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Original Article

Changes in Plasma Anti-Annexin A5 Antibody After Acute Myocardial Infarction

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ABSTRACT

Background: Anti-annexin A5 antibodies (aANVAs) are thought to have the capacity to cause thrombosis and to be a risk factor for cardiovascular diseases. The present study aimed to detect changes in aANVAs in acute myocardial infarction (AMI) on admission and 10, 40, and 70 days after the acute phase.

Methods: Forty-five patients with confirmed AMI were selected for analysis. Plasma aANVAs were measured by enzyme-linked immunosorbent assay (ELISA) on admission and 10, 40, and 70 days after AMI.

Results: Significant positive cases of aANVAs were observed during the follow-up (P<0.001). The positive cases of aANVAs were more during a 70-day period than on admission (P=0.004) and 10 days (P=0.008) and 40 days (P=0.016) following AMI. No significant increase was found in the plasma concentration of aANVAs during the follow-up (P>0.05).

Conclusions: A significant increase was observed in aANVAs-positive cases on the 70th post-AMI day. The increase may indicate the existence of a hyperactive coagulopathy state during this period. ***(Iranian Heart Journal 2022; 23(3): 69-76)***

KEYWORDS: Cardiovascular disease, Acute myocardial infarction, Annexin, Anti-annexin A5

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Myocardial infarction (MI) and its complications such as heart failure have remained the major cause of death in the world. Revascularization with thrombolytic agents or interventional procedures is the current and popular management for patients with MI.[1] Some factors such as prothrombotic agents may play a significant role in these patients. Endothelial markers such as the tissue plasminogen activator, the von-Willebrand factor, and fibrinogen have been

suggested as prothrombotic factors related to an increased risk of MI.[2,3]

Annexins belong to a family of ubiquitous cytoplasm calcium-binding proteins that might play a role in signal transduction. Annexin A5 or annexin V (ANV) is a cellular protein in the annexin group that is the only annexin with a pseudo-substrate site property.[4] ANV has phospholipids with a negative charge that could interact with calcium and prevent the action of prothrombinase and Tensa complexes. Therefore, ANV could block coagulation reactions that are dependent on the appearance of phospholipids.[5]

Recurrent pregnancy loss and repeated thrombosis in anti-phospholipid syndrome (APS) may result from the defective anticoagulation process. Since ANV has anticoagulant properties, it could be an effective protein in APS.[4] In addition to the first discovered antithrombotic effects of ANV,[6,7] it is also known to have possible diagnostic properties in visualizing cell death,[8] including the assessment of atherosclerotic plaque vulnerability.[9] Increased endogenous ANV plasma levels have been reported as a cause of MI.[10] ANV uptake in the infarct area is used to visualize cell death *in vivo* in these patients.[11]

The anti-apoptotic and anti-inflammatory effects of ANV could hint at a possible role for recombinant ANV as a therapeutic agent to reduce infarct size after myocardial ischemia-reperfusion injury in animal models, to decrease post-ischemic left ventricular remodeling, and to improve cardiac function by suppressing the cardiac inflammatory response.[12]

Anti-annexin A5 antibodies (aANVAs) have a procoagulant effect and induce endothelial cell apoptosis.[13] These autoantibodies may block ANV functions and lead to a thrombotic state and fetal loss.[14] Additionally, aANVAs have been found in various rheumatologic diseases such as APS,[4] systemic lupus erythematosus,[15] scleroderma,[16] Takayasu's arteritis,[17] and Behçet's disease.[18] In addition, a few studies have shown them in renal hypertension,[19] type I diabetes mellitus (DM),[20] cerebrovascular diseases,[21] and acute myocardial infarction (AMI).[22]

Shojaie et al[22] showed a significant association between aANVAs and AMI in the first 24 hours following an acute event. They claimed that positive aANVAs tests in patients with AMI indicated an increased coagulation potential independent of traditional cardiovascular risk factors.

In the current study, we aimed to study changes in plasma aANVAs among patients with AMI on admission and 10, 40, and 70 days after AMI.

