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Association between Blood Copper Levels and the Incidence of Ischemic Heart Disease

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Abstract

BACKGROUND: Ischemic heart disease is one of the interrelated disease among cardiovascular disease group. Pathophysiological model of ischemic heart disease and myocardial ischemia is caused by obstructive atherosclerotic plaque, which involves the narrowing of small blood vessels that oxygenate the heart muscle by the build-up of plaque. Diet plays an important role in ischemic heart disease. Copper, an essential trace metal micronutrient, is required for myocardial angiogenesis action. Copper deficiency leads to cardiac mitochondrial structural defect and interference in oxidative phosphorylation.

AIM: This study aims to examine the association between blood copper levels and the incidence of ischemic heart disease.

METHODS: A total of 30 patients in cardiovascular clinic in Universitas Sumatera Utara Hospital in Medan, Indonesia from September 2021 to January 2022 were included in this cross-sectional study, with descriptive analytics. Demographic data, smoking behavior, supplement consumption, anthropometry measurements, body mass index, and medical history were collected. Food frequency questionnaire (semiquantitative FFQ) was used to obtain food recall data. Blood level of copper was analyzed in Prodia Clinical Laboratory.

RESULTS: Out of 30 patients in this study, 70% were male with a mean age of 60.6 years old. Research subjects who had risk factor of smoking were as much as 33.3%. Comorbidities such as dyslipidemia and diabetes mellitus were apparent, which were 63.3% and 30%, respectively. Sixty percent of the subjects were sedentary with mean body mass index 25.9 kg/m^2. Median level of copper consumed daily was 1400 mcg/day and mean blood copper level was 1034,5 mg/L. Based on the blood copper level analysis of the subjects, we found an insignificant negative correlation between blood copper level with the incidence of ischemic heart disease ($r = –0.050$; $p < 0.795$).

CONCLUSION: This study found no association between blood copper levels and the incidence of ischemic heart disease.

Introduction

Over the past decade, cardiovascular disease (CVD) has emerged as the single most important cause of death worldwide. In 2010, CVD caused an estimated 16 million deaths and led to 293 million disability-adjusted life-years (DALYs) lost – accounting for approximately 30% of all deaths and 11% of all DALYs lost that year [1]. The East Asia and Pacific region is the most populated low-income and middle-income region in the world, with nearly 2 billion people; approximately 49% of the region is urban. The region is divided into three distinct sub-regions: Southeast Asia, East Asia, and Oceania [2]. CVD caused more than 4.5 million deaths in the EAP region in 2010, accounting for 35.2% of all deaths in the region. More than half of those deaths resulted from ischemic heart disease, whereas only 31% were due to stroke. In Southeast Asia and East Asia, CVD accounts for the largest percentage of total DALYs lost in the regions (26 million and 67 million, respectively) [3].

Cardiovascular disease (CVD) is a group of interrelated diseases that include atherosclerosis, hypertension, ischemic heart disease, peripheral vascular disease, and heart failure. Atherosclerotic cardiovascular disease (ASCVD) involves the narrowing of small blood vessels that oxygenate the heart muscle by the build-up of plaque. The plaque, known as atherosclerosis, can rupture, causing a blood clot to form that blocks the artery or travels somewhere else in the body, causing blockage at that site. The result can be a myocardial infarction (MI), which is also called a heart attack or stroke [4].

The causes, associations, and effects of heart disease are multi-factorial in origin and include nutrition and genetics. The literature reveals an increased incidence of heart disease in subpopulations with risk factors such as increased age, male gender, high serum cholesterol or triglyceride level, use of tobacco products

or oral contraceptives, high systolic and/or diastolic blood pressures, diabetes, family history, obesity, gout, premature arcus cornea, and a diagonal ear lobe crease [5].

