Factor XII level in patients with acute Myocardial Infarction

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Abstract:

Background: Acute myocardial infarction (AMI) is one of the most common diagnoses in hospitalized patients in industrialized countries. Factor XII is one of the contact activation factors. Hageman factor (FXII) activates coagulation through the intrinsic pathway. It concurrently activates the fibrinolytic system. This mechanism exists to limit clotting by cleaving fibrin, thereby solubilizing the fibrin clot.

Aims of the study: To study the level of intrinsic coagulation factor (FXII: C) in patients with acute myocardial infarction.

Subjects, Materials and Methods: Thirty patients were included in this study (9 female, 21 male) that were just admitted to the Coronary Care Unit in Al-Yermouk Teaching Hospital and diagnosed as having acute myocardial infarction. Blood samples were taken from these patients. Their age range is 48-68 years. Thirty healthy subjects (9 female, 21 male) age and sex matched with the patients were included as a control group. FXII: C activity was assayed for both patients and control groups.

Statistical Methods: The student T-test and correlation study were used for statistical tests.

Results Mean FXII: C (71.03±11.46) is significantly (P value <0.001) lower in acute myocardial infarction group than control group (119.00%±8.52).

Conclusion FXII: C level was significantly lower in patients with acute myocardial infarction than control group.

Key words: acute MI, factor XII assay

Introduction:

Acute myocardial infarction (AMI) is one of the most common diagnoses in hospitalized patients in industrialized countries. The predominant cause of coronary artery disease is atherosclerosis, a process that starts early in life and progress slowly and silently for decades. [1, 2]

Normal haemostasis involves the physiologic balance of procoagulant factors and anticoagulant factors that maintains liquid blood flow and the structural integrity of the vasculature. [3] Factor XII is one of the contact activation factors. [4]

Hageman factor was first discovered in 1955 when a routine preoperative blood sample of the 37-year-old railroad brakeman John Hageman was found to have prolonged clotting time in test tubes, even though he had no hemorrhagic symptoms. Hageman was then examined by Dr. Oscar Ratnoff who found that Mr. Hageman lacked a previously unidentified clotting factor. [5] Dr. Ratnoff later found that the Hageman factor deficiency is an autosomal recessive disorder, when examining several related people which had the deficiency. Paradoxically, pulmonary embolism contributed to Hageman's death after an occupational accident. Since then, case series clinical studies have identified an association of thrombosis and Factor XII deficiency, though the pathophysiology of the relationship is unclear. Hepatocytes express blood coagulation factor XII. [6]

The gene for factor XII is located on chromosome 5q33-qter and spans approximately 12 kb. It contains 14 exons. The intron–exon structure of the gene is similar to the plasminogen activator family of serine proteases. Portions of the gene are homologous to domains found in fibronectin and tissue-type plasminogen activator. [7]

Coagulation factor FXII is a single-chain 80-kD glycoprotein, composed of 596 amino acids, circulates in plasma as a serine protease zymogen. [8]

Hageman factor is a protein synthesized by the liver that circulates in an inactive form until it encounters collagen, basement membrane, or activated platelets (e.g., at a site of endothelial injury). [9] Proteolytic cleavage of factor XII is mediated by charged surfaces (glass, kaolin, cellite, dextran sulfate, endotoxin, urates, crude collagen, and sulfatides), autoactivation, and kallikrein. Prekallikrein, factor XI, factor VII, plasminogen, and complement C1 are proteolytically cleaved by activated factor XII (factor XIIa) into their active forms.[10] Activated Hageman factor (factor XIIa) initiates four systems involved in the inflammatory response: (1) the kinins system, producing vasoactive kinins; (2) the clotting system, inducing the activation of thrombin , fibrinopeptides, and factor X, all with inflammatory properties; (3) the fibrinolytic system, producing plasmin and inactivating thrombin; and (4) the complement system, producing the anaphylatoxins C3a and C5a. While activated Hageman factor induces clotting, it concurrently activates the fibrinolytic system. This mechanism exists to limit clotting by cleaving fibrin, thereby solubilizing the fibrin clot. [9]

An antithrombotic role for factor XII as a platelet aggregation inhibitor or plasminogen activator has been proposed. C1 esterase inhibitor is the major inhibitor of factor XIIa. Antithrombin III (AT-III),

alpha-2-antiplasmin, and alpha-2-macroglobulin also inhibit factor XIIa. [10]

The traditional view is that low levels of factor XII increase the risk of venous thrombosis. Indeed, in one study factor XII levels were lower in patients who had a venous thrombosis compared with healthy blood donors. [11] Several case reports pointed out the occurrence of myocardial infarction in persons with factor XII deficiency. [12, 13]

Aims of the study:

To study the level of intrinsic coagulation factor (FXII: C) in patients with acute myocardial infarction.

