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ORIGINAL ARTICLE

Eotaxin-1 Levels in Patients with Myocardial Infarction

Mehmet Kalayci [1] and Evrim Gul [2]

[1.]*Department of Medical Biochemistry, Elazig Fethi Sekin City Hospital, Elazig, Turkey*
[2.]*Department of Emergency Medicine, Faculty of Medicine, Firat University, Elazig, Turkey*

SUMMARY

Background: **Acute myocardial infarction is one of the leading causes of morbidity and mortality worldwide. Eotaxin-1, an eosinophil-specific chemoattractant, has been shown to be overexpressed in human atherosclerotic lesions. Eotaxin-1 levels are higher in coronary artery disease patients than in healthy individuals. In this study, we aimed to determine the eotaxin-1 concentrations of patients with myocardial infarction and to investigate the role of eotaxin-1 in myocardial infarction.**
Methods: **The study included 42 patients diagnosed with AMI (patients with suspected AMI based on history, physical examination, ECG, and biochemical markers and confirmed by angiography) and 40 healthy controls. Plasma eotaxin-1 levels were determined by enzyme-linked immunosorbent assay (ELISA).**
Results: **Eotaxin-1, troponin-I, CK, and CKMB levels were statistically higher in the patient group than in the control group. ROC analysis demonstrated that eotaxin-1 gave a sensitivity of 93% and a specificity of 48% once the cutoff value was 341.6 pg/mL. Additionally, the ROC analysis showed that troponin I yielded a specificity of 100% and a sensitivity of 91% when the cutoff value was 0.025 µg/L.**
Conclusions: **Eotaxin-1/eosinophils appear to have a role in coronary artery disease independent of known risk factors. Accordingly, this study and recent studies suggest that eotaxin-1 may be useful in the diagnosis of AMI in addition to other cardiac markers.**
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Correspondence:
Mehmet Kalayci, MD
Department of Medical Biochemistry
Elazig Fethi Sekin City Hospital
Elazig 23300
Turkey
Phone: +90 5541182255
Email: dr_mehmetkalayci@msn.com
Orcid ID: 0000-0001-9122-9289

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KEY WORDS

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INTRODUCTION

Acute myocardial infarction (AMI) continues to be one of the leading causes of mortality and morbidity in developed and developing countries [1]. Approximately 17.8 million people died from cardiovascular diseases worldwide in 2017 [2,3].
Myocardial injury is defined as the presence of elevated cardiac troponin values (cTn) with at least one value above the 99th percentile upper reference limit (URL). AMI is diagnosed when there is clinical evidence of acute myocardial ischemia with acute myocardial injury and an elevation and/or a decrease in cTn values with at least one value above the 99th percentile upper reference limit and at least one of the following: myocardial

ischemia symptoms; new ischemic ECG changes; development of pathological Q waves; visualization of new regional wall motion disorder or new loss of myocardium in a pattern consistent with ischemic etiology; identification of coronary thrombus by angiography or autopsy [4].

Currently, cardiac troponin (cTn) is the most sensitive and specific biochemical biomarker for the diagnosis of myocardial necrosis. Troponin I and troponin T are considered the gold-standard for the diagnosis of AMI. Increased plasma cTn content has been demonstrated in many cardiovascular diseases other than acute myocardial infarction, including acute or chronic heart failure, aortic dissection, myocarditis, cardiomyopathy, atrial fibrillation, and stroke [5]. Usually, AMI is caused by coronary artery disease and occurs as a result of rupture or erosion of the atherosclerotic plaque [4,6].

Eotaxin-1 is an eosinophil-specific chemoattractant found to be highly expressed at sites of vascular pathology [7]. In a study, eosinophil accumulation was detected in thrombi taken from patients presenting with AMI. Eotaxin-1 exerts its effects by activating CCR3 receptors, which are also present on basophils and T lymphocytes. Although eosinophils are rarely detected in atherosclerosis, increasing evidence suggests that eotaxin-1 may be involved in the atherosclerotic process. Overexpression of eotaxin, CCR3 mRNA, and protein has been demonstrated in atherosclerotic plaques [8]. This indicates the role of eotaxin in vascular inflammation. The fact that eosinophils play a pathological role in the heart has been suggested. Eotaxin-1 has been shown to be produced mainly by cardiac fibroblasts with interstitial localization in the heart [9].

