Makara J. Health Res., 2015, 19(2): 67-74
doi: 10.7454/msk.v19i2.5178

Zinc and Copper Levels in Patients with Primary Hypertension and Normotension

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Abstract

One of the causes of primary hypertension is an exposure to free radicals. The formation of free radicals in the body can be prevented by taking antioxidants. Zinc and copper are cofactors of endogenous antioxidant enzyme superoxide dismutase. This study aimed to analyze the differences of zinc and copper levels in primary hypertensive and normotensive patients. This was an analytical observational study with cross sectional design and simple random sampling method. Subjects were patients aged 40-70 years at Haji General Hospital consisting of 15 primary hypertensive patients and 15 normotensive individuals (comparison group). Data was collected through interviews and laboratory test of blood samples. Zinc and Copper serum concentrations were measured by AAS. Data were analyzed by chi-square and independent samples t-test. The results showed that the mean levels of zinc and copper in primary hypertensive patients was lower than normotensive. However, statistically there was no difference in zinc serum levels (p=0.852) in the two groups, and there was a significant difference in copper serum levels (p=0.032). It can be concluded that there were differences in copper serum levels between the two groups but not with the levels of zinc.

Abstrak

Kadar Zinc dan Cuprum pada Penderita Hipertensi Primer dan Normotensi. Salah satu pencetus hipertensi primer yaitu paparan radikal bebas. Pembentukan radikal bebas dalam tubuh dapat dicegah dengan antioksidan. Peran *zinc* dan *cuprum* adalah sebagai kofaktor dari enzim antioksidan endogen superoksida dismutase (SOD). Penelitian ini bertujuan untuk menganalisis perbedaan kadar serum *zinc* dan *cuprum* pada kelompok hipertensi primer dan normotensif. Penelitian ini merupakan penelitian observasional analitik dengan desain *cross sectional* dan metode *simple random sampling*. Subjek adalah orang yang berusia 40-70 tahun, mengunjungi Rumah Sakit Haji Surabaya, dan terdiri dari 15 responden hipertensi primer dan 15 responden normotensif (kelompok pembanding). Data dikumpulkan melalui wawancara dan uji laboratorium klinis sampel darah. Konsentrasi serum *zinc* dan *cuprum* diukur dengan metode AAS (Spektrofotometer Serapan Atom). Data dianalisis dengan *chi-square* dan *independent T-test*. Hasil penelitian menunjukkan bahwa tingkat rata-rata serum *zinc* dan *cuprum* responden pada kelompok hipertensi primer lebih rendah daripada kelompok normotensif. Namun, secara statistik tidak ada perbedaan yang signifikan (p=0,852) kadar serum *zinc* antara kelompok hipertensi primer dan kelompok normotensi, tapi ada perbedaan yang signifikan pada kadar serum *cuprum* (p=0,022). Kesimpulannya adalah ada yang berbeda dari kadar serum *cuprum* antara dua kelompok sedangkan untuk kadar serum *zinc* tidak berbeda.

Keywords: copper levels, normotensive, primary hypertension, sod (superoxide dismutase), zinc levels

Introduction

Hypertension problem is still prevalent among the people in the world and in Indonesia. Data from Global Burden of Disease (2010) stated that hypertension has become a major risk factor of cardiovascular disease.[1] Primary hypertension that is not treated immediately will develop into degenerative diseases. A Survey done by Riskesdas (2007) showed that the cause of death in Indonesia was dominated by degenerative disease including stroke (26.9%), hypertension (12.3%), diabetes mellitus (10.2%) and ischemic heart disease (9.3%).

The prevalence of hypertension in Indonesia tends to increase. Riskesdas (2013) reported that a tendency of

hypertension prevalence that based on diagnoses and anti-hypertension drug increased from 7.6% (2007) to 9.5% (2013). East Java was one of the provinces with hypertension prevalence (37.4%) higher than the national prevalence (31.7%).

