Serum Concentrations of Lipids and Apolipoprotein E in Angiographically Defined Coronary Artery Disease Patients

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Abstract

Apolipoprotein (apo) E is an important component of plasma lipoproteins and influences lipoprotein metabolism through its action as a receptor ligand. The association of serum apo E concentrations and coronary artery diseases (CAD) was investigated in 100 CAD patients (71 men, 29 women, mean age 62.0 years) and 155 healthy volunteers (87 men, 68 women, mean age 50.6 years). Patients with CAD had lower serum apo E concentrations (5.1±1.3 mg/dL) than the healthy volunteers (5.9±1.8 mg/dL, $p < 0.001$). There were no significant differences between the number of disease vessels and the concentration of serum apo E. Serum apo E concentrations may have an anti-atherosclerotic effect and the serum apo E levels could be a useful parameter for defining cardiovascular risk factor.

Key word : Serum Apolipoprotein E, CAD, Angiographically Defined

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Apolipoprotein E (apo E) is a 299 amino acid, arginine-rich glycoprotein. Many different cell types throughout the body synthesize Apo E. The major site of synthesis and secretion is liver parenchymal cells, which secrete apo E in association with very-low-density lipoprotein (VLDL) particles. In addition, apo E is a protein component of several other classes of plasma lipoprotein such as chylomicron and intermediate-density lipoproteins (IDL) (1). Only a minute amount of apo E is found in low-density lipoproteins (LDL). Apo E serves as a ligand for LDL receptor and apo E receptor. It was found

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that the amount of apo E and the location of apo E on the lipoprotein particles are important for the interaction between apo E and its receptor[2]. The mechanisms by which apo E prevents atherosclerosis development may be related to its function in lipoprotein clearance, but other more direct effects on the vessel wall may well contribute to arterial protection. As a ligand of the LDL and apo E receptors, apo E is the key mediator in the clearance of triglycerides (TG)-rich lipoproteins. It also appears to be responsible for the removal of apo-E-containing HDL from the blood circulation[3]. Apo E secreted by macrophages inhibits the unregulated uptake of oxidized-VLDL *via* the scavenger receptor and prevents transformation of macrophage into foam cells [4]. In case-control studies, apo E concentrations in VLDL and LDL were higher in patients with coronary artery disease (CAD) patients[5-8]. There was no evidence of correlation between serum concentrations of apo E and severity of CAD patients.

The aim of this study was to assess the interrelationship between serum apo E and lipid profiles in normal healthy subjects and angiographically confirmed coronary artery disease (CAD) patients.

MATERIAL AND METHOD

Study subjects

The study population comprised 100 Thai patients (71 men, 29 women) with angiographically confirmed coronary artery narrowing exceeding 50 per cent who were referred for coronary angiography at Her Majesty Cardiac Center, Faculty of Medicine, Siriraj Hospital, Bangkok, Thailand. Additionally, 155 healthy volunteers (87 men, 68 women) were recruited as normal healthy subjects. Informed consents were obtained from all patients and controls who participated in the study. The study was performed in accordance with the roles and regulations established by the hospital's ethic committee.

Blood samples and analysis

Fasting blood samples were drawn and allowed to clot; the serum was separated by high-speed centrifugation for 15 min. Serum were frozen at -70°C until analysis. Total cholesterol, triglycerides were measured by using established enzymatic methods on a Hitachi 717 automated analyzer (Roche Diagnostics, Thailand). High-density lipoprotein cholesterol (HDL-C) was determined by using the direct method on Hitachi 717. Low-density lipoprotein cholesterol (LDL-C) was calculated by using Friedewald's equation. Serum apo E concentrations were determined by immunoturbidimetry, using a kit from Wako Pure Chemical, Japan. The samples were assayed according to the manufacturer's recommendations. The detection limit of the method was 0.63 mg/dL to 10.0 mg/dL. Sera were diluted with double-distilled water when the apo E concentration exceeded 10.0 mg/dL. Control sera (lyophilized Wako Control, included in the kit) were analyzed in each series of measurements. The within run imprecision of apo E measurement was tested; it varied from 2.8 to 4.5 per cent. The day-to-day reproducibility was evaluated to be 3.8 to 5.2 per cent.

