دراسة عوامل الخطورة الجديدة للمرضى المصابين بقصور دورة الشرايين التاجية

رسالة مقدمة الى

كلية الطب – جامعة بابل كجزء من متطلبات نيل درجة الماجستير في علم الكيمياء الحياتية السريرية

من قبل الطالب

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١٤٢٨هـ ٢٠٠٧م

A STUDY ON NEW RISK FACTORS IN PATIENTS WITH ISCHEMIC HEART DISEASE

A thesis

Submitted to the committee of the College

of Medicine – Babylon University in partial fulfillment of the requirements for the degree of Master of Science in clinical biochemistry

BY

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الخلاصة

تمت دراسة ٨٥ مريض مصاب باعتلال شريان القلب التاجي لدراسة التغيرات في الفايبرينوجين في البلازما ، الفيريتين في المصل ، الحديد في المصل وحامض اليوريك في المصل لايجاد احتمالية استخدام هكذا متثابتة كمبنى في تشخيص امراض القلب التاجية .

ان المرضى التي تمت دراستهم كانوا ٦٢ ذكرا و ٢٣ انثى وكانت مجموعة السيطرة (٥٠) . المرضى المصابين بالسكري ، فرط ضغط الدم ، التاريخ العائلي الموجب للاصابة بامراض الشريان التاجي ، السمنة ن المدخنين قد تم استثناهم من الدراسة . ان تشخيص مرض الشريان التاجي قد تم بالتاريخ المرضي ن الفحص السريري تخطيط القلب الكهربائي ، فحص القلب بالامواج فوق الصوتية ، والتحاليل المختبرية .

قسم المرضى الى مجموعتين : الاولى اشتملت المصابين بمرض الشريان التاجي الحاد (٥٥ حالة) والاخرى اشتملت المصابين بمرض الشريلن التاجي المزمن (٣٠ حالة) .

ان تنتائج الدراسة اظهرت ان هناك زيادة معنوية في الفايبرينوجين في البلازما لدى كل المرضى في مجموعة مرض الشريان التاجي الحاد ($P<0.05$ في الذكور و$p<0.05$ في الاناث) بينما ليست هناك تغيرات معنوية لدى اولئك المصابين باحتشاء العظلة القلبية المزمن ($p>0.05$) من الجنسين ، في حين ان هناك زيادة معنوية عند المرضى الذكور المصابين بالذبحة الصدرية المزمنة ($p<0.05$).

ان مستوى الفيريتين والحديد في المصل قد وجد انها تزداد في ١٧ حالة بينما وجد انها تقل في المرضى الاخرين ($p>0.05$) عدا المرضى الذكور المصابين بامراض القلب التاجية الحاد ، حيث اظهورا تغير معنوي بالمقارنة بمجموعة السيطرة ($p>0.05$) .

فيما يخص حامض اليوريك في المصل ، عكست النتائج زيادة في المستوى فقط لدى ١٧ حالة مصابة بمرض القلب التاجي المزمن تركيز طبيعي لحمض اليوريك في المصل .

عند رسم الفايبرينوجين في البلازما ضد الفيريتين في المصل ينتج علاقة ايجابية في مجموعة السيطرة (معامل الترابط = ٠.٠٨ للذكور) ، (معامل الترابط = ٠.١٦ للاناث) ، بينما في حالات مرض القلب التاجي الحاد، اظهر المرضى الذكور علاقة ايجابية (معامل الترابط = ٠.٥) ، واظهر المرضى الاناث علاقة سلبية (معامل الترابط = ٠.٣٣) ، في حين ان كلا الجنسين المصابين بالذبحة الصدرية المزمن اظهروا علاقة سلبية (معامل الترابط = ٠.٥٩) ، بينما المرضى المصابين باحتشاء العضلة القلبية المزمن اظهروا علاقة ايجابية (معامل الترابط = ٠.٢٣).

عند رسم الفيريتين في المصل ضد الحديد في المصل علاقة ايجابية معنوية في كل المرضى المصابين بامراض القلب التاجية (معامل الترابط = ٠.٨٥ كلا مجموعتي الحاد والمزمن) وكذلك مجموعة السيطرة اظهرت علاقة ايجابية (معامل الترابط = ٠.٩).

اعتمادا على هذه النتائج ، نستنتج ان الفايبرينوجين في البلازما والفيريتين في المصل وبقدر اقل حمض اليوريك في المصل يمكن ان تستخدم وان تعتبر كمؤشر لتكهن المرض او تقدم مرض القلب التاجي .

الخلاصة

تمت دراسة (٨٥) مريض يعانون من مرض قصور الشرايين التاجية القلبية ، ولمعرفة التغيرات الحاصلة لهم في بلازما فايبرانوجين ، ومصل الفرتايين ، ومصل الحديد ، ومصل حامض اليوريك لإيجاد إمكانية استخدام مثل هذه المقاييس كمؤشرات في التشخيص السريري لهذا المرض .

شملت الدراسة (٦٢) ذكراً و(٢٣) أنثى مصابين بهذا المرض ومجموعة ضابطة شملت (٥٠) مريضاً . ليس لدى المرضى جميعهم تاريخ عائلي يحمل مرض قصور الدورة التاجية ، ولا يدخنون ، ولا يعانون من مرض السكري ، ولا ضغط الدم ، ولا يعانون من السمنة بشكل نسبي . تم إجراء تشخيص مرض قصور الشريان التاجي القلبي من خلال الفحص السريري والسجل التاريخي والفحوصات المختبرية و تخطيط القلب والتصوير فوق الصوتي.

تم تقسيم المرضى إلى مجموعتين ، المجموعة الأولى بواقع (٥٥) حالة من المرضى الذين يعانون من مرض قصور الشريان التاجي القلبي الحاد ، بينما تألفت الثانية من (٣٠) حالة يعانون من مرض قصور الشريان التاجي المزمن .

وأظهرت نتائج الدراسة زيادة في بلازما فايبرانوجين بالنسبة لمرضى مجموعة المرض الحاد جميعهم (P < ٠.٠٠٠٧) في الذكور وفي الإناث (P < ٠.٠٠١) ، بينما كان المستوى طبيعي (P > ٠.٠٥) في مجموعة المرض المزمن الذكور والإناث مع سداد العضلة القلبية ، بينما المرضى الذكور المصابين بالذبحة الصدرية المزمن (P < ٠.٠٠٦) . وفيما يتعلق بمصل الفرتين ومصل الحديد ازداد في بعض الحالات (١٧) حالة وانخفض في المرضى الآخرين (P < ٠.٠٥) في كل المرضى ماعدا الذكور المصابين بمرض قصور الشريان التاجي القلبي الحاد ، حيث أظهرت فروق واضحة عند المقارنة مع المجموعة الضابطة (P < ٠.٠٥) .

أما بالنسبة لحامض اليوريك ، فقد أظهرت النتائج زيادة في مستويات (١٧) حالة فقط من الذين يعانون من مرض قصور الشريان التاجي القلبي الحاد ، بينما أظهرت في مرضى قصور الشريان التاجي المزمن تركيز طبيعي لمصل حامض اليوريك (P < ٠.٠٥) .

ومن خلال الرسم البياني لبلازما فايبرانوجين مقابل مصل الفرتايين ظهرت علاقة تبادلية إيجابية في المجموعة الضابطة ، بينما ظهرت في مجموعة مرضى قصور الشريان التاجي القلبي الحاد (الذكور) علاقة تبادلية إيجابية بينما كشفت علاقة سلبية في المرضى الإناث لنفس المجموعة ، وفيما يخص مجموعة مرضى قصور الشريان التاجي المزمن ولكلا الجنسين والذبحة الصدرية المزمن كشفت علاقة تبادلية سلبية ، بينما كانت العلاقة التبادلية إيجابية لمرضى MI المزمن .

ومن خلال الرسم البياني لمصل الفرتايين مقابل مصل الحديد كشف علاقة تبادلية إيجابية واضحة لكل المرضى الذين يعانون من مرض قصور الشريان التاجي القلبي (

ولكلا المجموعتين الحاد والمزمن) وكذلك أظهر علاقة تبادلية إيجابية في المجموعة الضابطة .

اعتماداً على هذه النتائج ، نستنتج أن بلازما فايبرانوجين ومصل الفرتايين وبنسبة أقل مصل حامض اليوريك يمكن اعتبارها كمؤشرات لتشخيص أو توقع حدوث مرض قصور الشريان التاجي القلبي .

بسم الله الرحمن الرحيم

وَقُل رَّبِّ زِدْنِي عِلْماً

صدق الله العلي العظيم

سورة طه (اية ١١٤)

Certification

We certify that this thesis was prepared under our supervision at the Biochemistry Department- College of Medicine – Babylon University as a partial requirements for the degree of Master of Science in Clinical Biochemistry.

Professor
Dr. Mounam M. AL – Shouk

Lecturer
Dr. Kadhim Jawad AL - Hamdany

In view of the available recommendations, I forward this thesis for debate by the Examining Committee.

Head of Biochemistry Department

Lecturer

Dr. Kadhim Jawad AL – Hamdany

/ /٢٠٠٧

Examining Committee

We, the examining committee, certify that we have read this thesis entitled : *(A study on new risk factors in patients with ischemic heart disease)*

And have examined the student Haider Abd Jabbar AL – Ammar in its contents, and that in our opinion it is accepted as a thesis in partial fulfillment of the requirement for the degree of Master of Science in Clinical biochemistry.

