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Original Research Article

Elevation of serum E-selectin in Thai hyperlipidemia adults

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ABSTRACT

Background: Hyperlipidemia is an important risk factor of cardiovascular diseases (CVD), whose pathogenesis involves vascular endothelial dysfunction. Therefore, a specific marker of endothelial dysfunction, serum E-selectin, was assessed in Thai hyperlipidemia adults.
Methods: Subjects who had no history of hypertension, diabetes and other serious illness were recruited and classified as normolipidemia (n=100) and hyperlipidemia (n=100), by using the levels of blood lipids (hyperlipidemia: total cholesterol >200 mg/dl, low density lipoprotein cholesterol (LDL-C) >130 mg/dl, and triglyceride >150 mg/dl). Clinical data were collected, and laboratory analysis was done. Serum levels of uric acid, fasting blood glucose (FBS), blood urea nitrogen (BUN), and creatinine were measured by the dry chemistry automate analyzer. Serum E-selectin was measured by using the enzyme-linked immunosorbent assay.
Results: The hyperlipidemia subjects had significantly higher serum E-selectin levels than the normolipidemia subjects (18.98±11.58.56 versus 8.85±4.02 ng/ml). E-selectin was significantly correlated with blood lipids; total cholesterol, triglyceride, LDL-C, and HDL-C (r=0.477, 0.441, 0.453, and -0.191, respectively). Moreover, significant correlations of E-selectin with uric acid and fasting blood glucose were also found (r=0.155 and 0.166, respectively).
Conclusions: Serum E-selectin levels increased in hyperlipidemia and correlated with uric acid and fasting blood glucose, reflecting the association between hyperlipidemia and pathogenesis of CVD, Therefore, it emphasizes the importance of hyperlipidemia management.

Keywords: Hyperlipidemia, E-selectin, Endothelial dysfunction

INTRODUCTION

Hyperlipidemia (dyslipidemia) is defined by the abnormalities of blood lipids, including total cholesterol, triglyceride, and low-density lipoprotein (LDL-C). The prevalence of hyperlipidemia has been increasing globally, including in Thailand.[1] The Thai National Health Examination Survey IV in 2009 revealed that 66.5% of Thai population had hyperlipidemia, based on the national cholesterol education program (NCEP) adult treatment panel-III (ATP-III) guidelines.[2] Hyperlipidemia is an important risk factor of cardiovascular diseases (CVD), leading to mobility and motility of the affected individuals.[3,4] The other risks factors of CVD include diabetes, hypertension, and metabolic syndrome.[5-8]

Pathogenesis of CVD involves hyperlipidemia, accumulation of lipids and inflammation of blood vessels, and vascular endothelial dysfunction.[9,10] Vascular endothelial dysfunction is associated with release of cytokines, chemokines, and adhesion molecules, including monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and P-selectin.[9] Markers of endothelial dysfunction have been shown to have an association with risk factors of CVD, including diabetes, hypertension, and metabolic syndrome.[5-8] Therefore, markers of endothelial

dysfunction have been considered as a candidate for monitoring marker and therapeutic targets in CVD and other related diseases, especially in diabetes.[11,12] Among this, E-selectin is considered as a specific marker of vascular endothelial dysfunction.[5,13] It was shown that E-selectin is associated with inflammation and CVD.[14] Moreover, relationships between E-selectin, diabetes and metabolic syndrome have been reported.[7,8]

High prevalence of hyperlipidemia in Thai population indicates a risk of CVD. However, the influence of hyperlipidemia, independent of other risk factors, such as diabetes and hypertension, on endothelial dysfunction was not clearly established. Therefore, this study aimed to assess serum levels of E-selectin in subjects with hyperlipidemia alone.

METHODS

Subjects

A cross-sectional descriptive study was approved by the ethical review sub-committee board for human research involving sciences, Thammasat University, no. 3 (project no. 131/2561). Two hundred subjects at the age of 35-60 years, who came for an annual health check-up at the public health service center 27, health department, Bangkok, Thailand and had no history of hypertension, diabetes, and other serious illness, were included in this study. They were divided into two groups, one with hyperlipidemia (total cholesterol >200 mg/dl, LDL-C >130 mg/dl or triglyceride >150 mg/dl) and the other with normolipidemia (total cholesterol <200 mg/dl, LDL-C <130 mg/dl and triglyceride <150 mg/dl). Active smokers and subjects with diabetes mellitus, hypertension, kidney diseases, and ongoing illness were excluded from the study.

