

Evaluation of the Intrinsic Pathway of Coagulation in a Sample of Iraqi 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. Increased plasma hemostatic markers were noted in acute myocardial infarction, indicating that the blood coagulation system is highly activated in those patients.

Aims of the study: To study the level of intrinsic coagulation factors including (FVIII:C, FIX:C, FXI:C, FXII:C) in patients with acute myocardial infarction.

Type of the study: Cross-sectional study.

Methods: Thirty patients (their age range is 48-68 years) were included in this study (9 female, 21 male) who were just admitted to the coronary care unit in AL-Yarmouk Teaching Hospital and diagnosed as having acute myocardial infarction patients, blood samples were taken from those patients.

Twenty healthy subjects (6 female, 14 male) age and sex matched with the patients were included as a control group. The following investigations were done for both groups: 1. Packed cell volume (PCV%) by microhaematocrit method. 2. FVIII:C assay. [by parallel line bioassay of coagulation factors]. 3. FIX:C assay. 4. FXI:C assay. 5. FXII:C assay.

Results Mean FVIII:C (162.63 ± 17.22) was significantly ($P < 0.05$) higher in patients with acute myocardial infarction than control group (94.70 ± 9.34). 1-Mean FIX:C (151.20 ± 14.20) was significantly ($P < 0.05$) higher in acute myocardial infarction group than control group (94.10 ± 8.51). 2-Mean FXI:C (146.30 ± 7.87) was

significantly ($P < 0.05$) higher in acute myocardial infarction group than control group (102.00 ± 7.91). 3-Mean FXII:C (71.03 ± 11.46) was significantly ($P < 0.05$) lower in acute myocardial infarction group than control group (119.00 ± 8.52). 4-There is inverse relationship between FXI:C and FXII:C in acute myocardial infarction group ($P < 0.736$).

Conclusions Patients with acute myocardial infarction had significantly higher levels of FVIII:C, FIX:C, FXI:C than controls. FXII:C level was significantly lower in patients with acute myocardial infarction than control group. There is an inverse relationship between FXI:C and FXII:C in patients with acute myocardial infarction.

Keywords: acute MI, Intrinsic pathway, FVIII:C, FIX:C, FXI:C, FXII:C

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

Recent studies have found that elevated levels of coagulation factors are associated with an increased risk of thrombosis.⁽³⁾

Increased plasma hemostatic markers were noted in acute myocardial infarction, indicating that the blood coagulation system is highly activated in those patients.⁽⁴⁾

The contribution of the intrinsic pathway of the coagulation cascade is suggested by a number of clinical studies which demonstrated correlation between elevated levels of factor VIII and factor IX and the risk of coronary heart disease,⁽⁵⁾ and ischemic stroke.⁽⁶⁾

Several clinical surveys revealed a significantly reduced risk of myocardial infarction and 5-fold lower mortality from ischemic heart disease in factor VIII-deficient patients with hemophilia A, suggesting that the lowering of factor VIII level reduces development of thrombotic complications in atherosclerosis.⁽⁷⁾

Transgenic mice overexpressing human factor IX at persistently high levels died at much younger ages

than their cohorts expressing lower levels, or nontransgenic control animals. The median survival age of animals was inversely related to the circulatory levels of human factor IX.⁽⁸⁾

Factor XI acts as a procoagulant and an antifibrinolytic factor, and dysregulation may increase the risk of thrombosis. However, factor XI deficiency does not confer protection against myocardial infarction.⁽⁹⁾ Elevated levels may even increase the risk, similarly to the increased risk of venous thrombosis.⁽¹⁰⁾

While Hageman factor (FXII) is inducing clotting, it is concurrently activating the fibrinolytic system. This mechanism exists to limit clotting by cleaving fibrin, thereby solubilizing the fibrin clot.⁽¹¹⁾

An antithrombotic role for factor XII as a platelet aggregation inhibitor or plasminogen activator has been proposed.⁽¹²⁾ Several case reports pointed out the occurrence of myocardial infarction in persons with factor XII deficiency.^(13, 14)

Aims of the study:

- To evaluate the intrinsic coagulation factors activity (FVIII:C, FIX:C, FXI:C, FXII:C) and relation between them in patients with acute myocardial infarction.