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College of Medicine

Babylon University

DEDICATION

TO

MY PARENTS

MY Devoting Wife

MY SONS

Ahmed and Ali

List of errors

Page No.	No. of row	Error	Correction
Title	Last line	١٤٢٧ A.H	١٤٢٨ A.H
٩	Table ٤	Choledocholityiasis	Choledocholithiasis
١٧	٩	ICAM-I	ICAM-١
١٩	٢	activity	activiting
٢٦	١١	IHC	IHD
٢٨	١١	Fully- streak	Fatty- streak
٢٩	١٩	Iron accurate	Iron cumarate
٤٤	٢	١/٠	١/١٠
٤٤	١٠	acidy	acid
٥٧	٥	μ mol/dL	μ mol/L
٥٩	٤	Elevated a reduced	Show a reduced
٥٩	١٠	lipopteins	lipoproteins
٥٩	٢١	blood duration	blood loss
٦٢	٣	unregulated	upregulated
٦٣	Fig. ١٠	r = ٠.٥٩	r = ٠.٠٨
٦٣	Fig. ١١	r = ٠.٥٩	r = ٠.١٦
٦٤	Fig. ١٢	r = ٠.٥٩	r = ٠.٥
٦٤	Fig. ١٣	r = ٠.٥٩	r = ٠.٠٧
٦٥	Fig. ١٤	r = ٠.٥٩	r = ٠.٣٣
٦٥	Fig. ١٥	r = ٠.٥٩	r = ٠.٠٤
٦٦	Fig. ١٧	r = ٠.٥٩	r = ٠.
٧٣	Paragraph ٣ Line ٤	likely	unlikely
III	١٥	P < ٠.٠٠٠٧	P < ٠.٠٥
III	١٦	P < ٠.٠٠١	P < ٠.٠٥
III	١٩	P < ٠.٠٠٦	P < ٠.٠٥

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Praise worthy be to God for the accomplishment of this thesis

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Haider

ABBREVIATIONS

Abbreviation	*Details*
AA genotype	**Adenine adenine genotype**
ACE	**Angiotensen converting Enzyme inhibitor**
ADP	**Adenosine diphosphate**
AHA	**American heart association**
Arg-Gly	**Arginine-glycine**
BECAIT	**Bezafibrate effect on coronary atherosclerosis intervension trial**
BMI	**Body mass index**
CAD	**Coronary artery disease**
CCS	**Canadian cardiac society**
CHD	**Coronary heart disease**
CK	**Creatine kinase enzyme**
CVD	**Cardiovascular disease**
DIC	**Disseminated intravascular coagulation**
EBCT	**Electron beam computed tomography**
ECG	**Electrocardiography**
EDTA	**Ethylene diamine tetra acetic acid**
ELISA	**Enzyme linked immunosorbent assay**
FGA	**Fibrinogen alpha**
FGB	**Fibrinogen beta**
FGG	**Fibrinogen gamma**
GG genotype	**Gycine gycine genotype**
HDL	**High density lipoprotein**
HMG-COA reductase	**Hydroxy-٣-methyl Glutaryl-COA**
ICAM-١	**Intercellular adhesion molecule - ١**
IHD	**Ischemic heart disease**
Kg/m^{٢}	**Kilogram per meter sequar**
LDL	**Low density lipoprotein**
MI	**Myocardial infarction**
MRI	**Magnetic resonance imaging**
mRNA	**Messenger Ribonucleic acid**
NO	**Nitric oxide**
O$_{٢}^{\cdot}$	**Superoxide radical**
OC	**Oral contraceptive pills**
OH$^{\cdot}$	**Hydroxil radical**
PET	**Positron emission tomography**
PF	**Plasma fibrinogen**
Redox	**Reduction oxidation**
STEMI	**ST-elevation myocardial infarction**
SUA	**Serum uric acid**
UK	**United kingdom**

Summary

Eighty five patients with coronary heart disease were studied for the changes in their plasma fibrinogen, serum ferritin, serum iron and serum uric acid to find the possibility of using such parameters as a predictor in the diagnosis of coronary heart disease.

The patients studied were ٦٢ males and ٢٣ females and the control group was ٥٠. Patients who have diabetes mellitus, hypertension, positive family history of CHD, obesity, smoking were excluded from the study. The diagnosis of coronary heart disease was made by history, clinical examination, electrocardiography, echocardiography, and laboratory investigations.

The patients were divided into ٢ groups the first one included those with acute coronary heart disease (٥٥ cases), while the second one consisted of patients with chronic coronary heart disease (٣٠ cases).

The results of the study revealed that there is significant increase in plasma fibrinogen in all the patients in acute group ($p < ٠.٠٥$ in male, p ٠.٠٥ in female), while those (male & female) with chronic myocardial infarction there is non-significant changes ($p > ٠.٠٥$) whereas there is significant increase in plasma fibrinogen level in male patients with chronic angina ($p < ٠.٠٥$). Regarding serum ferritin and serum iron, there is non-significant difference in comparison to that of control group ($p> ٠.٠٥$), except those males with acute CHD, shows significant difference in comparison to that of control group ($p<$ ٠.٠٥).

Concerning serum uric acid, the results reflected an significant increase only in ١٧ cases with acute coronary disease, ($p< ٠.٠٥$) whereas all patients

with chronic CHD showed a non-significant changes in serum uric acid concentration (p > ٠.٠٥).

The plotting of plasma fibrinogen against serum ferritin revealed a positive correlation in control group (r) = ٠.٠٨ in male, (r)=٠.١٦ in female, while in acute CHD, the males patients showed a positive correlation (r)=٠.٠٥ and the females patients reflected a negative correlation (r)=٠.٣٣, whereas in chronic CHD group both males and females patients with chronic angina revealed a negative correlation (r)=٠.٥٩, while those with chronic MI revealed a positive correlation(r)=٠.٢٣.

The plotting of serum ferritin against serum iron revealed a significant positive correlation (r)=٠.٨٥in all patients with coronary heart disease (both acute and chronic group) and also in control group showed a positive correlation (r)=٠.٩ .

On the bases of these results, it was concluded that plasma fibrinogen and serum ferritin and for lesser extent serum uric acid in high levels could be used and regarded as a marker for the prediction &/or progression of coronary heart disease.

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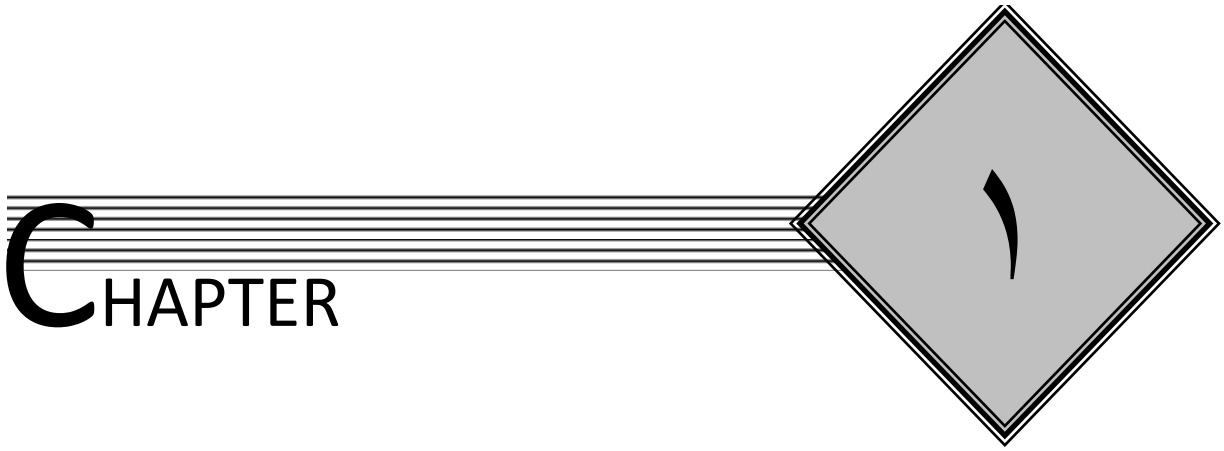


Introduction

SECTION I / Coronary Heart Disease

Ischemia refers to a lack of oxygen due to inadequate perfusion of the myocardium, which causes an imbalance between oxygen supply and demand. The most common cause of myocardial ischemia is obstructive atherosclerotic disease of pericardial coronary arteries. (١)

١. Epidemiology

Coronary heart disease (CHD) is the most common form of heart disease and single most important cause of premature death in Europe , the Baltic states ,Russia ,North and South America ,Australia and New Zealand, by ٢٠٢٠ it is estimated that it will be the major cause of death in all regions of the world. (٢)

In the UK (population ٥٩ million) ١ in ٣ men and ١ in ٤ women die of CHD, an estimated ٣٣٠,٠٠٠ people have a myocardial infarct each year and approximately ١.٣ million people have angina . the death rate of CHD in the UK is amongst the highest in western Europe (more than ١٤٠٠٠٠ people) but are falling , particularly in younger age groups ; in the last ١٠ years CHD mortality has fallen by ٤٢٪ among UK men and women aged ١٦-٦٤ . (٢)

In the United States men are more often affected than women by an overall ratio of ٤:١, but before age ٤٠ the ratio is ٨:١ and after age ٧٠ it is ١:١ in men , the peak incidence of clinical manifestation is at age ٥٠-٦٠ ; in women at age ٦٠-٧٠. (٣)

Coronary heart disease (CHD) remains the most common cause of death regardless of significant advancement in its prevention and treatment .(٤)

Obesity and type two Diabetes mellitus are increasing and are a prevalence powerful risk factor for CHD. With urbanization in the developing world, the prevalence of risk factors for CHD is increasing rapidly in these regions. (١)