Clinical data collection and laboratory analysis

Clinical data were collected as following, age, gender, body weight, height, and blood pressure. Body mass index (BMI) was calculated from body weight and height. For analysis of biochemical substances, blood samples were collected after 12 hours of fasting, and serum was isolated for laboratory analysis. The routine blood tests, including blood lipid profile (total cholesterol, triglyceride, LDL-C, and HDL-C), fasting blood glucose (FBS), blood urea nitrogen (BUN), creatinine, and uric acid were performed by using the FUJIFILM FDC NX500 dry chemistry automate (FUJIFILM Europe GmbH Medical Systems Division, Germany). The remaining serum was kept frozen at -70^{o}C until the analysis of serum E-selectin. Determination of E-selectin was done by using the enzyme-linked immunosorbent assay (R&D systems, Minneapolis, MN, USA). Briefly, diluted samples or standards (93, 187.5, 375, 750, 1500, and 3000 pg/ml) were added into anti-E-selectin antibody-precoated wells, followed by the incubation at room temperature for 2 hours. After washing off the unbound materials, the conjugated anti-E-selectin antibody was added into the well and further incubation at room temperature for 2 hours was done. Then, the unbound was washed off, followed by 20-minute incubation with the streptavidin-HRP. Then, the unbound materials were washed off, the substrate solution was added, and incubation in dark was done for 20 minutes, followed by the addition of the stop solution. The absorbance at 450 nm was then measured. The concentration of E-selectin was calculated from the standard curve.

Statistical analyses

Subjects with hyperlipidemia and normolipidemia were studied. Descriptive statistics was used for clinical data (age, sex, and BMI) and biochemical laboratory results (lipid profile, FBS, BUN, creatinine, and uric acid). Numerical data were shown as mean±standard deviation. Qualitative data were shown as frequency and percentage. E-selectin and other laboratory results were compared between two groups (normolipidemia versus hyperlipidemia) by using the student's t-test. Pearson's correlation was used to test correlations between numerical variables. The p value <0.05 was considered statistically significant. The statistical analysis was done by using statistical package for the social sciences (SPSS0 version 12 (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical data of the subjects

Two hundred subjects were included in the study and were divided into two groups, as described in the Materials and Methods, the hyperlipidemia (n=100) and the normolipidemia (n=100) groups. The clinical data were shown in Table 1. The hyperlipidemia and normolipidemia groups were 50.94±6.70 and 51.33±6.70 years old, respectively. For sex distributions, the female-to-male ratio was 55.0% to 45.0% in the hyperlipidemia group and 66.0% to 34.0% in the normolipidemia group. Both hyperlipidemia and normolipidemia groups had healthy BMI. The average BMI was at 21.74 and 22.22 kg/m^2 for the hyperlipidemia and normolipidemia groups, respectively. All the subjects had normal systolic and diastolic blood pressure (109.41±9.23/69.69±6.92 and 110.10±9.60/70.00±6.93 mmHg for hyperlipidemia and normolipidemia group, respectively).

Blood lipids, E-selectin and other laboratory results in hyperlipidemia

Concentrations of blood lipids in hyperlipidemia and normolipidemia groups were shown in Figure 1. Hyperlipidemia subjects had total cholesterol, triglyceride, LDL-C, and HDL-C of 241.62±28.09, 209.24±58.33, 168.68±31.96, and 50.38±12.88 mg/dl, respectively. For the normolipidemia group, the concentrations of total cholesterol, triglyceride, LDL-C, and HDL-C were 174.57±16.26, 81.64±27.05, 105.47±16.65, and

