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The relationship between vitamin A and ferritin towards malondialdehyde level among Javanese male smokers

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ABSTRACT

Cigarette smokes produce a large number of oxidants and promote secretion of ferritin by alveolar macrophages which are potential to encourage the lipid peroxidation. Malondialdehyde (MDA) is used as a parameter of lipid peroxidation. The study was aimed to evaluate the relationship between blood level of vitamin A and ferritin and MDA among Javanese male smokers. Sixty men who lived in Purworejo District, Central Java, Indonesia comprising 30 smokers as case group and 30 nonsmokers as control group were involved in this study. Blood sample was obtained from cubiti vein and then centrifuged to obtain plasma or serum. Blood levels of vitamin A, ferritin and MDA were measured by HPLC, ELISA and spectrophotometric methods, respectively. The result showed that the blood vitamin A, ferritin, and MDA levels in smokers were 25.09 ± 9.51μg/dL, 35.50 ± 24.17ng/dL, 1.15 ± 0.42μg/L, respectively, whereas in non smokers, they were 26.11 ± 9.19 μg/dL, 38.60 ± 15.25 ng/dL, 1.06 ± 0.50 μg/L, respectively. There was no significant difference of the blood vitamin A, ferritin, and MDA levels between smokers and the non smokers ($p > 0.05$). The linear regression analysis indicated that there was negative relationship between blood vitamin A and MDA levels although it was not significant ($p = 0.052$), while blood ferritin and MDA levels had a significantly positive relationship ($p = 0.010$). In conclusion, the low level of blood vitamin A among cigarette smokers does not lead to high blood MDA level, while high level of blood ferritin among smokers leads to high blood MDA level.

ABSTRAK

Merokok menghasilkan banyak oksidan dan memacu sekresi feritin makrofag alveolar yang potensial meningkatkan peroksidasi lipid. Malondialdehid (MDA) digunakan sebagai parameter peroksidasi lipid. Penelitian ini bertujuan mengevaluasi hubungan antara kadar vitamin A dan feritin dalam darah dengan MDA pada perokok laki-laki Jawa. Enam puluh laki-laki, terdiri dari 30 perokok sebagai kelompok kasus dan 30 bukan perokok sebagai kelompok kontrol, yang tinggal di Kabupaten Purworejo, Jawa Tengah, Indonesia terlibat dalam penelitian. Sampel darah diperoleh dari vena cubiti dan disentrifuse untuk mendapatkan plasma dan serum. Kadar vitamin A, feritin dan MDA darah ditetapkan berturut-turut dengan metode HPLC, ELISA dan spektorfotometri. Hasil penelitian menunjukkan kadar vitamin A, feritin dan MDA darah bukan perokok berturut-turut adalah 25,09 ± 9,51μg/dL, 35,50 ± 24,17ng/dL, 1,15 ± 0,42μg/L sedangkan laki-laki perokok berturut-turut adalah 26,11 ± 9,19 μg/dL, 38,60 ± 15,25 ng/dL, 1,06 ± 0,50 μg/L. Tidak ada perbedaan bermakna kadar vitamin A, feritin dan MDA darah perokok dan bukan perokok ($p > 0.05$). Hasil analisis regresi linier menunjukkan adanya kecenderungan hubungan negatif antara kadar vitamin A dengan MDA darah meskipun tidak bermakna ($p = 0,052$). Namun demikian terdapat hubungan positif yang bermakna antara kadar feritin dan MDA darah ($p = 0,010$). Dapat disimpulkan, kadar vitamin A yang rendah dalam darah perokok tidak menyebabkan kadar MDA darahnya tinggi, sedangkan kadar feritin yang tinggi dalam darah menyebabkan kadar MDA darah tinggi.

Keywords: vitamin A - ferritin - malondialdehyde - cigarette - smokers

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INTRODUCTION

The World Health Organization (WHO) estimates that there are around 1.3 billion smokers in the world, in which 1 billion of them are men. This represents about one third of the global population who are in age 15 and more. They also estimate that around 84% of smokers live in developing countries.[1] In Indonesia cigarette consumption is increasing more rapidly than that of anywhere else in the world. Among the world's 1.1 billion smokers, 6.6% are Indonesians.[2]

Large concentrations of oxidants are present in cigarette's tar and smoke. Each puff of cigarette smoke contains approximately 10^{14} of free radicals molecules in the tar phase and 10^{15} of that in the gas phase. It is also stated that cigarette smoking was a well-known risk factor for both cardiovascular disease and cancer. Cigarette smokes contain a large number of oxidants, leading to the hypothesis that many adverse effects of smoking result from oxidative damage. Cigarette smokes also contain large amounts of free radicals that could directly initiate and propagate the process of lipid peroxidation.[3]

