ABSTRACT

BACKGROUND
Atherosclerosis, the underlying cause of heart attack, stroke and peripheral disease, is a main cause of morbidity and mortality worldwide. Hypercholesterolemia and hypertriglyceridemia are independent factors in the development and progression of atherosclerosis. The atherosclerotic index (AI) is a strong indicator of cardiovascular heart disease. The objective of this study was to determine the relationship between lipid serum level and AI in subjects 50-70 years of age.

METHODS
A study of cross-sectional design was conducted among male and female subjects 50-70 years of age. The inclusion criteria were: healthy, and capable of active communication. The exclusion criteria were: subjects not completing the study, currently consuming antihyperlipidemic drugs. Lipid profile comprising total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, and malondialdehyde (MDA), was analyzed using commercial kits. Systolic and diastolic blood pressure and body mass index was measured in all subjects. Atherogenic index was calculated from (total cholesterol – HDL cholesterol) / HDL cholesterol. Multiple linear regression was used to analyze the data.

RESULTS
Mean age of the subjects was 60.6 ± 3.30 years and there was a significant relationship of LDL cholesterol and triglycerides with AI (β=0.009; p=0.000 and β=0.008; p=0.000, respectively). Triglyceride level was the most influencing factor for AI (β=0.008; Beta=0.616; p=0.000).

CONCLUSIONS
Higher triglyceride levels increase AI in subjects 50-70 years of age. Subjects with high serum triglyceride level but without symptoms of cardiovascular disease should be examined for the development of coronary artery blockage.

Keywords: Atherosclerotic index, lipid profile, malondialdehyde, 50-70 years of age

Higher triglyceride serum level increases atherosclerotic index in subjects 50-70 years of age

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Kadar trigliseride serum yang tinggi meningkatkan indeks aterosklerotik pada usia 50-75 tahun

ABSTRAK

LATAR BELAKANG
Aterosklerosis merupakan penyebab dari stroke, serangan jantung dan penyakit vaskular yang dapat menimbulkan kematian. Hiperkolesterolemia dan hipertrigliseridemia merupakan faktor risiko penting terjadinya aterosklerosis. Indeks aterosklerotik (IA) merupakan indikator kuat untuk penyakit kardiovaskular. Penelitian ini bertujuan untuk menentukan hubungan antara kadar lipid serum dan indeks aterogenik pada usia 50-70 tahun.

METODE

HASIL
Usia rata-rata subjek penelitian adalah 60,6 ± 3,30 tahun dan terdapat hubungn yang bermakna antara kolesterol-LDL dan trigliserid dengan IA, masing-masing dengan (β=0.009; p=0.000 dan β=0.008; p=0.000. Kadar trigliserid merupakan faktor yang paling besar terhadap IA (β=0.008; Beta=0.616;p=0.000).

KESIMPULAN
Semakin tinggi kadar trigliseride serum semakin tinggi skor IA pada subjek berusia 50-70 tahun. Pada subjek dengan kadar trigliserid serum tinggi, walaupun tidak ditemukan gejala penyakit kardiovaskular harus dilakukan pemeriksaan lebih lanjut untuk memastikan ada tidaknya penyumbatan pembuluh darah terutama di arteria coronaria.