٢. Pathophysiology

Knowledge concerning the pathophysiology of atherosclerosis and the clinical presentation of coronary artery diseases are accumulating rapidly . Abnormal lipid metabolism or excessive intake of cholesterol and saturated fats especially when superimposed on a genetic predisposition – initiates the atherosclerosis process. (٢)

Table (١): Coronary heart disease : clinical Manifestation and pathology. (٢)

Clinical problem	Pathology
Stable Angina	Ischemia due to fixed atheromatous stenosis of one or more coronary arteries
Unstable Angina	Ischemia caused by dynamic obstruction of coronary artery due to plaque rupture with superimposed thrombosis and spasm
Myocardial infarction	Myocardial necrosis caused by acute occlusion of coronary artery due to plaque rapture and thrombosis
Heart failure	Myocardial dysfunction due to infarction or ischemia
Arrhythmia	Altered conduction due to ischemia or infarction
Sudden death	Ventricular arrhythmia , systole or massive MI

The initial step is '' fatty streak'' or subendothelial accumulation of lipids and lipid-laden monocytes (macrophage) .Low density lipoprotein (LDLs) are the major atherogenic lipid. High–density lipoprotein (HDLs), in contrast , are protective and probably assist in the mobilization of LDLs. The pathogenetic role of other lipids , including triglycerides , is less clear. LDLs undergo in situ oxidation ,which makes them more difficult to mobilize as well as locally cytotoxic . Macrophage migrate into the subendothelial space and take up lipids, giving them the appearance of ''foam cell''. As the plaque progresses smooth muscle cells migrate into the lesion. At this stage, the lesion may be hemodynamically insignificant, but the endothelial function is abnormal and its ability to limit the entry of lipoproteins into the vessel wall is impaired . If the plaque remains stable, a fibrous caps forms , the lesion becomes calcified , and the used lumen slowly becomes narrowed .(٣)

Although many atherosclerotic plaques remain stable or progress only gradually, others may rupture , with a resulting extrusion of lipids and tissue factor that result in cascade of events culminating in intravascular thrombosis. The outcome of these events is determined by whether the vessel becomes occluded or whether thrombolysis occurs , either spontaneously or as the result of treatment ,and whether the plaque subsequently becomes stabilized. The result may be partial or complete vessel occlusion (causing the symptoms of unstable angina or myocardial infarction) , or the plaque may become reestablished , often with more severe stenosis. (٥)

A number of infectious agents, including Chlamydia pneumonia ,cytomegalovirus , and Helicobacter pylori , have been indirectly implicated in initiating or accelerating the inflammatory response. (٦)

٣. Risk Factors

An American heart association (AHA) prevention conference in ١٩٩٩ classified risk factors into three categories . The traditional “conventional” risk factors appear to have a direct causal role in atherogenesis . Predisposing factors, including obesity, family history of early onset CHD , and sedentary lifestyle , mediate some risk through the causal factors but may also have independent effects . The term 'conditional risk factors' was used for factors that have an association with increased risk for coronary artery disease although their causative , independent , and quantitative contribution to CHD are not well documented . These factors may enhance risk in the presence of the causative risk factors , hence the term 'conditional' . A fourth category that can be added is that of “ emerging “ risk factors which need further confirmatory studies .(٧)

Table (٢): Categories of risk factors for coronary heart disease CHD.(٧)

Conventional	***Predisposing***	***Conditional***	***“ Emerging “***
Cigarette smoking	Overweight and obesity,	Homocysteine	Lipoprotein– associated phospholipaseA٢
elevated blood pressure	physical inactivity	fibrinogen	pregnancy – associated plasma phosphatase

Elevated serum cholesterol	Male sex	Lipoprotein (a)	Asymmetric dimethylarginine
low HDL cholesterol	family history of early onsetCHD	small LDL partical size	Myeloperoxidase
diabetes mellitus	Socioeconomic factors	c-reactive protein	Nitrotyrosine
	behavioral factors	Serum ferritin	measures of oxidative stress
	Insulin resistance		Candidate gene polymorphisms

٤. Prevention

Although many risk factors for CHD are not modifiable, it is now clear that interventions such as smoking cessation , treatment of dyslipidemia , and lowering of blood pressure can both prevent CHD and delay its progression and complication after its manifest .(٨)

A series of clinical trials has demonstrated the efficacy of lowering LDL cholesterol with HMG-CoA reductase inhibitors (statins) in preventing death , coronary events , and strokes . Beneficial results have been found in patients who have already experienced coronary events (secondary prevention) , in those at particulary high risk for events (diabetics and patients with peripheral artery disease) and those with elevated LDL cholesterol without multiple risk factors .(٧)

Furthermore there is now more clear evidence that reduction of LDL cholesterol can prevent CHD and stroke in patients without clinically manifest atherosclerosis (primary prevention) and LDL levels as low as ١٣٠mg /dl .(٩)

Treatment of abnormally low HDL levels or elevation of lipoprotein (a) and small ,dense LDL particles is more difficult ,but oral niacin in high doses (٢-٣g/d or more) may be effective .Atrial in post infarction patients has demonstrated an increase in HDL levels with gemfibrozil (٦٠٠ mg twice daily) .(١٠)

Since LDL oxidation appears to play a role in the atherogenicity of lipid molecule that have passed into the vessel wall , antioxidant therapy (Vit. E therapy) has been advocated as a preventive measure . (١١)

Elevated plasma homocysteine levels are associated with an increased risk of vascular events , although homocysteine levels can be reduced with dietary supplements of folic acid (١mg/dl) in combination with vitamin $B_٦$ and vitamin $B_{١٢}$. (١٢)

Antiplatelete therapy is another very effective preventive measure . Aspirin (٣٢٥ mg every other day) in males over the age of ٥٠ reduces the incidence of myocardial infarction. (١٣)

Control of blood pressure has now been shown to prevent infection in older patients. The role of exercise remains controversial . Although individuals who exercise for at least ٣٠ minutes a week are at lower risk for subsequent coronary events . (١٤)

The heart outcomes prevention evaluation (HOPE) trial demonstrated that the ACE inhibitor ramipril reduces fatal and nonfatal vascular events by ٢٠ – ٥٠٪ in patients at high risk, including diabetic with additional risk factors or patients with clinical coronary, cerebral or peripheral arterial atherosclerotic disease. Thus, the role of ACE inhibitors in secondary prevention appears to be expanding beyond patients with heart failure or left ventricular systolic dysfunction. (١٥)

٥. Diagnosis

٥.١ Clinical Manifestations :

٥.١.١ Angina pectoris:

Angina pectoris is typically a retrosternal chest discomfort, usually perceived as pain but often described as pressure or heaviness . The patient may clench a fist when describing the sensation (Levine sign) . Discomfort often radiates to the neck , the left shoulder, the left arm , or the lower jaw and occasionally to the back . Chest discomfort is classified as typical angina if it : (١٦)

١. Has the qualities described above and last at least several minutes.
٢. Is provoked by exertion or emotion.
٣. Is relieved by rest or nitroglycerin .

Chest discomfort that has any two of these characteristics is called a typical angina . Chest discomfort meeting only one or none of these characteristics is classified as noncardiac chest pain . (١٦)

The severity of angina can be expressed by the Canadian Cardiac Society functional classification (CCS) as shown in table (٣) below.

Table (٣): Grading of Angina according to CCS classification. (١٦)

Class	*Description of stage*
I	"Ordinary physical activity does not cause angina" Such as walking or climbing stairs. Angina occurs with strenuous, rapid, or prolonged exertion at work or recreation.
II	"slight limitation of ordinary activity " . Angina occurs on walking or climbing stairs rapidly, walking uphill, walking or stair climbing after meals, in cold, in wind, or under emotional stress, or only during the few hours after a wakening.
III	"marked limitation of ordinary physical activity". Angina occurs on walking ١ to ٢ blocks on the level and climbing, flight of stairs under normal conditions and a normal pace.
IV	"inability to carryon any physical activity without discomfort" Anginal symptoms may be present at rest.

٥.١.٢ Acute Coronary Syndrome (Unstable Angina And Non – ST Segments Elevation MI) .

The patient with unstable angina or non – ST segment elevation MI seeks medical attention because he or she has recognized either that new symptoms have appeared or that a previously stable pattern of symptoms has become unstable. Patients with non-ST segment elevation MI also may be present with a pattern of increasing anginal episodes at lower levels of activity , but these patients are more likely to experience a prolonged episode of discomfort at rest .In many patients , the clinical presentation is indistinguishable from acute ST-segment elevation MI , whereas other patients many have nonspecific symptoms . Nausea, sweating, or shortness of breath and epigastric pain may accompany episodes of acute myocardial ischemia. In elderly or diabetic patients, these symptoms may be the only indication that myocardial ischemia is present . (١٧)

Approximately two thirds of patients describe the new onset of angina or a change in their anginal pattern in the month preceding infarction.(١٨)

However, in approximately one fourth of patients, myocardial infarction is associated with only mild symptoms or no symptoms at all.(١٩)

٥.١.٤ ST- Segment Elevation Myocardial Infarction

Traditionally , the diagnosis of acute MI has rested on the triad of ischemic chest discomfort , ECG abnormalities , and elevated serum cardiac markers . Acute MI was considered present when at least two of the three were present . with their increasing sensitivity and specificity , serum cardiac markers (e. g, troponin I or T) have assumed a dominant role in confirming the diagnosis of acute MI in patients with suggestive clinical and/or ECG features. (١٧)

STEMI generally occurs when coronary blood flow decreases abruptly after thrombotic occlusion of coronary artery previously affected by atherosclerosis. Slowly developing, high grade coronary artery stenosis do not usually precipitate STEMI because of the development of a rich collateral network overtime . Instead, STEMI occurs when coronary artery thrombus develops rapidly at a site of vascular injury.(٢٠)

٥.٢ Differential Diagnosis of Chest Pain

In primary care office practice, most patients with chest pain do not have myocardial ischemia. Although atypical chest pain may be ischemia, many alternative diagnoses should be considered before tests for CHD are performed. (٢١)

Table (٤) : Differential diagnosis of chest pain. (٢١)

Type	Cause
Non ischemic cardiovascular	Aortic dissection Pericarditis
Pulmonary	Embolus Pneumothorax Pneumonia Pleuritis
Gastrointestinal	Esophageal *Esophagitis , spasm , reflux* Biliary *Colic , choledocholithiasis , cholangitis* Peptic ulcer Pancreatitis

Chest wall	Costochondritis , fibrositis , rib fracture Sternoclavicular arthritis , Herpes zoster (before rash)
Psychiatric	Anxiety disorder Hyperventilation Panic disorder Primary disorder Affective disorder (e . g . , depression) Somatoform disorders Thought disorders (e . g . , fixed delusions) .

