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Original Article

Correlation Between Apolipoprotein E and Recurrent Acute Coronary Syndrome

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ABSTRACT

Background: Heart disease manifestation due to plaque disruption in the coronary arteries is acute coronary syndrome (ACS). Apolipoprotein-E (Apo-E) is a multifunctional protein with central roles in lipid transportation and metabolism. We analyzed the correlation between the Apo-E blood concentration and recurrent ACS.

Methods: This cross-sectional study recruited 90 patients who visited the outpatient cardiology clinic at Airlangga University Hospital. The patients were divided into 3 groups: without ACS, single ACS, and recurrent ACS. The Apo-E blood concentration was measured using the enzyme-linked immunosorbent assay in the Tropical Disease Center of the Airlangga University Laboratory.

Results: The median Apo-E concentration was 3.6 (1.32-14.9) µg/mL in the recurrent ACS group, 4.01 (2.61-18.54) µg/mL in the single ACS group, and 3.95 (1.19-43.51) µg/mL in the group without ACS. The Kruskal-Wallis test showed no differences in Apo-E between the groups. The χ^2 test demonstrated no correlation concerning Apo-E between the single ACS and recurrent ACS groups. The Fisher exact analysis showed no correlation between the Apo-E concentration and dyslipidemia.

Conclusions: Our results showed no correlation between the Apo-E concentration and recurrent ACS. ***(Iranian Heart Journal 2023; 24(4): 63-69)***

KEYWORDS: Cardiovascular disease, Risk factor, Dyslipidemia, Apo-E

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Cardiovascular disease (CVD) is the leading cause of mortality in the world. The World Health Organization (WHO) reported that 32% of global deaths were caused by CVD, with 85% of the deaths due to heart attack and stroke. Coronary heart disease is one of the CVD disorders. [1] The manifestation of

coronary heart disease is acute coronary syndrome (ACS), consisting of unstable angina, ST-elevation myocardial infarction (STEMI), and non-STEMI. [2]

Patients surviving an initial heart attack (eg, ACS) have a greater risk of recurrent attacks. In the first year, 10% of these patients will have a recurrent heart attack. If there are no cardiovascular incidents in the first year, then 3.6% of these patients will suffer a recurrent attack in the second year, and 5.6% will experience an attack in the fourth year. [3] The mortality of a recurrent attack is higher than that of an initial heart attack. [4]

Secondary prevention is performed with 2 goals: preventing mortality and morbidity from cardiovascular events and improving the quality of life. Prevention efforts include risk factor management, optimal pharmacological therapy, and non-pharmacological prevention. The identification of risk factors is the first step in optimal prevention efforts. [5]

One of the potential risk factors to be analyzed is the blood concentration of apolipoprotein-E (Apo-E), which is a multifunctional protein. Many studies have shown the principal role of Apo-E in lipid transportation and metabolism regulation. Apo-E is synthesized by the liver and is a part of various lipoproteins. Genetic variations at the *Apo-E* gene locus are correlated with the plasma Apo-E concentration. More than half of plasma Apo-E is in high-density lipoprotein (HDL). [6]

Low Apo-E concentrations affect reverse cholesterol transportation, platelet aggregation, and oxidative processes that have atherogenic potential. [7] Low risks of coronary heart disease and diabetes mellitus are linked to high HDL and Apo-E. [8, 9] The correlation between recurrent ACS and Apo-E is the hypothesis of the present research, which aimed to analyze the relationship between the Apo-E concentration and recurrent ACS.

METHODS

Subjects

The current cross-sectional analytic observational study was performed on patients who visited the cardiology outpatient clinic at Airlangga University Hospital between October 2021 and January 2022. The recruited patients were divided into 3 groups: recurrent ACS, single ACS, and without ACS.

The recurrent ACS group consisted of patients with a history of 2 or more ACS incidents between January 2016 and December 2020. The single ACS group consisted of patients with a history of 1 ACS incident. The group without ACS was composed of subjects without a history of ACS.