Kata kunci: Indeks aterogenik, profil lipid, malondialdehid, usia 50-70 tahun

INTRODUCTION
Cardiovascular disease is a worldwide major cause of morbidity and mortality. (1-3) Atherosclerosis is considered to be a basic cause of heart attacks, stroke and peripheral vascular disease. Hypercholesterolemia and hypertriglyceridemia are important risk factors for the genesis of atherosclerosis and the progressivity of atherosclerotic lesions. (1) The initial event in the induction of atherosclerosis is the accumulation of cells containing excessive amounts of lipids in the arterial wall. It has been demonstrated that increased production of reactive oxygen species (ROS) substantially influences the chronic inflammatory response in atherosclerosis. (4) ROS reacts with a number of biomolecules, such as lipids, carbohydrates, proteins, and nucleic acids, resulting in cellular dysfunction. Normally there is a balance between the production of free oxygen radicals and the antioxidant defense system. Antioxidant-oxidant imbalance induces oxidative stress as a result of excessive ROS formation. Oxidative stress is
known to be a component of the mechanism of cellular and molecular tissue damage in a broad spectrum of human disease.\(^5\) Increased ROS causes vasoconstriction since it reduces the production of nitric oxide (NO), and increases platelet aggregation and neutrophil adhesion to vascular endothelium.\(^6-8\)

The risk factors of atherosclerosis, such as hypercholesterolemia, diabetes mellitus, smoking, hypertension, and age, may increase the production of free ROS, not only by endothelial cells but also by smooth muscle and adventitial cells. Free radicals are extremely reactive chemical compounds, and the majority of molecules in the body may become a target of these free radicals, but lipids are their most frequent target. The cell membrane is a source of polyunsaturated fatty acids and is extremely vulnerable to attack by free radicals, a process known as lipid peroxidation. Lipid peroxidation leads to the formation of reactive products, some of which interact with proteins and DNA, thus giving rise to toxic and mutagenic components.\(^9\)

One of the standards used to evaluate the condition of the vascular wall resulting from the atherosclerotic process is the atherogenic index (AI), which is calculated from the difference between total cholesterol concentration and HDL cholesterol concentration, divided by HDL cholesterol concentration. The atherogenic index is a strong indicator of cardiovascular disease.\(^1,3\) The study of Nwagha et al.\(^10\) shows a positive correlation of LDL cholesterol and triglycerides with AI in postmenopausal women. A study on women with preeclampsia showed an association between serum MDA level and AI.\(^11\) The study of Prakash and Rao found that hypertriglyceridemia is a risk factor for the atherogenic process, and high LDL cholesterol and triglyceride levels increase the risk of cardiovascular disease.\(^2\) Lafta\(^3\) found a positive association between increased triglycerides and incidence of cardiovascular disease.

Malondialdehydes (MDA) are lipid peroxidation products and indicators of oxidation-induced damage. MDA have been used for many years as biomarker of lipid peroxidation. The progress of the atherosclerotic process is associated with oxidative stress and can be followed up with measurement of MDA.\(^1,11\) The study of Yang et al.\(^1\) compared hyperlipidemic and normolipidemic groups and showed a significantly positive association between MDA and AI. The investigators found a higher atherogenic index in the groups with increased lipid peroxidation levels. No information could be found in the literature of studies showing an association of lipid and MDA levels with AI in healthy adults. The objective of the present study was to determine any association between lipid levels (total cholesterol, HDL cholesterol, LDL cholesterol), and MDA on the one hand and atherogenic index on the other in healthy subjects 50-70 years of age.

**METHODS**

**Study design**

This study used an experimental cross-sectional design and was conducted at the Mampang Prapat Subdistrict Health Center, South Jakarta from February until May 2014.

**Study subjects**

Males and females aged between 50 and 70 years and residing at 2 kelurahan (villages) in Mampang Prapat Subdistrict, South Jakarta, were recruited into this study. The inclusion criteria were: capable of active communication, literate, physically healthy and not requiring help from others, and agreeing to sign informed consent. The exclusion criteria were: not participating in this study up to its completion, and current consumers of anti-lipid drugs.

The sample size was determined with the formula:

\[ N = \left\lfloor \frac{(Z_\alpha - Z_\beta)^2}{0.5 \times \ln(1-r)/(1-r)} \right\rfloor + 3 \]

where \(Z_\alpha = 1.96\), \(Z_\beta = 0.84\), and \(r = 0.61.\)\(^1\)
The minimum sample size required was 18, but in this study it was increased to 80, which is the sample size for using one commercial kit.

