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Original Article

Subclinical Atherosclerosis and Impaired Cardiac Autonomic Control in Pediatric Patients with Vitamin B12 Deficiency

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ABSTRACT

Objective: Homocysteine (Hcy) is an independent risk factor for predisposing to atherosclerosis and endothelial dysfunction. Hcy levels increase with Vitamin B12 deficiency. The aim of this study was to investigate the association between carotid intima-media thickness (CIMT) and the autonomic modulation of heart rate variability (HRV) in early detection of atherosclerosis and impaired cardiac autonomic control in pediatric patients with Vitamin B12 deficiency. **Materials and Methods:** Sixty patients with Vitamin B12 deficiency (14.4 ± 1.72 years, 36 female) and 40, age, sex, and body mass index-matched healthy controls (13.4 ± 1.86 years, 24 female) had performed 24-h Holter monitoring, carotid ultrasonography, and echocardiography. Linear regression models assessed associations between to HRV parameters and CIMT and the blood markers. **Results:** We defined Vitamin B12 deficiency as a serum level, 200 pg/mL. Hcy ($P < 0.001$) and CIMT ($P < 0.001$) levels were significantly higher in the patient group compared with the control group. Hcy level was found to be the most important independent variable affecting CIMT. Each 1 degree increase in Hcy, it was observed that the CIMT value increased by 0.01 mm (B = 0.01; $t = -2.39$; $P < 0.05$). Low-frequency power (LF), high-frequency power (HF) ($P < 0.001$), and the square root of the mean of the squared differences of two consecutive RR intervals (rMSSd) ($P = 0.04$) were significantly lower in the B12 deficient patients. Furthermore, Hcy level was found to be the most important independent variable affecting LF, HF, and rMSSd. **Conclusions:** Subclinical atherosclerosis was associated with cardiovascular autonomic imbalance in pediatric patients with Vitamin B12 deficiency. Homocysteinemia may be an important marker for the prediction of future cardiovascular disease.

Keywords: *Atherosclerosis, cardiac autonomic control, child, homocysteinemia, Vitamin B12*

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Introduction

Homocysteine (Hcy) is a nonprotein amino acid derived by demethylation of methionine, an essential sulfur-containing amino acid obtained from dietary proteins.[1] Hcy-mediated enhanced lipid peroxidation and generation of free radicals result in inflammation and acute endothelial dysfunction, which accelerates atherosclerotic process predisposing to cardiovascular disease (CVD). The carotid intima-media thickness (CIMT) is a surrogate marker for systemic atherosclerosis and is associated with an increased risk of stroke and myocardial infarction,[2] coronary, and cerebrovascular events in patients without clinical CVD.[3] Vlachakis *et al*. first suggested a role of atherosclerosis in diminishing baroreceptor sensitivity.[4] Heart rate variability (HRV) analysis is a commonly used noninvasive method to measure alterations in baroreflex and autonomic tone.[5,6]

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We hypothesized that atherosclerosis can increase in Vitamin B12 deficiency and we want to evaluate the effect of increased atherosclerosis on autonomic dysfunction. This process can be demonstrated by the increase in CIMT and differences in HRV analyzes.

Materials and Methods

This prospective study covers the period between January 2017 and May 2017. Sixty pediatric patients' ages ranging from 11 to 17 years with B12 deficiency and forty age- and sex-matched healthy controls who underwent a cardiovascular assessment for an innocent murmur were included in this study. This study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee. The detailed consent forms were signed by the parents of all participants before participating in the study.

Anthropometric and blood pressure measurements of the cases were performed. Patients with a history of diabetes mellitus, cardiac, endocrinologic or neurologic disease, alcohol or substance abuse, renal dysfunction, and pernicious anemia were excluded from the study. The CIMT and left ventricular mass index were evaluated by echocardiography. Twenty-four hours ambulatory electrocardiogram recordings were taken with Biomedical Instruments Holter recorder. The recordings were reviewed to confirm 18 h of clear recording which included the data from the whole night, and the beat classifications were manually checked and corrected. Time-domain and long-term frequency-domain HRV were calculated using the software present in the system ECGLab Holter Analysis System Version 1.0 (Biomedical Instruments, China). In time-domain analysis; the standard deviation of all the RR intervals, the square root of the mean of the squared differences of two consecutive RR intervals (rMSSD), and the percentage of the beats with consecutive RR interval difference of >50 ms (pNN50) were calculated. In frequency-domain analysis; low-frequency power (LF) (the area under the spectral curve from 0.04 to 0.15 Hz), high-frequency power (HF) (the area under the spectral curve from 0.15 to 0.40 Hz), and the ratio LF/HF were calculated. In frequency-domain analysis, LF was accepted as equivalent to the sympathetic plus parasympathetic components of autonomic function, HF was accepted as representing the parasympathetic component of autonomic function, and LF/HF as depicting the sympathovagal balance of the autonomic function.[7]