The role of free radicals in the pathogenesis of smoking-related diseases has been substantiated by detailed descriptions of the biochemical mechanisms involved. A free radical is a reactive molecule that contains one or more unpaired electrons. Free radical formation is a normal consequence of a variety of essential biochemical reactions, without which we could not live. However, free radicals are relatively unstable and have a tremendous potential to damage cells and tissues. Consequently, these highly reactive molecules require antioxidants in the form of enzymes and small molecular weight substances for detoxification.[4]

Oxidation of low density lipoprotein (LDL) and lipid peroxide can be prevented by antioxidants. The antioxidants bind free radicals and form a stabile compound. Therefore, a good antioxidant status in the body can prevent some diseases related with lipid peroxidation.[5] Antioxidants naturally occurred in the body are tocopherol (vitamin E), ascorbic acid (vitamin C), retinol (vitamin A), and â carotene. Those antioxidant vitamins inhibit free radicals by giving electron from their atom to free radicals, to bind single free radicals, without becoming new free radicals.[6,7] The role of vitamin A as an antioxidant is important in the immune function since it will protect cell's membrane from oxidative damage by free radicals.[6,8,9]

Smokers have been reported to promote secretion of ferritin by alveolar macrophages. Gas and tar phases of cigarettes increase ferritin releasing.[4] Therefore, ferritin is also a prooxidant that can increase the lipid peroxidation. Oxidation process causes cholesterols or fatty acids to be charged with free radicals. Those fatty acids charged with free radicals will react with metal ion, such as ferri or cuprum, as a process of lipid peroxidation. Lipid peroxidation forms a toxic aldehyde, such as malondialdehyde (MDA). These aldehyde compounds attack tiol and amino group from protein. Therefore, they cause the damaging of cells.[10] As a result, it has been used widely as the index of oxidative damage.[5] For that reason, this research may illustrate the relationship between vitamin A and ferritin towards lipid peroxidation (MDA) among smokers.

MATERIALS AND METHODS

Subjects

This was a case control study involving 60 individuals consisting of 30 smokers as case group and 30 nonsmokers as control group. Subjects were Javanese men who lived in Purworejo District, Central Java. The inclusion criteria of the case group were 1) male; 2) physically healthy; 3) has normal blood glucose level; 4) has normal total cholesterol level; 5) has normal triglyceride level; 6) smokes at least one cigarette per day; and 7)

has smoked for at least three months long. The inclusion criteria of control group were the same with the case group, except that subjects did not smoke. The exclusion criteria of research subject were that both control and case group do not use vitamin A supplementation. This study was preceded by obtaining ethical clearance from the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, and informed consent from all subjects.

Sample collection

Blood sample as much as 5 mL was obtained from cubiti vein of the subjects using disposable syringe. The blood sample was collected into tubes, an EDTA tube and a no-EDTA tube for each subject. Immediately, the blood sample was brought to laboratory and centrifuged in order to obtain plasma or serum samples.

Plasma vitamin A level measurement

Twenty uL of plasma sample was extracted with 50 uL mixture of ethanol and butanol (50:50) containing 5 mg BHT/mL. The mixture was then vortexed for 1 minute and centrifuged at 12.000 rpm for 3 minutes. Twenty uL of supernatant was taken and injected onto HPLC for analysis. An HPLC system including a solvent delivery pump (model LC-10ADv; Shimadzu), a controller (model SCL-10A*vp*; Shimadzu), an autoinjector (model AOC 20i; Shimadzu) and a UV-vis detector (model SPD-10A VP UV; Shimadzu) was used in this study. Acetonitrile: aquabidest (85:15) was used as mobile phase for eluting vitamin A with flow rate of 1.0 mL/minutes on a C-18 column (4.6 x 25 mm; 5µm). Absorbance was set at ë of 325 nm.

Plasma ferritin level measurement

Serum ferritin level was measured by simple sandwich ELISA as previously described by Erhardt *et al.*[11] after modification. Twenty µL of standard or serum samples or controls were dispensed into appropriate antibody-coated microtiter wells. One hundred µL of enzyme conjugate reagent was added into each wells. The solution in wells were mixed for 30 seconds to have completed mixing. The mixture of solution was then incubated at room temperature (18-22 ^{o}C) for 60 minutes. The wells were washed with distilled water to remove unbound-labeled antibodies. One hundred µL of a solution of 3,3',5,5'-tetramethylbenzidine (TMB) was added into each well, gently mixed for 5 seconds and incubated at room temperature in the dark for 20 minutes, resulting in the development of a blue color. The color development was stopped with the addition 100 µL of stop solution (1N HCl) to each well and it was made sure that all blue color changed to yellow color completely. The color optical density was read with ELISA reader (Bench mark; Bio Rad) at λ 450 nm within 30 minutes. The serum ferritin concentration was directly proportional to the color optical density of the samples.