Ninety to ninety five percent of hypertension cases are primary hypertension and 5-10% cases are secondary hypertension.[4] Primary hypertension is a condition in which there is an increase in blood pressure (above 140/90 mmHg) in adults with at least three consecutive measurements, and the cause is not known for certain.[4,5,6] Most primary hypertension occurs without symptoms and definitive signs. It is caused by multi factors, and there is an interaction between genetic and environmental factors.[7] In addition, there are many behavior risk factors that can cause hypertension including food consumption (high in salt and fat, low in fiber), alcohol consumption, smoking habits, physical inactivity and lack of exercise, and poor stress management.[7]

It has been argued that free radicals play a role as a trigger factor of hypertension.[8] Free radicals and ROS (Reactive Oxygen Species) are generated from the oxidation reaction of complex sub cellular level. If there is not enough antioxidant system in the body, oxidative stress can occur, and it can damage critical components of body cells. Cell damage caused by ROS occurs due to damage to proteins that can intrude endogenous antioxidant enzyme activity, and it can cause DNA damage and lipid damage that affects the structure of cell membranes.[10]

One of the endogenous antioxidants enzymes involved in the degradation process of intracellular ROS compounds are Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx).[10] Zinc and copper are cofactors of superoxide dismutase (SOD). Zinc stabilizes the protein structure of the enzyme Cu-Zn-SOD, while copper catalyzes enzyme Cu-Zn-SO.[11]

Several previous studies related to the role of trace elements toward hypertension showed that zinc and copper deficiency is associated with decreased potential antioxidant.[12] In addition, zinc deficiency affects immune cells, increases oxidative stress and inflammatory cytokines in which all are involved in the pathogenesis of primary hypertension.[13]

Based on the problems and the importance of the role of zinc and copper as endogenous antioxidants in reducing hypertension by binding free radicals, this study was conducted. Hopefully, after knowing the levels of zinc and copper, deficiency improvement of each trace element can be done with adequate consumption of zinc and copper.

Methods

This is an analytic observational study that describes the relationship between the independent (serum zinc and copper levels) and dependent variables (hypertension) through hypothesis testing. This study used Comparative Cross Sectional Design. At this survey researchers approached or measured different samples while at the same time identified risk factors in the subjects group. This study was conducted in Haji General Hospital Surabaya in June-August 2014. Laboratory tests were carried out in the Laboratory Installation of Haji General Hospital Surabaya and Surabaya Regional Medical Laboratory.

Subjects were respondents aged 40-70 years old who visited Haji General Hospital Surabaya. Inclusion criteria for the study were as follows: aged 40-70 years old, male and female, normal level of albumin, willing to follow the study and to sign the informed consent, willing to have blood drawn and were not taking supplements of zinc and copper before study. As for the exclusion criteria: pregnant and lactating women, suffering from other diseases (e.g, diabetes mellitus, stroke, coronary heart disease, kidney disease, preoperative cancer, and preoperative chronic infectious disease).[14] The sampling technique in this study was simple random sampling method. Furthermore, the subjects were divided randomly into primary hypertensive groups and normotensive groups, each with 15 respondents. Structured interviews and blood sampling were also conducted for selected subjects.

This study protocol was reviewed by the Ethics Committee of Haji General Hospital Surabaya regarding the protection of human rights and well-being in medical research. Before the data was collected, the the objectives, benefits, and impact of their participation in the study were explained to the subjects. Then, the subjects were asked to sign an informed consent as evidence of the willingness of the participants in the study.

Respondent characteristic data were obtained through interviews to know their age, gender, family history of hypertension, smoking, exercise habits and body mass index. Assessment of serum levels of zinc and cuprum and SOD was obtained from the results of laboratory tests.

Zinc and copper serum concentrations were measured by the Atomic Absorbant Spectrophotometry (AAS) method which was expressed in units of μmol/L. Zinc status was classified into normal (10-15 μmol/L) and deficient (if <10 μmol/L) (Gibson, 2005). Cut-off point for Copper serum level in a normal range was between 8.8 to 13.5 μmol/L for men and from 10.7 to 26.6 μmol/L for women (Gibson, 2005). While, SOD levels

were measured by ELISA (Enzyme Linked Immune Sorbent Assay).

