

Ethnic difference in serum antibodies to oxidized low density lipoprotein in healthy Malaysian subjects

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Abstrak

Latar Belakang: Sebagian populasi lebih rentan untuk terserang penyakit arterosklerosis dibandingkan dengan populasi lain. Etnis India mempunyai mortalitas yang tinggi untuk penyakit jantung koroner (PJK) dibandingkan dengan etnis Cina dan Melayu. Antibodi terhadap oxLDL (Ab-oxLDL) diproduksi sebagai respons imun dan insidens PJK di berbagai etnis berhubungan dengan respon imun ini. Tujuan penelitian ini adalah untuk mengukur kadar Ab-oxLDL dan kadar lipid di kalangan 3 etnis mayoritas di Malaysia.

Metode: Penelitian ini melibatkan 150 subjek sehat di Malaysia yang terdiri dari 50 etnis Melayu, 50 etnis Cina dan 50 etnis India. Kadar Ab-oxLDL diukur dengan menggunakan metode enzim immunoassai dan kadar trigliserida dan kolesterol diukur dengan menggunakan metode enzimatik. HDL-kolesterol diukur menggunakan metode presipitasi dan LDL-kolesterol dihitung menggunakan formula Friedewald.

Hasil: Kadar Ab-oxLDL [adjusted mean (95% CI)] paling tinggi dikalangan etnis Melayu [1404 (1202-1607) mU/mL] diikuti oleh etnis Cina [1026 (829-1223) mU/mL] dan India [954 (744-1163) mU/mL] ($P = 0.006$), sementara kadar HDL-C paling tinggi dikalangan etnis Cina [1.53 (1.44-1.61) mmol/L] diikuti oleh etnis Melayu [1.44 (1.35-1.53) mmol/L] dan India [1.35 (1.26-1.45) mmol/L] ($P = 0.035$).

Kesimpulan: Hasil penelitian membuktikan bahwa kadar Ab-oxLDL dan HDL-C berbeda-beda berdasarkan etnis dan Ab-oxLDL mungkin mempunyai sifat antiaterogenik dikalangan etnis Melayu. (*Med J Indones 2012;21:18-22*)

Abstract

Background: Some populations are more susceptible to atherosclerotic diseases than others. Indians had a higher mortality due to coronary heart disease (CHD) than Chinese and Malays. Antibodies to oxidized low density lipoprotein (Ab-oxLDL) are produced as an immune response to oxidized low density lipoprotein (oxLDL). The difference in prevalence of CHD among the ethnic groups may be related to the immune response. The objectives of this study were to determine the serum Ab-oxLDL levels and lipid profile among the three major Malaysian ethnic groups.

Methods: The participants of this study were 150 healthy subjects consisting of 50 Malays, 50 Chinese and 50 Indians. Serum Ab-oxLDL was measured by enzyme immunoassay method. Serum triglycerides and total cholesterol were measured by enzymatic methods. Serum high density lipoprotein cholesterol (HDL-C) was measured by precipitation method and low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula.

Results: AboxLDL level [adjusted mean (95% of CI)] was highest in Malays [1404 (1202-1607) mU/mL] followed by Chinese [1026 (829-1223) mU/mL] and Indians [954 (744-1163) mU/mL] ($P = 0.006$) and HDL-C level was highest in Chinese [1.53 (1.44-1.61) mmol/L] followed by Malays [1.44 (1.35-1.53) mmol/L] and Indians [1.35 (1.26-1.45) mmol/L] ($P = 0.035$).

Conclusion: Our results indicate that Ab-oxLDL and HDL-C levels differed by ethnic and AboxLDL may have antiatherogenic properties among Malaysian ethnic groups. (*Med J Indones 2012;21:18-22*)

Keywords: Antibodies to oxLDL, coronary heart disease, ethnic groups, LDL, lipid profile

Oxidative modification of low density lipoprotein (LDL) takes place *in vivo* and an enhanced uptake of oxidized low density lipoprotein (oxLDL) via scavenger receptor leads to accumulation of cholesterol ester promoting foam cell formation and fatty streak. Therefore, formation of oxLDL is a key event in the development of atherosclerosis.¹ Several studies have suggested that circulating oxLDL level is an independent predictor for coronary artery disease.^{2,3} OxLDL particles are immunogenic and an immune response to oxLDL can induce the formation of the antibodies.

