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ORIGINAL ARTICLE

Impaired global and segmental myocardial deformation assessed by two-dimensional speckle tracking echocardiography in patients with vitamin B12 deficiency

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Abstract

Background: *Contrary effects of vitamin B12 deficiency have been shown on the cardiovascular system. Aim of our study was to analyze left ventricular (LV) myocardial deformation, by using the two dimensional (2D) speckle tracking echocardiography (STE) in patients with vitamin B12 deficiency and normal LV ejection fraction.*

Methods: *Twenty-five patients with vitamin B12 deficiency (B12 levels < 200 pg/mL; mean age: 29.6 ± 8.2 years, 15 female), and 27 healthy controls (B12 levels > 200 pg/mL; mean age: 30.1 ± 6.9 years, 13 female) were included in the study. 2D echocardiography images were transferred to a workstation for further offline analysis. Longitudinal peak systolic (LPSS) and global strain (LGS) was obtained from 4 chamber and apical long axis (APLAX) views.*

Results: *Standard echocardiographic parameters and tissue Doppler imaging (TDI) velocities were compared between the groups. All LPSS values in the patient group except for apical 4C septal wall longitudinal strain were significantly decreased than those in the control group. There was a positive correlation between B12 levels and strain values except apical 4C septal wall strain values.*

Conclusions: *We found that in patients with vitamin B12 deficiency, global and segmental myocardial deformation was impaired and this impairment was correlated with the levels of vitamin B12.* (Cardiol J 2014; 21, 1: 60–66)

Key words: left ventricular myocardial deformation, speckle tracking echocardiography, strain, vitamin B12

Introduction

Vitamin B12 (cobalamin) which functions as a coenzyme in two metabolic processes — the conversion of methylmalonyl-CoA to succinyl-CoA and the remethylation of homocysteine to methionine — is an essential nutrient for proliferation and metabolism of normal cell activity [1–4]. Vitamin B12 deficiency is common and is one of the major public health problems. The diagnosis of vitamin B12 deficiency has traditionally been based on low serum vitamin B12 levels, usually less than 200 pg/mL

(150 pmol/L), along with clinical evidence of disease. In the case of deficiency, conversion of methylmalonyl-CoA is blocked (inside the mitochondria), which in turn can result in increased lipogenesis [2]. Vitamin B12 deficiency impairs fatty acid oxidation and energy metabolism in heart through epigenomic mechanisms related to imbalanced acetylation/methylation. In addition, decreased vitamin B12 is associated with increased plasma homocysteine levels. High homocysteine level in blood (hyperhomocysteinemia), which is an independent risk factor for atherosclerotic disease, disrupts endothelial functions and causes activation of procoagulant factors, malfunction of anticoagulant mechanisms [5]. Hyperhomocysteinemia has been suggested as a risk factor for cardiovascular disease, blood clotting abnormalities, atherosclerosis, myocardial infarction and ischemic stroke [5].

Further, anemia due to vitamin B12 deficiency may result in heart failure (HF). Low hemoglobin levels in anemia are associated with an elevated cardiac output at rest, cardiomegaly, and, frequently, heart murmurs, due to an increase in stroke volume [6]. It has been postulated that excessive work load chronically imposed on heart by the cardiac output may result in left ventricle (LV) dysfunction. LV diastolic dysfunction occurs earlier in the development of overall cardiac dysfunction, and its presence is an independent risk factor for mortality among adults with anemia [7]. Therefore, it is very likely that B12 deficiency affects cardiovascular system partly through hyperhomocysteinemia and partly through its direct effects.

Echocardiography is an important diagnostic tool for the detection of HF. With the echocardiographic methods developed in recent years decreased heart function can be determined before the occurrence of clinical HF and with the heart's structure remaining yet intact. Two dimensional (2D) speckle tracking echocardiography (STE) has recently emerged as a novel echocardiographic technique for rapid, bedside the analysis of regional LV strains in the longitudinal, radial, and circumferential directions [8]. Subclinical changes in LV function in anemia can be identified by quantifying myocardial strain, a dimensionless measurement of deformation, expressed as a fractional or percentile change from an object's original dimension [9]. 2D STE has been shown to provide complementary information about the clinical assessment of cardiac function [10, 11]. It may assess myocardial function and detect early subclinical myocardial involvement in many heart diseases, as well as quantify regional myocardial function in ischemic heart disease.