The diagnosis of an ACS history was based on the patient's clinical symptoms, electrocardiogram (ECG), and troponin test in medical records from January 2015 through December 2020. The MINI VIDAS was used to perform highly sensitive (hs) troponin I tests. The test was considered positive if the hs troponin I level was >19 ng/L. [10] If the hs troponin I level was ≤ 19 ng/L, ACS criteria were fulfilled based on ≥ 20 minutes of chest pain complaints with or without ST elevation in ECG. Recurrent ACS was defined if the time span between the initial and subsequent ACS incidents exceeded 28 days. [2] Subjects with chemotherapy and radiotherapy were excluded.

Data for age, sex, smoking history, blood glucose level, lipid profile, and blood pressure were obtained from medical records. Blood glucose levels were used to determine diabetes mellitus. Dyslipidemia was confirmed with the lipid profile test. Hypertension was confirmed based on blood pressure examinations.

The study protocol was approved by the Ethics Committee of Airlangga University Hospital on April 5, 2021 (approval code: 119/KEP/2021). Written informed consent was obtained from the entire study population.

Blood specimen collection was carried out after the subjects signed informed consent forms. In a BD Vacutainer (BD Diagnostics) without anticoagulants, 3 mL of blood was taken. The serum was separated from the blood after complete clotting for 2 hours and centrifuged at 3500 rpm for 20 minutes. The serum was stored in a freezer at −20 ℃ and examined simultaneously. The Apo-E concentration was measured with the enzyme-linked immunoassay method (Bioassay Lab reagent) with the Bio-Rad washer and reader. The Apo-E laboratory tests were performed in the Tropical Disease Center of Airlangga University Laboratory.

Statistical Analysis

The ANOVA and Kruskal-Wallis tests were conducted to compare differences in age and the Apo-E concentration, respectively, between the groups. The bivariate χ^2 or Fisher exact test was utilized to determine the correlation between sex, a history of diabetes mellitus, hypertension, dyslipidemia, and smoking and the incidence of ACS. The blood Apo-E concentration was categorized into 3 groups: low, normal, and high. Low Apo-E was defined if the concentration was < 3 µg/mL, normal if the concentration was 3-7 µg/mL, and high if the concentration was > 7 µg/mL. The correlation between the Apo-E concentration and ACS incidence was analyzed with the χ^2 test, while the Fisher exact test was applied concerning dyslipidemia. A confidence interval (CI) of 95% was used for this study. Thus, a *P* value < 0.05 was considered statistically significant. All the statistical analyses were conducted with SPSS 26.

RESULTS

The present study was performed on 90 subjects divided into 3 groups: recurrent ACS, single ACS, and non-ACS (30 subjects in each group). The characteristics of the study population are shown in Table 1. The ANOVA analysis showed no difference in age between the 3 groups (*P* = 0.555). There was a relationship between the incidence of recurrent ACS and hypertension (*P* = 0.035), diabetes mellitus (*P* = 0.042), and dyslipidemia (*P* = 0.003). The results demonstrated no relationship between the incidence of recurrent ACS and sex (*P* = 0.112) and smoking history (*P* = 0.14). The median Apo-E concentration was 3.6 µg/mL (range = 1.32-14.9 µg/mL) in the recurrent ACS group, 4.01 (2.61-18.54) µg/mL in the single ACS group, and 3.95 (1.19-43.51) µg/mL in the non-ACS group. The Kruskal-Wallis analysis showed no difference in the blood Apo-E concentration between the 3 groups (*P* = 0.683).

The distribution of the Apo-E concentration in the ACS groups can be seen in Figure 1. Normal Apo-E levels were found dominantly in the recurrent ACS group (50%), the single ACS group (63.33%), and the non-ACS group (63.33%). High Apo-E levels were found more frequently in the recurrent ACS group (30%), followed by the single ACS (20%) and non-ACS (16.67%) groups. The proportion of low Apo-E in the recurrent ACS and non-ACS groups had the same value (20%) compared with 16.67% in the single ACS group. The χ^2 analysis showed no relationship between the blood Apo-E concentration and recurrent ACS (*P* = 0.76).