Carotid atherosclerosis imaging and conventional echocardiography

The subclinical vascular disease scanning protocol for evaluation of CIMT and detection of carotid plaques was done according to the standardized protocol of the American Society of Echocardiography.[8] The mean CIMT value of the right common carotid artery was calculated. All the images were stored for offline analysis. The CIMT measurements were performed using software (EchoPAC PC version 113; GE Vingmed Ultrasound, IMT Package). This measurement procedure is less operator-dependent and does not require intense time according to the manual technique. The methodology is trustworthy among physicians because it is reproducible[9] [Figure 1a]. LV volumes and LV ejection fraction (LVEF) were measured using the modified Simpson method [Figure 1b]. LV mass was calculated using the formula that has been proposed by Devereux *et al*.[10] The Left Ventricular Mass Index was calculated by dividing the LVM (g) by the height in meters. Left ventricular hypertrophy (LVH) was defined as LVMI above 51 g/height.[11]

Analytical methods

Blood samples were obtained from each patient for the analysis of serum B12 levels, folic acid, Hcy, ferritin, hemogram, blood urea nitrogen, creatinine, cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase (LDH) levels were measured in all groups. The diagnosis of Vitamin B12 deficiency was defined when serum concentration was below 200 pg/ml, normality range varied from 200 to 900 pg/ml and excess 900 pg/ml, and a normal level of folic acid.[12] The method used to determine Vitamin B12 concentrations was a radioimmunoassay from Beckman Coulter's Unicel DXİ 800 analyzer.

Statistical analysis

The numerical parameters were reported as mean ± standard deviation. Student's *t*-test was used to compare the mean values of normal distribution variables. The groups were compared with Wilcoxon signed-rank test because HRV parameters were not normally distributed. Multivariable linear regression models were used to assess the association between HRV parameters and mean CIMT. Sequential multivariable models for each outcome were created based on our assessment of the covariates likelihood of being a confounder in the relationship between HRV and CIMT. All statistical tests were two-sided, and $P < 0.05$ was considered significant. Statistical analysis was carried out using SPSS 22 .0 (SPSS Inc.,Chicago, IL, USA) was used for data management and analysis.

Results

We studied 60 consecutive, newly diagnosed patients referred from the Department of Pediatric

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Table 1: Demographical, hematological, and conventional echocardiographic parameters of the two groups

	B12 deficient group (n=60)	Control group (n=40)	P
Age (years)	14.4±1.72	13.4±1.86	NS
Sex (male/female)	24/36	16/24	NS
Height (cm)	159.1±13.6	155.5±10.6	NS
Weight (kg)	51.7±14.04	49.2±14.01	NS
BMI (kg/m^2)	20.07±3.2	20.1±3.86	NS
MCV (fl)	86.6 (83.6-88.6)	83.7 (81.2-86.2)	<0.05
HDL (mg/dl)	44.4 (36.6-51)	49.25 (43.0-58.0)	<0.05
LDH (mg/dl)	161±33	175±32	<0.05
Folic acid level (ng/ml)	8.22±4.7	10.2±5.1	NS
Homocysteine (mmol/L)	21±8.06	10±2.54	<0.001
Serum Vitamin B12 (pmol/L)	100 (85-115)	238 (210-277)	<0.001
LVM (g)	58.20±9.05	56.90±7.05	NS
LVMI (g/m$^{2.7}$)	42.70±8.21	40.14±7.60	NS
CIMT (mm)	0.87 (0.69-1.21)	0.68 (0.52-0.80)	<0.001

NS=Non-significant; BMI=Body mass index; MCV=Mean corpuscular volume; HDL=High density lipoprotein; LDL=Low density lipoprotein; LVM=Left ventricular mass; LVMI=Left ventricular mass/body surface area of the normal children according to the height; CIMT=Carotid İntima media thickness

Table 2: Correlation between Vitamin B12 and other variables

Parameters	Vitamin B12 (n=100)	
	r	P
Homocysteine	−0.58	<0.001
Folate	0.24	<0.05
HDL	0.29	<0.05
LVMI	0.046	NS
CIMT	−0.27	<0.05

NS=Nonsignificant; HDL=High-density lipoprotein; LVMI=Left ventricular mass/body surface area of the normal children according to the height; CIMT=Carotid intima-media thickness

Hematology of our hospital (24 male, 36 female, mean age 14.4 ± 1.72 years). Ten of the patients had symptoms or signs which suggested the presence of autonomic dysfunction like orthostatic hypotension. Forty age- and sex-matched healthy children (16 male, 24 female; 13.4 ± 1.86 years) were used as controls. Table 1 presents the demographical, hematological, and conventional echocardiographic parameters of the two groups. The transthoracic echocardiography and 12-lead electrocardiography were normal in all participants enrolled in the study. In addition, no plaques or stenoses were observed at the vessel lumen in groups.