Antibodies to oxLDL (Ab-oxLDL) are detected in atherosclerotic lesions as well as in the serum.⁴ Levels

of Ab-oxLDL were significantly elevated in patients with type 2 diabetes mellitus,⁵ myocardial infarction,⁶ coronary artery disease,⁷ peripheral vascular disease⁸ and rheumatoid arthritis.⁹

Coronary heart disease (CHD) is a multifactorial disease. A variety of risk factors such as dyslipidaemia, hypertension, diabetes mellitus along with environmental influences and genetic susceptibility, can predispose to CHD event. Some populations are more susceptible to CHD than others. Previous studies provided evidence that Indians had a higher rate in terms of coronary heart disease mortality than Chinese and Malays.¹⁰ Apart from the conventional risk factors such as a high level of

low density lipoprotein cholesterol (LDL-C) and a low level of high density lipoprotein cholesterol (HDL-C), ethnic difference in prevalence of CHD may be related to the levels of Ab-oxLDL. Therefore, the objectives of this study were to compare serum levels of Ab-oxLDL and lipid profile among three ethnic groups: Malay, Chinese, and Indian.

METHODS

Subjects

The present study was a pilot study to observe the differences in serum levels of Ab-oxLDL and lipid profile among three ethnic groups. Sample size was estimated based on single proportion formula using 95% of confidence interval, 5% of precision and 50% population proportion. For this study, the minimum sample size required was 47 subjects, using 10% of the sample size calculated and taking into account a 20% dropout rate. Thus, 50 subjects were included in each ethnic group, making a total number of 150 subjects.

This study was approved by the Research Ethics Committee (Human), Universiti Sains Malaysia and it was carried out in Universiti Sains Malaysia, Health Campus, Kelantan. The information form, consent form and questionnaire were distributed to students and staff, and then the completed questionnaire were analysed. A total of 150 subjects consisting of 50 Malays, 50 Chinese and 50 Indians were selected for this study. Written consent was obtained from each participant. Our study subjects were clinically healthy Malaysian adults more than 18 years of age. Either smokers or subjects who are taking alcoholic beverages regularly were excluded in this study. We also excluded those who had a history of cardiovascular disease and chronic diseases such as diabetes mellitus and hypertension. Obese subjects and pregnant women were not allowed to participate in this study. We also excluded subjects who received any regular medication and who were on diet therapy to reduce weight. Weight and height were measured and body mass index (BMI) was calculated prior to sample collection.

Sample collection and processing

From each subject, 3 mL of venous blood was collected in the morning after an overnight fast (9-12 hours). After completing coagulation process, the tubes were centrifuged and serum was separated from blood. Serum samples were stored at -70°C for further analysis for lipid profile and Ab-oxLDL.

Measurement of lipid profile

Serum concentrations of triglycerides and total cholesterol were measured by enzymatic colorimetric methods (RANDOX laboratories, United Kingdom) on Hitachi 912 autoanalyzer. For the determination

of HDL-C, apoB-containing lipoproteins in the serum were precipitated with phosphotungstic acid and magnesium ions, and the cholesterol was measured in the supernatant after centrifugation (RANDOX laboratories, United Kingdom). LDL-C was calculated by Friedewald formula.

Measurement of Ab-oxLDL level

Level of Ab-oxLDL was measured by enzyme immunoassay method using commercial kit (oLAB, Biomedica, USA). All reagents and samples were made at room temperature ($18-26^{\circ}\text{C}$) before use in the assay. All steps were followed according to the manufacturer's instruction. All standards, controls and samples were used in 1:5 pre-dilution and in 1:50 end-dilution in the assay. In brief, the transfer of the pre-diluted materials into the coated microtiterstrip was made complete within 15 minutes. The coated microtiterstrip was covered and was incubated at 37°C for 1.5 hours. After washing, 100 μl Conjugate was added into each well. Next incubation was done at room temperature for 30 minutes followed by the washing step. Then, 100 μl of substrate was added into each well and the microtiterstrip was incubated at room temperature ($18-26^{\circ}\text{C}$) for 15 minutes in the dark. Lastly, 50 μl Stop Solution was added into each well and absorbance was immediately measured at 450 nm using ELISA reader. Ab-oxLDL titer was expressed in mU/mL defined by the manufacturer.