Based on the dyslipidemia category, the distribution of the Apo-E concentration can be seen in Figure 2. The dyslipidemia group was composed of 77 subjects, most of whom had normal Apo-E levels (62.34%), while the proportions of low and high Apo-E levels were not much different (19.48% and 18.18%). In the non-dyslipidemia group, with 13 subjects, high and normal Apo-E levels exhibited almost similar distributions (46.15% and 38.46%), with 2 individuals (15.39%) having low Apo-E levels. The Fisher exact analysis showed no significant

relationship between Apo-E levels and dyslipidemia (P = 0.11).

Table 1. Characteristics of the study population

Characteristic	Group			
	Recurrent ACS (n = 30)	Single ACS (n = 30)	Without ACS (n=30)	*P* value
Age, y				
Mean ± SD	61.53±8,57	59.10 ± 8.16	59.63 ± 10.33	0.555*
Sex (n, %) male female	22 (73.3) 8 (26.7)	23 (76.7) 7 (23.3)	16 (53.3) 14 (46.7)	0.112**
Hypertension (n, %)	29 (96.7)	24 (80)	20 (66.7)	0.035‡
Diabetes mellitus (n, %)	14 (46.7)	14 (46.7)	21 (70)	0.042**
Dyslipidemia (n, %)	28 (93.3)	25 (83.3)	15 (50)	0.003‡
Smoking history (n, %)	5 (16.7)	6 (20.0)	1 (3.3)	0.140‡
Apo-E median (min – max) : µg/mL	3,6 (1.32 -14.9)	4,01 (2.61 -18.54)	3,95 (1.19-43.51)	0.683‡‡

* ANOVA; ** the χ^2 test; ‡ the Fisher exact test; ‡‡ the Kruskal-Wallis test
ACS: acute coronary syndrome; Apo-E: apolipoprotein-E

