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Original Article

THE IMPACT OF COBALAMIN DEFICIENCY ON HEART FUNCTION; A STUDY ON ABNORMALITIES IN ELECTROCARDIOGRAPHY PATTERNS

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ABSTRACT

Objective: Cobalamin deficiency may cause a lack of dietary methyl donors, which alter the heart metabolism. Cobalamin deficiency is common in patients with malnutrition, gastric ulcers, diabetes mellitus, and alcoholism. Most studies on cobalamin deficiency are focused on its relationship with oxidative stress and atherogenesis. Therefore, this study aims to find the correlation between cardiomyocyte's energy metabolism in cobalamin deficiency and the risk of heart abnormalities through analysis of electrocardiography (ECG) patterns.

Methods: Adult male Sprague-Dawley rats (aged 24-28 w) were divided into 2 groups: the control group and cobalamin-deficient group. The control group was given standard diet while the treatment group received a modified diet, type AIN-93M (deficient in cobalamin), for a period of 16 w. ECG was performed in both groups on the last day of the 16-week period. Enzyme-linked immunosorbent assay (ELISA) test was also performed to evaluate plasma Hcy and B12 levels in each group at the end of the treatment period.

Results: At the end of the 16-week period, higher Hcy level and lower plasma B12 level were observed in the treatment group when compared to the control group. ECG patterns showed sinus rhythms in both groups, with a higher QRS amplitude and duration in the treatment group. Two of the seven rats in the treatment group developed cardiac arrhythmia.

Conclusions: Cobalamin deficiency impairs the heart's energy metabolism with left ventricular enlargement and arrhythmia.

Keywords: Cobalamin deficiency, ECG abnormality, arrhythmia

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INTRODUCTION

Heart failure (HF) is the terminal stage in cardiovascular disease, marked by the inability of the heart to pump blood with normal efficiency.1 In Asia, mortality and morbidity due to heart failure (HF) is still high [2]. In the ADHERE-AP (Asian-Pacific) Study, it was reported that HF patients registered in South-East Asia were generally younger (median age of 60 y for Indonesia) as compared with those in East Asia (median age of 77 y for both Hongkong and Taiwan) and in Australia (median age of 77 y). According to the data from Indonesia Basic Health Research (RISKESDAS) 2013, the prevalence of HF in Indonesia is at 0.13% for the total population of approximately 229, 696 people [1].

Most studies on cobalamin deficiencies are still focused on its relationship with oxidative stress and atherogenesis. Recent studies have shown the many classic risk factors for HF, such as smoking, hypertension, dyslipidemia, obesity, and diabetes mellitus [3]. One of the risk factors for HF is dietary intake imbalance. A recent study in nutrition and epigenomic/nutrigenomic reveald that some micronutrients are related to cardiovascular metabolism through epigenetic processes [4]. The micronutrients that may be involved in epigenetic processes are called methyl nutrients such as cobalamin/vitamin B12 [4, 5]. Methyl nutrients act as a source of methyl donors needed for DNA methylation, chromatin modification and various enzymatic reactions [4]. These epigenetic processes affect the activity in mitochondrial cardiomyocytes which regulates the heart's energy metabolism [6, 7]. Thus, deficiency in cobalamin may lead to lack of dietary methyl donors, which will alter the heart metabolism [6, 7]. Cobalamin deficiency may also contribute to an increased plasma Hyc level (Hyc)/hyperhomocysteinemia [8]. Solomon *et al.* [8] observed that high levels of Hyc are related to oxidative stress, dyslipidemia, and atherogenesis. The prospective Framingham Heart Study observed that the incidents of congestive heart failure (CHF) almost double in individuals with a circulating Hyc concentration above the sex-specific median [9]. These conditions also may contribute to the risk factors and severity of HF [10].

The prevalence of cobalamin deficiency is high (about 20%) in many developing countries especially in Asia [11]. The populations susceptible to cobalamin deficiency are the elderly, pregnant and breastfeeding women, children, and young adults [11, 12]. In Indonesia, although there is no precise data about the prevalence of cobalamin deficiency, the risk is still high because most of its population of a low grade socio-economic status. This is related to the ability to consume meat and other foods with cobalamin content. The risk of B12 deficiency is also high in patients with chronic gastric ulcers who take a proton pump inhibitor as long-term therapy [13]. A recent study in endocrinology shows that patients with type 2 diabetes mellitus who use metformin as an oral hypoglycemic agents, especially at a higher dose and for a long duration, have developed a risk of cobalamin deficiency [14]. Functional cobalamin deficiency along with megaloblastic anemia is also found in alcohol-dependent patients [15].