The data was analyzed with two mean difference test (independent samples T-test) for ratio scale data, Mann Whitney test for ordinal scale data, followed by Chi Square test to determine the prevalence ratio. Furthermore, to determine the relative prevalence of each independent variable odds ratio test was conducted. Normality of data distribution was tested with the Kolmogorov-Smirnov test.

Result and Discussion

The study involved 30 respondents consisting of 15 people included in hypertensive group and 15 people in normotensive group. According the age, most hypertensive respondents were 60-70 years old (46.6%). The average age of the respondents in the normotensive group was 45.2±5.54 years with a minimum age of 40 years and maximum age of 59 years. While in the hypertensive group, the average age of respondents was 55.67±8.88 years with a minimum age of 40 years and maximum age 65 years. There were more male respondents than women in the hypertensive group with a mean value of systolic blood pressure of 145.67±9.04 mmHg and diastolic blood pressure of 93.33±6.17 mmHg (Table 1).

Triggering factors of hypertension were divided into factors that cannot be modified and the factors that can be modified. Age, gender, and family history of hypertension were factors that cannot be modified, while the factors that can be modified included smoking, lack of exercise and body mass index (Table 2).

Relationship between Age with Hypertension. This study indicated that respondents aged 55 years and older were more common in the hypertension group (60%) compared to the normotensive group (13.3%). Based on the results of the analysis the correlation between age and hypertension was $p<0.05$. Respondents aged 55 years and older had 9.75 greater risk for hypertension than respondents aged 54 years and below (Table 2).

Age is a risk factor for hypertension that cannot be controlled. It has been argued that the incidence of hypertension increases with age. In old age, blood vessels lose elasticity. A decrease in the elasticity of the connective tissue and the relaxation of vascular smooth muscle result in decreased distension ability and tensile strength of the blood vessels so that the ability of the aorta and large arteries to accommodate the volume of blood pumped by the heart decreases, causing a decrease in cardiac output and increased peripheral resistance resulting in increased blood pressure.[4]

In addition, the aging process is also associated with the free radical theory, which argues that fisiolofis function changes because of the accumulation of macromolecular oxidative irreversible changes due to free radicals.[9] This accumulation increases with age so that more cell damage occurs, caused by oxidative stress. If this condition lasts for a long time, it can cause hypertension.[18] Research conducted by Winarsih et al. (2013) revealed that SOD activity decreased in the elderly. This proves that the free radical levels increases with age.

Relationship between Sex with Hypertension. The results showed no significant relationship between sex with hypertension (p=0.273). In the hypertensive group there were more male respondents (53.3%) than female respondents (46.7%). There were also male respondent (53.3%) in the hypertensive group compared with normotensive group (33.3%).

Table 1. Distribution Characteristics of Respondents

Variable	Hipertension		Normotension		Total	
	n	%	n	%	n	%
Age						
40-49	4	26.7	12	80	16	53.4
50-59	4	26.7	3	20	7	23.3
60-70	7	46.6	0	0	7	23.3
Sex						
Male	8	53.3	5	33.3	13	43.3
Female	7	46.7	10	66.7	17	56.7
Diastolic blood pressure (mmHg)						
Sistolic	145.67±9.04		107.53±7.06			
Diastolic	93.33±6.17		71.2±4.16			

Based on the results of the analysis, it was found that that gender cannot be concluded as a risk factor for hypertension (OR=2.286; CI 95%: from 0.522 to 10.011; p>0.05). It was suspected that both respondents—both men and women—had the same opportunities to be exposed to various external risk factors such as hypertension, lifestyle shift in diet, and physical inactivity (Table 2).

