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Original Article

Low maternal vitamin B12 status during pregnancy is associated with reduced heart rate variability indices in young children

Sambashivaiah Sucharita*†, Pratibha Dwarkanath†, Tinku Thomas†, Krishnamachari Srinivasan†, Anura V. Kurpad*† and Mario Vaz*†

**Department of Physiology, St. John's Medical College, Bangalore 34, India, and †St. John's Research Institute, St. John's National Academy of Health Science, Bangalore 34, India*

Abstract

Vitamin B12 plays a vital role in neuronal development, particularly in myelinogenesis. Demyelination of the autonomic nervous system occurs early in vitamin B12 deficiency. However, the impact of maternal vitamin B12 deficiency during pregnancy on neuronal function in the offspring is poorly documented. The objective of this study was to assess cardiac autonomic nervous activity in children born to mothers with low vitamin B12 status during pregnancy using heart rate variability (HRV) indices in the frequency domain. Seventy-nine healthy children between 3 and 8 years of age were evaluated from an ongoing birth cohort. The blood sample of the mother had been stored and was analysed for plasma vitamin B12 following enrolment of the child. Subjects were divided, based on the median maternal first trimester vitamin B12 status (114 pmol L^{-1}), into lower ($n = 40$) and higher ($n = 39$) vitamin B12 status groups. A lead II electrocardiogram was recorded in the supine posture and subjected to HRV analysis. Low-frequency HRV in absolute units was reduced significantly in children of the lower vitamin B12 status group ($P = 0.03$) and was 53% that of the higher vitamin B12 status group. There was a significant association between low-frequency and total power HRV with cord blood vitamin B12 levels ($\rho = 0.31$ and 0.30, both $P = 0.03$). In summary, children born to mothers with a lower vitamin B12 status have a reduced cardiac sympathetic activity. The long-term implication of this needs to be evaluated by follow-up studies.

Keywords: vitamin B12, autonomic nervous system, maternal, children, myelin, neuropathy.

Correspondence: Dr S. Sucharita, Department of Physiology, St. John's Medical College, Bangalore 56003, India. E-mail: sucharita@sjri.res.in

Introduction

The most crucial developmental period for the human central nervous system is gestation. During this period, neurons proliferate and supporting tissues are formed (de Graaf-Peters & Hadders-Algra 2006). Any physiological insult during this period will therefore likely have important consequences. Vitamin B12 deficiency is not uncommon among pregnant Indian mothers (Yajnik *et al*. 2008) and this may be relevant as case reports of pregnant mothers with pernicious anaemia have suggested an increased occurrence of neurological deficits in infants (Garewal 1988). Children born to mothers with vitamin B12 deficiency during pregnancy have depleted vitamin B12 stores and also receive an inadequate amount of vitamin B12 through breast milk (Allen 1994). Most of the published research on the relationship between vitamin B12 deficiency in children and maternal B12 deficiency is limited to case studies of infants of

mothers with pernicious anaemia or vegan mothers (Korenke *et al*. 2004; Weiss *et al*. 2004). Apart from more obvious neurological deficits, these infants are at risk for delayed developmental milestones (Casella *et al*. 2005).

Studies have also shown that severe vitamin B12 deficiency is associated with a reduced sympathetic component of the autonomic nervous system (ANS) using heart rate variability (HRV) indices (Beitzke *et al*. 2002). The ANS plays an important role in the regulation of the cardiovascular system, and increasing evidence suggests that alterations in autonomic regulation could lead to increased cardiovascular risk in adults (Greiser *et al*. 2009). HRV is a simple, sensitive and non-invasive technique of assessing cardiac autonomic function (Akselrod *et al*. 1981; van Ravenswaaij-Arts *et al*. 1993; Malik 1998) and relies upon the mathematical processing of the variation in the time intervals between heart beats (Kamath & Fallen 1993; Parati *et al*. 1995). Reduced HRV has been reported not only in cardiovascular pathology (e.g. post-myocardial infarction, congestive heart failure and post-cardiac transplant) but also in non-cardiological states, such as diabetic neuropathy and depression, and also predicts susceptibility to sudden infant death syndrome and poor survival in premature babies (Harper *et al*. 1982; Schwartz *et al*. 1992; Galland *et al*. 2006; Sucharita *et al*. 2011). In addition, reduced HRV has been associated with all-cause and cardiovascular mortality in prospective cohort studies (La Rovere *et al*. 1998; Schwartz & La Rovere 1998). HRV is also considered an index of central autonomic integration (Thayer & Lane 2000) and has been studied in various psychopathological states (Porges 1995). Changes in the parasympathetic component of HRV have been reported in a variety of behavioural problems (Porges *et al*. 1996) and in cognitive deficits (Eisenberg & Richman 2011) in children and adolescents.