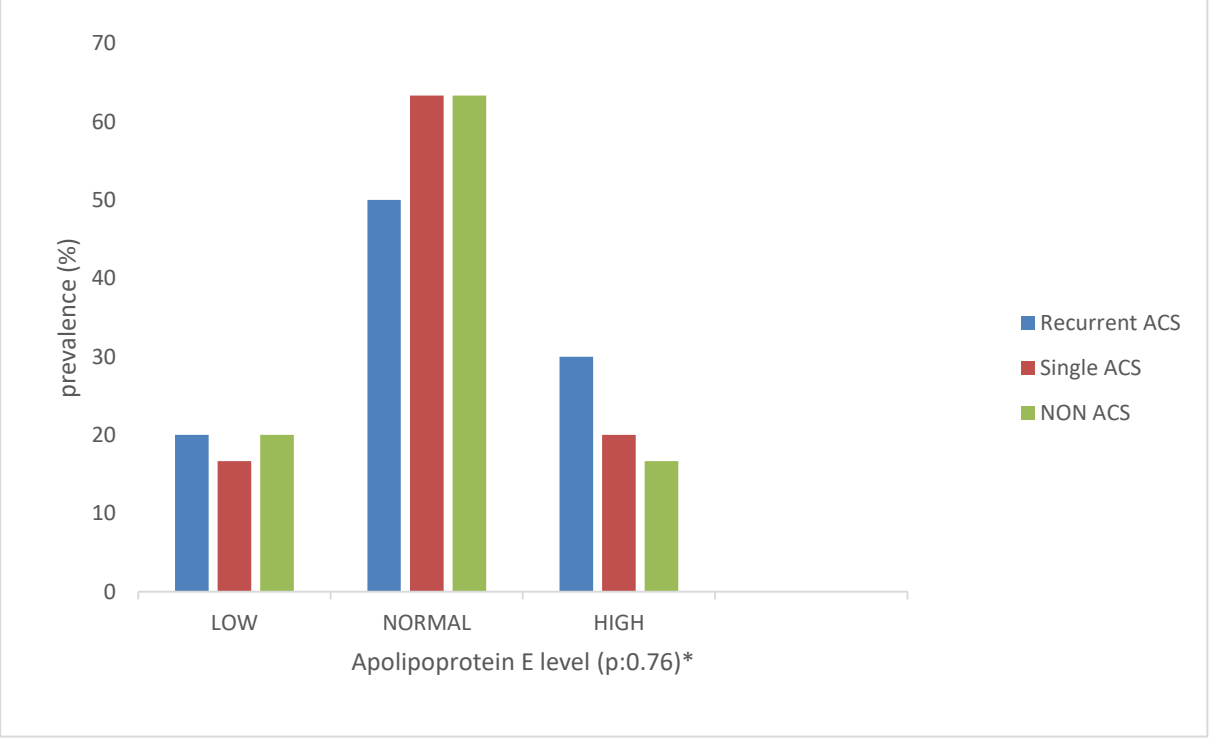

Figure 1: The image depicts the distribution of the Apo-E concentration in the ACS category.
* the χ^2 test
ACS: acute coronary syndrome

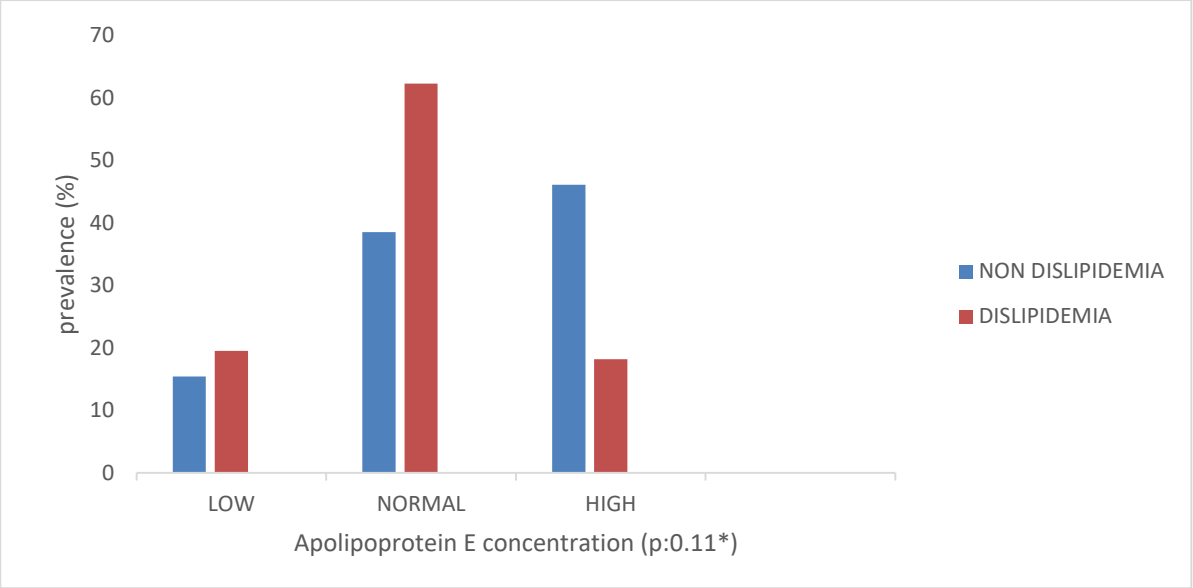

Figure 2: The image illustrates the distribution of the Apo-E concentration in the dyslipidemia category.
*The Fisher exact test
ACS: acute coronary syndrome

DISCUSSION

In the present study, we found no significant difference in the blood Apo-E concentration between recurrent ACS, single ACS, and non-ACS groups. The relationship between blood Apo-E levels and recurrent ACS was also nonsignificant. Several other studies have shown conflicting results vis-à-vis the relationship between Apo-E and CVD.

A nonsignificant relationship between Apo-E levels and CVD was also reported in some other studies. A meta-analysis conducted by Sofat et al [11] (2016) is a case in point since the Apo-E concentration was found to be unable to predict CVD events. Basu et al [12] (2019) reported the same results among patients with type 1 diabetes mellitus.