Serum malondialdehyde level measurement

Malondialdehyde was measured using three-times spectrophotometry technique (TBARS assay) as previously described by Pyles *et al.*[12] One mL serum sample was added with 4 mL thio barbituric acid reagent and incubated in the water bath at 90 ^{o}C for 80 minutes. Afterward, it was cooled on ice for 10 minutes and added with 4 mL butanol. The mixture was then vortexed and centrifuged at 3000g for 15 minutes. The supernatant was collected into another tube and read with spectrophotometer (Junior® II Spectrophotometers, Models 6120; Parkin Elmer-Colemen 55) at ë of 510, 532 and 560 nm.

Data analysis

The normality distribution of data was assessed using Kolmogorov-Smirnov test. Differences in

continuous variables between smokers and nonsmokers groups were compared using independent sample t-test. The correlation of beta carotene and ferritin to malondialdehyde was measured using linear regression. For all analysis, $p < 0.05$ was considered to be statistically significant.

RESULTS

This study involved 60 subjects consisting of 30 smokers as case and 30 non smokers as control. The characteristics of the subjects are shown in TABLE 1. The glucose level, triglyceride and total cholesterol of the smokers were not significantly different compared to non smokers ($p>0.05$), while the age of smokers was younger significantly than non smokers ($p<0.05$).

TABLE 1. Characteristics of smokers and non smokers

Variables	Smoker (n 30)	Non smoker (n 30)	95% CI	p
Glucose level (mg/dL)	96.7±13.2	101.4±15.1	-12.0 – 0.26	0.202
Triglyceride (mg/dL)	105.6±28.2	103.3±34.1	-13.9 – 18.5	0.776
Total chloresterol (mg/dL)	171.5±24.2	166.7±31.5	-9.8 – 19.3	0.514
Age (year)	38.7±10 1	45.5 ±8 1	-11.5 – 2 1	0 006

The blood vitamin A, ferritin, and MDA level among Javanese smokers and non smokers are shown in TABLE 2. No significant difference of blood vitamin A, ferritin and MDA were observed between Javenese smokers and non smokers

TABLE 2. Vitamin A, ferritin, and MDA level among Javanese smokers and non smokers

Variables	Means ± SD		p
	Smokers	Non smokers	
Vitamin A (μg/dL)	25.09±9.51	26.11±9.19	0.675
Ferritin (ng/dL)	35.50±24.17	38.60±15.25	0.557
MDA (μmol/L)	1.15± 0.42	1.06± 0.50	0.450

The linear regression (Pearson correlation) analysis of blood vitamin A level and blood MDA level is provided in TABLE 3 and FIGURE 2. The linear regression of blood vitamin A level and blood MDA level among Javanese smokers showed a negative correlation ($R^2=0.128$; $p=0.052$) indicating that 12.8% of blood MDA level of smokers was influenced by blood vitamin A status.

TABLE 3. The relationship between blood vitamin A level and blood MDA level among smokers

Independent variable	Dependent variable: MDA (μmol/L)		
	Coefficient	R^2	p
Vitamin A (μg/dL)	-0.358	0.128	0.052

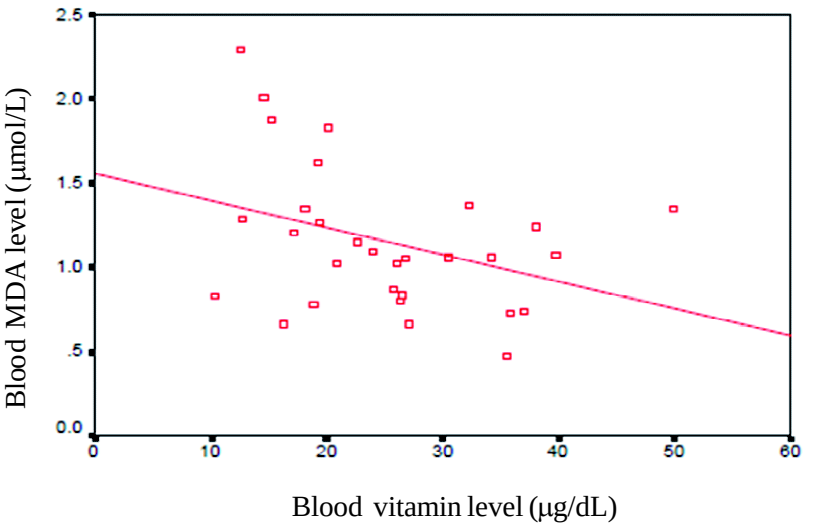

FIGURE 2. The linear regression of blood vitamin A level and blood MDA level among smokers

The linear regression (Pearson correlation) analysis of blood ferritin level and blood MDA level is provided in TABLE 4 and FIGURE 2. The linear regression of blood ferritin level and blood MDA level among Javanes smokers were significantly positive correlated (R^2=0.216; p=0.010) indicating that the blood ferritin level influenced 21.6% of the MDA level.