٥.٣ Physical Examination:

Physical examination during chest discomfort can provide a high degree of diagnostic certainty when abnormal findings wax and wane with the appearance and disappearance of chest pain. A transient S_3 or S_4 gallop, mitral regurgitation murmur, or paradoxical splitting of the second heart sound indicates that left ventricular function is altered during pain and strongly argues for an ischemic cause of pain. The patient with acute MI often appears anxious and in distress. Vital signs are often normal, but sinus tachycardia is not uncommon. The pulse may be rapid or slow if arrhythmias are present. Either hypotension caused by left or right ventricular dysfunction or arrhythmia or hypertension caused by adrenergic discharge may be present. The murmur of ischemic mitral regurgitation may be present . If a left bundle branch block is present, abnormal splitting of the second heart sound may be heard . (٢٢)

٥.٤ Investigation

٥.٤.١ Electrocardiography

The resting ECG is normal in about a quarter of patients with angina. In the remainder, abnormalities include old myocardial infarction (Q wave), nonspecific ST–T changes, atrioventricular or intraventricular conduction defects , and changes of left ventricular hypertrophy . During anginal

episode , the characteristic ECG changes is horizontal or down sloping ST segment depression that reverses after the ischemia disappear.

T-wave flattening or inversion may also occur. Less frequently, ST segment elevation is observed ; this finding suggests severe (transmural) ischemia and often occurs with coronary spasm (unstable angina) or (STEMI) . (٢٣)

٥.٤.٢ Exercise Electrocardiography

An exercise ECG is helpful in detecting the presence of CAD and in assessing prognosis . The test is generally safe , with MI and death occurring at a rate of less than ١ in ٢٥٠٠ tests . The principal role of the exercise ECG is to assess the risk of future cardiac events . (٢٤)

A – indication of Exercise ECG: (١)

Exercise ECG testing employed

١. to confirm the diagnosis of angina.
٢. to determine the severity of limitation of activity due to angina.
٣. to assess prognosis in patients with known CAD, including those recovering from MI , by detecting groups at higher or low risk.
٤. to evaluate responses to therapy.
٥. less successfully to screen asymptomatic population for silent CHD.

B – Contraindications to exercise ECG include: (١٧)

١. acute myocardial infarction within the previous ٢ days.
٢. serious arrhythmias.
٣. severe aortic stenosis.
٤. symptomatic heart failure.
٥. acute pulmonary embolism.
٦. acute myocarditis.
٧. acute pericarditis.
٨. acute aortic dissection.

٥.٤.٣ Echocardiography

Echocardiography can image the left ventricle and reveal segmented wall motion abnormalities, which may indicate ischemia or prior infarction. It is a convenient technique for assessing left ventricular function, which is an important indicator of prognosis and determinant of therapy. Echocardiography may be useful in identifying patients with MI in the emergency department. (٢٥)

Echocardiography is probably most useful in patients with left bundle branch block or abnormal ECG without ST-segment elevation whose symptoms are atypical and in whom the diagnosis is uncertain.(٢٦) An intravenous echocardiography contrast agent can enhance the accuracy of stress echocardiography. (٢٧)

٥.٤.٤ Radionuclide Angiography

This procedure images the left ventricle and measure its ejection fraction and wall motion .In coronary disease , resting abnormalities usually indicate stress induced ischemia.(٣) Perfusion imaging with both thallium and sestamibi in the emergency department has been reported to be both sensitive and specific in the evaluation of patients in whom the diagnosis is uncertain. (٢٨)

The radionuclide techniques can also identify the area of hypoperfusion and thereby identify the abnormal coronary artery. (٢٩)

Myocardial perfusion scintigraphy and positron emission tomography (PET) are another two nuclear medicine studies which provide additional information about the pressure, location and extent of CAD. (٣٠)

٥.٤.٥ Newer Imaging Modalities

A-Computed Tomography (CT):

CT scan can image the heart and, with contrast medium , the vascular system , but the relatively slow speed of most instruments limit its utility . Ultrafast or electron beam CT (EBCT) involves a specially designed instrument with high temporal resolution. It provides excellent assessment of cardiac structure and function . (٣٠)

B – Cardiac Magnetic Resonance Imaging (MRI) :

MRI is an evolving modality that provides high – resolution images of the heart and great vessels without radiation exposure or use of iodinated contrast media. It provides excellent anatomic definition, permitting assessment of pericardial disease, neoplastic disease of the heart, myocardial thickness, chamber size, and many congenital heart defects.(٣١)

٥.٦ Laboratory Findings:

٥.٦.١ Creatine kinase enzyme: any injury to myocardial cells results in the release of intracellular enzymes into circulating blood, permitting their detection by blood tests. Traditionally creatine kinase enzyme (CK) and an isoenzyme, CK-MB, found in high concentration in myocardial cells, have been used to diagnose myocardial infarction (MI) in its earliest stages . (٣٢)

٥.٦.٢ Myoglobin: is a low-molecular-weight hem protein found in cardiac muscle; its advantage for diagnosis is that it is released more rapidly from infarcted myocardium that is CK-MB it may be detectable within ١ to ٢ hours after the onset of acute MI. However myoglobin is also found in skeletal muscle, and the lack of specificity is a drawback .(٣٢)

٥.٦.٣ Troponins I and T : is a cardiac-derived troponin I (cTnI) and troponin T (cTnT), protein of the sarcomer, have amino acid sequences distinct from their skeletal muscle isoforms. Cardiac troponin I and T are not normally present in the blood; an increase in serum levels of troponin occurs early after myocardial cell injury. An elevated cardiac troponin level on admission is a predictor of subsequent cardiac events. The role of troponin assays in the evaluation of patients in the emergency department and elsewhere is likely to increase as a result of the proposed revision of the diagnostic criteria of myocardial infarction.(٣٣)

SECTION II / Plasma Fibrinogen

The fact that an acute myocardial infarction leads to hyperfibrinogenemia has been known since ١٩٥٠. (٣٤)

Fibrinogen is a blood protein that plays a critical role in normal and abnormal clot formation mechanism referred to as coagulation. A process of checks and balances, i.e an interaction between clotting factors and naturally occurring anticoagulants, normally results in healthy levels of fibrinogen and normal coagulation. If, however fibrinogen levels increase above normal, a blood clot becomes a threat; if fibrinogen level decrease below normal, a hemorrhage can result. Though the reference range used by most laboratories is ٢٠٠-٤٠٠ mg/dl, it is crucial to keep serum fibrinogen under ٣٠٠ mg/dl, a level considered safe.(٣٥)

Clinical significance:

١.Fibrinogen is the principal plasma protein affecting the sedimentation rate. Fibrinogen concentration rises several folds during inflammation or tissue necrosis .Estrogen ingestion, diabetes, obesity or pregnancy may also induce increased levels.

٢.Evidence has shown that plasma levels above the reference range constitute a significant independent risk factor for both coronary artery and cerebrovascular diseases.

٣.A decreased fibrinogen level in plasma is generally associated with a disturbance of liver metabolism (cirrhosis, ictrus…) or with fibrinolysis and DIC (disseminated intravascular coagulation) . (٣٦)

١. Biochemistry and Pathophysiology

Fibrinogen is a globular glycoprotein found in the plasma with molecular weight of ٣٤٠ KDa and is synthesized by the liver.(٣٧)

It comprises of three pairs of non-identical polypeptide chains (alpha, beta, and gamma chains), linked to each other by disulphide bonds. (٣٨)

Fibrinogen has a biological half-life of about ١٠٠ hr. As a clotting factor, fibrinogen is an essential component of blood coagulation system, being the precursor of fibrin, and the conversion occurs by cleaving of Arg-Gly peptide bonds of fibrinogen. (٣٩)

Fibrinogen plays a vital role in number of physiopatholgical processes in the body, including inflammation, atherogenesis and thrombogenesis. Proposed mechanisms include the infiltration of the vessel wall by fibrinogen, haemorrheological effects due to increasing in blood viscosity, increased platelet aggregation and thrombus formation. Furthermore, plasma fibrinogen is also a prominent acute-phase reactant; it augments the degranulation of platelets in response to adenosine diphosphate (ADP), when taken up by the granules. Thus, elevated concentration of fibrinogen, perhaps secondary to inflammation or infection (Chlamydia pneumoniae or helicobacter pylori) implicated in cardiovascular risk may operate, by increasing the reactivity of platelets.(٤٠)

Elevated plasma fibrinogen appears to suppress thrombin formation in plasma but also to enhance fibrin deposition in other organs. One explanation for this potential paradox is that thrombin, once formed, may be sequestered on fibrin (ogen) and is then either rapidly cleared from the circulation or becomes associated with fibrin deposited at the vascular wall. (٤١)

The mechanisms by which plasma fibrinogen mediate risk for CHD include :(٧)

١. increased plasma viscosity

٢. increased platelet aggregability .