METHODS

Subjects

The present longitudinal descriptive study was conducted on 74 consecutive patients with AMI. Nonetheless, during the follow-up, 5 cases with coronary artery bypass graft surgery, 5 cases with percutaneous coronary intervention, 1 case with left ventricular clots, and 18 cases due to poor cooperation were excluded from the study. Finally, the study was continued with 45 patients with confirmed AMI. The patients who presented with typical chest pain to the emergency room of Paymoneah Hospital, a referral center affiliated with Jahrom University of Medical Sciences, in the south of Iran, were selected after a confirmed diagnosis of AMI according to their history, electrocardiograms, cardiac enzyme levels, and cardiologist visits based on previous studies.[22] All the patients were followed up for 70 days. The study protocol was approved by the Ethics Committee of Jahrom University of Medical Sciences (ethics code: JUMS.REC.1388.37), and informed consent was obtained from all the patients before enrollment.

A questionnaire, including demographic data and information about the past medical and drug history, was filled out for each patient. The exclusion criteria consisted of the presence of severe liver disease, malignancy, recent or new cardiac surgery, recent or new angioplasty, anticoagulant drug use, hemolysis, a history of deep vein or arterial thrombosis, and inflammatory and rheumatologic diseases such as collagen vascular disease, systemic lupus erythematosus, and APA syndrome. A fasting plasma glucose level of 126 mg/dL or higher or the use of diabetic medications was defined as DM.

Laboratory Analysis

For aANVA assessment, blood samples were collected immediately after admission and before the administration of anticoagulation drugs. Other samples were taken 10, 40, and 70 days after AMI. The samples were centrifuged, and plasma was stored at –70 °C for future analysis. In addition, fasting blood samples were drawn from all the subjects. Serum lipid profiles, including total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol, as well as other biochemical factors, including glucose, were measured via routine biochemical methods.

Moreover, aANVAs were determined by human anti-annexin V platinum ELISA, as described by the manufacturer (Affymetrix, eBioscience, BMS247). Briefly, MicroWell strips were washed twice with approximately 400 µL of Wash Buffer per well through the aspiration of MicroWell contents between washes. Next, 50 µL of Assay Buffer (1x) and 50 µL of each sample in duplicate were added to the sample wells. Then, 50 µL of the horseradish peroxidase (HRP) conjugate was added to all the wells. The strips were covered with an adhesive film and were incubated at room temperature (18–25 °C) for 2 hours. The MicroWell strips were washed 3 times, and 100 µL of the TMB substrate solution was pipetted to all the wells. Thereafter, the MicroWell strips were incubated at room temperature (18–25°C) for about 10 minutes. However, the quick pipetting of 100 µL of the STOP Solution into each well stopped the enzyme reaction. The absorbance of each MicroWell was read at 450 nm as the primary wavelength and optionally 620 nm as the reference wavelength. Plasma concentrations of 18 ng/mL or lower were reported as normal.

Statistical Analysis

The SPSS software, version 18.0 (Chicago), was used to analyze the data. Numerical and qualitative parameters were reported as the mean ± the standard deviation and percentages, respectively. The normality of the data was checked using the Kolmogorov–Smirnov test. The differences between the groups were evaluated using the Student *t*, χ^2, repeated measure, Cochran, McNemar, and Friedman tests. The Pearson correlation test was performed to evaluate the correlation between the data. A *P* value of less than 0.05 was considered significant.

RESULTS

The study was conducted on 45 patients, composed of 34 men (75.6%) and 11 women (24.4%) at a mean age of 60.7±10.0 years. The study population's demographic data are presented in Table 1. Nineteen patients (42.2%) were smokers. Prediabetes and DM were reported in 18 (40.0%) and 15 (33.3%) patients, respectively. The mean serum levels of triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol were within the normal range. However, the level of fasting blood sugar was above the normal range (119.1±31.4 mg/dL).

Significant positive cases of aANVAs were observed during the follow-up (*P*<0.001).

The positive cases of aANVAs were more during a 70-day period than on admission (*P*=0.004) and 10 days (*P*=0.008) and 40 days (*P*=0.016) after AMI (Table 2). No significant increase was found in the plasma concentration of aANVAs during the follow-up (*P*>0.05).