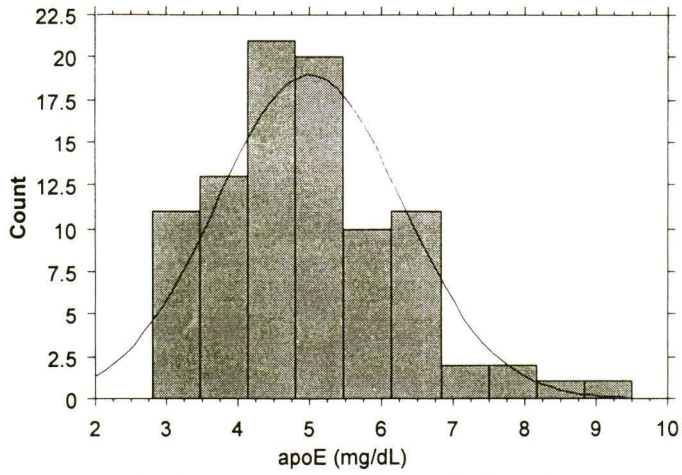
Statistical analysis

All results are expressed as the mean and standard derivation (SD). Unpaired *t*-tests were carried out to compare the parameter between different groups. StatView (Abacus, USA.) statistical packages were used for statistical analysis. The significant p-valve was <0.05.

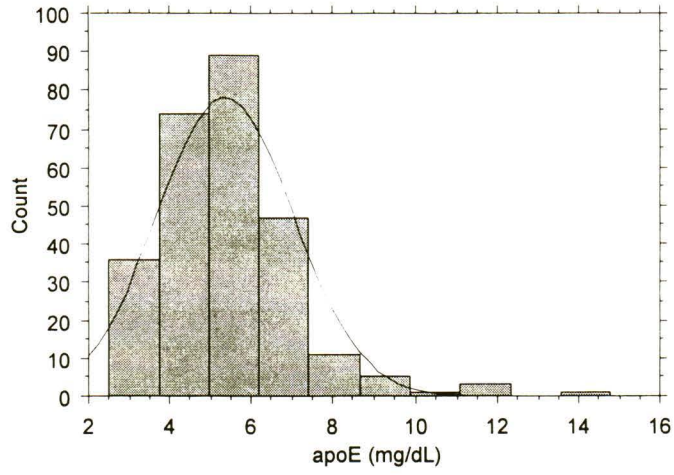
Table 1. Age, lipid profiles and serum apo E concentration of CAD patients and normal healthy controls.

	CAD patients (n = 100)	Normal healthy controls (n = 155)	P value
Age, years	62.0 ± 9.6	50.6 ± 12.7	<0.0001
TC, mg/dL	200.1 ± 42.9	210.6 ± 46.5	NS
TG, mg/dL	148.8 ± 76.1	128.3 ± 83.2	0.047
HDL-C, mg/dL	39.2 ± 12.9	49.1 ± 16.7	<0.0001
LDL-C, mg/dL	131.1 ± 39.8	135.3 ± 41.1	NS
Apo E, mg/dL	5.1 ± 1.3	5.9 ± 1.8	<0.001

NS = no significant difference



(a) Serum apo E (mg/dL) in CAD patients



(b) Serum apo E (mg/dL) in healthy volunteers

Fig. 1. Frequency distribution of serum apo E concentrations in (a) CAD patients and (b) normal healthy subjects.

RESULTS

Age, lipid profiles and apo E concentrations are presented in Table 1. In the present study, the mean age of the CAD patients was slightly higher than the normal healthy subjects but total cholesterol and LDL-C of both groups were the same. Serum concentrations of triglycerides, HDL-C and apo E were lower in the CAD patients when compared with the control subjects. The frequency distributions of serum apo E are shown in Fig. 1. The authors also studied the association between serum apo E concentrations and the severity of the disease. There was no significant association between number of diseased vessels and the concentration of serum apo E as shown in Fig. 2.

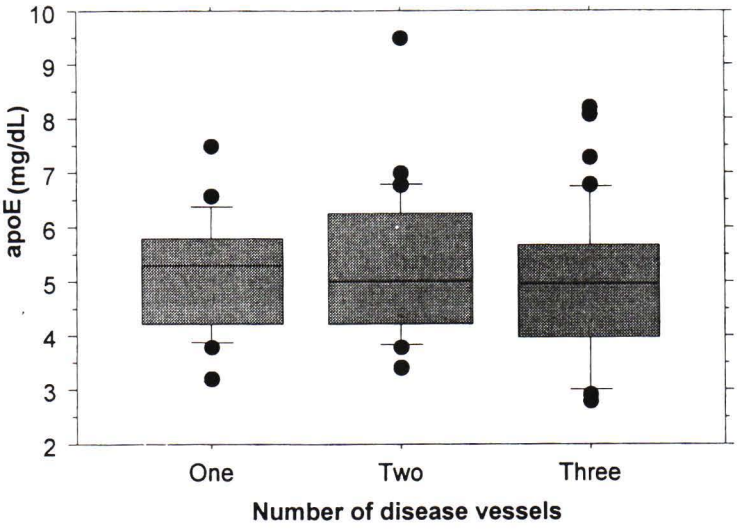


Fig. 2. Box plot of serum apo E concentrations in CAD patients who were confirmed with one, two and three diseased vessels by coronary angiography.