Assessment of regional LV function with 2D STE in patients with vitamin B12 deficiency has not yet been studied. The aim of our study was to assess the ability of subtle differences in the LV strain patterns in the longitudinal directions to characterize features of subclinical LV dysfunction in patients presenting with B12 deficiency.

Methods

Study population

Evaluated at different clinics of our hospital, 27 control subjects (group I, 13 female, mean age 30.1 ± 6.9 years; B12 levels > 200 pg/mL) with normal vitamin B12 levels and 25 patients (group II, 15 female, mean age 29.6 ± 8.2 years) with similar characteristics but with vitamin B12 deficiency (< 200 pg/mL) were included in the study. Patients with established coronary artery disease, echocardiography evidence of regional or global wall motion abnormalities, atrial fibrillation, valvular heart disease, diabetes mellitus, hypertension (systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg or drug use for the disease), chronic obstructive airway disease, renal failure, hypertrophic cardiomyopathy, patients receiving replacement therapy with vitamin B12 previously, and anemia for different reasons like folate or iron deficiency were excluded from the study.

Study protocol was approved by Institutional Ethics Review Board and conforms to the principles stated in the Declaration of Helsinki. All the patients were informed about the procedure to be performed, and their written consents were obtained.

Echocardiographic measurements

Echocardiographic examinations were performed in all cases using Vivid E9 Dimension echocardiography device (Vingmed Ultrasound, GE, Horten, Norway) with 3.5 MHz single matrix transducer while the patient was lying in the left lateral decubitus position. All the images were stored in digital media (Echopac Workstation; Vingmed Ultrasound GE) for later measurements. Echocardiographic recordings were obtained from standard parasternal long and short axis, apical long axis (APLAX) and apical 4 chamber (4C) views. Echocardiographic evaluation and measurements were performed according to the guidelines of American Society of Echocardiography [12]. Left atrium, LV end-diastolic and end-systolic, and aortic root dimensions were measured from parasternal long-axis view. The LV

volumes and ejection fraction were obtained by the modified biplane Simpson method [12]. Mitral and tricuspid inflow recordings were taken by pulsed wave Doppler ultrasound. From these recordings diastolic E, and A wave velocities were measured and E/A was calculated.

Tissue Doppler echocardiography

During imaging in the apical 4C view, tissue velocity mode of the device was selected, and pulsed wave Doppler sample volume was placed at the level of lateral mitral rings and TDE images were obtained. Myocardial peak systolic (S), early diastolic (E'), late diastolic (A') velocities were measured [13] and E/E' was calculated.

2D speckle tracking echocardiography

2D echocardiography images were obtained from APLAX and apical 4C views. All images were obtained during breath hold, and stored in cine-loop format from three consecutive beats. The frame rate for images was between 40 and 80 frames/s. All data were transferred to a workstation for further offline analysis. After defining the endocardial border manually, an epicardial tracing was automatically developed by the software system for each view. If the automatically obtained tracking segments were adequate for analysis, the software system was allowed to read the data, whereas analytically inadequate tracking segments were either corrected manually or excluded from the analysis. Strain measurements were reported as the longitudinal peak systolic (LPSS) and global strain (LGS) automatically developed by the software system for APLAX and 4C views.

For the assessment of intra-observer variability, the analyses were repeated twice by the same observer within 1 week. For the inter-observer variability assessment, a second independent observer repeated the analyses.

Statistical evaluation

For statistical evaluation SPSS 11.5 (SPSS Inc. Chicago USA) statistical software program was used. Continuous variables were expressed as means ± SD, and categorical variables as percentages. One sample Kolmogorov-Smirnov test was used for the normality test of all variables. For the comparison of parametric variables independent sample t test, and for categorical variables χ^2 test were used. Binary logistic regression analysis was performed to identify the independent predictors of strain values in the groups. Standardized β-regression coefficients and their significance from logistic regression analysis were reported. Correlations between variables were tested by Pearson correlation tests. Linear regression analysis was performed to identify the independent predictors of global strain by including the parameters which were correlated with the global strain in bivariate correlation analysis. Standardized β-regression coefficients and their significance from linear regression analysis were reported. In all assessments 2-sided $p < 0.05$ was accepted as statistically significant.