Subjects:

This study was carried out in Al-Yermouk Teaching Hospital during the period from October 2008 to March 2009.

Thirty (30) patients were included in this study (9 female, 21 male) .They were just admitted to the coronary care unit and diagnosed as having acute myocardial infarction . Their age range is 48-68 years.

For each patient a questionnaire form was filled with the following information: age, sex, cigarette smoking, alcohol consumption, history of hypertension, history of diabetes. Patient on anticoagulant were excluded from the study.

Thirty healthy subjects (9 female, 21 male) age and sex matched with the patients were included as a control group.

Sample collection:

Before receiving any medication blood samples were taken from each patient. Two (2) ml of blood were withdrawn in syringes by clean venipuncture, 1.8 ml dispensed in plastic tubes containing 0.2 ml trisodium citrate dihydrate 32 g/L.

The citrated blood was centrifuged without delay at 2500g for 15 minutes to prepare platelet poor plasma, the plasma was stored without delay at -40 C° to perform factor assay for factors XII: C.

Pooled fresh plasma was obtained from apparently normal healthy twenty 20 donors, and stored without delay at -40C° to be used as control (calibration curve).

Factor XII: C assay

The assay is an APTT based parallel line bioassay. [14] The assay consisted of the measurement of the clotting time, in the presence of cephalin and activator of a system in which all the factors are present, constant and in excess except factor XII which was derived from the sample being tested.

Reagents:

1-STA® - Deficient factor XII: freeze dried citrated human plasma from which factor XII has been removed by immuno-adsorption.(REF 00722)

2-STA® - PTT.(REF 00480)

3-Owren –Koller Buffer, (REF 00360)

4-STA®-CaCl2 0.025M (REF00367)

5-Frozen citrated platelet poor plasma stored at - 40°C from patients and normal pooled plasma.

6-System control [N]+[P](REFOO617):control plasma, normal and abnormal levels.

Normal range: 60-150 %.

Calibration curve:

The frozen citrated platelet poor plasma aliquots of control pooled plasma are thawed. The following dilutions of the control plasma in Owren –Koller buffer were made:

Dilution: 1:10 1:20 1:40

Factor XII activity: 100% 50% 25%

The dilutions were prepared in the following way:

1-In a plastic plane tube mix 0.9 ml of Owren –Koller buffer + 0.1 ml of control plasma to obtain a dilution of 1:10.

2-In each of a second and a third plastic plane tubes 0.5 ml of Owren-Koller buffer was added.

3-In the second tube 0.5 ml from the first tube was added to 0.5 ml buffer to obtain a dilution of 1:20.

4-In the third tube 0.5 ml from the second tube was added to the 0.5 ml buffer to obtain a dilution of 1:40.

5-The dilutions and the time were plotted on a log - log paper to obtain a straight line.

Patients' plasma:

The frozen citrated platelet poor plasma aliquots of patients' plasma are thawed. Dilutions of the patients' plasma in Owren –Koller buffer were made in the same way of that of the control plasma:

Dilution: 1:10 1:20 1:40

Factor XII activity: 100% 50% 25%

Procedure:

In a glass test tube set in water-bath at 37±0.5C°, 0.1ml of STA®-Deficient XII, 0.1 ml of test sample (standard, patient or control), and 0.1 ml of cephalin were added .Tube content were mixed and incubated for 3 minutes .After 3 minutes 0.1 ml of 0.025 M calcium chloride CaCl2 prewarmed at 37C° was added to the mixture. A stop watch was started simultaneously and time was recorded in seconds.

Blank and standard plasma were tested at the beginning of each run to verify the validity of the results.

Using a log-log paper, factor XII activity was plotted on the X-axis, and the corresponding clotting times (seconds) on the Y-axis. Then the best fit calibration line was drawn. The times that the test patients' plasmas had taken to clot and those of the controls were interpolated on the calibration line to determine their respective factor XII levels (%).

Results:

Thirty Patients (9 females, 21 males) with documented acute myocardial infarction were included in this study. Their ages range between 48-68 years. Information about history of hypertension, diabetes, cigarette smoking and alcohol consumption were taken from each patient as shown in **table (1).**

Table (2) shows the types of acute MI according to site. **Figure (1)** shows the distribution of factor XII level in patient group and control group. **Table (3)** shows the mean of factor XII level in patients and controls.

Table (1) shows distribution of patients according to history of hypertension, diabetes, cigarette smoking, and alcohol consumption.