Our own studies have shown that HRV is reduced in chronically undernourished adults; at that time, we suggested that vitamin B12 deficiency was a possible cause, but did not test this (Vaz *et al*. 2003). We are not aware of any data that have evaluated maternal vitamin B12 status and its impact on autonomic function in children beyond infancy. The objective of the present study was to assess the autonomic nervous activity (HRV indices) in children born to mothers with low vitamin B12 status during pregnancy and to compare them with children of mothers with higher vitamin B12 status.

Material and methods

Subject recruitment

Ninety-seven healthy children between 3 and 8 years of age were recruited from an ongoing pregnancy and birth cohort at the Medical College and Hospital. At the time of the enrolment of the child into the present study, the vitamin B12 status of the mother during pregnancy was not known. The blood sample of the mother had been stored as part of an ongoing birth cohort study and was analysed for plasma vitamin B12 following enrolment of the child. Seventy-nine children had their mother's stored blood samples available and were included in the final analysis. The study was approved by the Institution Ethics Review Board. Parental consent was obtained and every child also gave assent to the study; details of the procedures

Key messages

- Vitamin B12 deficiency is common among pregnant Indian mothers.
- The impact of maternal vitamin B12 deficiency during pregnancy on neuronal function in the offspring is poorly documented.
- Young children born to mothers with lower vitamin B12 status have reduced cardiac sympathetic activity, based on the HRV analysis. There was also a significant association between LF power spectra in absolute units, total power HRV and cord blood vitamin B12 levels.
- Early detection and intervention in pregnant women who are vitamin B12 deficient may help improve cardiac autonomic function in children.

in the form of images were explained to them by one of the investigators in the presence of a parent. Children were assigned to one of the two groups (higher and lower vitamin B12 status) based on the median maternal plasma vitamin B12 values in the first trimester (114 pmol L^{-1}). Median values were considered in order to obtain an equal distribution between the two study groups and to maximise statistical power. The cut-off value used is just lower than the lower limit of plasma vitamin B12 defined by the Institute of Medicine (i.e. 120 pmol L^{-1}) (Food and Nutrition Board, Institute of Medicine 1998). Details of the original recruitment into the cohort are explained in an earlier publication (Muthayya *et al.* 2006). Briefly, pregnant women (18–40 years) who were below 20 weeks of gestation and registered for antenatal screening at the Department of Obstetrics and Gynaecology at the Medical College Hospital were invited to participate in the study. Every effort was made to recruit women as early in their pregnancy to carry out baseline measurements at 12 weeks of gestation. Women with multiple pregnancies, those with a clinical diagnosis of chronic illness, such as diabetes mellitus, hypertension, heart disease and thyroid disease, those who tested positive for hepatitis B, hepatitis B surface antigen (HbSAg), HIV or syphilis venereal disease research laboratory test infections, or those who anticipated moving out of the city before delivery were excluded. Blood was drawn from subjects after an overnight fast by venipuncture using trained personnel and collected in both ethylene diaminetetraacetate and plain vacutainers (Beckton Dickinson, Franklin Lakes, NJ, USA) in the first trimester and at the end of each subsequent trimester whenever possible. Whole blood was used for the estimation of haemoglobin (Cell-Dyn 1700, Abbott Labs, Abbott Park, IL, USA) and plasma was stored at –80°C for vitamin B12 estimation. Cord blood was collected at birth, whenever possible, and was centrifuged; plasma was stored for vitamin B12 analysis of the total number of children selected.

Biochemical analysis

Vitamin B12 was determined using a chemiluminiscent immunoassay (Unicel DxI 600; Beckman Coulter, Brea, CA, USA). Inter-assay coefficient of variation (CV) for plasma vitamin B12 was 6.6–8.5%, and intra-assay CV for plasma vitamin B12 was 4.8–6.9%.