Results

A total of 50 patients with B12 deficiency were evaluated. Seven patients who did not have a good echocardiographic window or analytically inadequate tracking segments, 4 patients with cardiac involvement, 3 patients with arrhythmia and 11 patients with additional iron or folate deficiency were excluded from the study. A total of 25 patients with B12 deficiency and 27 sex- and age-matched controls were included in the study. Baseline clinical and demographic characteristics of both groups and their comparisons are summarized in Table 1. There were no statistically significant differences between the two groups with regard to age, gender, smoking, systolic/diastolic blood pressure and hyperlipidemia. There was statistically significant difference between the groups regarding B12 levels (264.85 ± 50.24 pg/mL in group I and 136.44 ± 31.95 pg/mL in group II, $p < 0.001$) (Table 1). All the laboratory characteristics between groups except for B12 levels were also similar. Standard echocardiographic parameters and tissue Doppler imaging (TDI) velocities were comparable between the groups (Table 2). All LPSS values in the patient group except for apical 4C septal wall longitudinal strain were significantly decreased co pared to those in the control group (Table 3). There was a positive correlation between B12 levels and strain values except for septal wall strain values (Table 4). There was no correlation of LPSS and LGS values with other demographic, clinical and laboratory variables ($p > 0.05$ for all) (data not shown). Independent predictors of plasma B12 levels were determined with linear regression analysis by including the parameters that were correlated with the strain values in correlation analysis and neither of the strain values was detected as an independent predictor of plasma B12 levels (Table 4).

Table 1. General characteristics in controls and patients.

	Controls (n = 27)	Patients (n = 25)	P
Age [years]	30.1 ± 6.9	29.0 ± 8.0	0.808
Female sex	13 (48%)	15 (60%)	0.419
BMI [kg/m^2]	25.7 ± 4.4	27.8 ± 6.1	0.165
SBP [mm Hg]	115.0 ± 16.5	113.8 ± 18.5	0.854
DBP [mm Hg]	71.7 ± 11.1	71.7 ± 13.0	1.000
Dyslipidemia	6 (22%)	3 (12%)	0.330
Smoking	11 (40%)	6 (24%)	0.238
Fasting blood glucose [mg/dL]	94.6 ± 20.5	98.3 ± 20.5	0.516
Blood urea nitrogen [mg/dL]	26.1 ± 8.7	22.0 ± 6.6	0.064
Serum creatinine [mg/dL]	0.74 ± 0.19	0.65 ± 0.17	0.093
Hemoglobin [g/dL]	14.3 ± 1.7	13.3 ± 2.2	0.059
Mean corpuscular volume [fL]	84.9 ± 4.7	83.1 ± 3.7	0.141
Platelet count [$10^3/\mu L$]	244.3 ± 53.4	242.1 ± 65.3	0.895
White blood cells count [$10^3/\mu L$]	9.63 ± 2.85	9.49 ± 3.65	0.884
Serum levels of vitamin B12 [pg/mL]	264.9 ± 50.2	136.4 ± 32.0	**< 0.001**

Statistically significant results are given in **bold**; BMI — body mass index; SBP — systolic blood pressure; DBP — diastolic blood pressure

Table 2. Standard echocardiographic parameters in controls and patients.

	Controls (n = 27)	Patients (n = 25)	P
LVEDV [mL]	100.4 ± 18.4	102.9 ± 26.6	0.699
LVESV [mL]	31.0 ± 8.4	32.6 ± 11.9	0.582
Stroke volume [mL]	69.4 ± 13.4	70.2 ± 17.6	0.862
LVEF [%]	69.2 ± 5.4	68.4 ± 7.0	0.652
Fractional shortening [%]	39.0 ± 4.4	38.4 ± 5.8	0.655
Mitral E peak velocity [cm/s]	88.5 ± 15.4	85.8 ± 14.7	0.522
Mitral A peak velocity [cm/s]	64.2 ± 15.9	65.8 ± 11.0	0.688
Mitral E/A ratio	1.44 ± 0.34	1.33 ± 0.27	0.204
Tissue Doppler, lateral S [cm/s]	11.5 ± 2.3	12.2 ± 2.6	0.294
Tissue Doppler, lateral E' [cm/s]	17.4 ± 2.4	16.3 ± 3.3	0.184
Tissue Doppler, lateral A' [cm/s]	9.63 ± 2.45	9.29 ± 2.56	0.633
E/E' ratio	5.15 ± 0.95	5.40 ± 0.95	0.385

LVEDV — left ventricular end-diastolic volume; LVESV — left ventricular end-systolic volume; LVEF — left ventricular ejection fraction

Discussion

For the first time, our study evaluated LV myocardial functions in patients with B12 deficiency by 2D STE in addition to conventional echocardiographic examination. We found that global and segmental longitudinal myocardial deformation was impaired and this impairment was correlated with the levels of vitamin B12 in patients with B12 deficiency, though we could not reveal independent association of vitamin B12 levels and strain values in the linear regression analysis (Table 4).