It has been argued that the incidence of hypertension is higher in men than women up to the age of about 55 years. At the age between 55-74 years the risk of hypertension in men and women is almost the same; however, after the age of 74, women are at higher risk of developing hypertension.[19] This is related to the role of the estrogen. Estrogen may function as an antioxidant. Estrogen prevents oxidation of LDL (Low Density Lipoprotein). LDL cholesterol is more easily penetrated by the plaque in the blood vessel walls when oxidized. Moreover, estrogen also plays a role in the vasodilatation of blood vessels and blood vessels elasticity. With reduced estrogen at menopause, women become vulnerable to the risk of hypertension.[5]

Relationship between Smoking with Hypertension. Most respondents did not have smoking habits. Only 5 people out of all respondents smoked, and the fifth respondents (33.3%) had hypertension. While in the normotensive group, none of the respondents had smoking habit. The analysis of the Fisher's Exact Test showed that there were significant differences in smoking habits (p=0.042) between the hypertensive and normotensive groups (Table 2).

Table 2. Factors Associated with Hypertension

Variable	Hypertension		Normotension		OR (CI 95%)	p
	n	%	n	%		
Age						
≥ 55	9	60	2	13.3	9.750 (1.592-59.695)	0.014*
≤ 54	6	40	13	86.7		
Total	15	100	15	100		
Sex						
Male	8	53.3	5	33.3	2.286 (0.522-10.011)	0.273
Female	7	46.7	10	66.7		
Total	15	100	15	100		
History of Hypertension						
Yes, parents	10	66.7	4	26.7	5.50 (1.145-26.412)	0.033*
No	5	33.3	11	73.3		
Total	15	100	15	100		
Smoking habit					-	
Yes, Once	5	33.3	0	0		0.042**
Never	10	66.7	15	100		
Total	15	100	15	100		
Exercise						
Yes, Often	8	53.3	13	86.7	5.688 (0.939-34.457)	0.059
Never	7	46.7	2	13.3		
Total	15	100	15	100		
BMI (Body Mass Index)						
Exceeding normal	11	73.3	1	6.70	38.5 (3.74-395.41)	0.002*
Normal	4	26.7	14	93.3		
Total	15	100	15	100		

*) Chi-Square test, significant (p<0.05)
**) Test Fisher's Exact Test, significant (p<0.05)

Some previous cross-sectional studies have also found an association between smoking and hypertension. RISKESDAS 2007 showed that smoking is a risk for hypertension. In theory, this is likely due to the chemical nicotine in cigarettes and the carbon monoxide inhaled through cigarette smoke into the blood flow, which damage the endothelial of blood vessels and cause increased heart rate and blood pressure.[19] Exposure to cigarette smoke is a form of free radicals which in the long term can increase lipid peroxidation, triggering oxidative stress, and can damage the endothelial lining of blood vessels causing hypertension. A study by Rust (2004) proved that the SOD activity in smokers is lower than non-smokers.[20]

Relationship between Family History with Hypertension. A family history of hypertension may also be a risk factor for hypertension. In this study, respondents who had a family history of hypertension from their parents were more common in hypertension group (66.7%) than the normotensive group (26.7%). The presence of family history of hypertension increased the risk of developing hypertension by 5.5 times compared to no family history of hypertension (OR=5.5; CI95%: 1145-26412; p<0.05).

Theoretically, primary hypertension is usually associated with genetic factors. Someone may have a greater chance of suffering from hypertension if parents have hypertension. Hypertension tends to be a hereditary disease, although it has not been proven with diagnostic tests. Duprez (2008) reported that a normal person with a family history of hypertension has decreased parasympathetic nerve activity. Changes in the autonomic nervous can be derived through genetic role in the incidence of hypertension.[22]

Relationship between Exercise with Hypertension. Physical activity or exercise done regularly for 30-45 minutes can reduce the risk of hypertension.[19] Sports is associated with the management of hypertension because it can decrease the peripheral resistance of blood circulation that can reduce hypertension. In addition, exercise can also improve the elasticity of the endothelium of blood vessel walls by inhibiting the formation of free radicals and increasing the production of nitric oxide. Thus the formation of arteriosclerosis progression may be inhibited and prevent hypertension.[23]

In this study, lack of exercise habits can not be concluded as a risk factor for hypertension (OR=5.688; CI 95%: 0.939-3.4457; p=0.059). This means—not in accordance to the theory—that respondents in this study who had less physical activity did not suffer from hypertension.