DISCUSSION

The measurement of serum apo E concentration is not a routine test in the clinical chemistry laboratory, in contrast to the assay of apo A1 and apo B. The serum apo E concentration is determined in part by the genetic variation at the apo E locus; however, a large amount of variability remains unexplained by this genetic factor, suggesting that other genetic and environmental components are major determinants of serum apo E concentration[9,10]. The protective role of apo E against atherosclerosis has been remarkably strengthened by the generation of transgenic animal models of apo E deficiency [11]. Apo E-deficient mice develop severe hypercholesterolemia and premature diffuse atherosclerotic vascular disease, which can be prevented by bone marrow transplantation[12-14]. We demonstrated that serum apo E concentrations in CAD patients were significantly lower than in healthy subjects. However, we cannot address the association between serum apo E concentrations and the severity of the diseases. Our study shows the same results as Genest et al, Corbo et al, and Shen et al(6,15-17). Most of these investigators reported low apo E levels in CAD patients when compared with controls. Unfortunately, these study groups did not investigate the association of serum apo E concentrations and the severity of the diseases.

From this point of view, there may be benefit from an intravenous injection of synthetic apo E in CAD patients who are detected with low apo E concentrations in their sera. There were three animal studies that tested this hypothesis[3,18,19]. The treatments resulted in more than 60 per cent reduction of cholesterol in the aorta and remarkably decreased the surface of the aorta covered by macroscopic plaques. Quantitative data are consistent with the hypothesis that serum apo E concentrations may have an anti-atherogenic role and suggest that serum apo E levels could be a useful parameter for defining risk in CAD patients.

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REFERENCES

1. Mahley RW, Palaoglu KE, Atak Z, et al. Turkish Heart Study: lipids, lipoproteins, and apolipoproteins. J Lipid Res 1995; 36: 839-59.
2. Mazzone T. Apolipoprotein E secretion by macrophages: Its potential physiological functions. Curr Opin Lipidol 1996; 7: 303-7.
3. Mahley RW. Apolipoprotein E : Cholesterol transport protein with expanding role in cell biology. Science 1988; 240: 622-30.
4. Shimano H, Yamada N, Katsuki M, et al. Overexpression of apolipoprotein E in transgenic mice : Marked reduction in plasma lipoproteins except high density lipoprotein and resistance against diet-induced hypercholesterolemia. Proc Natl Acad Sci U S A 1992; 89: 1750-4.
5. Chivot L, Mainard F, Bigot E, et al. Logistic discriminant analysis of lipids and apolipoproteins in a population of coronary bypass patients and the significance of apolipoproteins C-III and E. Atherosclerosis 1990; 82: 205-11.
6. Genest JJ Jr, Bard JM, Fruchart JC, Ordovas JM, Wilson PF, Schaefer EJ. Plasma apolipoprotein A-I, A-II, B, E and C-III containing particles in men with premature coronary artery disease. Atherosclerosis 1991; 90: 149-57.
7. Luc G, Fievet C, Arveiler D, et al. Apolipoproteins C-III and E in apoB- and non-apoB-containing lipoproteins in two populations at contrasting risk for myocardial infarction: The ECTIM study. Etude Cas Temoins sur 'Infarctus du Myocarde. J Lipid Res 1996; 37: 508-17.
8. Syvanne M, Rosseneu M, Labeur C, Hilden H, Taskinen MR. Enrichment with apolipoprotein E characterizes postprandial TG-rich lipoproteins in patients with non-insulin-dependent diabetes mellitus and coronary artery disease: a preliminary report. Atherosclerosis 1994; 105: 25-34.
9. Braeckman L, De Bacquer D, Rosseneu M, De Backer G. The effect of age and lifestyle factors on plasma levels of apolipoprotein E. J Cardiovasc Risk 1998; 5: 155-9.
10. Boer JM, Feskens EJ, Schouten EG, Havekes LM, Seidell JC, Kromhout D. Lipid profiles reflecting high and low risk for coronary heart disease: Contribution of apolipoprotein E polymorphism and lifestyle. Atherosclerosis 1998; 136: 395-402.
11. Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci USA 1992; 89: 4471-5.
12. Linton MF, Atkinson JB, Fazio S. Prevention of atherosclerosis in apolipoprotein E-deficient mice by bone marrow transplantation. Science 1995; 267: 1034-7.
13. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb 1994; 14: 133-40.
14. Reddick RL, Zhang SH, Maeda N. Atherosclerosis in mice lacking apo E. Evaluation of lesional development and progression. Arterioscler Thromb 1994; 14: 141-7.
15. Corbo RM, Vilardo T, Ruggeri M, Gemma AT, Scacchi R. Apolipoprotein E genotype and plasma levels in coronary artery disease. A case-control study in the Italian population. Clin Biochem 1999; 32: 217-22.
16. Corbo RM, Scacchi R, Vilardo T, Ruggeri M. Polymorphisms in the apolipoprotein E gene regulatory region in relation to coronary heart disease and their effect on plasma apolipoprotein E. Clin Chem Lab Med 2001; 39: 2-6.
17. Shen X, Xia Y, Sass C, Visvikis S, Siest G. Association of apolipoprotein E polymorphism and concentration with serum lipids and apolipoprotein level in the Chinese from Shanghai. Clin Chem Lab Med 1998; 36: 615-9.
18. Yamada N, Shimano H, Mokuno H, et al. Increased clearance of plasma cholesterol after injection of apolipoprotein E into Watanabe heritable hyperlipidemic rabbits. Proc Natl Acad Sci USA 1989; 86: 665-9.
19. Yamada N, Inoue I, Kawamura M, et al. Apolipoprotein E prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbits. J Clin Invest 1992; 89: 706-11.