Several other studies have shown different results. Mooijart et al [13] (2006) stated that high levels of Apo-E led to increased C-reactive protein, indicating a higher risk of CVD. The increased risk occurs in subjects more than 85 years of age. Mendivit et al [14] (2013) showed that Apo-E levels were related to coronary heart disease. The Apo-E examination in their study was performed only on isolated VLDL and LDL. Corsetti et al [15] (2011) concluded that Apo-E levels could predict CVD events only in women but not men.

To examine the Apo-E concentration in this study, we drew upon polyclonal antibodies, which could not distinguish between Apo-E isoforms. The gene that influences Apo-E synthesis has 3 alleles: ε_2, ε_3, and ε_4. There are 3 types of isoforms in Apo-E according to the alleles in the *Apo-E* gene: E2, E3, and E4. [16] The Apo-E laboratory test with monoclonal antibodies according to the Apo-E isoform target may yield different results. The variation in the Apo-E laboratory test with monoclonal antibodies can be interpreted as why Apo-E was not related to recurrent ACS and dyslipidemia in this study.

Apo-E levels in our study showed no association with dyslipidemia. Li et al [17] (2021) showed different results insofar as Apo-E levels were related to the incidence of ischemic heart disease and LDL cholesterol. Apo-E laboratory tests in the

study were carried out on separate isoforms, namely Apo-E2, Apo-E3, and Apo-E4.

The structure and modification of Apo-E affect its biological activity. The *Apo-E* gene polymorphisms that encode Apo-E2, Apo-E3, and Apo-E4 affect the resulting Apo-E structure. Apo-E3 is the most common isoform type found. Apolipoprotein E2 exhibits a lower affinity for the LDL receptor; therefore, Apo-E clearance becomes slower, and the plasma Apo-E level rises. The liver, then, responds by regulating LDL receptors to decrease cholesterol levels. Apolipoprotein E4 diminishes Apo-E; then, cholesterol increases in the plasma. The ε_4 allele is also associated with high levels of LDL cholesterol. [7]

Mooijart et al, [13] in their study on subjects older than 85 years, showed different results. Increased levels of Apo-E in the normal allele or wildtype $\varepsilon_3\varepsilon_3$ genes were associated with high total cholesterol, LDL cholesterol, triglycerides, and low HDL cholesterol. It should be considered that the role of Apo-E in dyslipidemia and CVD is based more on protein function than the concentration of Apo-E.

There are several major risk factors for recurrent ACS. Some factors such as age, sex, and heredity cannot be changed. Modifiable factors include smoking, dyslipidemia, hypertension, lack of physical activity, obesity, and diabetes mellitus. [18,19] Apo-E is widely associated with dyslipidemia as a minor risk factor for CVD. Dyslipidemia itself is influenced by many conditions, including various genetic factors, diet, nutrition, lifestyle, and anti-lipid therapy.

Our results may have been affected by our small sample size. We faced the challenge of recruiting matched subjects for each group because of difficulties in finding ACS subjects without major risk factors, such as hypertension, diabetes mellitus, and dyslipidemia.

Our results demonstrated no significant difference in the Apo-E concentration between the recurrent ACS, single ACS, and non-ACS groups. The correlation between the Apo-E concentration and recurrent ACS was nonsignificant. We decided to perform examinations with Apo-E isoforms, such as E2, E3, and E4, in a future study.

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Conflict of Interest
The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

REFERENCES

1. World Health Organization (WHO). Cardiovascular diseases (CVDs). Available from: *https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)*. Accessed December 2nd 2022.

2. Collet JP, Thiele H, Barbato E, Barthelemy O, Bauersachs J, Bhatt DL,et al. Group ESCSD 2020 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J. 2020: 1-79.