It seems possible in the future that there may be some biomarkers of acute coronary syndrome which have potential clinical value. A number of studies have been conducted to find new cardiac markers. In this study, we aimed to determine and compare the eotaxin-1 concentrations of patients with myocardial infarction and the control group.

MATERIALS AND METHODS

A total of 42 patients presenting to the Emergency Department of the Medical Faculty Hospital with the complaint of chest pain and were diagnosed with AMI and 40 healthy controls were included in the study. Patients had a history, physical examination, ECG, and biochemical markers which indicated AMI and their diagnosis had been confirmed by angiography. In the emergency department, blood samples were taken from patients with suspected AMI at the time of admission. Patients who received a definite diagnosis as a result of angiography were included in the study. All participants were informed about the study and approval was obtained from the Ethics Committee of Firat University (dated 19.07.2018, meeting no. 13, decision no. 28).

In the study, samples were taken from the control and patient groups in a tube containing aprotinin (BD Vacutainer K_3EDTA/Aprotinin, Plymouth, UK). The obtained blood samples were centrifuged at 4,000 rpm for 10 minutes, and the plasmas were put into small volume tubes to be studied and stored at -70°C until the study day.

Cholesterol, HDL, LDL, triglyceride, CK, and CKMB levels were analyzed in a Siemens Advia 1800 autoanalyzer (Siemens Healthcare Diagnostic Inc., Tarrytown, NY, USA), Troponin I levels were analyzed in a IQT90 FLEX immunoassay analyzer (Radiometer Medical ApS, Bronshoj, Denmark).

Plasma eotaxin-1 levels were studied using the Human Eotaxin-1 ELISA kit (Sunred Biological Technology, catalog no.: 201-12-0113, Shanghai, China) in accordance with the operating procedures specified in the kit catalogues. Washing and incubation of the plates were done by CombiWash device (Human Diagnostics, Wiesbaden, Germany). Absorbance measurements were performed on the Chromate 4300 Microplate Reader (Awareness Technology, Palm City, FL, USA). The intra-assay and inter-assay coefficient of variation for plasma eotaxin-1 were found to be < 10% and < 12%, respectively. The sensitivity of the eotaxin-1 kit was 2.156 pg/mL. The recovery value of the eotaxin-1 was 95 - 104%. The assay range of the eotaxin-1 kit was 2.5 - 720 pg/mL.

The data obtained in the study were given as mean ± standard deviation. Before comparing the groups, normality of the data distribution was checked with the Kolmogorov-Smirnov test. Chi-squared test was used for non-parametric parameters. Student's *t*-test and Mann-Whitney U tests were used for comparisons between groups, and Pearson's correlation was used to examine the relationships between the parameters within the groups. p-values < 0.05 were considered to be the lowest level of significance.

RESULTS

Demographic and biochemical data of the groups are given in Table 1. There was no statistical difference between the groups in terms of gender distribution ($p > 0.05$). In addition, significant differences were found in CK, CK-MB, troponin I, LDL, and HDL levels between the groups (Table 1).

Plasma eotaxin-1 levels were determined as 413.75 ± 152.8 pg/mL in the control group and 494.34 ± 162.6 pg/mL in the patient group. Plasma eotaxin-1 levels in the patient group were statistically higher than the control group ($p < 0.05$) (Table 1).

A positive correlation existed between eotaxin-1 levels with troponin I ($r = 0.277$, $p = 0.075$), CK ($r = 0.254$, $p = 0.104$), and CKMB ($r = 0.260$, $p = 0.097$) in the patient group. However, these correlations were not statistically significant.

According to the ROC analysis, eotaxin-1 yielded a sensitivity of 93% and a specificity of 48% once the cutoff

Table 1. Demographic and biochemical data of the control and patient groups.

	Control (n: 40)	AMI (n: 42)	p-value
Gender (F/M)	14/26	13/29	1.00
Age (years)	51.8 ± 7.0	57.5 ± 8.9	**0.030**
Cholesterol (mg/dL)	201.4 ± 22.3	214.0 ± 44.6	0.318
HDL (mg/dL)	45.4 ± 9.8	40.1 ± 6.3	**0.045**
LDL (mg/dL)	125.1 ± 18.9	140.8 ± 41.5	**0.039**
Triglyceride (mg/dL)	152.5 ± 49.8	167.7 ± 63.4	0.230
CK (U/L)	131.4 ± 18.5	339.5 ± 195.6	**0.000**
CKMB (U/L)	10.4 ± 5.9	44.52 ± 25.4	**0.000**
Troponin I (µg/L)	0.01 ± 0.002	1.87 ± 4.1	**0.000**
Eotaxin-1 (pg/mL)	413.7 ± 152.8	494.3 ± 162.6	**0.004**

