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Relation between β-carotene and ferritin upon malondialdehyde in Javanese male smoker

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ABSTRACT

Smoking of cigarette can cause additional free radicals. Oxidative damage is resulted from the accumulation of free radicals in the body. Malondialdehyde (MDA) is the end product of free radicals, a marker of oxidative damage. β-carotene is pro-vitamin A, an antioxidant known to quench singlet oxygen. Ferritin is thought to release excessive iron in smokers, thereby increasing the oxidative stress. The aim of the study is to evaluate the relation between β-carotene and ferritin toward MDA level in Javanese smokers. This study was carried out in a case-control, cross sectional design nested with cluster sampling. Participants were Javanese smokers and non-smokers in Purworejo district, Central Java. Samples and data were obtained secondarily. The results were analyzed using independent samples t-test and linear regression. The results showed that there was very weak negative correlation between β-carotene with MDA (R square = 0.013; p value =0.320) and very weak positive correlation between ferritin with MDA (R square = 0.043; p value = 0.067). There was no statistically significant relation of β-carotene with MDA. Ferritin level was marginally influential upon MDA level as the marker of lipid peroxidation between smokers and non-smokers. Aging apparently became the confounding variable by influencing the lipid peroxidation more efficiently than smoking itself (p = 0.013). In conclusion, there was no significant relation between β-carotene and ferritin with MDA in smokers.

Key words : smoking – cigarette – β-carotene – ferritin – malondialdehyde

INTRODUCTION

Smoking is a common practice worldwide especially in the low and middle class population in developing countries. The smoking male population is 50% in developing contries compared to only 35% in developed countries. Diseases related to cigarette smoking is the second most common causes of mortality in the world. Moreover smoking itself is the fourth most common risk factor for diseases worldwide.[1]

Cigarette smoke is considered a harmful substance due to its content of various chemicals (approximately 3800 chemicals) and existing free radicals. This is thought to be the pathogenicity of cigarette.[2] Free radicals naturally are always formed in human body, only in the amount and rate that the body is able to cope with. Lipid bilayer cell membrane could be attacked by reactive oxygen species (ROS). Terminal carbonyl compounds, including MDA, are resulted from this reaction.[3] Malondialdehyde is the end product of lipid peroxidation.[4] Lipid peroxidation occurs in three different sequences. First is non-enzymatic, free radical-mediated chain oxidation. Second is non-radical oxidation, and the third is enzymatic reaction.[5]

Human body protects itself from excessive oxidative stress by antioxidant system. When the antioxidants present in the body are insufficient to outbalance the free radicals, pathological consequences might develop.[6] When MDA level is high, the antioxidant level would be low.[7] β-carotene, a carotenoid antioxidant (pro-vitamin A) has been hoped to be the preventive nutrient for cancer, especially in cigarette smokers. β-carotene is best known in its ability to quench singlet oxygen. It is also known to eliminate peroxyl radical by terminating the radical reactions by binding to the attacking free radicals. In this process, the β-carotene is destroyed.[8]

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Free radical activity from long-term smoking in young cigarette male smokers is sufficiently scavenged.[9] Oxidative stress is present in elderly male who were chronic smokers (among Chinese population) nevertheless it is also observed in young adult male who were long-term smokers.[10] Increased circulating products of lipid peroxidation are found circulating in non-smoking elderly. Aging is considered to increase oxidative stress.[11]

Ferritin content is supposedly low in the plasma. When tissue injury occurs due to oxidative stress (due to cigarette smoking, toxins, burns, etc.), transition metals, primarily Fe, is released, causing formation of free radicals.[12] The objective of this study was to evaluate the relation between beta-carotene and ferritin with MDA in Javanese male smokers.