Anthropometric measurement

All participants underwent a detailed anthropometric assessment. Maternal anthropometric measurements performed during the first trimester are presented in the present analysis. A digital balance (Soehnle, Frankfurt, Germany) was used to record the weights of all mothers to the nearest 100 g. Measurements of height were made using a stadiometer to the nearest 0.1 cm. Mid-upper arm circumference was measured using a plastic measuring tape, and biceps, triceps and subscapular skinfolds (Holtain calipers, Crymych, UK) were used to assess the body composition in mothers using published prediction equations (Durnin & Womersley 1974). Maternal body mass index was calculated using weight and height at baseline (kg m^{-2}).

General physical examination of the child was performed on the day of the visit by a paediatrician. Anthropometry of the child was assessed on the same day that HRV was measured and included height, weight, and waist and hip circumferences. The circumferences were measured using a standard non-stretchable tape measure at the narrowest point between the iliac crest and ribcage (waist) and at the level of the greater trochanter (hip).

Heart rate variability

Autonomic evaluation was assessed using power spectral analysis of HRV. Subjects were instrumented for lead II electrocardiogram. Measurements were performed after a 30-min rest in supine posture for 10 min. Details of the signal processing and mathematical calculations have been discussed earlier (Vaz *et al.* 2003). Briefly, spectral analysis was performed using a fast Fourier transform. The frequency resolution was 0.0078 and the highest frequency evaluated was 0.4 Hz. The spectra obtained for the different data sets were averaged to reduce variance and to sharpen reproducible central peaks. Power was calcu-

Table 1. Socio-demographic, anthropometric, biochemical and haematological profile of mothers and children in the lower and higher vitamin B12 status groups

Parameters	Lower vitamin B12 status group ($n = 40$)	Higher vitamin B12 status group ($n = 39$)
Mother's data		
Age (year)	24 ± 3	24 ± 4
Weight (kg)	52.1 ± 8.1	53.1 ± 10.3
Height (cm)	156.0 ± 6.1	156.0 ± 4.8
Body mass index (kg m^{-2})	21.5 ± 3.8	21.8 ± 4.2
Percentage fat (%)	27.4 ± 4.6	28.3 ± 6.0
Haemoglobin (gm dL^{-1})	11.8 ± 1.1	11.6 ± 1.2
Serum vitamin B12 (pmol L^{-1})	82.2 (68.0–101.1)	157.1** (138.7–193.3)
Child's data – characteristics at birth		
Gestational age at birth (weeks)	39.1 ± 1.4	38.5 ± 1.3
Birthweight (kg)	2.8 (2.6–3.2)	3.0 (2.6–3.1)
Vitamin B12 levels in cord blood (pmol L^{-1})	92.2 ($n = 20$) (73.6–136.7)	184.1** ($n = 20$) (145.0–266.6)
Child's data – current status		
Age (year)	5.3 ± 1.4	5.7 ± 1.3
Height (cm)	109.1 ± 1.0	112.1 ± 1.4
Weight (kg)	17.2 ± 3.4	18.1 ± 5.5
Sum of skinfold (mm)	25.5 ± 7.3	28.9 ± 8.8
Waist hip ratio	0.88 ± 0.05	0.87 ± 0.03

Grouping based on the first trimester median vitamin B12 level of 114 pmol L^{-1}. Data are mean ± standard deviation/median (mother's serum vitamin B12, cord blood vitamin B12 and birthweight) (interquartile range). **$P < 0.01$, compared with lower vitamin B12 status group.

lated in the two bands. The 0.04–0.15 Hz band of RR power [referred to as the low-frequency (LF) band] is believed to reflect, at least in part, sympathetic nerve activity to the heart and partly parasympathetic activity, while the 0.15–0.4 Hz band [high-frequency (HF) band] reflects parasympathetic nerve activity to the heart. In addition to the absolute power, data for HRV are also presented as normalised units, as recommended (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996), where the power in the LF and HF bands is expressed as a percentage of the total power minus the power of the very-low-frequency band (0.0–0.04 Hz) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996).