This study aims to assess the correlation between cardiomyocyte's energy metabolism in cobalamin deficiency and the risk of heart abnormalities through analysis of ECG patterns. We hypothesized that vitamin B12 deficiency cause's hyperhomocysteinemia and induces abnormality of the heart's electrical activity.

MATERIALS AND METHODS

Animals

All experimental procedures using animals were approved by the medical research committee of Faculty of Medicine, Universitas Indonesia (219/UN2. FI/ETIK/2017).

Fourteen adult male Sprague-Dawley rats aged 24-28 w (provided by The Research and Development division, Ministry of Health, Republic of Indonesia) were divided into 2 groups: the control group (C; n = 7) and cobalamin-deficient group (D; n 7). Both groups were maintained on a 12 light-dark cycle with free access to food and water. During a 16-week study period, the control group was given a standard diet type, AIN-93M (Harlan Teklad, USA) normal formula

while the treatment group received a modified diet based on the same food, AIN-93M but with cobalamin limited. To inhibit unintentional uptake of cobalamin, 5% per kg pectin was added to the modified deficient diet. The rats body weight and food consumption were monitored every month (every 4-week period).

ECG

ECG is a process to record the heart's electrical activity in a period of time using electrodes on the skin [16]. These electrodes detect tiny changes in electrical currents from the heart muscles (depolarization and repolarization) during the heart's contraction [16]. Abnormality in the heart's energy metabolism will result in abnormal electrical conduction, which is expressed as an abnormality in ECG patterns.

ECG was recorded in both groups on the last day of the 16-week period. Before the procedure was carried out, the rats were given intraperitoneal anesthesia with a combined dose of ketamine (100 mg/kg) and xylazine (5 mg/kg). After anesthetization the rats were restrained on a square board using a small micropore adhesive tape. Electrodes used were acupuncture needles placed subcutaneously on four extremities, on the middle area of the chest and at the upper left axillary region (these ECG points represent lead measurements: I, II, III, aVL, aVR, aVF, V1, and V6). The ECG device used was type BTL-08 MT Plus (USA).

Sample measurements

Blood samples were taken at the beginning and end of the 16-week period. Blood was collected from the *retrobulbar plexus/sinus orbitalis* using a specialized cannula and then stored in the vacuette/vacutainer, which contained Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Centrifugation was performed immediately at a speed of 3000 rpm for 15 min at room temperature and then plasma was collected by pipetting into a 1.5 ml microtube. The samples were stored at-80 °C until further analysis was performed.

Plasma Hcy and cobalamin levels were quantified by *direct competitive* Enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies against Hyc and cobalamin, respectively. The reagents used were MBS766209 and MBS731816 (MyBioSource, USA). Intra and inter-assay CVs of the Hcy ELISA were<8% and<10%, respectively. Both CVs of the B12 ELISA were<10%. Quality control materials recommended by the manufacturers were used for all samples.

Statistical analyses

Body weight, plasma Hcy, plasma B12, the heart rate, and RS amplitude were analyzed using a t-test (independent t-test for analysis between groups and paired t-test for analysis within a group). The corrected QT interval (QTc) and QRS-T angle were analyzed using a Mann-Whitney test. All data were analyzed with SPSS 21.0 statistic software for Windows (SPSS Inc., USA).

RESULTS

Table 1: Experimental group characteristics at the beginning and end of the study

Variable	Control		Deficient	
	C_0	C16	D_0	D16
Number of samples	7	7	7	7
Body weight (g)	295.71±6.50	379.43±18.28[a]	303.29±10.56	363.00±17.94[b]
Hcy (µmol/l)	250.14±27.81	253.07±28.16	416.80±209.77	842.90±373.58[b,c]
B12 (ng/l)	425.60±68.46	405.51±67.43[a]	654.24±133.88[c]	388.26±113.29[b]

Data are mean±SD. [a] **p<0.001 vs C_0; [b] **p<0.01 vs D_0; [c] **p<0.01 vs C_{16}

Body weight

At the beginning of the study, the body weight of both the control group and treatment group was statistically homogenous. During the 16-week feeding period, both groups exhibited a significant gain in body weight (C0 vs C16, **p<0.001; D0 vs D16, **p<0.001) but no significant weight difference was observed between groups (table 1).