Among the participants, 38 (84.4%) and 7 (15.6%) cases had ST-elevation myocardial infarction (STEMI) and non–ST-elevation myocardial infarction (NSTEMI), respectively (Table 3). In the STEMI group, 81.6% of the subjects were men, which was significantly higher than the percentage of men in the NSTEMI group (42.9%) (*P*=0.028). The percentage of smokers in the STEMI group was significantly higher than that in the NSTEMI group (50% vs 0%; *P*=0.019). Nevertheless, the STEMI and non-STEMI groups did not differ statistically differently concerning type II DM, age, plasma concentrations of aANVAs, fasting blood sugar, triglyceride, total cholesterol, LDL cholesterol, HDL cholesterol, and positive cases of aANVAs during the follow-up (*P*>0.05).

There were no significant correlations between plasma aANVAs and cardiovascular risk factors, including type II DM, triglyceride, total cholesterol, LDL cholesterol, HDL cholesterol, and smoking. In addition, no correlations were noted between aANVAs and age and sex.

Table 1: Characteristics of the study population

Variables	N= 45	
Age (y)	60.7±10	
Sex (men/women)	34/11	
MI type (NSTEMI/ STEMI)	7/38	
Blood Sugar Group	Normal	12(26.7%)
	Prediabetes	18(40%)
	Diabetes mellitus	15(33.3%)
Smoking (Yes/No)	19/26	
Fasting blood sugar (mg/dL)	119.1±31.4	
Triglyceride (mg/dL)	133.4±72.2	
Total cholesterol (mg/dL)	185.8±39.9	
LDL cholesterol (mg/dL)	121.9±30.3	
HDL cholesterol (mg/dL)	44.1±9.4	

HDL, High-density lipoprotein; LDL, Low-density lipoprotein; NSTEMI, Non–ST-elevation myocardial infarction; MI, Myocardial infarction; STEMI, ST-elevation myocardial infarction

Table 2: Plasma concentrations of aANVAs and aANVAs-positive patients in the study population on admission and at 3 follow-ups

Variables	On admission	10 DAA	40 DAA	70 DAA	*P* value
aANVAs (ng/mL)	25.56±7.01	26.37±7.4	25.59±7.3	26.99±8.6	> 0.05
aANVAs-positive patients	8 (17.8%)	9 (20%)	10 (22.2%)	17 (37.8%)	< 0.001

aANVAs, Anti-annexin A5 antibodies; DAA, Days after admission

Table 3: Characteristics of the subjects with acute MI according to the type of infarction

Variables	NSTEMI, n=7	STEMI, n=38	*P* value
Age(y)	61.9±8.5	60.5±10.4	0.747
Sex (men/women)	3/4	31/7	0.028
Smoking (yes/No)	0(0%)	19(50%)	0.016
Diabetes mellitus type II	2(18.6%)	13(34.2%)	0.562
PC aANVA Ad	1(14.3%)	7(18.4%)	0.793
PC aANVA 10 DAA	1(14.3%)	8(21.1%)	0.681
PC aANVA 40 DAA	1(14.3%)	9(23.7%)	0.583
PC aANVA 40 DAA	3(42.9%)	14(36.8%)	1.00
Fasting blood sugar, (mg/dL)	114.3±33.5	119.9±31.4	0.666
Triglyceride (mg/dL)	112.6±65.7	137.3±73.4	0.411
Total cholesterol (mg/dL)	172.3±25.1	188.3±41.8	0.336
LDL cholesterol (mg/dL)	114.3±23.3	123.3±31.5	0.477
HDL cholesterol (mg/dL)	43.6±9.9	44.2±9.5	0.882

PC aANVAs, Positive cases of anti-annexin A5 antibodies; Ad, Admission; DAA, Days after admission; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; NSTEMI, Non–ST-elevation myocardial infarction; MI, Myocardial infarction; STEMI, ST-elevation myocardial infarction

DISCUSSION

ANV, as a calcium-binding protein, plays a role in cell proliferation and exocytosis and has anti-inflammatory and anticoagulant properties.[23,24] The potent anticoagulant effect of ANV is derived from the inhibition of prothrombin activation and thrombus formation.[25] The anti-apoptotic and anti-inflammatory effects of ANV could suggest a possible role for recombinant ANV as a therapeutic strategy to protect the myocardium after myocardial ischemia-reperfusion injury in animal models.[12]

Previous studies have reported a rise in aANVAs in various abnormalities.[15,26] Further, a few studies have reported an association between aANVAs and AMI.[22,23] Roldán et al[23] analyzed the presence of anti-annexin A5IgG antibodies in patients with MI under the age of 45 by comparison with healthy subjects. They detected only 2 patients with positive aANVAs. However, we detected 8 patients (17.8%) with positive aANVAs on admission, which is higher than the percentage reported by Roldan and colleagues. The discrepancy in the results could be due to ethnic differences.