TABLE 4. The relationship between vitamin A and MDA among smokers

Independent variable	Dependent variable: MDA (µmol/L)		
	Coefficient	R^2	P
Ferritin (ng/dL)	0.465	0.216	0.010

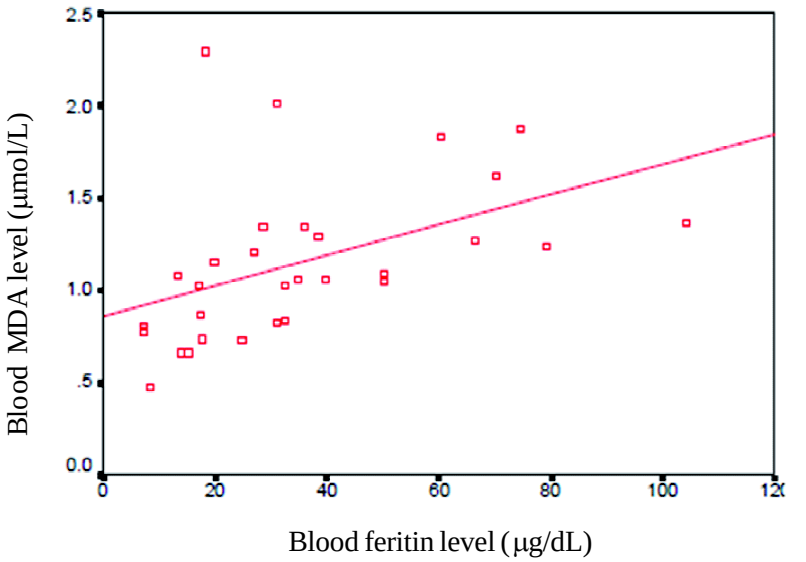

FIGURE 2. The linear regression of blood ferritin level and blood MDA level among smokers

DISCUSSION

Cigarette smoke contains approximately 10^{14} of free radicals molecules in the tar phase and 10^{15} of that in the gas phase. The free radicals can directly initiate and propagate the process of lipid peroxidation.[3] A free radical is a reactive molecule that contains one or more unpaired electrons. Free radicals are also relatively unstable and have a tremendous potential to damage cells and tissues. Smoking has been reported to promote secretion of ferritin by alveolar macrophage. Gas and tar phases of cigarettes affect ferritin releasing.[4] Ferritin is an iron storage in most of body cells. When the ferritin is released from the cell, the iron in the ferritin will be easy to be oxidized by oxidant. This research showed that blood MDA level was significantly influenced by ferritin which means that lipid peroxidation is influenced by ferritin level.[13] Previously, the relationship between ferritin and lipid peroxidation has been demonstrated[14] and it can be concluded that ferritin was a good source of iron for lipid peroxidation catalysis.

This study also reported that there was a negative correlation between blood vitamin A and MDA levels, however its correlation was not significant ($p>0.05$). Vitamin A is stated as one of antioxidants in the body.[15] Antioxidant is defined as substance that can prevent quality deterioration due to oxidation process.[16] Vitamin A antioxidant activity is giving its atom's electron to free radical through binding single free radical without becoming a new free radical.[4] The role of vitamin A as an antioxidant is important in immune function since it will protect cell's membrane from oxidative damage by free radicals. In other words, vitamin A is able to destroy free radicals by protecting cell and tissue structural integrity. Vitamin A is also able to bring back both the integrity and function of mucous surface.[6,8]

The insignificant correlation between vitamin A and MDA can be interfered by some conditions experienced by the case subject but we did not consider it. The conditions included liver function and lipid metabolism of subject. Over 90% of the vitamin A in the animal was stored in the liver. It was also stated that in nature, vitamin A was found largely as an ester, and consequently was highly soluble in organic solvent but not in aqueous solutions. Vitamin A is lipid soluble. Therefore, the consideration of liver function and lipid metabolism in the body is important in determining vitamin A level.[17]

The age of subjects is thought to influence the amount of blood MDA level. In this study, the result was that there was significant differences ($p=0.006$) of age distribution among smokers (38.7 ± 10.1) and non smokers (45.5 ± 8.1). This result showed that the age of non smokers was higher than that of smokers. This condition can contribute to the higher blood MDA level in non smokers than in smokers. As what has been stated[18] there is an age associated increase in steady state concentration of lipid peroxidation products. Therefore, the oxidative damage is senescence-associated alteration.

CONCLUSION

The low blood level of vitamin A among smokers does not lead to high blood MDA level. However, the high blood level of ferritin among smokers leads to high blood MDA level.

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