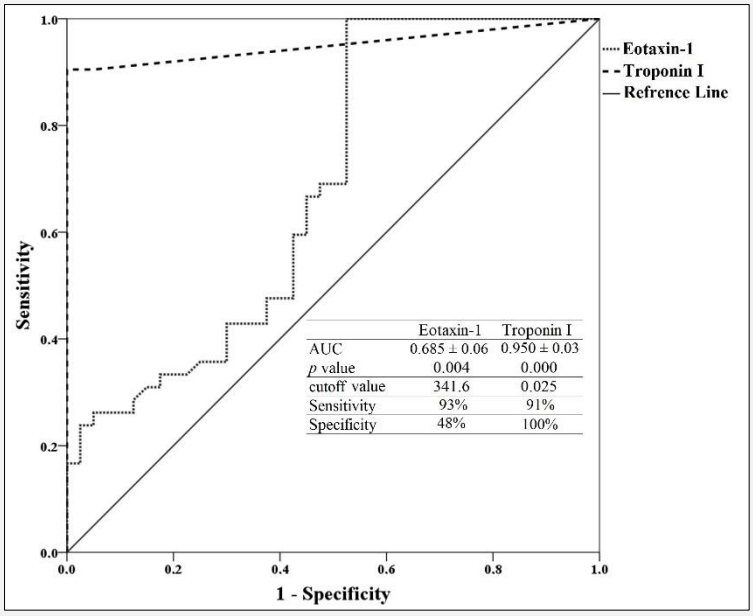

Figure 1. ROC analysis of eotaxin-1 and troponin I tests.

value was 341.6 pg/mL. Additionally, troponin I gave a specificity of 100% and a sensitivity of 91% when the cutoff value was taken as 0.025 µg/L (Figure 1).

DISCUSSION

Cardiovascular diseases (CVD) continue to be the leading cause of the disease burden globally. CVD cases increased from 271 million in 1990 to 523 million in 2019. The number of CVD-related deaths has increased from 12.1 million in 1990 to 18.6 million in 2019. In addition, coronary heart disease (CHD) is one of the leading causes of morbidity and mortality worldwide [10]. The most common form of CHD is acute myocardial infarction. Myocardial infarction can cause damage or death of heart muscle tissue due to prolonged ischemia and hypoxia [11]. Therefore, early diagnosis is vital. The patient's history and clinical findings, electrocardiographic examination, and biochemical markers

have been the main methods for diagnosing MI, but ECG changes occur in only 57% of AMI patients [12]. Therefore, we need accurate, easily accessible, and cost-effective cardiac markers for better diagnosis of AMI, which mostly occurs as a result of coronary artery disease. Endothelial dysfunction, plaque formation of lipids and smooth muscle, and inflammation cause atherosclerotic plaque. Rupture or erosion of the atherosclerotic plaque causes narrowing of the arteries and obstruction of blood flow, resulting in AMI [13]. Prospective studies suggest that eosinophils may play a role in coronary atherosclerosis [14,15]. Eosinophil count is thought to be associated with increased risk and prevalence of CHD [16]. An increase in blood eosinophil counts for at least 5 days has been reported after acute myocardial infarction (AMI), mostly at the infarct site [17]. It has been shown that eotaxin-1, an eosinophil-specific chemoattractant, is overexpressed in human atherosclerotic lesions. Eotaxin-1 levels are higher in coronary artery disease patients than healthy individuals [7,18,19].

This study examined levels of eotaxin-1, which has been shown to be produced primarily by cardiac fibroblasts with interstitial localization in the heart [9]. Eotaxin-1 levels were found to be higher in AMI patients compared to the healthy control group. In the ROC analysis, when the cutoff value for eotaxin-1 was 341.6 pg/mL, the sensitivity was calculated as 93% and specificity was 48%. When the cutoff value for troponin I was 0.025 µg/L, the sensitivity was measured as 91% and specificity was 100%. In addition, a positive correlation was found between eotaxin-1 levels and troponin I in the patient group. These findings are consistent with previous studies [7,18-20]. In a study conducted in 2020, Li et al. reported that eotaxin-1 has an important role in sepsis-induced myocardial damage. They also stated that there is a positive correlation between circulating eotaxin-1 levels and the severity of myocardial damage in septic patients [21].