57.67±12.19 mg/dl, respectively. The hyperlipidemia group had normal blood glucose, BUN and creatinine level (Figure 2). However, the hyperlipidemia group had significantly higher FBS levels than the normolipidemia group (91.66±5.87 versus 88.67±5.94 mg/dl). In addition, uric acid concentrations were greater in the hyperlipidemia than the normolipidemia group (6.18±1.51 and 5.10±1.11 mg/dl, respectively). Moreover, when classifying the data by gender, it was found that 14.7% of normolipidemia and 46.7% in hyperlipidemia males had serum concentrations of uric acid higher than 7.0 mg/dl, while 47.3% of hyperlipidemia and 9.1% of normolipidemia females had serum uric acid higher than >6.0 mg/dl (Table 2). In addition, serum levels of E-selectin in the hyperlipidemia and normolipidemia groups were 18.98±11.58.56 and 8.85±4.02 ng/ml, respectively (Figure 2E). When compared with the normolipidemia group, the hyperlipidemia group had significantly higher E-selectin levels ($p<0.05$).

Table 1: Clinical data of the hyperlipidemia and normolipidemia subjects.

Variable (unit)	Normo-lipidemia	Hyper-lipidemia
	n=100	n=100
Age (year)	51.33±6.70	50.94±6.70
Gender		
Female (%)	66.0	55.0
Male (%)	34.0	45.0
BMI (kg/m^2)	22.22±2.08	21.74±2.23
Systolic blood pressure (mmHg)	110.79±8.88	109.41±9.23
Diastolic blood pressure (mmHg)	70.3±6.97	69.69±6.92

Data shown as mean±standard deviation

Correlations among E-selectin and other laboratory results

Blood lipids showed significant correlations among themselves. Total cholesterol had strong positive correlations with LDL-C and triglyceride at r=0.915 and 0.693, respectively (Table 3). Moreover, LDL-C and triglyceride were moderately correlated (r=0.601). However, a significant negative correlation was observed between total cholesterol and HDL-C (r=-0.163), LDL-C and HDL-C (r=-0.301), and triglyceride and HDL-C (r=-0.392). Moreover, serum levels of E-selectin were tested for correlations with the other laboratory results. It was showed that E-selectin had a significantly positive correlation with the blood lipid profile (Table 3). Significant correlations of E-selectin with total cholesterol, triglyceride, LDL-C, and HDL-C were at r=0.477, 0.453, 0.441, and -0.191, respectively. Interestingly, E-selectin was also significantly correlated with FBS and uric acid at r=0.166 and 0.155, respectively (Table 3).

In addition, FBS and uric acid also had significant correlations with the blood lipid profiles (Table 3), with an exception for FBS and HDL-C. In addition, uric acid was also positively correlated with FBS results with r=0.147.

Table 2: Hyperuricemia in the hyperlipidemia and normolipidemia subjects, classified by sex.

Classification	Hyperuricemia (%)	
	Male (uric acid > 7.0 mg/dL)	Female (uric acid > 6.0 mg/dL)
Hyperlipidemia	46.7	47.3
Normolipidemia	14.7	9.1

Data shown as mean±standard deviation

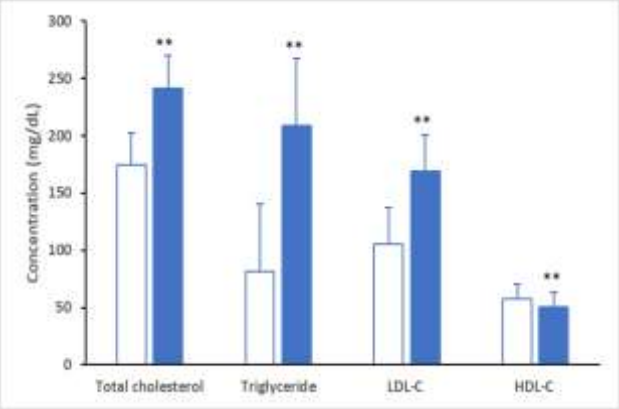

Figure 1: Concentrations of blood lipids in the hyperlipidemia and normolipidemia subjects.
Data shown as mean±standard deviation; **p<0.001

Table 3: Correlations among blood lipids, FBS, uric acid, and E-selectin.