3. Jernberg T, Hasvold P, Henriksson M, Hjelm H, Thuresson M, Janzon M. Cardiovascular risk in post-myocardial infarction patients: nationwide real world data demonstrate the importance of a long-term perspective. European Heart Journal. 2015; 36:1163-70.

4. Law MR, Watt HC, Wald NJ. The underlying risk of death after myocardial

infarction in the absence of treatment. Arch Intern Med. 2002; 162:2405-10.

5. Pekkanen J, Linn S, Heiss G, Suchindran CM, Leon A, Rifkind BM, et al. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. New Engl J Med. 1990; 322(1700).

6. Frieden C, Wang H, Ho CMW. A mechanism for lipid binding to Apo-E and the role of intrinsically disordered regions coupled to domain-domain interactions. Proc Natl Acad Sci U S A. 2017; 114(24): 6292-97.

7. Davignon J. Apolipoprotein E and atherosclerosis beyond lipid effect. Arterioscler Throm Vasc Biol. 2005; 25:267- 69.

8. Aroner SA, Yang M, Li J, Furtado JD, Sacks FM, Tjønneland A, et al. Apolipoprotein C-III and high-density lipoprotein subspecies defined by apolipoprotein C-III in relation to diabetes risk. Am J Epidemiol. 2017; 186(6):736-44.

9. Jensen MK, Aroner SA, Mukamal KJ, Furtado JD, Post WS, Tsai MY, et al. HDL subspecies defined by presence of apolipoprotein C-III and incident coronary heart disease in four cohorts. Circulation. 2018; 137(13):1364-73.

10. Zorbozan N, Atikeler GF. Evaluation of high-sensitivity cardiac troponin measurement procedure performance in serum: the vidas 3 high sensitivity cardiac troponin I. International Journal of Biochemistry 2020; 3(3):161-65.

11. Sofat R, Cooper JA , Kumari M , Casas JP , Mitchell JP , Acharya J, et al. Circulating apolipoprotein E concentration and cardiovascular disease risk: Metaanalysis of results from three studies. Plos Medicine. 2016; 1002146

12. Basu A, Bebu I, Jenkins AJ, Stoner JA, Zhang Y, Klein RL, et al. Serum apolipoproteins and apolipoprotein-defined lipoprotein subclasses: a hypothesis-generating prospective study of cardiovascular events in T1D. Journal of Lipid Research.2019; 60.

13. Mooijaart SP, Jimmy FP, van Heemst D, Havekes LM, de Craen AJM , Slagboom PE , et al. Apo-E plasma levels and risk of cardiovascular mortality in old age. Plos Medicine. 2006; 3(6):0874-83.

14. Mendivil CO, Rimm EB, Furtado J, Sacks FM. Apolipoprotein E in VLDL and LDL With Apolipoprotein C-III is Associated With a Lower Risk of Coronary Heart Disease. Journal of the American Heart Association. 2013; 2(000130)

15. Corsetti JP, Gansevoort RT, Bakker SJL, Navis G, Sparks CE, Dullaart RPF. Apolipoprotein E predicts incident cardiovascular disease risk in women but not in men with concurrently high levels of high-density lipoprotein cholesterol and C-reactive protein. Metabolism Clinical and Experimental. 2012; 61:996-1002.

16. Zhong L, Xie YZ, Chao TT, Wang Z, Li X, Shen RC, et al. A Rapid and cost effective method for genotyping apolipoprotein E gene polymorphism. Molecular Neurodegeneration. 2016; 11(2).

17. Li MY, Kwok MK, Schooling CM. Investigating effects of plasma apolipoprotein E on ischemic heart disease using mendelian randomization study. Nutrients. 2021; 13(2215)

18. Aditya M, Wahyuni CU, Isfandiari MA. Risk factor analysis of recurrent acute coronary syndrome. Jurnal Berkala Epidemiologi. 2018; 6(3):192-99.

19. Govender RD, Al-Shamsi S, Soteriades ES, Regmi D. Incidence and risk factors for recurrent cardiovascular disease in middle-eastern adults: a retrospective study. BMC Cardiovascular Disorders.2019; 19(253).