Statistical analysis

The normality of the data was examined using Kolmogorov–Smirnov test. The data are expressed as mean (standard deviation) when normally distributed. When skewed, data are represented as median (quartile 1, quartile 3). Comparison between groups was assessed using an independent *t*-test/Mann–Whitney *U*-test. A comparison of within trimesters was performed using repeated measure analysis of variance (RMANOVA). Correlation of vitamin B12 status of the mothers in each trimester with that in cord blood was examined using Pearson's correlation coefficient and this, together with HRV parameters, was examined using Spearman's rank correlation coefficient (ρ). Results were considered significant if $P < 0.05$. All statistical analyses were performed using SPSS (v13, SPSS, Chicago, IL, USA).

Results

Anthropometric and haematological data of the mothers and children included in the study, stratified by vitamin B12 status, were comparable (Table 1). Cord blood vitamin B12 levels were significantly reduced in the lower maternal vitamin B12 status group, although the number of available cord blood samples was restricted ($n = 40$). Mean serum vitamin

Table 2. Heart rate variability parameters of the children in the lower and higher vitamin B12 status groups

Parameters	Lower vitamin B12 status group ($n = 40$)	Higher vitamin B12 status group ($n = 39$)
Low-frequency power (LF) (0.04–0.15 Hz) (m s^2)	646.3 (287.1–1098.9)	989.3* (532.5–1519.7)
High-frequency power (HF) (0.15–0.4 Hz) (m s^2)	509.0 (230.1–1086.9)	865.9 (353.2–1969.4)
Total power (0–0.4 Hz) (m s^2)	1554.5 (719.9–2942.2)	2240.4 (1202.3–3959.8)
Normalised LF	57.6 (45.5–68.6)	57.1 (42.3–66.9)
Normalised HF	48.4 (41.5–60.0)	50.3 (41.9–62.7)
Ratio LF/HF	1.23 (0.76–1.65)	1.15 (0.67–1.68)
Heart rate (bpm)	98.8 (89.6–105.3)	94.4 (87.6–102.7)

LF, low frequency; HF, high frequency; bpm, beats per minute. Data are median (interquartile range). *$P < 0.05$ compared with lower vitamin B12 status group.

B12 levels of the mothers during the three trimesters were as follows: first trimester ($n = 79$): 127.3 ± 64.1; second trimester ($n = 49$): 106.1 ± 44.6; third trimester ($n = 64$): 116.8 ± 44.5. The vitamin B12 levels between the three trimesters were compared in a subset of 31 mothers using RMANOVA and was found to be not statistically significant ($P = 0.62$). There was a significant correlation in the serum vitamin B12 levels at the first, second and third trimesters with cord blood vitamin B12 levels (r = 0.62–0.81).

Table 2 summarises the HRV data of children in the two groups (based on the first trimester vitamin B12 status of the mother). LF HRV in absolute units was reduced significantly in children of mothers with lower vitamin B12 ($P = 0.03$) and was 53% that of the children of mothers with higher vitamin B12 status. Along similar lines, total and HF (absolute units) power of HRV tended to be lower in children of mothers with lower vitamin B12 status ($P = 0.05$ and $P = 0.09$, respectively). There were no differences in the normalised units of HRV indices between the two study groups.

The correlation (ρ) between cord blood vitamin B12 levels and LF and total power HRV was 0.31 and 0.30, respectively, while the correlation with HF HRV was 0.25.

Discussion

Data from the present study indicate that LF power spectra in absolute units, often used as an indicator of cardiac sympathetic activity, were significantly reduced in young children born to mothers with lower vitamin B12 status during pregnancy. There was a significant association between LF power spectra in absolute units, total power HRV and cord blood vitamin B12 levels.

While reduced HRV has been used as a predictor of cardiovascular risk and as a prognostic marker in adult population (Dekker *et al*. 2000), it is difficult to speculate on the role of reduced cardiac sympathetic activity in children born to vitamin B12 deficient mothers at this stage. Long-term follow-up of these children could help us understand this better. These studies would help us understand whether the lower HRV in childhood tracks into adulthood, as does occur for several other functional variables such as physical activity, body composition, cardiorespiratory fitness, and among others (Power & Parsons 2000; Boreham *et al*. 2004; Dwyer *et al*. 2009; Brisbois *et al*. 2012). This is important as lower HRV is associated with increased all-cause and cardiovascular mortality in adulthood (Tsuji *et al*. 1996) and there is some suggestion that HRV should be considered a therapeutic target (Routledge *et al*. 2002).