Acute ischemic injury is often associated with myocardial inflammatory response, myocyte apoptosis, and cardiac arrhythmia. Chronic ischemia leads to further injury, including myocardial infarction, remodeling, and contractile dysfunction. In both acute and chronic condition, the trigger is reduced vital supplies including oxygen in the coronary circulatory system. The initial molecular response of myocardial tissue to the reduction in oxygen supply is the accumulation of hypoxia-inducible factor-1a (HIF-1a) [6], [7], [8] and HIF-2a [9], [10], [11], [12]. HIF-1 a dimerizes with HIF-1b or ARNT (aryl hydrocarbon receptor nuclear translocator) to make up the master transcription factor HIF-1 that regulates a variety of genes involved in cellular metabolism and function [13], [14]. Of these, genes are those involved in angiogenesis and arteriogenesis [15]. The coordination of these gene products would increase the density of capillaries to counteract the deficiency in oxygen supply.

Copper is an essential trace metal micronutrient, less abundant than other metals such as iron and zinc, but widely utilized as a catalytic or structural cofactor by enzymes and proteins that are highly relevant to cardiac physiology and pathology. Copper regulates various important biological processes, including mitochondrial oxidative phosphorylation, iron mobilization, connective tissue crosslinking, antioxidant defense, melanin synthesis, blood clotting, and neuron peptide maturation [16]. It has long been known that copper is essential for cardiac metabolism and function [17], [18], [19]. Copper deficiency leads to cardiac mitochondrial structural defect and disturbance in oxidative phosphorylation [20], [21], and cardiac hypertrophy eventually leading to heart failure [22], [23], [24]. Epidemiological studies have identified the link between copper deficiency and ischemic heart disease [25], [26], [27]. Molecular and cellular mechanisms of action of copper in cardiac metabolism and function have been extensively explored [28], although comprehensive understanding remains elusive.

Copper is required for HIF-1 transcriptional activity. Deprivation of copper from cells leads to suppressed expression of vascular endothelial growth factor (VEGF) and other genes that are regulated by HIF-1, although the accumulation of HIF-1a is not affected under hypoxic condition. There are multiple sites that potentially require copper for activation of HIF-1, including HIF-1a synthesis, stabilization, translocation from cytosol to nucleus, binding to the HRE sequence of target genes, and HIF-1 transcriptional complex formation [29].

Methods

This cross-sectional descriptic analysis study included 30 consecutive patients age ≥ 18 years old (both men and women) in cardiovascular clinic in Universitas Sumatera Utara Hospital in Medan, Indonesia from September 2021 to January 2022. This research was carried out after obtaining approval from the USU Medical Research Ethics Commission. Patients were asked to answer questionnaire that would provide information about demographic data, smoking behavior, supplement consumption, and medical history. Food frequency questionnaire (semiquantitative FFQ) was used to obtain food recall data. After completing questionnaires, patients underwent history taking, physical examinations, and ECG recording. Anthropometry measurements, including body weight, body height, and waist circumference, were performed twice and the data acquired was the average of the each measurement results. The results of these measurements are used to determine body mass index (BMI). All patients had their blood samples drained by analyst, which then collected and analyzed for the level of copper in Prodia Clinical Laboratory.

Statistical analysis

All statistical analyses were carried out using the SPSS statistical software, version 20. Data were presented in univariate and bivariate configuration. Analytical test was performed using Spearman Correlation Test.

Results

We enrolled 30 patients whom complied to both inclusion and exclusion criteria and signed for informed consent. The characteristics of the subjects evaluated consisted of age, gender, occupation, and level of education. Majority of the subjects were male with a mean age of 60.6 years. Occupations of the subjects were housewives (23.3%), retired lecturers (10.0%), civil servants (13.3%), entrepreneurs (46.7%), private employees (3.3%), and farmers (3.3%). Level of the education varied from elementary school (10.0%), junior high school (6.7%), high school (50.0%), diploma (3.3%), undergraduate (23, 3%), graduate (3.3%), and postgraduate (3.3%). Research subjects who had risk factor of smoking were as much as 33.3%. Comorbidities such as dyslipidemia and diabetes mellitus were apparent, which were 63.3% and 30%, respectively (Table 1).

