

## Annexin V Levels as an Indicator of Myocardial Cell Death after Myocardial Infarction

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### Abstract

**Background:** Myocardial ischemia is associated with apoptosis of cardiomyocyte and because of apoptotic cell death is characterized by externalization of Phosphatidylserine on the cell membrane, so it is amenable to targeting by Annexin V.

**Objective:** To compare plasma concentrations of Annexin V in patients who had an early infarct with patients without infarction. And to analyze the plasma concentration of Annexin V in relationship to cardiovascular risk factors.

**Patients and Methods:** A Case-control study of 100 patients (case) who are diagnosed with Myocardial Infarction (MI) and admitted to the coronary care unit of Baqubah Teaching Hospital and another 100 patients homogenous in terms of age and gender and who attended the hospital for other cause than myocardial infarction is selected as the control group during a period between the first of April and the first of July 2019. A special questionnaire used to collect the required information, an early morning blood sample is taken to measure the level of Annexin V by ELISA, Student's t-test, ANOVA test and Chi\_square test to find an association and differences between variables.

**Results:** The results showed that The mean Annexin V level is significantly higher in cases (10.48155ng/ml) than control (1.28803ng/ml) with p-value =0.001 and a sample taken within 24 hours after MI is significantly higher in the mean level of Annexin V then the sample taken after 24 hours of MI.

**Conclusion:** Generally, the measurement of Annexin V level has provided a good diagnostic test to evaluate myocardial infarction.

**Keywords:** Myocardial infarction, Annexin V, Phosphatidylserine, Apoptosis

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### Introduction

Myocardial Infarction occurs as a result of many factors includes; environmental factors and individual predisposing factors [1, 2]. Genetic and pharmacological manipulations indicate that cell death is an important

component in the pathogenesis of myocardial infarction. Cells die primarily by apoptosis or necrosis. Apoptosis has long been recognized as a highly regulated process [3]. To decrease myocardial damage due to these

processes, PCI is the preferred reperfusion strategy [4].

However, reperfusion injury results from post-ischemic reperfusion due to the formation of reactive oxygen species that lead to direct cell damage, cell death, and production of inflammatory cytokines[5]. Many changes occur during reperfusion of the myocardium after ischemic damage. Necrosis and apoptosis appear to be ongoing during ischemia, while apoptosis is boosted by the reperfusion event [6, 7]. Myeloid cells can influence immune responses through binding and ingestion of dying cells that lead to suppressing or enhancing inflammation which indicates a close interaction between inflammation and cell death [8]. This effect of apoptosis and inflammatory changes that lead to decrease in cellular damage might result in new clinical therapies [9]. Annexin V is a calcium-dependent glycoprotein with anticoagulant capacity in vitro, therefore Annexin V inhibits Tenase complexes (X-ase complex) and prothrombinase that decrease plaque aggregation and adhesion. Circulating Annexin V released from vascular wall cells(smooth muscle cells, endothelial cells) or from secretory cells of the liver and spleen; once it is in the plasma, it binds to endothelial cells or blood cells [10]. Annexin V form an antithrombotic shield around the phospholipids, replacing their coagulation factors, and capable of inhibiting the prothrombinase and Tenase complex that activates more factor X which lead to thrombin formation, and reducing plaque adhesion and aggregation. Also, Annexin V possesses high apoptotic cell affinity, since these cells produce a large amount of

phospholipids, particularly phosphatidylserine [11]. Annexin V has gained broad attention over the last decade because of the anti-thrombotic effects of Annexin V [12] and diagnostic properties in visualizing cell death [13] including assessment of atherosclerotic plaque vulnerability [14]. There is a study in Iraq that justify the level of Annexin V increased in patients with recent acute MI related to infarction site and increased in patients with heart failure that complicated with hypertension [15], and this study is the second one ,which deals with patients with acute MI and patients without. The aim of this study is to determine the plasma concentration of Annexin V in patients who had an early infarct. Comparing the plasma concentration of Annexin V in patients who had an early infarct with patients without infarction. And to relate the plasma concentration of Annexin V to the time passing, and cardiovascular risk factors.