There are studies indicating that there is no relationship between circulating eotaxin levels and coronary atherosclerosis. In their study, Mosedale et al. found that there is no difference in eotaxin levels between patients with coronary heart disease and the control group [22]. To date, 3 eotaxins have been identified, namely eotaxin-1, eotaxin-2, and eotaxin-3 [23]. Eotaxin-1 levels have been investigated in studies which showed that eotaxin-1 levels are higher in patients with coronary artery disease than healthy individuals. In the study of Mosedale et al., it is not clear which type of eotaxin was studied [22]. In another study, Sheikine et al. concluded that the presence of a polymorphism in the eotaxin gene does not have a clear relationship between plasma eotaxin-1 levels, biochemical risk indicators, and the degree of coronary artery stenosis [24]. Patients who had myocardial infarction, were treated in coronary intensive care unit, and survived were included in the aforementioned study. Plasma eotaxin levels were analyzed several years after AMI. We think that no difference was detected as plasma eotaxin samples were not taken at the time of myocardial infarction. In addition, it should be considered that different experimental designs, different characteristics of the subjects included in the study, ethnic differences, and medical treatments may cause these differences.

Increasing evidence and results of this study support that eotaxin-1 may play a role in the atherosclerotic process. Increased levels of eotaxin-1 in atherosclerotic lesions indicate a potential role of eotaxin in vascular inflammation [25]. Gene and sequence variants that affect the eosinophil count and a polymorphism in the eotaxin gene that affects the eosinophil count are thought to be associated with an increased risk of myocardial infarction [19,26]. In addition, eotaxin plays a role in endothelial inflammation and vascular smooth muscle cell migration [27]. Eotaxin is overexpressed in injured arteries and smooth muscle cell-rich areas of atherosclerotic plaques. This suggests that eotaxin may regulate smooth muscle cell migration and contribute to the progression of atherosclerosis [28].

Eotaxin-1/eosinophils appear to have a role in coronary artery disease independent of known risk factors. Accordingly, recent studies have suggested that eotaxin-1 may be useful in the diagnosis of AMI in addition to other cardiac markers, but these studies are limited. Therefore, the ability of eotaxin-1 to detect cardiac damage needs to be explored and clarified with larger samples.

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References:

1. Liakos M, Parikh PB. Gender Disparities in Presentation, Management, and Outcomes of Acute Myocardial Infarction. Curr Cardiol Rep 2018;20:64 (PMID: 29909444).

2. The WHO CVD Risk Chart Working Group. World Health Organization cardiovascular disease risk charts: revised models to estimate risk in 21 global regions. Lancet Glob Health 2019;7:e1332-45 (PMID: 31488387).

3. Virani SS, Alonso A, Benjamin EJ, et al. Heart Disease and Stroke Statistics-2020 Update: A Report From the American Heart Association. Circulation 2020;141(9):e139-e596 (PMID: 31992061).

4. Thygesen K, Alpert JS, Jaffe AS, et al. Fourth Universal Definition of Myocardial Infarction (2018). Circulation 2018;138(20): e618-e651 (PMID: 30571511).

5. Wang XY, Zhang F, Zhang C, Zheng LR, Yang J. The Biomarkers for Acute Myocardial Infarction and Heart Failure. Biomed Res Int 2020;2020:2018035 (PMID: 32016113).

6. Reindl M, Reinstadler SJ, Feistritzer HJ, et al. Acute myocardial infarction as a manifestation of systemic vasculitis. Wien Klin Wochenschr 2016;128(21-22):841-3 (PMID: 27624326).

7. Emanuele E, Falcone C, D'Angelo A, et al. Association of plasma eotaxin levels with the presence and extent of angiographic coronary artery disease. Atherosclerosis 2006;186(1):140-5 (PMID: 16084515).

8. Jiang P, Wang DZ, Ren YL, Cai JP, Chen BX. Significance of eosinophil accumulation in the thrombus and decrease in peripheral blood in patients with acute coronary syndrome. Coron Artery Dis 2015;26(2):101-6 (PMID: 25340316).

9. Diny N, Hou X, Barin JG, et al. Macrophages and cardiac fibroblasts are the main producers of eotaxins and regulate eosinophil trafficking to the heart. Eur J Immunol 2016;46(12):2749-60 (PMID: 27621211).