**Anthropometric measurements**

Anthropometric assessment comprised determination of weight, height, and body mass index (BMI). Height was measured using a portable microtoise with an accuracy of 0.1 cm. Weight was measured using Sage portable scales with an accuracy of 0.1 kg. Body mass index was calculated from the formula weight (kg) / height (m²) and categorized as follows: normal (< 23.0 kg/m²), overweight or obesity (≥ 23.0 kg/m²), and obesity according to the criteria determined for Asians. (12)

**Laboratory investigations**

From each subject, a 10-ml blood sample was drawn from the cubital vein using plain tubes after an overnight fast of 12-14 hours and centrifuged at 3000 rpm for 15 minutes. The serum was separated and placed into an automatic analyzer.

All determinations of lipid profile components (total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations) were performed spectrophotometrically using an Advia 1800 system and the respective Bayer reagent kits. All procedures produce a complex of a given color, the intensity of which can be spectrophotometrically determined and is proportional to the blood concentration of the lipid component to be assessed.

The principles of the lipid profile determinations are as follows. For total cholesterol determination, cholesterol esters in the sample are hydrolyzed by cholesterol esterase into free cholesterol. This is then oxidized by cholesterol oxidase into cholest-4-en-3-on and peroxides. The peroxides are reacted with 4-aminophenazone and phenol to form the colored 4-(p-benzoquinone-monoimino)-phenazone.

For LDL cholesterol determination, reagent No. 1 solubilizes chylomicrons, HDL and VLDL cholesterol to yield free cholesterol, which is subsequently oxidized into peroxides. The peroxides are then reacted with 4-aminoantipyrine. Next, reagent No. 2 releases the LDL from the LDL cholesterol produced in the first reaction so that the LDL is oxidized to form peroxides. These latter are reacted with N,N-bis(4-sulphobutyl)-m-toluidine-disodium (DSBmT) into a blue-violet colored complex.

For HDL cholesterol determination, HDL cholesterol is separated from other lipid components and solubilized by a specific detergent. The HDL cholesterol is then oxidized and released from its esters, thus forming peroxides. These are reacted with 4-aminoantipyrine and DSBmT using peroxidase catalyst to form a red-colored complex.

For triglyceride concentration determination, the triglycerides in the sample are hydrolyzed into glycerol and free fatty acids. The glycerol is then phosphorylated to yield glycerol-3-phosphate, which is then oxidized into dihydroxy-acetone-phosphate, forming peroxides. The peroxides are reacted with 4-aminophenazone to form the colored 4-(p-benzoquinone-monoimino)-phenazone.

The atherogenic index was calculated from the formula: (total cholesterol – HDL cholesterol) / HDL cholesterol. (13) Plasma MDA concentration was measured using thiobarbituric acid reactive substances (TBARS) according to the Satoh method. The pink-colored chromogens that are formed by the reaction of barbituric acid with MDA are measured at 530 nm. (13) The determination was performed at Prodia Laboratories using high-performance liquid chromatography (HPLC).

**Ethical clearance**

Ethical clearance was issued by the Research Ethics Commission, Faculty of Medicine, Trisakti University.

**Data analysis**

The Kolmogorov-Smirnov test was used to test for normality of the data distribution. Since the data were normally distributed, multiple
regression analysis was used to determine the variables which the greatest influence on AI. A p-value of <0.05 was declared to be statistically significant.

RESULT

The 80 study subjects who met the inclusion and exclusion criteria were between 50 to 70 years of age, comprising 20 males (25%) and 60 females (75%), with mean age of 60.06 ± 3.30 years. With regard to educational level, 6 subjects (7.5%) had no education, 8 subjects (10%) did not finish primary school, 25 subjects (31.3%) finished primary school, 15 subjects (18.8%) were junior high school graduates, 25 subjects (31.3%) were senior high school graduates, and 1 subject (1.3%) was of academic level. With regard to employment, 38 subjects (47.5%) were employed and 42 subjects (52.5%) were unemployed. Mean systolic blood pressure was 137.44 ± 20.93 mmHg and mean diastolic blood pressure 84.33 ± 11.08 mmHg. Mean body mass index was 25.94 ± 3.48 kg/m² (Tables 1 and 2).