Inadequate dietary intake, inability to absorb from the intestinal tract, and metabolic and transport disorders which result from being unable to use the vitamin may cause the lack of B12. The incidence of B12 deficiency appears to increase with age. HIV-infected and vegetarians who are not taking in proper amounts of B12 are also prone to deficiency [3]. We do not know the reason for vitamin deficiency in this younger age group in our study, though absorption disorders associated with genetic factors, bacterial infections and parasitic infestations would be plausible mechanisms.

In our study we found that all LPSS values in the patient group except for apical 4C septal wall longitudinal strain were significantly decreased compared to those in the control group, and there

Table 3. Comparison of left ventricle longitudinal peak systolic and global strain values in controls and patients and regression analysis between the groups.

	Controls (n = 27)	Patients (n = 25)	P	Beta regression coefficient	P
LPSS, apical 4C, lateral wall	−19.90 ± 4.53	−13.86 ± 4.58	**< 0.001**	−0.217	0.187
LPSS, apical 4C, septal wall	−20.19 ± 3.75	−19.88 ± 3.35	0.753		
LGS, apical 4C	−20.58 ± 3.23	−17.45 ± 3.58	**0.002**	−193.984	0.999
LPSS, APLAX, posterior wall	−20.09 ± 3.77	−16.92 ± 3.85	**0.004**	−0.223	0.423
LPSS, APLAX, septal wall	−20.41 ± 3.02	−17.02 ± 5.16	**0.005**	−0.377	0.088
LGS, APLAX	−20.80 ± 2.94	−17.62 ± 3.56	**0.001**	−193.689	0.999

Binary logistic regression analysis was performed to identify the independent predictors of strain values; standardized β-regression coefficients and their significance from logistic regression analysis were reported; statistically significant results are given in **bold**; $p < 0.05$ was considered statistically significant; LPSS — longitudinal peak systolic strain; 4C — four chamber; LGS — longitudinal global strain; APLAX — apical long axis

Table 4. Correlation and regression analysis between B12 levels and strain values.

	Pearson correlation coefficient	P	Beta regression coefficient	P
LPSS, apical 4C, lateral wall	0.457	**0.001**	5.235	0.077
LPSS, apical 4C, septal wall	−0.008	0.953		
LGS, apical 4C	0.341	**0.013**	6.564	0.512
LPSS, APLAX, posterior wall	0.301	**0.032**	5.235	0.077
LPSS, APLAX, septal wall	0.356	**0.010**	8.226	0.078
LGS, APLAX	0.368	**0.007**	114.36	0.086

Pearson correlation coefficients were used for comparing continuous variables and strain values; linear regression analysis was performed to identify the independent predictors of strain values; standardized β-regression coefficients and their significance from linear regression analysis were reported; statistically significant results are given in **bold**; $p < 0.05$ was considered statistically significant; LPSS — longitudinal peak systolic strain; 4C — four chamber; LGS — longitudinal global strain; APLAX — apical long axis

was a positive correlation between B12 levels and strain values except apical 4C septal wall strain values. Further prospective studies with larger sample size are needed to elucidate the mechanism of non-uniform myocardial deformation of the absence of serum vitamin B12 levels and interventricular septum strain association.