Relationship between Body Mass Index with Hypertension. Body Mass Index (BMI) is the most practical method of determining the level of overweight and obesity in adult respondents under the age of 70 years (WHO, 2006). In this study, BMI was classified according to WHO (2004): thin (<18.5 kg/m^2), normal (18.5 to 24.9 kg/m^2), overweight (≥25 kg/m^2), pre-obesity (25 to 29.9 kg/m^2), and obese (≥30 kg/m^2).

The results showed, the average value BMI in hypertensive group was 26.43±3.52 kg/m^2 higher when compared to the normotensive group 23.71±1.36 kg/m^2. Based on the analysis, we found a significant relationship ($p<\alpha$) between BMI with hypertension. Respondents with BMI more than normal (overweight or obese) had a 38.5 higher risk of developing hypertension compared with respondents who had a normal BMI (Table 2).

A previous study (Bay et al, 2007), which compared data from SHIELD and NHANES, also stated that the increase in BMI is generally associated with the prevalence of hypertension (p<0.001). There were as many as 46-55% of hypertensive patients who were obese, and 80-85% were overweight.[20] In theory, obesity that is followed by increasing fat metabolism will lead to increased production of ROS (Reactive Oxygen Species) in circulation and in adipose cells. ROS can stimulate inflammation and induce cell apoptosis. Increased ROS in adipose cells leads to the disruption of the balance of oxidation-reduction reactions, resulting in a decrease in antioxidant enzyme in the circulation. This situation is called oxidative stress. Oxidative stress is believed to have an important role in the pathophysiology of hypertension.[24]

Thus, it can be argued that another factor that causes hypertension is the presence of free radical exposure. Free radicals can be resisted by antioxidants. The body has natural antioxidant enzymes such as superoxide dismutase (SOD). This enzyme is catalyzed by zinc and copper. The difference between the levels of zinc, copper and SOD in hypertensive and normotensive groups can be seen in Table 3.

Relationship between Zinc with Hypertension. Zinc is an essential component in the structure and function of the cell membrane. Zinc functions as an antioxidant and protects the body against lipid peroxidation. Zinc is needed for enzyme superoxide dismutase (SOD) activity. SOD disables superoxide anion and peroxides that are free radicals. Copper and zinc induce superoxide dismutase (SOD) to clean up free radicals.[12]

In this study, the mean serum level of zinc in the hypertension group (9.96±2.88 mol/L) was lower than the normotensive group (10.9±5.08 mol/L).

Table 3. Distribution of Zinc, Copper, and Superoxide Dismutase Concentrations on Participants in Hypertensive and Normotensive

Variable	Mean	SD	p value
Concentration of zinc (µmol/L)			
Hypertensive	9.96	2.88	0.852
Normotensive	10.9	5.08	
Concentration of Copper (µmol/L)			
Hypertensive	41.37	18.39	0.022*
Normotensive	58.47	22.03	
Concentration of SOD (U/mL)			
Hypertensive	0.82	0.56	0.177
Normotensive	1.27	0.62	

Table 4. Distribution of the Status of Zinc and Copper on Participants

Variable	Hypertension		Normotension		PR (CI 95%)	p
	n	%	n	%		
Zinc Status					2.286	0.273
Deficiency (< 10 µmol/L)	10	66.7	7	46.7	(0.522-10.011)	
Normal (10-15 µmol/L)	5	33.3	8	53.3		
Copper Status					2.406	0.260
Deficiency (< 8.8 µmol/L)	11	73.3	8	53.3	(0.521-11.104)	
Normal (8.8-17.5 µmol/L)	4	26.7	7	46.7		

Serum zinc level of the hypertensive group was in the category of zinc deficiency (less than 10 mol/L). The normal value of serum zinc level is 10-15 mol/L.[15] This research found the same results as study done by Chiplonkar et al, (2004), which reported that the erythrocyte zinc level in hypertensive patients was lower than the normotensive.[15] However, the results of this study showed that there was no significant statistical association between zinc status (OR=2.286; 95% CI: 0522-10011; p>0.05) and the incidence of hypertension (Table 4).