Socio-demographic information		No	%	CONTROL
Sex	Male	21	70	21
	Female	9	30	9
Smoking	Yes	19	63.3	
	No	11	36.7	30
Alcohol consumption	Yes	8	26.7	
	No	22	73.3	30
Hypertension	Yes	18	60.0	
	No	12	40.0	30
Diabetes mellitus	Yes	12	40.0	
	No	18	60.0	30

Table (2): subgroups of patients according to types of myocardial infarction.

Type of MI	No. of patients
Anterior MI	12
Lateral MI	4
Septal MI	4
Inferior MI	8
Extensive MI	2

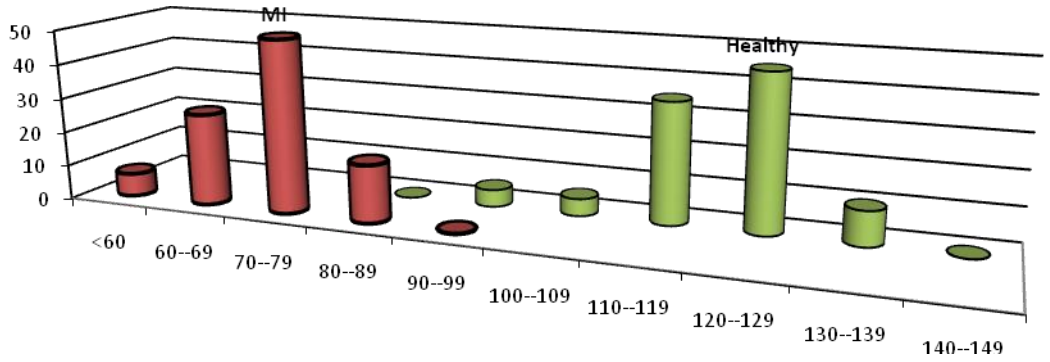

Figure (1): Factor XII level in patients and controls. The figure shows the number of patients and controls (Y axis) for values of factor XII activity (X axis).

Table (3): showing mean, standard deviation, range and P value of age and factor XII level in patients and control subjects.

	MI		Healthy		P value
	Mean±SD	Range	Mean±SD	Range	
Factor XII	71.03±11.46	(30-85)	119.00±8.52	(96-130)	0.0001*

Discussion:

Coronary artery disease is the major cause of mortality and morbidity in developed countries. [2]

The predominant cause of coronary artery disease is atherosclerosis, a process that starts early in life and progress slowly and silently for decades. [2]

Lower levels of factor XII result in enhanced thrombus formation by decreased fibrinolysis or effects on inflammation and complement activation. [15,16].

In the revised model of coagulation, factor XI is activated by thrombin, whereas factor XII is believed to have a profibrinolytic function rather than initiating coagulation via the intrinsic pathway.

This is illustrated by several case reports, linking a deficiency of factor XII with myocardial infarction and depression of factor XII dependent fibrinolytic activity with risk of re-infarction. (17) Also fibrinolytic activity is impaired in factor XII deficient patients which may explain the occurrence of thromboembolic complications in these patients. [18]

However, a recent report showed that factor XII–deficient mice were protected in a model of lethal pulmonary embolism and had a defective arterial thrombosis formation, suggesting that factor XII also contributes to thrombin and fibrin formation.[18] These results are in contradiction with the findings of this study. One of the explanations might be that humans have evolved to a different mechanism of coagulation than mice. The presence of atherosclerosis plays an important role in the occurrence of myocardial infarction in humans, whereas vessels of the mice used in the models clearly differ in this aspect. An alternative explanation is that the potential functions of factor XII only become apparent at certain concentrations of factor XII.

In this study, the concentration of factor XII: C in the subjects was not below 23%. In the mouse study, only homozygote-deficient mice were protected against arterial thrombosis.

The heterozygote mice showed similar results compared with mice containing normal levels of factor XII, with nearly all vessels containing thrombi, a slightly lower time to occlusion and slightly more platelets per surface area. [19]

Because the used models were not designed or sensitive for enhanced thrombus formation, it can therefore not be excluded that heterozygote mice had enhanced thrombus formation.

This study has found that mean FXII:C was significantly lower (P value <0.001) in acute myocardial infarction group (71.03±11.46) than control group (119.00%±8.52) as shown in table (3),that is similar to the results found by Leiden Group.[20]

Taken together and on the basis of the findings of this study, we can then speculate that lower levels of factor XII result in enhanced thrombus formation by decreased fibrinolysis or effects on inflammation and complement activation.[15,16]

Patients deficient for factor XII do not bleed, and, although factor XII is essential for contact activation and the activated partial thromboplastin time, it has been assumed that factor XII does not contribute to clot formation.

Conclusion:

FXII: C level was significantly lower in patients with acute myocardial infarction than control group.

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