Shojaie et al[22] showed significantly higher positive cases of aANVAs in patients with AMI than in healthy control subjects (at least 24 hours after the incident onset). The authors assumed that because patients had aANVAs on the first day of their MI, aANVAs must have been present prior to the event. In addition, they claimed that the marked difference with other studies could be due to the detection of all antibody subtypes against ANV. Their study design renders it difficult to interpret the results because the acute changes seen may have originated from tissue death. To address this issue, we decided to study changes in aANVAs over a period of 70 days. Therefore, we measured aANVAs in patients with AMI on admission and then 10, 40, and 70 days after AMI. In agreement with Shojaie et al,[22] we detected significantly high positive cases of aANVAs on admission.

We observed significantly high positive cases of aANVAs in patients with AMI during a period of 70 days in the post-acute phase. To the best of our knowledge, it is the first study to demonstrate a significant rise in these autoantibodies in the post-MI period. Still, the mean plasma concentrations of aANVAs over 10, 40, and 70 days were not significantly higher than that on admission. In addition, the plasma

concentration of aANVAs was not different between the NSTEMI and STEMI groups.

High levels of aANVAs in patients with AMI could indicate a procoagulant trend. The relationship between antiprothrombin (aPT) and IgG isotypes of aANVAs and thrombosis was investigated in patients with systemic autoimmune diseases. Lakos et al[27] showed that the frequency of thrombotic events in patients with aPT or aANVAs was higher than that in patients without APS. They suggested that the measurement of aPT and aANVAs might be 2 valuable factors for the diagnostic confirmation of APS and the evaluation of the risk of venous and arterial thrombosis in patients with systemic autoimmune diseases.

Lieby et al[28] studied 5 randomly selected monoclonal antiphospholipid antibodies (aPl) in a patient with APS to investigate their thrombogenic properties to induce fetal loss in pregnant mice. They reported that fetal loss was probably induced by the antibody directed against ANV.

We did not measure ANV, but we thought that the increase in the positive cases of aANVAs over time might be a body response to the elevated level of ANV after MI. Therefore, future studies should seek to detect ANV and aANVAs together to clarify the vague points. Our study is the first study to follow up changes in aANVAs among patients with AMI. Scintillatingly, we found that after 70 days of follow-up, aANVAs-positive results rose to 17 cases (37.7%), showing a significant increase in aANVAs along with the progression of MI.

Individuals who experience MI are at an increased risk of recurrent infarction. In this group, the annual death rate is at least 5 to 6 times that in patients without coronary heart disease.[29] Increased aANVAs may be one of several causes of the existence of the hypercoagulable state and recurrent thrombotic events in these patients.

However, some studies have demonstrated that aPL prevents the binding of ANV to the endothelium, positing that this is a novel mechanism of atherothrombosis that can occur with intravenous immunoglobulin. Elevations in immunoglobulin levels in the serum of normal healthy subjects increase the possibility of side effects reported in atherothrombosis and cardiovascular diseases.[30] Accordingly, our study could predict an increasing thrombogenicity in patients with MI.

We could not exactly evaluate the association between aANVAs and post-MI major cardiovascular events and prognosis because we excluded patients who had undergone coronary artery bypass graft surgery or percutaneous coronary intervention, as well as those who expired during the study. In addition, since there was no study on the variation of aANVAs in the post-MI course, we do not know how long changes in aANVAs among these patients tend to last. We found a rise in aANVAs during a 70-day period following AMI in a sample of the Iranian population. Given the relatively small size of our study population, we suggest designing large case-control studies with longer duration in other subgroups and populations.

CONCLUSIONS

The results of the present study showed a significant increase in the positive cases of aANVAs in a period of 70 days after AMI. Our data suggest that this increase may indicate the existence of a hypercoagulable state in the post-MI period. Further studies are recommended to evaluate the role of changes in aANVAs in patients with AMI.

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Conflict of Interest
None declared.

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