Analysis on nutritional status of the research subjects (Table 2) showed mean weight was 66.3 kg,

Table 1: Characteristics of research subjects

Variable	n = 30	p value
Age, year	60.6 ± 8.0	0.2821
Gender, n (%)		
Male	21 (70,0)	
Female	9 (30,0)	
Occupation, n (%)		
Housewives	7 (23,3)	
Retired lecturers	3 (10,0)	
Civil servants	4 (13,3)	
Entrepreneurs	14 (46,7)	
Private employees	1 (3,3)	
Farmers	1 (3,3)	
Level of education, n (%)		
Elementary school	3 (10)	
Junior high school	2 (6,7)	
High school	15 (50,0)	
Diploma	1 (3,3)	
Undergraduate	7 (23,3)	
Graduate	1 (3,3)	
Postgraduate	1 (3,3)	
Expense, Rupiah	3.000.00,00 (1.000.000,00–8.000.000,00)	0.001
Activity Levels, n (%)		
Sedentary	18 (60,0)	
Rather active	12 (40.0)	
Smoking, n (%)		
Active smoker or ex-smoker (<2 years)	10 (33.3)	
Non-smoker or ex-smoker (> 2 years)	20 (66.7)	
Comorbidities		
Dyslipidemia	19 (63.3)	
Diabetes Mellitus	9 (30.0)	
Blood copper level, ug/L	1034.5 ± 168.6	0.8071

[1]Shapiro–Wilk.

mean height was 160.2 cm, BMI 25.9 kg/m^2, median waist circumference was 95.0 cm. Sixty percent of the subjects were sedentary and 40% of the subjects were rather active.

Table 2: Nutritional status

Variable	(n = 30)	p value
Height, cm	160.2 ± 6.5	0.3751
Weight, kg	66.3 ± 12.2	0.1261
BMI, kg/m^2	25.9 ± 4.4	0.2411
Waist circumference, cm	95.0 (66.0–118.2)	0.0001

Description: BMI: body mass index, [1]Shapiro–Wilk.

Nutritional intake in research subjects (Table 3) was the median energy or calorie intake of 1707.8 kcal, protein intake of 72.4 grams, fat intake of 46.2 grams, carbohydrate intake of 233.3 g, and copper intake of 1400 mcg.

Table 3: Dietary intake

Variable	(n = 30)	p value
Energy, kkal	1707.8 (1045.5–3170.3)	0.007[1]
Protein, g	72.4 (49.7–166.9)	0.007[1]
Fat, g	46.2 (21.9–100.6)	0.017[1]
Carbohydrate, g	233.3 (126.7–464.7)	0.013[1]
Copper, mcg	1400 (800–4100)	0.000[1]

[1]Shapiro–Wilk.

Analysis on the dietary intake of the subjects (Table 4) showed that protein intake has a weak and significant negative correlation with the incidence of ischemic heart disease ($r = -0.386$; $p < 0.05$).

Table 4: Association between dietary intake and ischemic heart disease

Variable	R	p value
Energy, kkal	−0.278[1]	0.137
Protein, g	−0.386[1]	0.035*
Fat, g	−0.216[1]	0.251
Carbohydrate, g	−0.077[1]	0.685
Copper, mcg	−0.039[1]	0.831

[1]Spearman correlation; *Statistically significant.

Table 5 showed that the blood copper level of the subjects has a weak and insignificant negative correlation with the incidence of ischemic heart disease ($r = -0.050$; $p < 0.795$).

Table 5: Association between blood copper level and ischemic heart disease

Variable	R	p value
Blood copper level, ug/L	−0.050[1]	0.795

[1]Spearman correlation.

Discussion

The research subjects were patients with age group >18 years, where the incidence of ischemic heart disease in Asia is between 40 and 69 years old [30]. This is in accordance with the mean age of the subjects of this study, which is 60.6 years old. Risk factors for ischemic heart disease are smoking and other comorbidities. Smoking is one of the most important risk factors in the incidence of ischemic heart disease [30]. This study showed that 63.3% of subjects were active smoker or ex-smoker <2 years. Forty-six percent of men and 9% of women had ischemic heart disease [30]. Other than smoking, subjects of this study appeared to have comorbidities such as dyslipidemia and diabetes mellitus. Ohira and Iso's study stated that dyslipidemia and diabetes were associated with ischemic heart disease [30].