Mean total cholesterol was 205.53 ± 36.49 mg/dL, mean LDL cholesterol 140.08 ± 34.31 mg/dL, mean HDL cholesterol was 48.33 ± 8.56 mg/dL, mean triglyceride concentration was 141.90 ± 72.36 mg/dL, mean systolic blood pressure was 137.44 ± 20.93 mmHg, mean Table 2. Mean values of serum lipid profile, blood pressure and malondialdehyde in subjects (n=80)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid profile</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>201.53 ± 36.49</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>140.08 ± 34.31</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>48.33 ± 8.56</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>141.90 ± 72.36</td>
</tr>
<tr>
<td>Atherogenic index (%)</td>
<td>3.34 ± 0.92</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>137.44 ± 20.93</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84.33 ± 11.08</td>
</tr>
<tr>
<td>Malondialdehyde (µM)</td>
<td>0.35 ± 0.09</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.94 ± 3.48</td>
</tr>
</tbody>
</table>

diastolic blood pressure 84.34 ± 11.08 mmHg, mean malondialdehyde concentration (MDA) was 0.35 ± 0.09 µM, and mean atherogenic index was 3.34 ± 0.92 % (Table 2).

The results of multiple linear regression analysis showed a significant association between LDL cholesterol (p=0.000), triglycerides (p=0.000) and AI. There was no significant association between AI on the one hand and age (p=0.836), MDA, (p=0.867), and BMI (p=0.949), systolic blood pressure (p=0.477) and diastolic blood pressure (p=0.933) on the other. The variable triglyceride concentration had a great influence on AI (β=0.008;Beta=0.616; p=0.000) (Table 3).

DISCUSSION

Among the 80 study subjects a significant association was found between increases in AI and LDL cholesterol (p=0.000) and triglyceride concentration (p=0.000). These results are similar to the study results of Nwagha et al.(9) who found that increases in triglyceride and total cholesterol concentrations result a higher AI. Atherogenic index is a strong indicator of cardiovascular disease.(3,9)

In this study a nonsignificant association was found between AI and MDA. Similar results were found in the study of Jasim, (14) but different from
the results of the study of yang diperoleh Amirkhizi et al.,(15) Linna M et al.(16) and Emokpae et al. (17) who found a significant association between AI and MA. This may have been caused by the fact that the study subjects came from different populations. Our study was conducted on healthy subjects, whereas the study by Amirkhizi et al.,(15) Linna M et al.(16) and Emokpae et al. (17) were carried out on pre-hypertensive women, subjects with hyperlipidemia, and subjects with adult sickle cell nephropathy.

No significant association was found between AI and systolic and diastolic blood pressure. This differs from the results of the study by Amirkhizi F et al.(15) and Goyal et al. (18) who found an association between changes in lipid concentrations and hypertension.

Systolic blood pressure increases progressively with advancing age, in comparison with diastolic blood pressure. Oxygen is important and also toxic to human life. Within the cell, through enzymatic and nonenzymatic processes, oxygen receives an electron and is transformed into reactive oxygen species (ROS) that damages cellular lipids, which are broken up into smaller parts, such as MDA, 8-isoprostane, 8-isoprostaglandins-F2 and 8-hydroxy-2-O-deoxyguanosine. Oxidative stress occurs when there is an imbalance between ROS production and the antioxidant defense system. ROS is produced naturally in normal metabolism and plays an important role in biologic processes, such as the killing of bacteria.(19) Upon environmental stress, ROS levels increase dramatically and may damage the cellular structures, particularly when there is no defense mechanism by antioxidants, such as the enzyme SOD or the antioxidant vitamins A, C, E, and polyphenols.(18) Vascular changes may be mediated in several ways, therefore oxidative stress may result in the proliferation of vascular smooth muscle cells accompanied by hypertrophy and accumulation of collagen, causing thickening of the tunica media and narrowing of the vascular lumen. In addition, increased oxidative stress may damage the endothelium and disturb vascular relaxation, thus increasing vascular contractile capacity. All of these influences on the blood vessels may lead to hypertension.(20,21)