Parameters	Total C	TG	LDL-C	HDL-C	FBS	UA	E-sel
Total C	1						
TG	0.693**	1					
LDL-C	0.915**	0.601**	1				
HDL-C	-0.163*	-0.392**	-0.301**	1			
FBS	0.226**	0.188**	0.220**	NS	1		
UA	0.292**	0.391**	0.334**	-0.288**	0.147*	1	
E-sel	0.477**	0.453**	0.441**	-0.191**	0.166*	0.155*	1

Data were shown as mean±standard deviation; **correlation is significant at the 0.01 level (2-tailed); *correlation is significant at the 0.05 level (2-tailed); NS: no significant correlation

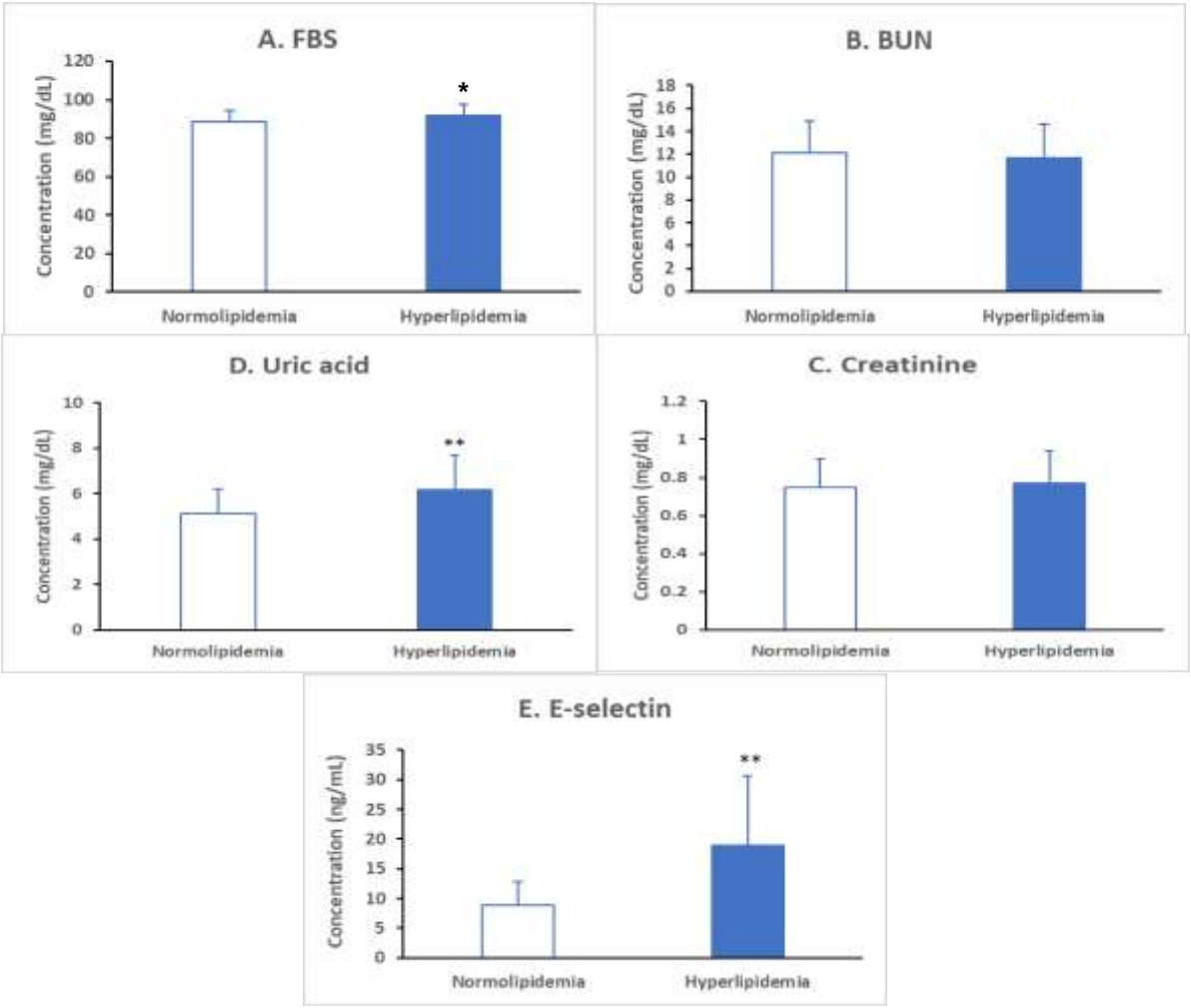

Figure 2. Laboratory results in the hyperlipidemia and normolipidemia subjects.