The mean BMI in this study was 25.9 kg/m2. Based on the WHO criteria for Asia Pacific, the nutritional status was categorized as obesity level I. In addition, the median waist circumference of the subjects was 95 cm, which based on the WHO classification for Asia, could be classified as central obesity if the waist circumference was 90 cm for men and 80 cm for women. Central obesity is part of the metabolic syndrome which can be used to identify individuals at increased risk of future cardiovascular disease events even in the absence of other metabolic disorders. A study by Thomsen and Nordestgaard stated that obesity with or without metabolic syndrome was associated with an increased risk of myocardial infarction and ischemic heart disease [31].

Certain food intakes are associated with the risk of ischemic heart disease or coronary heart disease. There is a negative association with sufficient strength of association between protein intake and ischemic heart disease. The intake of energy, fat, carbohydrates, and copper did not have a significant relationship with the incidence of ischemic heart disease. Voortman *et al.* reported that there was no significant relationship between macronutrient composition (intake of energy, carbohydrates, protein, and fat) with the incidence of ischemic heart disease, but there was a consistent negative association between protein and ischemic heart disease, although the results were not significant [32].

In this study, there was no significant relationship between blood copper levels and the incidence of ischemic heart disease. A study conducted by Klevay stated that copper deficiency in rats can cause an increase in cholesterol, blood pressure, homocysteine, isoprostane and uric acid, impaired glucose tolerance, and oxidative damage and thrombosis, whose pathogenesis is similar to ischemic heart disease that occurs in humans [33]. Research by Morrell *et al.* reported that insufficiency of copper levels can trigger dyslipidemia and increase oxidative stress that contributes to metabolic disease [34]. This study is also inconsistent with the research of He and Kang who reported an association between copper and ischemia of myocardium caused by suppression of myocardial angiogenesis due to disturbances in the transcriptional regulation of hypoxia-inducible factor-1α (HIF). -1α) due to copper deficiency resulting in reduced angiogenic capacity [35]. Based on the research conducted by Xiao *et al.*, copper can trigger the regeneration of the myocardium through reactivation of HIF-1 which regulates angiogenesis so that copper can be an approach in alternative therapy for ischemic heart disease [36]. Research by Liu and Miao also mentions that copper deficiency can affect many tissues such as the liver, small intestine, blood vessels, brain, and adipose tissue, including the heart, which the most sensitive tissues to copper deficiency. Copper plays a role in maintaining the activity of copper-dependent proteins, such as cytochrome c oxidase (CCO), superoxide dismutase-1 or -3 (SOD1/3), metallothionein (MT), ceruloplasmin (CP), and lysyl oxidase (LOX), which are proteins essential in the regulation of oxidative phosphorylation, iron mobilization, antioxidant defense, and cross-linking of connective tissue in the heart. Impaired copper metabolism, including copper deficiency and disorders of copper companion, copper transporter, or copper-binding protein causes cardiac dysfunction, myopathy, and an increased risk of death [16]. DiNicolatonio *et al.* also concluded that there is a relationship between copper deficiency and the incidence of ischemic heart disease [37]. A study conducted by Kodali *et al.* reported that genetic validation of copper has a negative association with ischemic heart disease, which means copper can reduce mortality and morbidity of ischemic heart disease [38]. A semiquantitative food frequency questionnaire dietary assessment was used for dietary assessment that required a memory recall for the past month so that it might be a bias information. Further, research with more accurate in assessing dietary intake such as food weighing could be done to minimize the bias.

Conclusion

This study is a descriptive study with a sample of 30. The results found that the majority of the subjects were male with an average age of 60.6 years. The median level of copper consumed daily was 1400 mcg/day and the mean of blood copper level was 1034.5 ug/L that showed the normal limit of person's copper status. This study found no association between blood copper levels and the incidence of ischemic heart disease. However, there was a significant weak association between dietary protein and the incidence of ischemic heart disease.

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