### Patients and Methods

The study design: The current study is a case-control study with analytical elements. The data collection was conducted between the first of April and the first of July 2019 among participants attending the coronary care unit of the medical department in Baqubah teaching hospital, Diyala city center, Iraq. The mean age of patients was  $61.7 \pm 11.6$  years with a range between 40-95 years and the mean age of controls was  $60.4 \pm 9.1$  years with a range between 44-81 years. Male to female ratio in control was 1.9:1 and the male to female ratio in the case was 2:1. A convenient sample of 100 patients (case) who were diagnosed with myocardial infarction (MI) and admitted to the coronary

care unit of the medical department in Baqubah teaching hospital. The other 100 patients homogenous in terms of age and gender and who attended Baqubah teaching hospital for other causes than MI were selected as the control group. A self-constricted questionnaire form prepared by the researcher and supervisor to collect information from the participants by direct interview with them. The questionnaire included information regarding selected variables like gender (female and male), age (<50years, 50-59 years, 60-69 years and  $\geq 70$  years), medical history of hypertension and diabetes mellitus, smoking history (current smoke, ex-smoker and never smoke), present alcohol intake (yes, no), occupational history (employee, free work and retired), family history of ischemic heart disease, drug history for a cardiac problem. Pulse rate and respiratory rate were measured by the researcher manually and the blood pressure of each participant was measured by mercury sphygmomanometer. Then the participants' weight, height, temperature, BMI, and electrocardiography (ECG) measured by trained staff. Venous samples were taken early in the morning for the patient and control groups. The plasma Troponin was determined by serology Ab/Ag (CTK Biotech rapid test made in USA 2019) and the plasma Annexin V concentration was determined by Human (ANXA5) ELISA kit enzyme-linked immunosorbent assay (Elabscience, made in china 2019).

The results were expressed in ng/ml. Analysis of all blood samples was performed in the Biochemistry Laboratory of Baqubah teaching hospital. Duration between MI

attack and blood samples drawn was also measured.

### Serum sample collection

The blood samples were drawn from the median cubital vein or from another vein when this method was not accessible. After that, 5 ml of blood was drawn and put in the gel tube and put in the cool box until transport to the laboratory room of the emergency department and separated by centrifuge for 15 min at  $1000 \times g$  at  $2 \sim 8^{\circ}C$ . After separation of whole blood, the serum sample was drawn by pipette and put into plain tube and stored in deep freezer  $\{-20C^{\circ}\}$ . An Annexin V was detected by using a human (ANXA5) ELISA kit (Elabscience). This kit applies to the in vitro quantitative determination of Human ANXA5 concentrations in plasma, serum, and other biological fluids.

### Statistical analysis

Statistical analysis of data was carried out using the Statistical Packages for Social Sciences (SPSS), Version 23. The P-value of  $\leq 0.05$  was considered to be statistically significant.

### Results

A total of 200 participants were enrolled in this study, 100 patients with a history of myocardial infarction (case), and 100 control participants. The mean age of patients was  $61.7 \pm 11.6$  years with a range between 40-95 years and the mean age of controls was  $60.4 \pm 9.1$  years with a range between 44-81 years. Male to female ratio in control was 1.9:1 and the male to female ratio in the case was 2:1.

**The difference in Annexin V level between studied groups:** The mean Annexin V level was significantly higher in case (10.48155

ng/ml) than control (1.28803ng/ml) with p- value =0.001.

**Table (1):** The difference in Annexin V level between studied groups

Variables	Mean	Std. Deviation	Median	Minimum	Maximum	P value
control	1.28803	.74283	1.17400	.12390	3.18781	0.001*
case	10.48155	5.28156	9.28571	2.98577	19.83471	

\*\*Student's t- test , significant  $\leq 0.05$

**Relation of Annexin V level to the duration of samples drawn (duration of MI):** The relation of Annexin V level to the duration of MI drawn was also measured, and the mean level of Annexin V when the blood

draw at <24 hours after myocardial infarction was significantly higher than the mean level of Annexin V when the blood draw after 24 hours of myocardial infarction.

**Table (2):** Relation of Annexin V level to duration of samples drawn (duration of MI)

		Mean Annexin V level $\pm$ SD	P value
Duration	<24 hours	13.6542 $\pm$ 6.15	0.043*
	$\geq$ 24 hours	10.2190 $\pm$ 5.093	

\*\*Student 's t- test , significant  $\leq 0.05$

**Relation of Annexin V level with some patient's character:** No significant difference in Annexin V level between different groups of age, gender, BMI,

smoking history, alcohol history and occupation (p=0.25, 0.22, 0.31, 0.75, 0.24 and 0.45 respectively).