٣. increased vascular smooth muscle proliferation .

٢. Fibrinogen and Inflammation

The process of inflammation is primarily mediated by its interaction with leucocytes through the surface receptors of the latter termed integrins. The two main receptors for fibrinogen on the surface of leukocytes include mac-١ (CD١١ b/CD١٨, alpha M beta٢) and alpha X beta ٢ (CD١١ C/CD ١٨, P١٥٠, ٩٥). Leukocytes (both monocytes and myeloceytes) can specifically induce mac-١ receptor to bind fibrinogen.(٤٢)

The ability of Mac-١ receptor to bind fibrinogen results from the maturational changes occurring in the receptor during the process of cell differentiation, and is not seen in resting leukocytes. The site on fibrinogen that interacts with Mac-١ is not shared by other integrins. (٤٣)

Fibrinogen is also aligned for intercellular adhesion molecule-١ (ICAM-١), and enhances monocyte-endothelial cell interaction by bridging the Mac-١ on monocytes to ICAM-١ on endothelial cells. (٤٤)

Thus ICAM-١ behaves as a cell surface ligand for alpha L beta ٢ and alpha M beta ٢ (MAC-١) integrins, and has a key role in leukocytes adhesion to the vascular endothelium. Furthermore, fibrinogen up regulates and increases the ICAM-١ proteins on the surface of endothelial cell, resulting in increasing adhesion of leukocytes cells on the surface of endothelial cells, even at high shear rates inflow condition. (٤٥) The fibrinogen binding to ICAM-١ on the endothelial cells also mediates the adhesion of platelets, hence the interaction of fibrinogen and cells expressing ICAM-١ is associated with cellular proliferation. (٤٦)

Fibrinogen, on binding to its integrin receptor on the surface of leucocytes also facilitates a chemotactic response, thus playing a vital role in the process of inflammation. (٤٧)

One of the proposed mechanisms by which fibrinogen includes pro-inflammatory changes in leukocytes includes an increase in the free intracellular calcium and increase expression of neutrophil activation markers. These processes result in an increase in phagocytosis, antibody-mediated leucocyte toxicity and delay in apoptosis. (٤٨)

Fibrinogen is also involved in the facilitation of both cell-cell interaction and the interaction of cell and extracellular matrix such as collagen. Thus, as explained above, fibrinogen is an important mediator of cell-cell interaction, adhesion and inflammation . (٤٩)

٣. Fibrinogen and Atherogenesis

Fibrinogen and its metabolites appears to cause endothelial damage and dysfunction by a number of mechanisms mentioned above. (٥٠)

Many human atherosclerotic lesions, showing evidence of fissure or ulceration, can contain a large amount of fibrin, which may either be in the form of mural thrombus, in layers within the fibrous cap, in the lipid- rich core, or diffusely distributed throughout the plaque. This phenomenon may be compounded by the decrease in arterial intimal fibrinolytic activity and plasminogen concentration observed in cardiovascular disease. (٥١)

It has been proposed that once in the arterial intima, fibrin stimulates cell proliferation by providing a scaffold along which cells migrate, and by binding fibronectin, which stimulates cell migration and adhesion.(٥٢)

Fibrin degradation products, which are present in the intima, may stimulate mitogenesis and collagen synthesis, attract leukocytes, and alter endothelial permeability and vascular tone. In the advanced plaque, fibrin itself may be involved in the tight binding of LDL and accumulation of lipid, resulting in the lipid core of atherosclerotic lesions. However, it cannot be overemphasized that

many of these observations are only associations, and a definite causal role for fibrinogen cannot be fully demonstrated. (٥٠)

٤. Fibrinogen and Thrombogenesis

Thrombogenesis is regulated by a fine balance between the coagulation and fibrinolytic pathways; subsequent to vessel wall trauma, tissue thromboplastin is released from the subendothelium. Tissue thromboplastin, in turn, triggers the extrinsic pathway of coagulation by activating factor VII to VIIa. Contact of blood with the foreign surface initiates the intrinsic pathway of coagulation, by activiting factor XII to XIIa, as well as platelets. Platelet aggregation, however, does not confer adequate stability, and therefore activation of the coagulation pathway is also necessary . (٥٣)

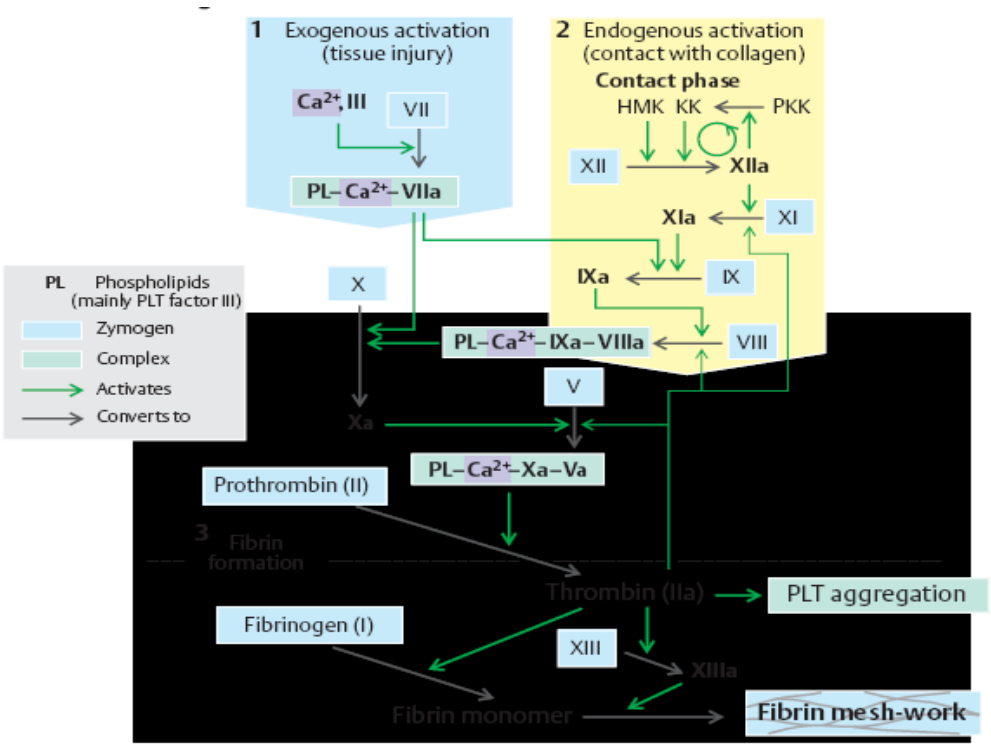


Fig. (١) The common pathway of the coagulation cascade . (٣٤)

The final common pathway of the coagulation cascade involves the activation of factor X to Xa, and the subsequent activation of prothrombin to thrombin, which is a protease enzyme, facilitates the cleavage of fibrinogen into fibrin monomers, which link, to each other, both sideways and end-to-end to form fibrin polymers. Activated factor XIII facilitates the cross linkage of fibrin polymers to form stable fibrin clot .Fibrinogen is also involved in the final common pathway of platelet aggregation. Fibrinogen cross-links the platelets by binding the glycoprotein IIb-IIIa receptor on the platelet surface.(٥٣)

٥. Factors That Effect Plasma Fibrinogen Levels

A-Genetic Influences

The evidence suggests that plasma fibrinogen levels are probably under genetic control, as polymorphisms account for some ٢٠-٥١% of variations in plasma fibrinogen levels.(٥٤)

The fibrinogen locus comprises three genes coding for fibrinogen gamma (FGG) fibrinogen alpha (FGA) and fibrinogen beta (FGB) clustered in a region of approximately ٥٠kb on the long arm of chromosome ٤ q٢٣ - q٣٢, the direction of transcription of the beta gene being in the opposite direction to that of the other two .(٥٥)

There is a single copy of each gene, the alpha gene in the midle flanked by the beta gene one side and gamma gene on the other. Variation in the fibrinogen locus contributes to the individual difference in the plasma fibrinogen levels. (٥٦)

However, the precise molecular mechanism (s) underlying the genetic heritability of plasma fibrinogen concentration remain unclear. Newer information stems from molecular biology, Polymorphism in the human fibrinogen gene with higher fibrinogen levels does not increase the risk for myocardial infarction (MI). (٥٧)

B. Extrinsic Influences :

١. ***Gender***: Most of prospective epidemiological studies showed that the plasma fibrinogen levels were higher in women than men, although it is well established that the incidence of cardiovascular disease among men is higher than that among women. Levels of HDL

cholesterol in women being higher than those in men may explain some, but not all, of sex differences in incidence of CHD.(٥٨)

٢. ***Age***: Plasma concentration of fibrinogen generally increases with age. (٥٩)

This age-related increase in plasma fibrinogen may be due to a slower rate of disposal of fibrinogen, rather than an increased production rate. (٦٠)

٣. ***Body Mass Index (BMI)*** : Plasma fibrinogen concentration has been positively correlated with BMI , the waist circumference, the hip circumference and waist- to-hip ratio in both sexes . (٦١)

Indeed plasma fibrinogen level is significantly higher amongst patients with a body mass index of > ٣٠ kg /$m^{٢}$, compared to those with BMI < ٢٥ kg/$m^{٢}$. (٦٢)