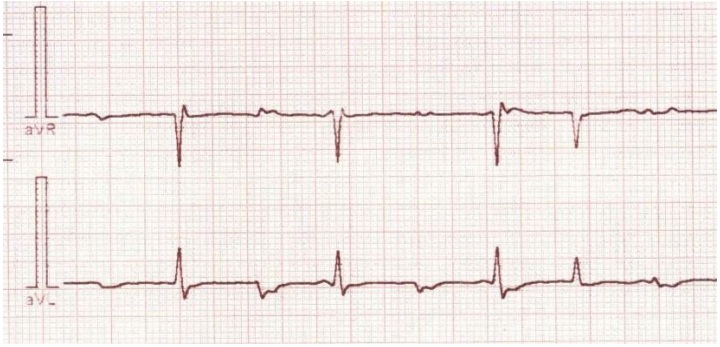
Plasma Hcy and B12 levels

All animals in the treatment group showed a significant increase in plasma Hcy concentration (D16 vs D0, p<0.01; D16 vs C16, **p<0.01) (table 1) and a significant decrease in plasma B12 concentration (D16 vs D0, **p<0.001) compared to the control group. This condition shows the efficacy of the vitamin deficient model used in this study. Nevertheless, the control group also had a significant decrease in plasma B12 levels (C0 vs C16, **p<0.001).

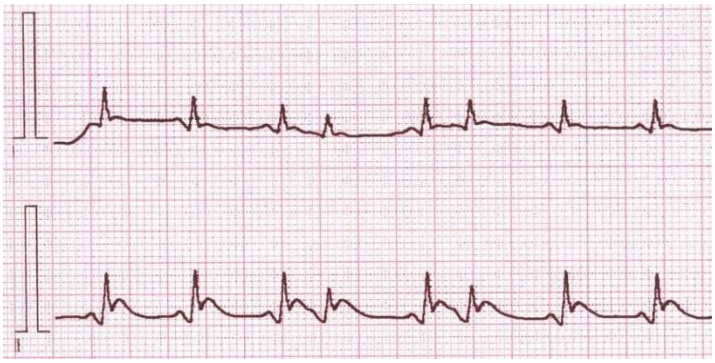
Table 2: Characteristics of ECG after 16 w of treatment

Variable	Control	Deficient
	(C)	(D)
Heart rate (pulse/min)	255.89±38.15	264.76±38.51
QTc (ms)	0.10±0.01	0.16±0.02*
RS Amplitude (mV)	1.11±0.45	2.38±1.47
QRS-T angle (degree)	19.84±23.13	58.71±50.59*

Data are mean±SD. *p<0.05 vs C



Rat 3



Rat 7

Fig. 1: Arrhythmia in the treatment group

ECG

In this study, four parameters of ECG that represented the activity of left ventricle were examined: (1) Heart Rate, (2) QTc, (3) RS amplitude, and (4) QRS-T angle. After a 16-week B12 deficient period, no significant increase in the heart rate was observed, whereas the QTc significantly increased (D vs C, *p<0.05), the RS amplitude increased, although significantly, and the QRS-T angle significantly increased (D vs C, *p<0.05) (table 2). We also observed that two of the seven rats in the treatment group develop cardiac arrhythmias (fig. 1).

DISCUSSION

The results from this study show that weight gain in both the control group and treatment group after a 16-week study period was consistent with normal growth and development in average Sprague-Dawley rats. Increasing body weight is closely related to advancing age (from "weaning" to "old") until the rats enter a senescence period, when the body mass starts to decrease [17, 18]. According to the results in this study, feeding rats with either a standard or cobalamin-deficient diet did not affect their weight gain.