ซีรั่มไลปิดและอะโปไลโปโปรทีน อี ในผู้ป่วยโรคหลอดเลือดหัวใจโคโรนารี่ชาวไทยที่ได้รับการตรวจยืนยันโดยการตรวจสวนหัวใจ

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อะโปไลโปโปรทีน อี (อะโป อี) ถือได้ว่าเป็นส่วนประกอบสำคัญของพลาสม่าไลโปโปรทีนและมีอิทธิพลต่อการเมตาบอลิซึมของไลโปโปรทีนโดยทำหน้าที่เป็นตัวจับไขมันส่งไปยังปลายทางที่ต้องการ คณะผู้วิจัยได้ทำการศึกษาถึงความสัมพันธ์ระหว่างความเข้มข้นของซีรั่ม อะโป อี กับผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่จำนวน 100 ราย (ชาย 71 ราย, หญิง 29 ราย, อายุเฉลี่ย 62 ปี) เปรียบเทียบกับอาสาสมัครสุขภาพดีจำนวน 155 ราย (ชาย 87 ราย, หญิง 68 ราย, อายุเฉลี่ย 50.6 ปี) ผลการวิจัยพบว่าในกลุ่มผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่มีระดับซีรั่ม อะโป อี ต่ำกว่าในกลุ่มอาสาสมัครสุขภาพดีอย่างมีนัยสำคัญทางสถิติ (5.1 ± 1.3 มก/ดล เปรียบเทียบกับ 5.9 ± 1.8 มก/ดล ค่าพี < 0.001) และเมื่อหาความสัมพันธ์ระหว่างความรุนแรงของโรคโดยดูจากจำนวนหลอดเลือดแดงโคโรนารี่ที่อุดตันกับระดับซีรั่ม อะโป อี ผลปรากฏไม่พบความสัมพันธ์ดังกล่าวผลการศึกษาแสดงให้เห็นว่า อะโป อี ในเลือดน่าจะมีส่วนช่วยในการต่อต้านการเกิดภาวะหลอดเลือดแดงแข็งได้ไม่มากก็น้อยและการตรวจหาระดับซีรั่ม อะโป อี คงจะมีประโยชน์ในการบอกถึงปัจจัยเสี่ยงต่อการเกิดโรคหัวใจและหลอดเลือดได้อีกทางหนึ่ง

คำสำคัญ : ซีรั่ม อะโปไลโปโปรทีน อี, โรคหลอดเลือดแดงโคโรนารี่, การตรวจยืนยันโดยการตรวจสวนหัวใจ

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