In our study we did not find a significant association between AI and BMI. This agrees with the results of the study by Ranjit et al. (22) who found no correlation of BMI and lipid profile with AI. In the present study there was also no significant association between AI and age. Advancing age increases the atherosclerotic process and thus AI. The lack of a significant association in this study may have been caused by the small sample size and the narrow age range. These results were in contrast with those of Nghawa UT et al.(10) who stated that advancing age increases AI in postmenopausal women.

Epidemiological studies have shown a positive association between total cholesterol concentration and coronary heart disease. In a number of patients the total cholesterol concentration is not an accurate predictor of the risk of coronary heart disease, because total

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>Beta</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>.005</td>
<td>.019</td>
<td>.836</td>
</tr>
<tr>
<td>MDA</td>
<td>-.144</td>
<td>-.015</td>
<td>.867</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>.009</td>
<td>.324</td>
<td>.000 **</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>.008</td>
<td>.616</td>
<td>.000 **</td>
</tr>
<tr>
<td>BMI</td>
<td>-.001</td>
<td>-.006</td>
<td>.949</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>-.004</td>
<td>.083</td>
<td>477</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>.000</td>
<td>-.009</td>
<td>913</td>
</tr>
</tbody>
</table>

Legend: B=regression coefficient; Beta=standardized coefficient; MDA=triglycerides, and malondialdehyde; LDL=low density lipoprotein; HDL=high density lipoprotein.
cholesterol is a combination of all types of cholesterol, not only atherogenic lipoproteins such as low-density lipoproteins (LDL), very-low density lipoproteins (VLDL), and intermediate lipoproteins (IDL), but also anti-atherogenic lipoproteins such as high-density lipoproteins.

Smaller LDL particles are more atherogenic than larger ones. Particles that are larger and contain much triglycerides and other smaller particles such as small VLDLs and IDLs, known as apo C-III containing lipoproteins, are extremely atherogenic.

A case-control study with prospectively collected samples found that small LDL particles and high triglyceride concentrations are the major independent risk factors for myocardial infarction. Several studies have demonstrated an inverse association between lipoprotein size and the ability to penetrate the endothelial barrier and enter the arterial intima. Chylomicrons and large VLDLs cannot enter the arterial wall, but small VLDLs and IDLs may do so. Therefore triglycerides rich in lipoproteins are termed atherogenic.\(^{(23)}\)

The design of our study was cross sectional, so that our study cannot explain any cause-and-effect association between triglyceride concentration and AI. The clinical implication of our study results is that increased serum triglyceride concentration is a risk factor for atherogenesis. Atherosclerosis is a basic cause of heart attacks, stroke, and peripheral vascular disease.\(^{(1)}\) In healthy persons with high serum triglyceride concentrations but no signs of cardiovascular or cerebrovascular disease, further investigations are necessary to determine the presence or absence of vascular obstruction, particularly in the coronary arteries. Further studies of longitudinal design are necessary to show a cause-and-effect association between serum triglyceride concentration and AI.

**CONCLUSIONS**

The present study succeeded in demonstrating that higher serum triglyceride concentration increases AI scores in healthy subjects 50–70 years of age. Subjects with high serum triglyceride level but without symptoms of cardiovascular disease should be examined for the development of coronary artery blockage.

**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

**ACKNOWLEDGMENT**

The investigators thank the Dean and Vice-Deans of Trisakti University for moral and material support in the conduct of this study. Thanks are also due to the Head of Mampang Health Center (Puskesmas Mampang) and staff and the study subjects who participated in the study.

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