Material and Methods: This is a prospective case control study. It was approved by the ethical committee of Iraqi Board of Pathology conforming to Declaration of Helsinki.

The study was carried out in the Laboratory Department of Al-Yarmuk Teaching Hospital during the period from October 2008 to March 2009.

Thirty 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. Oral informed consent was obtained from each patient or next of kin.

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.

Twenty healthy subjects (6 female, 14 male) age and sex matched with the patients were included as a control group.

Before receiving any medication blood samples were taken from each patient. Four 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 room temperature (20-25°C), 2500g for 15 minutes to prepare platelet poor plasma, the plasma was stored without delay at -40°C to perform factor assay for factors VIII:C, IX:C, XI:C and XII:C later.

Pooled fresh plasma was obtained from 20 apparently normal healthy donors (other than the control group), and separated in many plastic containers. They were stored without delay at -40°C. Each time one of them was used as control (calibration curve).

- FVIII:C assay, FIX:C assay, FXI:C assay, FXII:C assay were done by parallel line bioassay of coagulation factor.⁽¹⁵⁾
- The factor VIII assay, factor IX assay, factor XI assay and factor XII assay were determined using commercially available kits for each of factor VIII, factor IX, factor XI and factor XII (Diagnostica Stago /France).
- Normal activity (according to the instructions mentioned in the leaflets of the kits) for each of FVIII, FXI, FXII is 60-150%, while for factor IX it is 50-160%
- Blood for determining the packed cell volume was obtained by skin prick.
- Packed cell volume was performed manually by the use of the micro hematocrit method.⁽¹⁶⁾

Statistical Analysis: Computerized statistical analysis were performed using SPSS (statistical package of social sciences), version 15. The statistical significance of difference in the mean between two groups was assessed using independent t test and correlation study.

P value less than 0.05 was considered indicative of statistically significant difference.

Results: Thirty Patients (9 females, 21 males) with newly diagnosed 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. Table 1 shows distribution of patients according to risk factors. There was no significant difference in mean PCV% between patients with acute myocardial infarction (mean PCV% 41.20±3.34) and control group (mean PCV% 39.90±2.92). P value 0.163, as shown in table 2.

Mean FVIII:C was significantly higher (P value 0.0001) in patients with acute myocardial infarction (162.63%±17.22) than controls (94.70%±9.34). Also mean FIX:C was significantly higher (P value 0.0001) in acute myocardial infarction group (151.20%±14.20) than controls (94.10±8.51), as well mean FXI:C was significantly higher (P value 0.0001) in acute myocardial infarction (146.30%±7.87) group than controls (102.00%±7.91). All shown in table 2.

On the other hand, mean FXII:C is significantly lower (P value 0.0001) in acute myocardial infarction group (71.03%±11.46) than control group (119.00%±8.52), as shown in table 2. Relationship between FXI:C and FXII:C in acute myocardial infarction group, as shown in figure 1.

Discussion: The predominant cause of coronary artery disease is atherosclerosis⁽²⁾ which is the result of a complex interaction between: blood constituents, disturbed blood flow and vessel wall abnormalities.⁽¹⁷⁾

Inflammation with increased endothelial permeability and monocyte recruitment; growth, with smooth muscle cell proliferation and migration and matrix synthesis; degeneration, with lipid accumulation; necrosis, by toxic effect of oxidized lipids; calcification and thrombosis.⁽¹⁷⁾

This study demonstrates an increase level of FVIII during the early hours of documented acute myocardial infarction. Similar results were found by Rumley A et al,⁽¹⁸⁾ Gorog DA et al,⁽¹⁹⁾ Mircea P et al⁽²⁰⁾ and AL-Azzawi.⁽²¹⁾

Elevation of the FVIII is considered to be a systemic response related to the acute phase reaction.⁽²²⁾ Similar elevation in FVIII were reported in patient with other acute illnesses accompanied by an elevation of acute phase reactants.⁽²⁰⁾