The current findings are important given the notion that vitamin B12 deficiency is likely to be higher in India than Western populations because of a higher prevalence of vegetarianism and possibly reduced dietary availability, coupled with a higher intestinal parasitic load which may limit vitamin B12 absorption (Makharia 2006; Desai & Gupte 2007). Small studies in India suggest that vitamin B12 deficiency is not uncommon among pregnant Indian mothers (Pathak *et al*. 2007), and maternal intake and absorption of

vitamin B12 during pregnancy and lactation have a strong influence on the vitamin B12 status of the infant (Costello & Osrin 2003). These are critical periods as vitamin B12 availability during the perinatal period is known to influence the development of critical areas in the brain and impact on brain function (Dror & Allen 2008). Vitamin B12 is important for the formation of the myelin sheath, and myelination of the brain is most pronounced from mid-gestation through to the second year of life (Black 2008).

Experimental models provide further understanding of the role of vitamin B12 on neurological development. Studies show that it stimulates a network of cytokines and growth factors (neurotrophic) and inhibits tumour necrosis factor α (neurotoxic) in the developmental stage of the nervous system. Vitamin B12 deficiency is known to trigger an imbalance between these factors, leading to myelinolytic damage of neurons. This is particularly important during early fetal life. Reduced availability of vitamin B12 during pregnancy may affect the fetus by restricting myelination, dendritic arborisation and synaptic connectivity that occur early in life (Molloy *et al*. 2008) The tissue levels of neurotransmitters (e.g. serotonin, dopamine, norepinephrine and acetylcholine) may also be altered, resulting in neuroanatomical, neurochemical or neurometabolic changes (Black 2008). The functional consequences of these alterations vary depending the timing of the deficiency relative to the developing neurological processes.

Long-term vitamin B12 deficiency in adults is known to cause the well-known triad of anaemia, neuropathy and cognitive deficits (Reynolds 2006). Neuropathy may precede other deficits in an adult population (Chiew *et al*. 1989; Chalouhi *et al*. 2008). In recently published study on healthy elderly Indians, a reduction in vitamin B12 status was associated with reduced HRV, with a specific reduction in LF absolute power (Sucharita *et al*. 2012). Critically, this was reversible with oral vitamin B12 supplementation of 100 μg for 3 months. The autonomic nervous changes were not accompanied by sensori-motor peripheral neural involvement, either clinically or using nerve conduction studies. This supports the general notion that autonomic deficits precede sensori-motor deficits in peripheral neuropathies of various aetiologies (Vinik *et al*. 2003). None of the children in the present study, born to mothers with reduced vitamin B12 status during pregnancy in the current study, had any clinical neurological deficits during infancy or later.

One of the limitations of the study was that the vitamin B12 status of the children at the time of the HRV measurement was not known as the children did not consent to a blood sample. It is possible that some children may have changed their vitamin B12 status from what they were at birth. Stratification of the children by current vitamin B12 status would have allowed us to explore the relationship between vitamin B12 status of the mother during pregnancy and autonomic function in the child more fully. In addition, homocysteine and methyl malonic acid levels were not assessed in the mother. This is important as this could have helped uncover borderline vitamin B12 deficiency in the mother; the current misclassification, if any, would, however, have likely favoured a null rather than a significant association between maternal vitamin B12 status and childhood HRV.

In summary, maternal malnutrition continues to be an important public health issue in India, and early detection and intervention can help reduce some of the sequelae (Stabler & Allen 2004). The long-term implication of reduced cardiac sympathetic activity in children born to vitamin B12 deficient mothers is not known. Whether a reduced *in utero* exposure to vitamin B12 could limit the ability to enhance HRV in the long term, seen by some as a potential therapeutic target (Routledge *et al*. 2002), needs to be investigated. Extended follow-up studies on children born to mothers with reduced vitamin B12 status would be required to answer these questions.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

SS analysed and interpreted the data and wrote the initial draft of the manuscript. TT provided statistical guidance in data analyses. TT, PD, KS, AVK and MV assisted in the interpretation of results. All co-authors participated in manuscript preparation and critically reviewed all sections of the text for important intellectual content.

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