The mean CIMT values were 0.87 mm (0.69–1.21) and 0.68 (0.52–0.80) mm in the Vitamin B12 deficiency and control groups, respectively (P < 0.001). Children in the control and Vitamin B12 deficiency groups were compared regarding age, sex, weight, height, body mass index, lipid levels (cholesterol, triglycerides, LDL, and HDL), thyroid function, serum iron levels, iron-binding capacity, complete blood count, and C-reactive protein levels, which are known to be related to atherosclerosis and vascular changes. No significant differences were detected in the evaluated parameters, other than the mean corpuscular volume (P < 0.05), HDL (mg/dl) (P < 0.05), LDH (P < 0.05), Hcy (P < 0.001), B12 (P < 0.001), and CIMT (P < 0.001) values between groups. When the relationship between Vitamin B12 value and other parameters was examined in the study group, there were statistically significant correlations between Vitamin B12 and Hcy (r = −0.58, P < 0.001) and CIMT (r = −0.27, P < 0.05) in the negative direction. There were statistically significant

Table 3: Multiple regression analysis between B12 Deficiency and other variables

Independent variables	Vitamin B12 (pg/ml)					
	Univariate logistic regression			Multiple logistic regression		
	OR	95% CI	P	OR	95% CI	P
CIMT	31.79	4.53-222	<0.001	16.17	1.00-266	<0.05
Homocysteine	1.53	1.26-1.87	<0.001	1.59	1.4-2.02	<0.001
Triglyceride	1.008	1.00-1.016	0.50	1.01	1.0-1.02	<0.05
HDL	0.92	0.88-0.97	0.002	0.9	0.82-0.99	<0.05

OR=Odd's ratio; CI=Confidence Interval; HDL=High-density lipoprotein; CIMT=Carotid intima-media thickness

Table 4: Multiple regression analysis between carotid intima-media thickness and other parameters

	Beta	t	P
Constant		4.82	<0.001
Homocysteine	0.01	2.39	0.01
LDH	−0.002	−1.62	0.10
Vitamin B12	−0.001	−1.5	0.13

LDH=Lactate dehydrogenase

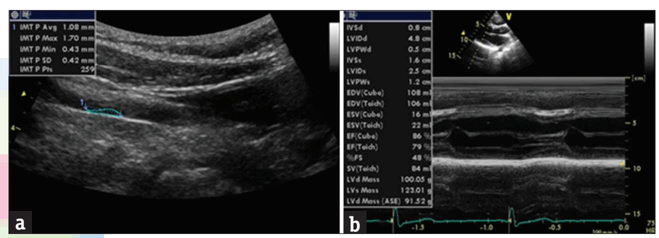

Figure 1: (a) Carotid intima-media thickness was measured on the posterior wall of the common carotid arteries 1–2 cm proximal to the carotid bifurcation and the proximal portion of the internal carotid (bulb) at a depth of 4 cm, by two-dimensional ultrasound (B-mode). The carotid intima-media thickness measurements were performed using software (EchoPAC PC version 113; IMT Package). (b) Left ventricular volumes and left ventricular ejection fraction were measured using the modified Simpson method

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Table 5: The heart rate variability parameters of the Vitamin B12 deficient group and the healthy controls

	B12 deficient group (*n*=60)	Control group (*n*=40)	*P*
Minimum heart rate[a]	51.5±5.7	49.4±3.8	NS
Maximum heart rate[a]	131.5±25.2	121.5±15.1	NS
Mean heart rate[a]	79.7±10.04	77.2±8.01	NS
Time-domain analysis			
SDNN (ms)	150.1±28.3	169.07±31.4	NS
rMSSD (ms)	49±9.42	53.41±11.34	0.04
pNN50 (%)	24.12±14.12	29.25±23.0	NS
Frequency-domain analysis			
LF (ms^2)	52.5±5.11	77.65±6.34	<0.001
HF (ms^2)	41.47±3.45	55.2±5.8	<0.001
LF/HF	1.29±0.31	1.41±0.48	NS

