74: 339-343.

13. Antony AC (2001) Prevalence of cobalamin (vitamin B-12) and folate deficiency in India--audi alteram partem. Am J Clin Nutr 74: 157-159.

14. Bamji MS (1983) Vitamin deficiencies in rice-eating populations. Effects of B-vitamin supplements. Experientia Suppl 44: 245-263.

15. Fu Q, Levine BD (2009) Autonomic Circulatory Control during Pregnancy in Humans. Semin Reprod Med 27: 330-337.

16. Krishnaveni GV, Hill JC, Veena SR, Bhat DS, Wills AK, et al. (2009) Low plasma vitamin B12 in pregnancy is associated with gestational "diabesity" and later diabetes. Diabetologia 52: 2350-2358.

17. Toru S, Yokota T, Inaba A, Yamawaki M, Yamada M, et al. (1999) Autonomic dysfunction and orthostatic hypotention caused by vitamin B12 deficiency. J Neurol Neurosurg Psychiatry 66: 804-805.

18. Mujawar SA, Patil VW, Daver RG (2011) Study of Serum Homocysteine, Folic Acid and Vitamin B12 in Patients with Preeclampsia. Ind J Clin Biochem 26: 257-260.

19. Sucharita S, Bharathi AV, Kurpad AV, Vaz M (2002) A Comparative study of tests of cardiac parasympathetic nervous activity in healthy human subjects. Physiol Meas 23: 347-354.

20. Vaz M, Bharathi AV, Sucharita S, Nazareth D (2003) Heart rate variability and baroreflex sensitivity are reduced in chronically undernourished, but otherwise healthy, human subjects. Clin Sci (Lond) 104: 295-302.

21. Troger RM, Rauh R, Mahlke C, Gottschalk T, Muck-Weymann M (2003) Agreement of two different methods for measurement of heart rate variability. Clin Auton Res 13: 99-102.

22. Malik M (1996) Heart rate variability: standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology, North American Society of Pacing and Electrophysiology. Circulation 93: 1043-1065.

23. Aparicio-Ugarriza R, Palacios G, Alder M, González-Gross M (2015) A review of the cut-off points for the diagnosis of vitamin B12 deficiency in the general population. Clin Chem Lab Med 53: 1149-1159.

24. Yang CC, Chao TC, Kuo TB, Yin CS, Chen HI (2000) Preeclamptic pregnancy is associated with increased sympathetic and decreased parasympathetic control of HR. Am J Physiol Heart Circ Physiol 278: 269-273.

25. Musa SM, Adam I, Lutfi MF (2016) Heart Rate Variability and Autonomic Modulations in Preeclampsia. PLoS One 11: 0152704.

26. Thornburg KL, Jacobson SL, Giraud GD, Morton MJ (2000) Hemodynamic changes in pregnancy. Semin Perinatol 24: 11-14.

27. Chapman AB, Abraham WT, Zamudio S, Coffin C, Merouani A, et al. (1998) Temporal relationships between hormonal and hemodynamic changes in early human pregnancy. Kidney Int 54: 2056.

28. Jarvis SS, Shibata S, Bivens TB, Okada Y, Casey BM, et al. (2012) Sympathetic activation during early pregnancy in humans. J Physiol 590: 3535-3543.

29. Hart EC, Charkoudian N, Wallin BG, Curry TB, Eisenach J, et al. (2011) Sex and ageing differences in resting arterial pressure regulation: the role of the β-adrenergic receptors. J Physiol 589: 5285-5297.

30. Jensen BL, Schmid C, Kurtz A (1996) Prostaglandins stimulate renin secretion and renin mRNA in mouse renal juxtaglomerular cells. Am J Physiol 271: 659-669.

31. Gibson PS, Powrie R, Peipert J (2001) Prevalence of syncope and recurrent presyncope during pregnancy. Obstetrics & Gynecology 97: 41-42.

32. Sucharita S, Thomas T, Antony B, Vaz M (2012) Vitamin B (12) supplementation improves heart rate variability in healthy elderly Indian subjects. Auton Neurosci 168: 66-71.

33. Sucharita S, Sowmya S, Thomas T, Kurpad AV, Vaz M (2013) Plasma vitamin B12, methylmalonic acid and heart rate variability in healthy young Indian adults. Int. J Vitam Nutr Res 83: 147-153.

34. Metz J (1992) Cobalamin deficiency and the pathogenesis of nervous system disease. Annu Rev Nutr 12: 59-79.

35. Toru S, Yokota T, Inaba A, Yamawaki M, Yamada M, et al. (1999) Autonomic dysfunction and orthostatic hypotention caused by vitamin B12 deficiency. J Neurol Neurosurg Psychiatry 66: 804-805.

36. Baker H, DeAngelis B, Gittens-Williams L, Barrett T (2002) Vitamin Profile of 563 Gravidas during trimesters of pregnancy. J Am Coll Nutr 21: 33-37.

37. Molloy AM, Kirke PN, Brody LC, Scott JM, Mills JL (2008) Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. Food and Nutrition Bulletin 29: 101-111.