Factor VIII:c levels are increased in an inflammation - sensitive manner.⁽²³⁾ It is an acute phase protein whose level may rise for a variety of non-specific reasons such as physical stress or adrenaline infusion or tissue break-down and repair.⁽²⁴⁾ Its causal relation to cardiovascular diseases may be due to a number of factors including: factor VIII, (along with factor V) is a key procoagulant factor and is capable of dramatically increasing the rate of factor IXa-catalyzed activation of factor Xa in a dose dependent manner,⁽²⁵⁾

several cross-sectional studies have demonstrated increased factor VIII levels in individuals considered at high risk for future events.⁽²⁶⁾

The degree of elevation in FVIII depend on certain factors such as the level of FVIII before infarction, as studies showed a relation between elevated FVIII level and development of IHD.⁽²⁷⁾

Regarding the increase in FIX level, similar results were found by M.C. Minnema,⁽²⁸⁾ and Vaziri N D.⁽²⁹⁾ FIX plays an important role in blood coagulation in that it is proteolytically activated by FXIa of the intrinsic pathway, as well as by the tissue factor-FVIIa complex in the extrinsic pathway.⁽³⁰⁾ Transgenic mice overexpressing human factor IX at persistently high levels died at much younger ages than their cohorts expressing lower levels, or nontransgenic control animals. The median survival age of animals was inversely related to the circulatory levels of human factor IX. Animals expressing high circulatory levels of hFIX had various degrees of myocardial fibrosis around cardiac vasculature, predominantly located in the myocardium of the left ventricle. Fibrosis replaced normal myocardium in multifocal patterns. The observed intramyocardial distribution patterns of fibrosis may be due to ischemic injury from occlusion of small vessel and subsequent reperfusion.⁽³¹⁾

Circulatory levels of both human (hFIX) and murine (mFIX) increase with advancing age.⁽³²⁾ The basic genetic mechanisms responsible for age-related increase and stable patterns of hFIX and hPC gene expression, respectively involve 2 critical genetic elements, ASE (age-related stability element) and AIE (age-related increase element).⁽³³⁾

FXI level is higher in patient with myocardial infarction than in control group, similar results were found by Carine J. M. Doggen,⁽³⁴⁾ M.C. Minnema⁽²⁰⁾, Merlo C.⁽³⁵⁾

Factor XI:c has procoagulant and antifibrinolytic effects; thus, an increased risk of myocardial infarction in the presence of high factor XI:c was to be expected and is in line with studies on venous thrombosis.⁽³⁴⁾

Thrombin is capable of activating factor XI in vitro,⁽³⁶⁾ and this would imply a factor XII-independent activation of factor XI. Thrombin dependent activation of factor XI constitutes an amplification pathway, because activation of factor XI leads, via the activation of factors IX and X, to the formation of additional thrombin.⁽³⁷⁾ This additional thrombin may be important for the activation of a carboxypeptidase B, called thrombin activatable fibrinolysis inhibitor (TAFI), resulting in the attenuation of fibrinolysis.⁽³⁸⁾ Hence, thrombin-dependent activation of factor XI may be a critical element in the onset of acute coronary syndromes.

Inhibition of factor XI in an experimental thrombosis model in rabbits leads to enhanced thrombolysis, thereby showing an antifibrinolytic effect of

factor Xia.⁽³⁹⁾ Additionally, factor XI-deficient mice were protective from an induced arterial occlusion, suggesting that therapeutic inhibition of factor XI may be a reasonable strategy for treating or preventing thrombus formation.⁽⁴⁰⁾

In contrast, factor XIIc among patients with myocardial infarction was lower than in control subjects, which is similar to the results found by Carine J.M. Doggen.⁽³⁴⁾

Lower levels of factor XII result in enhanced thrombus formation by decreased fibrinolysis or effects on inflammation and complement activation.⁽⁴¹⁾

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 reinfarction.⁽⁴²⁾ Also fibrinolytic activity is impaired in factor XII deficient patients which may explain the occurrence of thromboembolic complications in these patients.⁽⁴³⁾

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.⁽⁴³⁾ 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.⁽⁴⁴⁾ 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.⁽⁴¹⁾ 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.⁽⁴¹⁾

Figure (1) shows that FXII:C correlates inversely with FXI:C in patients with acute myocardial infarction, these results are in line with the current concept of coagulation and that in contrast to factor XI, factor XII is not involved in physiologic thrombin and fibrin formation.⁽⁴⁵⁾

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. In contrast, factor XI is involved in normal thrombin and fibrin formation, and elevated levels of factor XI have previously been found to be associated with an increased risk of venous thrombosis.⁽⁴⁵⁾

3. Screening for haemostatic changes in a large follow up study is suggested to predict the risk of myocardial infarction.
4. Evaluating the prognostic value of FVIII :C, FIX:C,FXI:C and FXII:C on future cardiovascular events and occurrence of complications .
5. Evaluations of the haemostatic factors in other arterial thrombotic diseases as cerebrovascular accidents.