**Table (3):** Relation of Annexin V level with some patient's character

Variables		Mean Annexin V level	P value
Age groups	< 50 years	11.0694	0.25**
	50-59 years	12.0149	
	60-69 years	9.8323	
	$\geq$ 70 years	9.38900	
Gander	Male	10.9293	0.22*
	Female	9.5724	
BMI	Normal	10.98123	0.31**
	Overweight	9.14402	
	Obese	9.23612	
Smoking history	Current smoker	10.62426	0.75**
	Ex-smoker	11.32149	
	Never smoke	10.48153	
Alcohol history	Yes	10.876	0.24*
	No	9.5643	
Occupational history	Employee	10.874	0.45**
	Free worker	10.784	
	Retired	9.9543	

\*Student's t- test , ANOVA test, significant  $\leq 0.05$

**Relation of Annexin V level with patients' medical history:** The mean Annexin V level has no significant difference with a history of hypertension and diabetes mellitus (p-value= 0.85, 0.86 respectively). Although the mean Annexin V level was higher in patients with a

positive family history of IHD than the patients with a negative family history of IHD, there was no significant difference in mean Annexin V level between them (p-value = 0.39).

**Table (4):** Relation of Annexin V level with patients' medical history

Variables		Mean Annexin V level	P value
History of HT	+VE	10.39290	0.85*
	-VE	10.59438	
History of DM	+VE	10.39483	0.86*
	-VE	10.58333	
Family history Of IHD	+VE	11.29430	0.39*
	-VE	10.22489	

\*Student T test , significant  $\leq 0.05$

## Discussion

In this study, the mean Annexin V level in patients with MI was 10.48155ng/ml and being significantly higher than control patients, similar to this finding in other study was done in Diyala in 2016 that show a high level of Annexin V in patients with MI as compared to healthy subjects (15). A Japanese study in 1996 showed that the plasma Annexin V concentration in normal healthy individuals was  $\{1.7 \pm 0.6 \text{ ng/ml}\}$  (mean  $\pm$  S.D.), while that in acute MI patients was elevated to  $\{13.2 \pm 6.8 \text{ ng/ml}\}$  (P-value less than 0.0001) at the time of initial blood drawing (16) and another Japanese study in 2003 that shows Annexin V concentration in acute myocardial infarction was  $11.0 \pm 4.9 \text{ ng/ml}$  (17). Another Swedish study in 2005 also show a higher level of Annexin V level in patients with Systemic lupus erythematosus and a history of cardiovascular disease were compared with 26 healthy women (population controls) (18). In contrast to those studies, another study that was done

in china 2002, that show the plasma level of Annexin V in young patients who have had an acute myocardial infarction and found a low level of plasma Annexin V in patients with premature AMI and this interpreted in that study by presence of a procoagulant trend, hypercoagulable condition and strongly suggests a genetic basis for the concentration of plasma Annexin V (11). Another study that was done in Iran in 2009 (19), was shown the concentration of plasma Annexin 5 in patients with acute MI ( $0.83 \pm 0.77 \text{ ng/ml}$ ) were significantly lower than those in the control group ( $4.12 \pm 2.88 \text{ ng/ml}$ )  $p = 0.002$  but also this study shown patients with acute myocardial infarction had more positive anti-Annexin 5 antibody  $\{20(45.5\)\}$  than the control group  $\{6(15.8\)\}$ ,  $p = 0.004$  and this interpreted by the low level of plasma Annexin V together with high level of anti-Annexin V antibody in patients with acute myocardial infarction may indicate the presence of a

hypercoagulable state. Similar to two separated Japanese studies(20,21), the current study shows the mean level of Annexin V when the blood draw at <24 hours after MI was significantly higher than the mean level of Annexin V when the blood draw after 24 hours of MI, this difference may be due to a lack of completion of the process of apoptosis seems to be related to a lack of Adenosine triphosphate and/or a conversion of apoptosis to necrosis by a secondary inflammatory response. In the current study, there was a lack of an association of Annexin V level with classic cardiovascular risk factors, and this was proved by all the above studies.

### Conclusions

The mean Annexin V level was significantly higher in patients with acute myocardial infarction than patients without.

The mean level of Annexin V when the blood draw at <24 hours after MI was significantly higher than the mean level of Annexin V when the blood draw after 24 hours of MI. Annexin V level had no association with classic cardiovascular risk factors. Measurement of Annexin V level provides a good diagnostic test to evaluate myocardial infarction and to be used with other markers.

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