It can be argued, therefore, that zinc is associated with the incidence of hypertension through the pathogenesis of atherosclerosis, including vascular endothelial dysfunction and inflammatory response. Reiterer et al. (2005) found an increase in LDL cholesterol and oxidative stress in rats with zinc deficiency. Prolonged oxidative stress can cause inflammation that contributes to atherosclerosis. In addition, zinc deficiency can reduce the function of Cu-Zn SOD enzymes that act as antioxidants. Zinc catalyzes the superoxide dismutase reaction. A decrease in the activity of this enzyme triggers excessive formation of free radicals, which in turn can damage the endothelium relaxation factor in the blood vessels.

Relationship Copper with Hypertension. The mean level of copper serum in hypertensive group (5.99±2.96 mol/L) was also lower than the normotensive group (8.78±3.30 mol/L). Copper serum level of the hypertensive group in the category of deficiency copper was 8.8-13.5 mol/L for men and 10.7-26.6 µmol/L for woman. This was reinforced by the results of the statistical analysis of Independent T-test which showed that a mean difference of copper serum level was significant (p<0.05) between hypertensive group and normotensive group. Taneja (2007) also reported that copper serum level decreased in hypertension. However, the existence of such differences did not show a significant relationship between cuprum status (OR=2.406; 95% CI: 0.521-1.1104; p>0.05) and the incidence of hypertension.

In theory, copper deficiency is associated with vascular defects such as aneurysms, heart enlargement and heart failure. Copper deficiency relative to the zinc result in decreased HDL (High Density Lipoprotein) and increased LDL (Low Density Lipoprotein) can cause atherosclerosis and causes hypertension.[12]

Relationship between Superoxide Dismutase with Hypertension. This study showed that the average level of superoxide dismutase in hypertensive group (0.82± 0.56 U/mL) was lower than the normotensive group (1.27±0.62 U/mL). However, the Independent T-test showed that there was no significant difference (p>0.05) of the mean level of superoxide dismutase between the normotensive and hypertensive groups (Tabel 3).

The superoxide dismutase enzyme is a primary antioxidant in the body that prevents the formation of

new free radicals by transforming free radicals into a molecule that decreases the negative effects before they could react. The role of zinc and copper as cofactors of superoxide dismutase (SOD) enzyme is to scavenge free radicals. Zinc and copper deficiency is associated with the activation of SOD. In this study, deficient zinc and cuprum was found in each group, both hypertensive or normotensive respondents. Although the amount of zinc and cuprum deficiency was worse in patients with hypertension but it was not statistically significant. Nevertheless, the presence of zinc deficiency and cuprum was also followed by superoxide dismutase which also tended to be deficient in patients with hypertension. According to Bertinato (2003), decreased levels of zinc and copper resulted in a decrease in the activity of superoxide dismutase.

It can be argued that the level of Superoxide dismutase activity in humans is more or less the same. What is different is capacity induction of superoxide dismutase, the body's ability to increase the amount of superoxide dismutase when it should respond to the rising number of oxygen radicals in the body. The older the person, more down superoxide dismutase activity occur. Besides, genetics also control superoxide dismutase.

Conclusions

There is no significant relationship between zinc and copper deficiency status with hypertension. Nonetheless, the average value of the concentration of zinc and copper serum in the hypertensive group was lower than the normotensive group. Superoxide dismutase level was lower in hypertension group. This study highlights the importance of endogenous antioxidants such as zinc and copper micro-minerals. Therefore, hypertensive patients should pay attention to the fulfillment of the nutritional needs with antioxidants to prevent free radicals. Frequent consumption of food sources of zinc and copper is essential to fulfill the needs of the micro-minerals in blood and to prevent hypertension.

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