٤. ***Metabolic Syndrome***: Metabolic syndrome is characterized by the presence of ٣ or more of the following metabolic markers : HDL cholesterol < ١.١٣ mmol /١ , triglyceride > ١.٨ mmol /l , glucose > ٥.٥ mmol/l, diastolic blood pressure > ٩٠ mmHg. Plasma fibrinogen increases with a number of components of metabolic syndrome, independent of major confounders. (٦٣)

٥. ***Physical Exercise :-***

a- Acute Exercise: changes in the plasma fibrinogen levels have been reported after acute exercise in the healthy one. (٦٤)

Acute exercise may cause a rise in plasma fibrinogen levels in patients with some vascular disease for example, those with chronic AF or stable chronic heart failure, when, exercised to exhaustion, plasma fibrinogen level increased significantly within ٢٠ minutes .(٦٥)

b- Regular Exercise: Regular exercise over a span of few weeks or months has shown a reduction in plasma fibrinogen levels both in healthy and diseased individuals. (٦٦)

٦. ***Seasonal Differences***: Plasma fibrinogen levels show a seasonal variation, with peak in winter, both in normal healthy adults and in patients with cardiovascular disorders. (٦٧)

٧. ***Vitamin C and Infection***: It has been suggested that a lower dietary intake of vitamin C and /or an increase in upper respiratory infections in winter season might be the underlying cause for the raised levels of acute- phase reactants, especially fibrinogen . (٦٨)

٨. ***Hormonal Status***: Both cross- sectional and longitudinal studies demonstrate that oral contraceptive (OC) pills use results in a significant rise in plasma fibrinogen levels, especially those pills with high estrogen concentration. (٦٩)

Conversely, plasma fibrinogen level returns to normal on discontinuation of the OC pill, usually within about ٣ three months. (٧٠)

٩. ***Smoking***: Cigarette smoking is strongly associated with increased plasma fibrinogen levels, and the adverse cardiovascular effects of smoking may partly be mediated through an increase plasma fibrinogen levels. (٧١)

Smoking potentiates thrombosis at the dysfunctional endothelium, at least partly by increasing the concentration of plasma fibrinogen and altering the activity of platelets. All these proatherogenic effects of smoking to injure the endothelium are also observed, to lesser extent, in passive smokers.(٧٢)

١٠. ***Race***: Across-sectional epidemiological studies, showed fibrinogen levels to be ٠.٢ g/l higher in blacks than in whites. (٧٣)

١١. ***Alcohol***: Moderate drinking appears to lower plasma fibrinogen concentration. The so-called French paradox may be at least partly explained in relation to the effects of alcohol on clotting factors. This U- shaped association was stronger amongst men than women. Consumption of wine and spirits was also associated with changes in plasma fibrinogen levels (because it contain phenolic compounds), whereas consumption of beer or cider was not. In women, for example, ١g of alcohol per day induces a٠.٠٠٨ g/l decrease in the mean plasma fibrinogen, while in men the decrease was ٠.٠٠٤ g/l within down slope of the U-shaped curve. (٥٩)

The precise mechanism by which alcohol influences plasma fibrinogen levels remain uncertain. Animal experiments have suggested that alcohol exerts its effects through the action on the genetic expression of plasma fibrinogen in the liver cells. (٧٤)

٦. Fibrinogen : Cause or Effect?

The concentration of plasma fibrinogen positively correlated with the severity of the underlying coronary heart disease; in some studies plasma fibrinogen levels are higher in patients with unstable angina than in patients with stable angina ,and higher in patients with severe vasospastic angina than in those with mild vasospastic angina and stable effort angina.(٧٥)

Furthermore raised plasma fibrinogen levels have prognostic implications, being a strong predictor of CHD, fatal or non-fatal, new or recurrent , and of death from an unspecified cause , for both men and women, and therefore, a predictor of accelerated coronary atherosclerosis.(٧٦)

Furthermore, the beneficial effect of statin and fibrates in reducing coronary artery diseases events and mortality cannot entirely be explained by their beneficial effect on lipid. In addition to lipid lowering, the modification of thrombus formation and degradation, alteration in inflammatory response, plaque stabilization and improved endothelial function are thought to be responsible for additional reduction of morbidity and mortality due to cardiovascular events.(٧٧)

Although statins appear to improve thrombogenicity and endothelial dependent vasoresponsiveness, there is lack of convincing evidence of a reduction in plasma fibrinogen levels with statins, in contrast to the fibrates. For example, in the bezafibrate coronary atherosclerosis interventions trial (BECAIT), the beneficial effect of bezafibrate on coronary events in young male survivors of MI, was attributed partly to the reduction in plasma fibrinogen levels, in addition to the beneficial effect on plasma lipid profile. (٧٨)

Certainty, the positive association between plasma fibrinogen levels and cardiovascular events is as strong as that for elevated cholesterol levels .(٧٩).

Higher levels of plasma fibrinogen markedly increase the predictive power of high serum LDL cholesterol; conversely, low plasma fibrinogen levels are associated with low coronary risk, even when LDL is raised. (٨٠)

Interestingly, plasma fibrinogen levels are also raised in people with family history of premature heart disease. Therefore, modification of cardiovascular risk factors may result in beneficial reduction of plasma fibrinogen levels and better cardiovascular outcome. (٨١)

٧. Fibrinogen as an Acute- Phase Reactant

Plasma fibrinogen is an acute-phase protein, and is therefore likely to increase with inflammation or tissue necrosis. Interpretation of raised fibrinogen may be complicated by its behavior as an acute-phase reactant. For example, plasma fibrinogen concentration is raised after acute stroke and acute MI, probably as an acute phase response. Nevertheless, measurement of plasma fibrinogen levels could potentially be more useful than those of other acute phase reactants such as C-reactive protein, as fibrinogen is probably more specific to vascular disease. (٨٢)

٨. Genetic Variation in Plasma Fibrinogen

Genetic variation in the fibrinogen gene may have implications in prognosis of patients with vascular disorders. For example, the data from the Edinburgh Artery Study provide evidence that a polymorphism of plasma fibrinogen gene is associated with a varying risk of peripheral atherosclerosis. (٨٣)

٤٥٥ AA genotype was associated with over twice the risk of peripheral artery disease (PAD), compared with the ٤٥٥ GG genotype.(٨٤)

It is important to appreciate that although several studies demonstrate a strong association between polymorphisms of the fibrinogen Beta chain gene and plasma fibrinogen concentration, only few have found a direct association between the former and the risk of ischemic heart disease. A substantial number of studies failed to find an association between polymorphism in the fibrinogen gene and cardiovascular risk. (٨٥)

Section III / Serum Iron And Serum Ferritin

Iron is both an essential mineral nutrient and an environmental toxin. (٨٦)

Iron is carried in the plasma bound to the protein transferrin (mol mass ٨٠٠٠٠). This molecule binds two atoms of Fe^{3+} and delivers iron to cells by interaction with the membrane transferrin receptor .(٨٧)

Serum iron levels vary throughout the day, morning levels are greatly assumed to be higher than afternoon or evening levels. Iron overload is much less common than iron deficiency and its diagnosis is not usually difficult once the possibility has been considered. Increased plasma [iron] with normal [transferrin] or total iron binding capacity TIBC, often lead to ١٠٠٪ saturation of transferrin. (٨٨)

١.The Common Causes of Iron Overload: (٨٨)

A-Excessive Intake

- Over supplementation with iron tablets.
- Repeated blood transfusion.

B- Excessive Absorption

- Haemochromatosis (Hereditary HFe).

Iron is required for oxygen transport and cellular oxidative metabolism. However ,iron store rises to levels above physiological requirements with aging ,and such elevated levels have been implicated both mechanistically and epidemiologically in the pathogenesis of a number of common diseases of aging , particularly atherosclerosis .(٨٩)

٢.The Iron Hypothesis of the Risk of Cardiovascular Disease:

In ١٩٨١, Sullivan proposed that iron depletion protects against ischemic heart disease. He argued that the difference in the incidence of heart disease between men and women could be explained by differences in levels of stored iron . He argued in support of his theory that myocardial failure occurs in patients with iron storage disorders and that there is accumulation of stored iron with age in men and after menopause in women.(٩٠)

The risk of heart disease in women increased equally by natural menopause or by surgical menopause (independent of oopheroctomy) , rather than hormonal factors , might be responsible for the protection of premenopausal women against ischemic heart disease (IHD) .(٩١)

Although anemia is a significant risk factor in IHD and higher mortality rate.(٩٢)

In men, iron stores, assessed by serum ferritin concentration , rise after adolescence , in women , iron stores remain low and only begin to rise after the age of ٤٥ years. (٩٣)

The maximal sex difference in serum ferritin levels is reached at approximately ٤٥ years of age . the maximal sex difference in heart disease is also reached at approximately ٤٥ years .(٩٤)

Besides explaining the sex difference of Coronary heart disease , the iron hypothesis could also explain the low prevalence of CHD in areas with a high prevalence of iron deficiency .However , this iron deficiency may be caused by insufficient diet , which is less atherogenic . The iron hypothesis could also explain the protective effect of medication that causes gastrointestinal blood loss, e.g. aspirin, or inhibit iron absorption, e.g. cholestyramine, and the risk of an increasing effect of oral contraceptives, which are known to decrease menstrual blood loss. (٩٥)

٣. Dietary Iron Intake and CHD:

Genetic, pathological, and environmental factors may contribute to iron-related cardiovascular risk. For each milligram of iron consumed, there was an increase of ٥% in the risk of Coronary heart disease. (٩٦)

An increased risk of myocardial infarction among men with a higher intake of hem iron (red meat), which was positively correlated with total iron stores, may be noticed. However, they could not find an association between total iron intake and CHD. (٩٧)