LV myocardial dysfunction, even though subclinical, is associated with worse prognosis because of leading to increased risk of cardiovascular complications detected in various diseases [14, 15]. Since standard echocardiography, with the assessment of LVEF and pulse wave Doppler mitral inflow parameters is often inadequate to identify early myocardial abnormalities, other diagnostic tools are needed in order to detect the onset of cardiac involvement [16–18]. With this respect, TDI and, more recently, 2D strain, have been shown to provide a more accurate evaluation of myocardial function. Tissue velocity measurements by TDI at the level of mitral and tricuspid annulus have been used for the diagnosis and monitoring of many cardiac diseases with higher sensitivity, and specificity [13, 19]. However, Doppler-only-based techniques are limited due to angle dependence of the signal and TDI has a limited utility in the segmental and regional evaluation of myocardial functions because of technical drawbacks. 2D STE techniques overcome some of these limitations by allowing tracking in any direction within the 2D image. Computerized 2D STE is a new method that provides quantitative data during evaluation process of segmental, regional, and global LV functions with apparent advantages over other echocardiography-based methods used in daily practice for LV systolic or diastolic function analysis with regard to angle independency and temporal and spatial resolution, notwithstanding 2D STE is inferior to tissue velocity imaging derived strain [20–22]. Our findings support these

data as we observed impaired longitudinal peak systolic strain in spite of preserved and comparable echocardiographic parameters including TDI velocities between the groups. However, the mechanism for spared diastolic function in case of affected systolic myocardial function in vitamin B12 deficiency remains to be established though we have not evaluated diastolic functions by 2D STE which was beyond our aim and it may be one of the limitations of our study.

Several studies have been reported on the impact of vitamin B12 on cardiovascular system. Taban-Shomal et al. [23] studied "the cardiac effects of prolonged vitamin B12 and folate deficiency in rats". They found that fractional shortening, LV dimension at end-diastole and end-systole, posterior wall thickness, perivascular collagen, mast cell number, and natriuretic peptide tissue levels were comparable in vitamin deficiency and control animals. Interstitial collagen, plasma natriuretic peptides, and tissue homocysteine levels were decreased in vitamin deficient animals. They concluded that vitamin B12 deficiency did not affect cardiac function and morphology. There is only very limited evidence that vitamin B12 deficiency predisposes to the risk of mortality and morbidity from cardiovascular diseases in adults. Conflicting effects of vitamin B12 deficiency on the cardiovascular system have been shown [24–29]. Looker et al. [25] and Zeitlins' et al. [26] studies showed an untoward effect of vitamin B12 that increased vitamin B12 levels were positively associated with increased total mortality and combined fatal and non-fatal cardiac disease. In another study there was no association between B12 concentrations and death from coronary heart disease or cardiovascular disease in a cohort without any cardiovascular disease at baseline [24]. Though our study is not a cardiovascular outcome study, with the findings of the present study we have revealed that the decreased serum vitamin B12 level is associated with subtle cardiac involvement evidenced with impaired strain values. Despite the link between the presence of vitamin B12 deficiency and strain values and significant association of serum vitamin B12 levels and strain values, we could not reveal independent association between the above-mentioned parameters. Several issues might have led to the absence of this independent association such as young study population (30.1 ± 6.9 years in group I and 29.6 ± 8.2 years in group II) with decreased overall cardiovascular risk, duration of vitamin B12 deficiency (which might be fairly short in our young study population), and small sample size. Gueant Rodriguez et al. [30] showed that

homocysteine predicts increased natriuretic peptides which are quantitative markers of cardiac dysfunction through a link with impaired mitochondrial fatty oxidation. Abnormally elevated plasma natriuretic peptides therefore provide independent evidence that a patient has cardiac dysfunction in spite of preserved LV ejection fraction.

Limitations of the study

One of the limitations of our study was that homocysteine or methylmalonic acid levels which would add to the value of our manuscript and associated with true low B12 levels were not investigated. Additionally, it should be noted that the present study had a relatively small sample size and larger sample size is needed to confirm/exclude the findings of the study. Furthermore, the apical 2 chamber views were inadequate to analyze in our imaging archive. Therefore, we could not analyze them and we could not calculate the global strain which was based on averaging three apical views traditionally. Since longitudinal strain values were more sensitive than other deformation values like radial or circumferential, we only studied the longitudinal strain. However, it would be better to show radial or circumferential deformations beside longitudinal fibers. Despite these limitations, this study brings attention to a novel, important hypothesis at the forefront of cardiology research by showing an association between LV myocardial deformation and B12 levels and also by determining subclinical LV dysfunction at an early stage using 2D strain as diagnostic tool.

Conclusions

Consequently, we detected that myocardial strain values were decreased in patients with vitamin B12 deficiency. Further prospective studies with longer follow-up periods and larger sample size are needed to confirm/exclude the findings of this study and to further elucidate its clinical importance.

Conflict of interest: none declared

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