Statistical analysis

The data were analyzed using computer software PASW Statistic version 18.0. For the anthropometric data, numerical data with normal distribution were presented as mean whereas categorical data were expressed as number (%). Differences of Ab-oxLDL and lipid profile among ethnic groups were tested using analysis of covariance and the results were presented as adjusted mean. *P* value less than 0.05 was considered statistically significant at 95% confidence interval.

RESULTS

Our study consisted of 150 healthy Malaysian subjects and the anthropometric data of the three ethnic groups are presented in table 1.

Table 1. Anthropometric data of the study subjects

	Total n = 150	Malays n = 50	Chinese n = 50	Indians n = 50
Sex				
Male	78 (52.0)	21 (26.9)	21 (26.9)	36 (46.2)
Female	72 (48.0)	29 (40.3)	29 (40.3)	14 (19.4)
Age (years)	25.0 (7.7)	21.3 (4.6)	24.5 (7.4)	29.2 (8.5)
BMI (kg/m ²)	21.8 (3.5)	21.3 (3.2)	20.6 (3.0)	23.4 (3.7)

Sex - n(%); Age & BMI - mean(SD)

Distribution of Ab-oxLDL levels

Figure 1 shows the distribution of serum Ab-oxLDL in total study subjects and its median (range) was 1095 (50 to 3010) mU/mL. Ab-oxLDL levels were observed significantly different among the three groups (Table 2). The Malay group had a higher adjusted mean level [1404 (95% of CI:1202-1607) mU/mL] compared to the Chinese group [1026 (829-1223) mU/mL] and Indian group [954 (744-1163) mU/mL] ($P=0.006$). Indians had the lowest level of Ab-oxLDL.

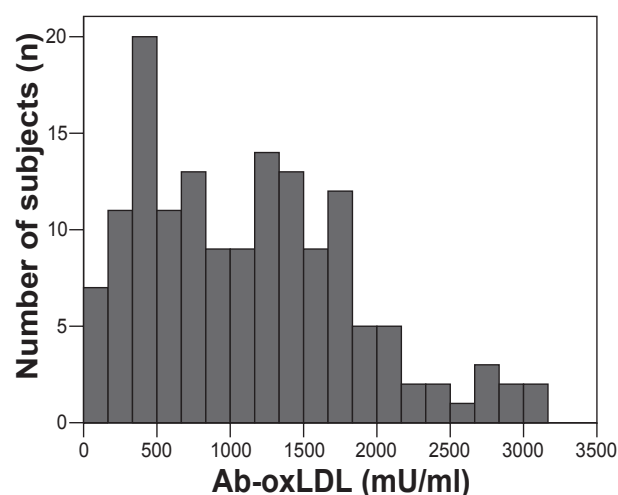


Figure 1. Distribution of serum Ab-oxLDL levels in the 150 subjects

Table 2. Distribution of Ab-oxLDL in the three ethnic groups

	Adj. Mean (95% CI) ^a			F-stat (df) ^a	P value
	Malay n=50	Chinese n=50	Indian n=50		
Ab-oxLDL (mU/mL)	1404 (1202-1607)	1026 (829-1223)	954 (744-1163)	5.22 (2,144)	0.006*

Ab-oxLDL, Antibodies to oxidized low density lipoprotein

^aAdjusted mean using analysis of covariance controlling for sex, age, BMI

*Significant difference among Malays, Chinese and Indians

Distribution of lipid profile variables

The adjusted mean (95% of CI) levels of lipid profile variables among the three ethnic groups are shown in table 3. Levels of serum triglycerides, total cholesterol and LDL-C were not different among the three groups. However HDL-C levels were significantly higher in the Chinese group [1.53 (95% of CI: 1.44-1.61) mmol/L] compared to the Malay group [1.44 (1.35-1.53) mmol/L] and the Indian group [1.35 (1.26-1.45) mmol/L] ($P=0.035$). Indians had the lowest level of HDL-C.