MATERIALS AND METHODS

Subjects

This was a case control study. Total of 79 Javanese males who were resident of Purworejo, Central Java were enrolled in this study, those consisted of 45 smokers and 34 non smokers. The inclusion criteria of the subjects were 1) male; 2) total cholesterol level < 220 mg/dL; 3) triglyceride level < 165 mg/dL; 4) blood glucose level < 126 mg/dL; 5) creatinine level < 23 mg/dL; 6) had complete minimum data (name, age, duration of smoking, number of cigarette smoked per day; 7) for smoker group: smoke at least 1 cigarette a day and has been smoking for no less than 3 months; 8) for nonsmoker group: not smoking. The exclusion criterion was any multivitamin consumption. This study was approved by the Medical and Health Research Ethical Committee of Faculty of Medicine, Gadjah Mada University, Yogyakarta. Informed consent was obtained from every subject.

Sample collection

Blood sample as much as 10 mL was obtained from the subjects after they had fasted for ≥ 10 hours. The blood sample was collected into 2 tubes, an EDTA tube and a non-EDTA tube, for each subject.

β-carotene measurement

β-carotene was measured using HPLC method described by Dietz *et al.*[13] after a modification. Twenty uL of plasma sample was extracted with 100 uL mixture of ethanol and butanol (50:50) containing 5 mg BHT/mL. The mixture was then vortexed and centrifuged at 12.000 rpm for 5 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. The mobile phase used for eluting β-carotene was acetonitrile:tetrahydrofurane:methanol:1% ammonium acetate (64.8:22:6.8:2.8). β-carotene was eluted on a C-18 column (4.6 x 25 mm; 5µm) with a retention time of 1.0 mL/minutes. Absorbance was measured at λ of 447 nm.

Ferritin measurement

Ferritin was measured using simple sandwich ELISA as previously described by Erhardt *et al.*[14] with modification. The measurement system utilized one rabbit anti-ferritin antibody for the solid phase (microtiter wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. 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 and incubated at room temperature 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 was added into each well and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development was stoped with the addition 100 µL of stop solution (1N HCl) to each well and the color changed to yellow. The color optical density was read with ELISA reader (Bench mark; Bio Rad) at 450 nm within 30 minutes. The concentration of ferritin was directly proportional to the color optical density of the samples.

Malondialdehyde measurement

Malondialdehyde was measured using three-times spectrophotometry technique (TBARS assay) as previously described by Pyles *et al.*[15] One mL serum sample was added with 4 mL thio barbituric acid reagent and incubated in the waterbath at 90°C for 80 minutes. Afterward it was cooled on ice for 10 minutes and added 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 λ of 510, 532 and 560 nm.

Data analysis

The normality distribution of data was assessed using Kolmogorov-Smirnov test. Differences in continuous variables between smoker and nonsmoker 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

A total of 79 subjects consisted of 45 smokers and 34 non-smokers. The blood β-carotene, ferritin and MDA levels of both smokers and non smokers can be seen in TABLE 1. Although smokers had lower β-carotene and higher ferritin and MDA levels than those non smokers but there were statistically no significant differences on the variables between smokers and non smokers. The independent t-test result of variables in both smokers and non smokers can be seen in TABLE 1.

TABLE 1. The independent t-test result of blood β-carotene, ferritin and MDA levels (mean ± SD) of both smokers and non smokers

Variables	Non smokers (n=34)	Smokers (n=45)	p
Age (years)	44.66±7.94	39.71±8.91	0.013
β-carotene (µg/dL)	111.17±63.91	106.51±52.73	0.730
Ferritin (ng/mL)	39.20±24.23	46.78±25.79	0.184
MDA (ng/mL)	1.08±0.41	1.14±0.50	0.579

The linear regression analysis of beta-carotene and MDA, ferritin and MDA were provided in TABLE 2 and FIGURE 1 and 2.