While on other hand, many studies confirmed that no correlation between iron intake and CHD was found, e.g. (Liao y, cooper RS, Mc Gee DL.١٩٩٤. Iron status and coronary heart disease). (Morrison HI, etal.١٩٩٤. serum iron and risk of fatal acute myocardial infarction), (Rauramaa R, etal ١٩٩٤. Association of risk factors and body iron status to carotid atherosclerosis in middle-aged eastern Finnish men),

٤.Exercise, Iron Levels, and CHD:

Exercise is associated with reduced mortality from cardiovascular disease .The reduction of mortality cannot be explained solely by improvements in risk factors such as lipid profile or blood pressure. (٩٨)

The mechanisms by which physical exertion could decrease body iron stores include : exercise acidosis with early release of iron from transferrin and iron loss through sweating. All studies on the relationship between iron stores , exercise , and cardiovascular disease, however ,will be influenced by the widespread use of iron, vitamins supplements , and antioxidants by athletes .(٩٩)

٥. Mechanisms of Iron Induced Cardiovascular Damage:

The mechanism by which iron may stimulate atherogenesis is unclear. It is suggested that the catalytic role of iron in lipid peroxidation may be an important factor in the formation of atherosclerotic lesions. Normal native LDL can cross the arterial wall without causing damage to the vessel wall. Iron-catalyzed free radical reactions cause oxidation of LDL , which occurs in endothelial cells , smooth muscle cells , lymphocytes , or macrophages .(١٠٠)

Unlike native LDL , oxidized LDL is recognized by so-called scavenger receptors on tissue macrophages and causing rupture in the intima of the vessel wall followed by accumulation of lipids in these cells and the formation of foam cells , the characteristic cells of fatty-streak lesions of early atherosclerosis .(١٠١)

Oxidized LDL also has chemotactic capacity that provides recruitment of circulating monocytes to the vessel wall and inhibits macrophages from leaving the intima of the arterial wall. Thereby, oxidized LDL has cytotoxic capacity that induces changes in the endothelial cells with loss of endothelial integrity, which could facilitate further accumulation of both circulating monocytes and LDL and thus promote the progression of the atherosclerotic lesion. (١٠٢)

The explanation for the detrimental effects of iron rests in its redox cycling capacity, iron catalyzes formation of reactive oxygen species through the Fenton and Haber –Weiss reactions. (١٠٣) as follows :

Fenton reaction : $H_2O_2 + Fe^{+2} \rightarrow {}^{-}OH + {}^{\cdot}OH + Fe^{+3}$

Heber weiss reaction : $O_2^{\cdot-} + Fe^{+3} \rightarrow Fe^{+2} + O_2$

$H_2O_2 + Fe^{+2} \rightarrow Fe^{+3} + {}^{\cdot}OH + {}^{-}OH$

Oxidative damage to all kinds of molecule, e.g., in lipid peroxidation, is mainly caused by the highly toxic hydroxyl radical $OH^{\cdot}$ (١٠٤).

This excess iron is taken up by the iron storage protein, ferritin. Storage iron has no known physiological role but can be released readily from ferritin to generate oxygen free radicals that are highly toxic to biomolcules. Reactive oxygen species initiate lipid oxidation required for atherogenesis. (١٠٥)

Cells are protected against iron-induced oxidative damage by the production of ferritin, which can be modified into hemosedrin intracellularly. (١٠٦)

Under physiological conditions, these proteins, as well as transferrin and lactoferrin, protect against iron toxicity. To perform a role in the iron-catalyzed Haber-weiss reaction, iron in most instances must be released from these proteins and chelated to low- molecular – weight forms such as iron citrate or iron cumarate. Super oxide ($O_2^{\cdot}$) produced during oxidative stress can mobilize iron from ferritin, and hydrogen peroxide (H_2O_2) is capable of releasing iron from hem. (١٠٧, ١٠٨)

Hem derived from lysed red blood cells is taken up rapidly by endothelial cells and releases its iron, thus promoting oxidant damage.(٢٤)

Although transferrin does not release its iron at a normal pH, at a low pH, which may be the case in arterial walls, iron can be released from transferring and induce Oxidation of LDL. (١٠٩)

The induction of ferritin expression occurred in parallel with the progression of the lesions. Furthermore, Prussian blue stain showed the presence of iron deposits in advanced lesions but not in the early lesion of rabbit and human aortas.(١١٠)

The production of apo-ferritin within the macrophages and endothelial cells could be a protective mechanism against the damaging effects of free iron or oxidized LDL. (١١١)

Other studies showed that ferritin production in bovine pulmonary

artery endothelial cells is stimulated by aspirin in therapeutically relevant Concentrations. Besides the well-known inhibitory effects on platelet aggregation, enhanced ferritin production in endothelial cells, which makes them more resistant to oxidative damage, might also be a mechanism by which aspirin prevents endothelial damage in cardio-vascular disease . (١١٢)

In later stages of the atherosclerotic process, more iron-loaded ferritin is found in the lesions as well as in smooth muscle cells. In condition of oxidative stress, this ferritin could serve as a source of free catalytic iron.(١١٠)

Studies have shown that iron can stimulate lipid peroxidation *in vitro* and *in vivo*. (١١٣)

This peroxidation was inhibited by desferrioxamine, an antioxidant iron- chelating agent that, in contrast to pro-oxidant iron chelators, such as ethylene diaminetetraaceticacid (EDTA), does not promote a Fenton reaction or lipid peroxidation. (١١٤)

Another study demonstrated that the iron chelator deferiprone (L١) prevented the oxidation of LDL and diminished the cytotoxic capacity of LDL *in vitro* and vivo.(١١٥)

٦. Ischemia/ Reperfusion Damage in Coronary Artery Disease:

Atherosclerosis can lead to coronary artery disease, causing myocardial ischemia and infarction. Reperfusion damage, caused by restoration of aerobic metabolism after a period of ischemia, is dependent on the presence of free radicals. During reoxygenation, Oxygen free radicals are produced. By catalyzing the Haber-Weiss reaction, iron plays a role in the generation of oxygen free radicals. vidence suggests that iron promotes the damage that occurs during ischemia and reperfusion, even in the absences of iron overload.(١١٦)

An iron-supplemented diet increased the degree of oxidative injury in ischemic rat hearts. (١١٧)

Some, but not all, studies of the effect of chelator desferrioxamine in ischemic myocardial events in animals show a beneficial effect of pretreatment with the drug on reperfusion damage. (١١٨)

There is some evidence that iron is mobilized during organ ischemia, thus being available for catalyzing free-radical generation.(١١٩)

SECTION IV / Serum Uric Acid

١. Epidemiology

Many epidemiological studies reported over the past ٥٠ years have confirmed nearly consistently an association of elevated serum uric acid (SUA) level with cardiovascular events; although not all have found that the correlation is independent of other risk factors. Several theories have been advanced, including those that implicate elevated SUA as a causative factor (for cardiovascular events) for instance, by increasing platelet reactivity. (١٢٠)

Over recent years there has been renewed debate about the nature of the association between raised serum uric acid concentration and cardiovascular events (CVE). Several large studies have identified the value, in populations, of serum uric acid concentration in predicting the risk of cardiovascular events, such as MI. (١٢١)

It has been difficult to identify the specific role of elevated SUA because of its association with established cardiovascular risk factors such as hypertension, diabetes mellitus, hyperlipidaemia and obesity. Indeed, it is not even clear at this stage whether uric acid has a damaging or protective effect in these circumstances. Increased understanding of the mechanisms underlying these associations may allow a clearer interpretation of the importance of elevated SUA concentration, and the potential value of specific urate-lowering treatment on cardiovascular disease. (١٢٢)

٢. Uric Acid Synthesis:

Purines arise from metabolism of dietary and endogenous nucleic acids, and are degraded ultimately to uric acid , which is catalyzed by the enzyme xanthin oxidase, which is responsible for the production of uric acid and damaging free radicals .

This is a central link in the association between SUA and myocardial ischemia, myocardial dysfunction and non-cardial function which is determined primarily by impaired peripheral blood flow. (١٢٣)

Uric acid is a weak acid (pK_a ٥.٨), distributed throughout the extracellular fluid compartment as sodium urate, and cleared from plasma by glomerular filtration. (١٢٤)

Around ٩٠٪ of filtered uric acid is reabsorbed from the proximal renal tubules, while active secretion into the distal tubule by an ATPase-dependent mechanism contributes to overall clearance. (١٢٥)

The reference range of SUA is variable, according to geographical distribution, gender and dietary sources. For an individual, urate concentration is determined by a combination of the rate of Purine metabolism (both endogenous and the exogenous) and the efficiency of renal clearance. Purine metabolism is influenced by dietary, as well as genetic factors regulating cell turnover. Uric acid is sparingly soluble in aqueous media, and persistent exposure to high serum levels predisposes to urate crystal deposition within soft tissues. (١٢٦)