Cobalamin is a cofactor that acts as a methyl donor for methionine synthase in the methionine cycle [4, 5, 19]. Cobalamin deficiency might impair conversion of Hyc to methionine. Thus, the concentration of Hyc will increase [5, 19]. In this study, we found a statistically significant increase in plasma Hcy concentrations both within the treatment group and between the two groups. These findings are consistent with previous studies on cobalamin which show that deficiency in cobalamin will induce hyperhomocysteinemia [20, 21].

Measuring plasma or serum cobalamin levels is a gold standard method to determine if a patient is suffering from cobalamin deficiency [22, 23]. There are many causes of cobalamin as such as a *Helicobacter pylori* infection, low dietary intancbike, gastric malabsorption, and genetic disorders that may result in a low plasma cobalamin concentration [22]. In this study, we found that plasma B12 concentrations in the treatment group was significantly decreased. This result is also consistent with previous studies, which states that one of the causes of cobalamin deficiency is low dietary intake and malabsorption (considering the addition of 5% pectin to inhibit unintentional uptake of cobalamin). Meanwhile within the control group, plasma B12 concentration also significantly decreased. This result seemed contradictory because the diet given was standard with no cobalamin deficiency. Even though the plasma B12 levels between the treatment group and the control group was not statistically significant at the end of the treatment, the plasma B12 levels in the treatment groups were still lower than those of the control group (plasma B12 levels in the control group decreased 20.11 pg/ml, while plasma B12 levels in the treatment group decreased 265.98 pg/ml).

ECG was performed to assess the heart's electrical activity. There were four parameters examined in this study. The first parameter was heart rate, in which there was no significant difference between the control group and treatment group. It seems that, diet does not affect heart rate specifically. The normal range of heart rate in adult Sprague-Dawley rats is 330-480 beats/min [24]. In this study, the heart rate in both groups was lower than normal. These findings are consistent with a previous study, in which a combination dose of ketamine-xylazine (±100 mg/kg and±5 mg/kg) decreased the heart rate [24]. Two of the seven rats in the treatment group developed cardiac arrhythmia. These findings are in line with another study, in which high levels of Hcy/hyperhomocysteinemia impaired the heart's electrical conductivity [25, 26].

The second parameter examined was the QTc. QTc is a measure of the combination of cardiac depolarization and repolarization as it encompasses both the QRS complex and the J-T interval [27]. Ventricular conduction delay is also often associated with (to a lesser degree) lengthening of the QT interval [27]. This study shows that QTc interval in the treatment group was significantly higher when compared to the control group. We may suggest that in the treatment group, the process of depolarization-repolarization was disturbed and might have resulted in delayed ventricular conduction. The most probable explanation was that high levels of Hyc induced the heart remodeling process. Delayed ventricular conduction is the first sign to appear in the heart muscle hypertrophy. This study result supports the pathophysiologic mechanism in cardiac hypertrophy related to hyperhomocysteinemia [28, 29].

The third parameter examined was the RS amplitude. The RS amplitude is an integral part of the QRS complex and it represents electrical activities in the heart's ventricles (especially left ventricle) [30]. Previous studies stated that the RS amplitude is closely related to the risk of developing hypertrophic cardiomyopathy; the higher the RS amplitude, the higher the risk of developing hypertrophic cardiomyopathy [30]. We did not observe any significant increase in the RS amplitude in the treatment group. However, we observed the tendency of increasing RS amplitude in the treatment group. According to this phenomenon, we believe the treatment group was in the process of developing minor hypertrophic cardiomyopathy. Future research with a longer study period is needed to confirm this theory.

The fourth parameter examined was the QRS-T angle. By ECG, one can measure a spatial angle between depolarization and repolarization, specifically an angle between the QRS vector and T vector, namely spatial QRS-T angle [31]. In previous studies, QRS-T angle has been shown to predict ventricular arrhythmia [32]. In this study, the treatment group showed a significant increase in the QRS-T angle. This result is in line with a previous study, as evident in the arrhythmia developed by two of the seven deficient rats. The probable mechanism in this finding is related to hyperhomocysteinemia-induced the heart electrical impairment and is closely related to the possibility of cardiac hypertrophy. In conclusion, this study showed that cobalamin deficiency impairs the heart's energy metabolism with left ventricular enlargement and arrhythmia.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

No potential conflicts of interest relevant to this article were reported.

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