[a]Beats per minute. *P* value lower than 0.05 was accepted as statistically significant. NS=Nonsignificant; rMSSD=Square root of the mean of the sum of the squares of differences between adjacent NN intervals; SDNN=Standard deviation of all NN intervals; LF=Low frequency; HF=High frequency

correlations between Vitamin B12 and folate (r = 0.24, $P < 0.05$) and HDL (r = 0.29, $P < 0.05$) in the positive direction [Table 2]. Multilinear regression analysis of B12 deficiency as the dependent variable; Hcy, CIMT, HDL, LDH, and triglycerides were independent variables of the patient group and found Hcy was the most important independent variable in Vitamin B12 deficiency ($P < 0.001$) [Table 3]. When the relationship between CIMT and other parameters was examined in the study group, a statistically significant positive correlation was found between CIMT and Hcy (r: 0.37; $P < 0.001$). In addition, there was a significant negative correlation between CIMT and Vitamin B12 (r: −0.27; $P < 0.05$). Hcy level was found to be the most important independent variable affecting CIMT as a result of multiple linear regression analysis [Table 4]. Each 1 degree increase in Hcy, it was observed that the CIMT value increased by 0.01 mm (B = 0.01; t = 2.39; $P < 0.05$) and explained about 17% of the total variability in CIMT (R^2 = 0.17; $P < 0.001$).

The HRV parameters of the groups were summarized in Table 5. In time-domain parameter analysis, rMSSd was significantly lower in the B12 deficient patients when compared to healthy controls. In frequency-domain analysis, LF and HF were significantly lower in the Vitamin B12 deficient patients. The LF/HF ratio did not show statistically significant difference. The Pearson correlation between CIMT and HRV parameters showed a significant negative correlation: −0.52 (95% confidence interval [CI]: −0.61 to −0.39), −0.45 (95% CI: −0.54 to −0.33), −0.31 (95% CI: −0.78 to −0.27) for LF, HF, and rMSSD, respectively.

Discussion

In this study, Hcy and CIMT were found to be higher as a cardiovascular risk and atherosclerosis indicator in children with B12 deficiency. Furthermore, HRV parameters were found to be lower as an indicator for impaired cardiac autonomic control. This suggests that high Hcy levels may exert an important cardiovascular and atherogenic risk by affecting endothelial dysfunction, vessel wall changes, biochemical parameters, and impaired cardiac autonomic control.

Several studies have demonstrated that elevated Hcy is an independent risk factor for predisposing to atherosclerosis and endothelial dysfunction.[13-15] Pawlak reported that Vitamin B12 is the most important determinant factor of Hcy in vegetarian studies.[16] Homocysteinemia is a reactive amino acid and initiates premature atherosclerosis by damaging endothelial cells.[17]

CIMT testing is recognized as a valid method for the noninvasive assessment of atherosclerosis. Şahpaz showed a significant increase in CIMT in patients using peritoneal dialysis with high Hcy levels.[18] A dramatic decrease has shown in Hcy levels and CIMT in hemodialysis patients with the administration of 5 mg folic acid, 0.4 mg Vitamin B12, and 50 mg Vitamin B6 after 6 months.[19]

HRV data suggest that an abnormality of autonomic nervous function, in the form of decreased sympathetic and parasympathetic activity. We revealed decreased rMSSD, LF, and HF in patients with B12 deficiency, all of which is in line with decreased parasympathetic modulation of the autonomic nervous system.[7] Pereira et al showed the relationship between CIMT and cardiovagal dysfunction assessed through frequency[20] and time-domain.[21] Morever, previously different studies showed that increased CIMT was associated with a significant decrease in baroreflex sensitivity in prehypertensives, hypertensives, and type 2 diabetic patients.[22,23]

However, we did not demonstrate any differences in LF/HF ratio between two groups. Since HF mainly reflects parasympathetic modulation and LF reflects both sympathetic and parasympathetic influence; LF/HF is a measure of the autonomic nervous system rather than sympathovagal imbalance. The LF/HF ratio is mathematically close to each other, and this parameter does not reflect distinct autonomic phenomenon.[24]

Conclusions

In conclusion, Vitamin B12 deficiency causing hyperhomocysteinemia may be important for the prediction of future CVD. Dietary supplementation with Vitamin B12, folate, and other substances can reduce

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Hcy levels. Such simple measures may reduce the risk of vascular disease in Vitamin B12 deficiency patients.

Study limitations

First, although normal CIMT and HRV values (as based on age) were identified for adult patients, standard ranges according to age are unknown for pediatric populations. Thus, we compared the CIMT and HRV values of children with B12 deficiency with those of a control group. As such, there is a need for more comprehensive, multicenter studies on this topic, particularly featuring larger sample sizes and longer follow-up times. Second, since this was a cross-sectional study, our results relied on data that were obtained at a certain time point. It will be more accurate to observe the effects of Vitamin B12 deficiency on vasculature by monitoring CIMT and on cardiovascular autonomic neuropathy by monitoring HRV over the long term.

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Conflicts of interest

There are no conflicts of interest.

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