Conclusions:

1. The results of this study demonstrate that patients with acute myocardial infarction have significantly higher levels of FVIII:C, FIX:C, FXI:C than control groups during the acute phase .
2. FXII:C level was significantly lower in patients with acute myocardial infarction than control group.
3. There was an inverse relationship between FXII:C level and FXI:C level in patients with acute myocardial infarction.

Recommendations:

1. Screening for other haemostatic factors and of fibrinolytic system such as plasminogen activators inhibitors and fibrin degradation products in patient with MI.
2. Study the effect of treatment (anticoagulants and thrombolytic agents) on the haemostatic changes in patients with acute MI.

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

		No	%
Sex	Male	21	70
	Female	9	30
Smoking	Smokers	19	63.3
	None	11	36.7
Alcohol	Alcohol consume.	8	26.7
	None	22	73.3
Hypertension	Hypertensive	18	60.0
	None	12	40.0
Diabetes mellitus	Diabetics	12	40.0
	None	18	60.0

Table (2): showing mean, standard deviation, range and P value of different parameters in patients and control subjects.

	MI		Healthy		P value
	Mean±SD	Range	Mean±SD	Range	
Age (years)	58.37±6.41	(48-68)	59.60±6.05	(48-68)	0.499
PCV%	41.20±3.34	(35-47)	39.90±2.92	(35-45)	0.163
Factor VIII%	162.63±17.22	(115-189)	94.70±9.34	(78-107)	0.0001
Factor IX%	151.20±14.20	(104-172)	94.10±8.51	(75-108)	0.0001
Factor XI%	146.30±7.87	(126-160)	102.00±7.91	(86-114)	0.0001
Factor XII%	71.03±11.46	(30-85)	119.00±8.52	(96-130)	0.0001

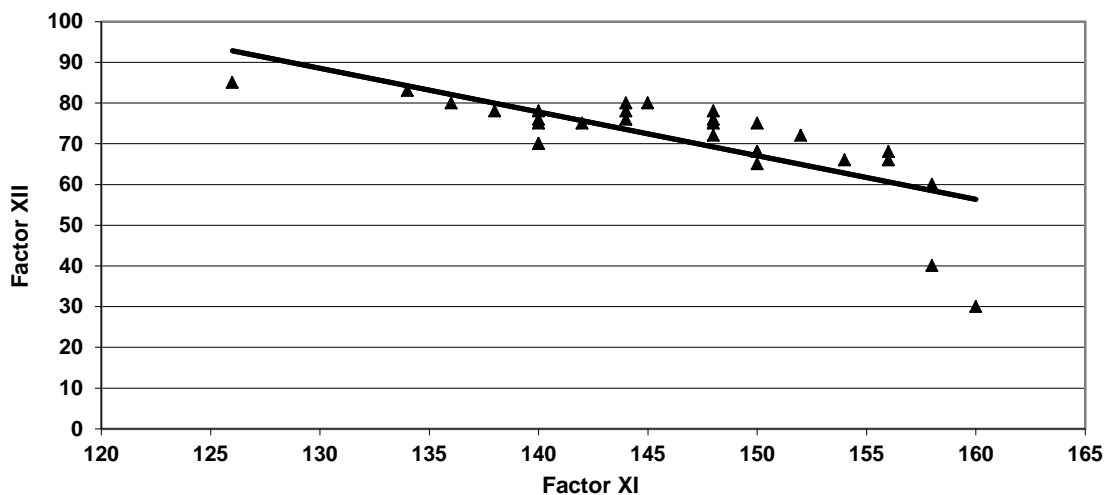


Figure (1): The correlation between Factor XI and Factor XII in MI. There is an inverse correlation between FXI:C and FXII:C.

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