TABLE 2. The relation between β-carotene with MDA and ferritin with MDA among subjects

Independent variable	Dependent variable (MDA in ng/mL)		
	R	R^2	p
β-carotene (µg/dL)	-0.133	0.013	0.320
Ferritin (ng/mL)	0.207	0.043	0.067

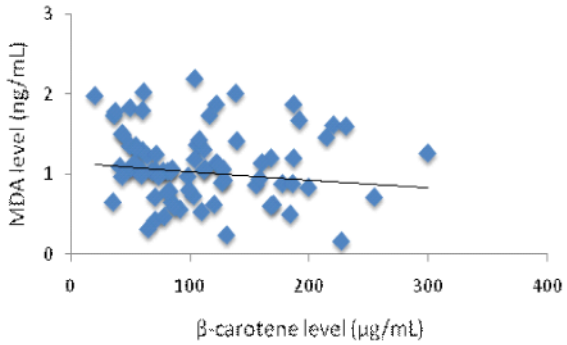

FIGURE 1. The linear regression of β-carotene and MDA among subjects

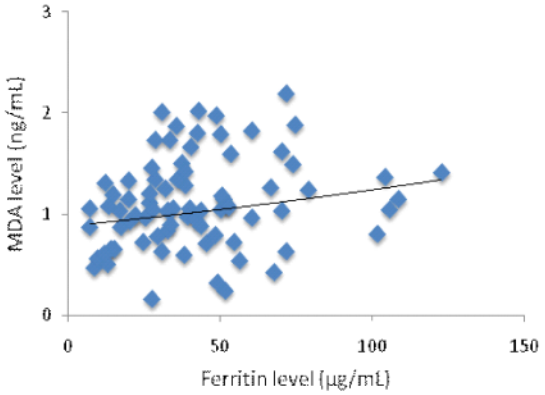

FIGURE 2. The linear regression of ferritin and MDA among subjects

DISCUSSION

It was shown in the results that MDA was not significantly influenced by neither β-carotene nor ferritin. Age, which was thought did not influence MDA was variable with the most significant

difference between smoker and non smoker groups. It meant that aging more efficiently influenced lipid peroxidation (seen through marker of MDA) than smoking activities. This relationship was also observed in a study by Goraca.[11]

In linear regression, it was shown that in this research there was some relation between β-carotene with MDA, likewise ferritin. β-carotene as expected as antioxidant was negatively related to MDA. When β-carotene level was high, the MDA level was low, providing agreement to a statement by Durak[7] that when MDA was high the antioxidant would be low. This relation, though, was very weak with R square = 0.013, meant that β-carotene only affected MDA as much as 1.3%. This very weak relationship was not shown to be significant in the population (p = 0.320).

Some possibilities of why beta-carotene did not successfully influence MDA negatively in the population might be the age factors, where the body could not cope to oxidative stress sufficiently anymore as in their youth, as proposed by Leonard.[9] Aging might affect the body natural capability to quench free radicals and its oxidative stress.

Ferritin as a prooxidant was shown to have positive influence toward MDA. This meant that when ferritin level is increased, the MDA level would likewise be increased. Unfortunately, the strength of this relationship was similarly very weak, with R square of 0.043 or 4.3% only. The p-value of this relation, though, marginally approached 0.05. This meant that perhaps ferritin might truly act as a prooxidant in smokers, both in this research and in the population as well.

A substance called polyhydroxybenzene in the cigarette smoke could contribute to higher ferritin release. This was achieved by the conversion of ferric form of iron (Fe^{+3}) to its ferrous form (Fe^{+2}).[16] Since high level of iron is released from its storage form and there is no physiologic output for loss of iron excess (except in females, during menstruation). Iron overload may take place and adds the burden of oxidative stress, especially in male smokers.

Ho *et al.*[10] reported that older Chinese male who were chronic smokers had more lipid peroxidation in comparison with younger Chinese male chronic smokers. Perhaps this finding explained the reason of hingher level of lipid peroxidation in smoker than in non-smoker groups, although there were more young adult smokers in the smokers groups. The finding of this research in conjunction to the finding of Ho *et al.*[10], in which older Javanese male smokers suffered more lipid peroxidation (shown through MDA as a marker), might indicated that Javanese might not much differ from Chinese.[10]

CONCLUSION

It could be concluded that β-carotene and ferritin did not influenced MDA significantly. Aging became counfounding factor and influenced lipid peroxidation more effectively than smoking itself.

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