Table 3. Distribution of lipid profile variables in the three ethnic groups

Lipid profile variables (mmol/L)	Adj. Mean (95% CI) ^a			F-stat (df) ^a	P value
	Malay n=50	Chinese n=50	Indian n=50		
TG	0.97 (0.83-1.11)	1.03 (0.89-1.16)	0.98 (0.84-1.12)	0.200 (2,144)	0.819
TC	5.04 (4.77-5.32)	4.85 (4.58-5.11)	4.87 (4.59-5.16)	0.568 (2,144)	0.568
HDL-C	1.44 (1.35-1.53)	1.53 (1.44-1.61)	1.35 (1.26-1.45)	3.439 (2,144)	0.035*
LDL-C	3.16 (2.90-3.43)	2.85 (2.60-3.11)	3.09 (2.82-3.36)	1.517 (2,144)	0.223

HDL-C- high density lipoprotein cholesterol; LDL-C - low density lipoprotein cholesterol; TC - total cholesterol; TG - triglycerides

^aAdjusted mean using analysis of covariance controlling for sex, age, BMI

*Significant difference among Malays, Chinese and Indians

DISCUSSION

Circulating Ab-oxLDL levels have been investigated in healthy subjects as well as in a variety of disease conditions. In the present study, Ab-oxLDL levels were found to be significantly different in the ethnic groups. The Malay group exhibited the highest level whereas Indians had the lowest level. Miller and colleague also reported a variation in Ab-oxLDL levels among Whites, Africans and South Asians.¹¹ Ethnic differences in immune response to oxLDL appear in part to be related to the development of CHD.

Increased intima-media thickness of common carotid artery (CCA-IMT) and high circulating oxLDL are related to increased risk of CHD and cerebrovascular disease. In healthy Japanese population, circulating Ab-oxLDL was inversely correlated with plasma oxLDL.¹² Chen et al. have also demonstrated an inverse relationship between Ab-oxLDL/oxLDL ratio and CCA-ITM in a clinically healthy population.¹³ A recent study has reported that circulating Ab-oxLDL, in hypertensive patients, was inversely correlated with high sensitivity C-reactive protein (hs-CRP) level which is an inflammation marker.¹⁴ Ab-oxLDL, derived from a patient with coronary artery disease, inhibited the uptake of oxLDL by macrophages.¹⁵ In addition, Habets and colleagues demonstrated that immunization of LDL receptor-deficient mice with oxidized low-density lipoprotein-pulsed matured dendritic cells reduced the progression of atherosclerosis.¹⁶ All data from these studies indicated that immune response to oxLDL may have a protective role in atherosclerosis. Therefore, an elevation in Ab-oxLDL levels may be considered one of protective markers for atherosclerosis. Low circulating Ab-oxLDL level in Indian group seen in our study may explain the higher CHD rate in Indians.

However, the clinical importance of circulating Ab-oxLDL is still controversial as a positive correlation between Ab-oxLDL and CRP was seen in patients with rheumatoid arthritis.¹⁷ Increased Ab-oxLDL levels were found in familial hypercholesterolemia compared to the control subjects and the titers were correlated with Achilles tendon thickness.¹⁸ On the other hand, other studies did not support the association between Ab-oxLDL level and cardiovascular disease. A cross-section study has reported that there was no correlation between circulating oxLDL and Ab-oxLDL in subjects above 40 years old.¹⁹ An association of Ab-oxLDL with either CHD or cardiovascular disease events was not found in a cohort study.²⁰ Furthermore, the level of circulating IgM Ab-oxLDL was lower in patients with CHD than in patients without CHD, suggesting that these antibodies might be inversely associated with the presence of atherosclerosis.²¹ Although our study demonstrated the ethnic difference in Ab-oxLDL levels in healthy subjects, a variety of factors is to be considered in measurement of Ab-oxLDL level such as physical activity and diet.

In the present study, Malays had the highest levels of total cholesterol and LDL-C whereas Chinese had the highest levels of HDL-C. However, a statistically significant difference was seen only in HDL-C where Indians had lower levels than Malays and Chinese. Tan et al. found that the highest LDL-C was seen in Malays and the lowest HDL-C was seen in Indians among three ethnic groups in Singapore.²² It is possible that a higher CHD rate in Indians may be partly due to low Ab-oxLDL although low HDL-C has been known to be a CHD risk factor.

In conclusion, we have highlighted the ethnic variation in levels of circulating Ab-oxLDL and HDL-C in healthy subjects. The results from this study, showing the lowest production of Ab-oxLDL in Indians who has the highest mortality rate of CHD, provide information for new therapeutic targets in the prevention and treatment of cardiovascular disease.

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