10. Roth GA, Mensah GA, Johnson CO, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990 - 2019: Update From the GBD 2019 Study. J Am Coll Cardiol 2020;76(25): 2982-3021 (PMID: 33309175).

11. Chen Z, Pan X, Yan G, Sheng Z, Ma G. miR-142 is a Sensitive Biomarker for the Diagnosis and Prognosis of Acute Myocardial Infarction. Clin Lab 2020;66(6) (PMID: 32538053).

12. Reyes-Retana JA, Duque-Ossa LC. Acute Myocardial Infarction Biosensor: A Review From Bottom Up. Curr Probl Cardiol 2021; 46(3):100739 (PMID: 33250264).

13. Jayaraj JC, Davatyan K, Subramanian SS, Priya J. Epidemiology of Myocardial Infarction. In: Pamukcu B, editor. Myocardial Infarction. 3rd edition: IntechOpen; 2019 DOI:10.5772/intechopen. 74768.

14. Niccoli G, Cosentino N. Eosinophils: a new player in coronary atherosclerotic disease. Hypertens Res 2012;35(3):269-71 (PMID: 22258026).

15. Tanaka M, Fukui M, Tomiyasu K, et al. Eosinophil count is positively correlated with coronary artery calcification. Hypertens Res 2012;35(3):325-8 (PMID: 22072111).

16. Niccoli G, Ferrante G, Cosentino N, et al. Eosinophil cationic protein: A new biomarker of coronary atherosclerosis. Atherosclerosis. 2010;211(2):606-11 (PMID: 20307883).

17. Liu J, Yang C, Liu T, et al. Eosinophils improve cardiac function after myocardial infarction. Nat Commun 2020;11(1):6396 (PMID: 33328477).

18. Niccoli G, Montone RA, Sabato V, Crea F. Role of Allergic Inflammatory Cells in Coronary Artery Disease. Circulation 2018; 138(16):1736-48 (PMID: 30354461).

19. Zee RY, Cook NR, Cheng S, et al. Threonine for alanine substitution in the eotaxin (CCL11) gene and the risk of incident myocardial infarction. Atherosclerosis 2004;175(1):91-4 (PMID: 15186951).

20. Wyss CA, Neidhart M, Altwegg L, et al. Cellular actors, Toll-like receptors, and local cytokine profile in acute coronary syndromes. Eur Heart J 2010;31(12):1457-69 (PMID: 20447947).

21. Li Y, Zhao Y, Qiu C, et al. Role of eotaxin-1/CCL11 in sepsis-induced myocardial injury in elderly patients. Aging (Albany NY) 2020;12(5):4463-73 (PMID: 32147601).

22. Mosedale DE, Smith DJ, Aitken S, et al. Circulating levels of MCP-1 and eotaxin are not associated with presence of atherosclerosis or previous myocardial infarction. Atherosclerosis 2005; 183(2):268-74 (PMID: 15894320).

23. Wenneras C, Matsumoto K, Bochner BS, et al. Eosinophil Trafficking. In: Lee JJ and Rosenberg HF, editors. First edition, Elsevier Inc., 2013:121-66. https://doi.org/10.1016/B978-0-12-394385-9.00006-7

24. Sheikine Y, Olsen B, Gharizadeh B, Jatta K, Tornvall P, Ghaderi M. Influence of eotaxin 67G>A polymorphism on plasma eotaxin concentrations in myocardial infarction survivors and healthy controls. Atherosclerosis 2006;189(2):458-63 (PMID: 16510147).

25. Haley KJ, Lilly CM, Yang JH, et al. Overexpression of eotaxin and the CCR3 receptor in human atherosclerosis: using genomic technology to identify a potential novel pathway of vascular inflammation. Circulation 2000;102(18):2185-9 (PMID: 11056090).

26. Gudbjartsson DF, Bjornsdottir US, Halapi E, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet 2009;41(3):342-7 (PMID: 19198610).

27. Jamaluddin MS, Wang X, Wang H, Rafael C, Yao Q, Chen C. Eotaxin increases monolayer permeability of human coronary artery endothelial cells. Arterioscler Thromb Vasc Biol 2009; 29(12):2146-52 (PMID: 19778943).

28. Kodali RB, Kim WJ, Galaria II, et al. CCL11 (Eotaxin) induces CCR3-dependent smooth muscle cell migration. Arterioscler Thromb Vasc Biol 2004;24(7):1211-6 (PMID: 15130922).