Data were shown as mean±standard deviation; *p<0.01, **p<0.001

DISCUSSION

The present study examined E-selectin, a marker of endothelial dysfunction, in the hyperlipidemia subjects, excluded those who had diabetes and hypertension. In addition, all subjects had healthy BMI. It was found that E-selectin was elevated in the hyperlipidemia subjects, suggesting possible effects of lipid abnormalities on endothelial dysfunction, which is a key player in the development of CVD. A correlation between E-selectin and blood lipids was also observed. This is consistent with previous reports showing that soluble E-selectin increases in Chinese dyslipidemia.[15] The present results also showed correlation between serum E-selectin and blood lipids, total cholesterol, triglyceride, LDL-C, and HDL-C. This agrees with previous report showing association between E-selectin and HDL-C as well as LDL-C.[16,17] This associations potentially cause leukocyte adhesion and inflammation of the vascular wall, leading to atherosclerotic lesion ant the development of CVD.[18] Therefore, the present study revealed that hyperlipidemia, without diabetes and hypertension, is a key player in CVD's pathogenesis.

In the present study, uric acid was elevated in the hyperlipidemia subjects and correlated with both E-selectin and blood lipids. Uric acid has been shown as an inflammatory marker and is correlated with the other inflammatory markers, including C-reactive protein (CRP).[19] Moreover, increased uric acid levels are associated with oxidative stress, inflammation, endothelial dysfunction, metabolic syndrome, atherosclerosis and CVD.[20, 21] The correlations may show the possible links between lipid abnormalities, inflammation, and endothelial dysfunction. This is supported by the previous reports showing the connection between uric acid, endothelial dysfunction, inflammation, metabolic syndrome, and CVD.[15,16,20,22,23] Metabolic syndrome included not only hyperlipidemia but also visceral obesity, insulin resistance, and/or hypertension. This study focused on hyperlipidemia and, therefore, emphasizes that lipid abnormality could be the key player in the pathogenesis of CVD, involving inflammation and endothelial dysfunction. This is supported by the previous report showing that cardiovascular events can occur long before the onset of insulin resistance, and the events involve lipid abnormalities.[24] Therefore, our results reinforce the

important role of lipid abnormalities in the development of CVD.

In addition, FBS was also associated with blood lipids and E-selectin. Previous reports have shown an association between diabetes and abnormalities of blood lipids, both of which are considered risk factors of CVD. In this study, even though FBS levels in the hyperlipidemia subjects were within the normal range (<100 mg/dl), FBS was significantly higher in the hyperlipidemia subjects, when compared with the normolipidemia subjects. In addition, it was shown that hyperlipidemia is an atherogenic factor and risk of type 2 diabetes.[25]

A cohort study revealed that risk factors of CVD, including high total cholesterol, increased before diagnosis of diabetes.[24] Another study showed that diabetic subjects have higher blood lipids at baseline than nondiabetic subjects and abnormalities of blood lipids impair blood glucose and/or glucose tolerance test, implying that abnormal lipid metabolism leads to impaired glucose metabolism and insulin resistance.[26,27]

It has been shown that CVD may happen before the diagnosis of diabetes. Consistent with this study, it was shown that the hyperlipidemia group had higher blood glucose. Therefore, it is likely that hyperlipidemia individuals have higher risks of type 2 diabetes.

This study did have a few limitations. The sample size in this study was relatively small. Therefore, it is possible that the included subjects did not represent the Thai population due to the sampling process. In addition, subclinical inflammation of the subjects might be present. Such conditions may interfere with the laboratory results. Nevertheless, we excluded the subjects who had possible subclinical inflammation, to diminish such interference on E-selectin levels.

CONCLUSION

In conclusion, this study reveals increasing levels of endothelial dysfunction marker E-selectin in hyperlipidemia. E-selectin was associated with uric acid and FBS, which may imply the occurrence of inflammation and tendency toward blood glucose impairment. These present results reinforce the role of lipid abnormalities on endothelial dysfunction and development of atherosclerosis and CVD, independent from insulin resistance, hypertension, and obesity. Moreover, the possible interrelationships among hyperlipidemia, endothelial dysfunction, inflammation, and blood glucose impairment were elaborated.

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