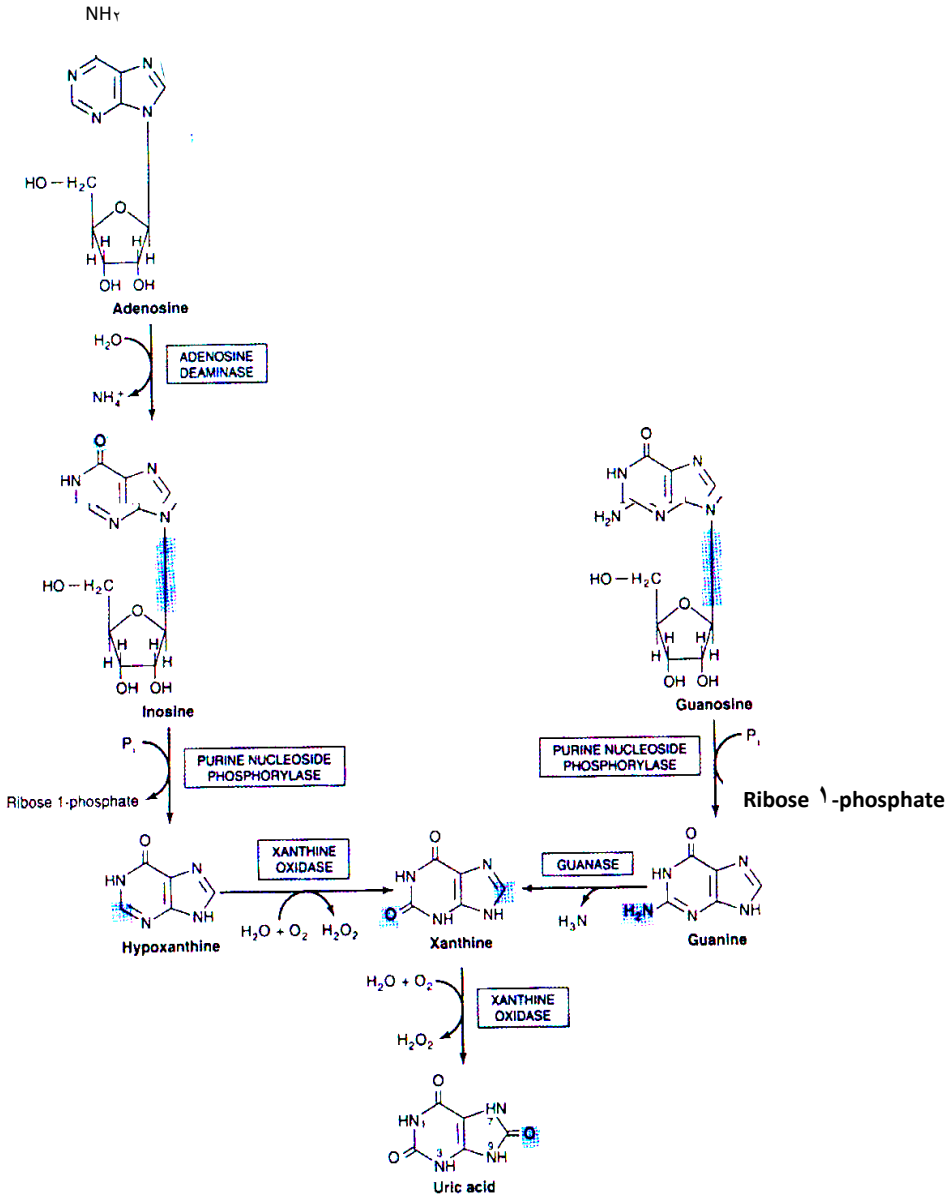


Figure (٢) Formation of uric acid from purine nucleosides by way of the purine bases hypoxanthine, xanthine, and guanine. (١٢٧)

In mammals other than higher primates, the enzyme uricase cleaves uric acid, forming highly water – soluble end products allantoin. However, since human lack uricase, the end product of purine catabolism in man is uric acid. (١٢٧)

In man, the urate oxidase gene located on chromosome ١ is not expressed due to two non – sense mutation. Loss of uric oxidase activity appears to have developed under evolutionary pressure. (١٢٨)

٣. Uric Acid as a Risk Factor for Cardiovascular Disease

An epidemiological link between SUA and an increased cardiovascular risk has been recognized for many years. (١٢٩)

Observational studies show that serum uric acid concentrations are higher in patients with established CHD compared with healthy controls.(١٣٠)

Elevated SUA concentrations are also found in healthy offspring of parents with coronary artery disease, indicating a possible causal relationship. However, hyperuricemia is also associated with possible confounding factors including elevated serum triglyceride and cholesterol concentrations, blood glucose, fasting and post-carbohydrate plasma insulin concentration, waist-hip ratio and body mass index. (١٣١)

About one quarter of hypertensive patients have co-existent hyperuricemia and interestingly, asymptomatic hyperuricemia predicts future development of hypertension, irrespective of renal function.(١٣٢)

Among patients with established hypertension, elevated serum uric acid concentration has been associated with a significantly increased cardio-vascular risk. (١٣٣)

Thiazide diuretics confer unequivocal benefits in treatment of hypertensive patients, and cause a significant reduction in cardio – vascular mortality. There is a significant relationship between SUA concentration and total cardio vascular mortality, which was independent of BMI, serum lipid concentration, smoking habit, blood pressure and age. (١٣٤)

Recent evidence suggests that uric acid may be an important causal agent in cardiovascular disease, for example, by inducing renal disease and hence hypertension. (١٣٥)

In contrast to these findings, several studies have suggested that the relationship between elevated SUA and cardiovascular risk does not persist after correcting for other risk factors. (١٣٦)

The epidemiological evidence suggests that SUA is an independent predictor of cerebro-vascular disease in subjects with hypertension and established vascular disease but not in healthy subjects. This evidence suggests that the influence of SUA on CHD is explained by the secondary association of SUA with other established etiological risk factors. There is no evidence so far to indicate that lowering SUA levels with drug treatment has a beneficial effect on CVD outcome. (١٣٧)

٤. Direct Impact of Uric Acid on Vascular Function:

The endothelium plays a central role in maintaining vascular tone through synthesis and release of nitric oxide (NO), a potent vasodilator.(١٣٨)

Reduction of NO bioavailability is an important early step in the development of atherosclerosis. (١٣٩)

The so-called endothelial dysfunction, associated with impaired endothelium-dependent vasodilatation, may arise from excessive free radical activity, which disrupts synthesis and accelerates degradation of nitric oxide. (١٤٠)

Thus increased oxidative stress appears to have an important role in the development and progression of atherosclerosis and is characteristic finding associated with its major risk factors, such as diabetes mellitus, hypertension, hypercholesterolemia and smoking. (١٤١)

Serum uric acid possesses antioxidant properties, and contributes about ٦٠٪ of free radical scavenging activity in human serum, uric acid interacts with peroxynitrite to form a stable nitric oxide donar, thus promoting vasodilatation and reducing the potential for peroxynitrite-induced oxidative damage. (١٤٢)

Thus, uric acid could be expected to protect against oxidative stresses. However, uric acid has been found to promote low – density lipoprotein (LDL) oxidation in vitro, a key step in the progression of atherosclerosis, and these effects are inhibited by vitamin C indicating an important interaction between aqueous anti – oxidants. (١٤٣, ١٤٤)

Uric acid can also stimulate granulocytes adherence to the endothium, and peroxide and superoxide free radical liberation. (١٤٥, ١٤٦)

Uric acid traverses dysfunctional endothelial cells and accumulates as crystal within atherosclerotic plaques. These crystals may contribute to local inflammation and plaque progression (hidden danger of SUA) , and the crystal accumulation may be greater in patients with elevated SUA.(١٤٧)

Thus, while uric acid appears to make a significant contribution to serum anti – oxidant capacity, it could also lead directly or indirectly to vascular inquiry. It is interesting to note that treatment of chronic heart failure patients with allopurinol (Xanthine oxidase inhibitor) restore endothelial function , this effect may have been due to an increase in recorded serum antioxidant capacity . (١٤٨)

٥. Uric Acid as a Marker of Subclinical Ischemia:

Adenosen is synthesized and released by cardiac and vascular myocytes. Binding to specific adenosine receptors causes relaxation of vascular smooth muscle and arteriolar vasodilatation. (١٤٩)

Adenosine makes a small contribution to normal resting vascular tone , since competitive antagonism at the adenosine receptor by methyl-xanthines , such as theophylline, reduces blood flow response to ischemia in the forearm vascular bed . (١٥٠)

Under conditions of hypoxia and tissue ischemia, vascular adenosine synthesis and release are up regulated, causing significantly increased circulating concentrations. (١٥١)

Cardiac and visceral ischemia promotes generation of adenosine, which may serve as an important regulatory mechanism for restoring blood flow and limiting the ischemia. (١٥٢)

Adenosine synthesized locally by vascular smooth muscle in cardiac tissue is rapidly degraded by endothelium to uric acid, which undergoes rapid efflux to the vascular lumen due to low intracellular pH and negative membrane potential. (١٥٣)

Xanthine oxidase activity and uric acid synthesis are increased *in vivo* under ischemic conditions, and therefore elevated SUA may act as a marker of underlying tissue ischemia . (١٥٤).

In the human coronary circulation, hypoxia , caused by transient coronary artery occlusion, leads to an increase in the local circulating concentration of uric acid. In conclusion therefore, elevated SUA may be a marker of local or systemic tissue ischemia and provides one possible explanation for a non-causal associative link between hyperuricemia and cardiovascular disease. (١٥٥)

Aims of the Study

The Research Main Guidelines Were Directed:

١. Detect the reference plasma fibrinogen, serum ferritin, serum iron and serum uric acid concentrations in patients with coronary heart disease.
٢. Make a comparison between the results of serum and plasma concentration of each parameter in acute and chronic coronary heart disease groups and clarify the correlation between them.
٣. Study the changes in level of each parameter mentioned above to find the possibility of using each of them as a predictor or a marker of clinical myocardial ischemia (coronary heart disease).

CHAPTER ٢

Section I/ Materials

١. Patients

٨٥ Patients with coronary heart disease were included in this study. None of those patients had a history of chronic disease or serious illnesses like diabetes mellitus or hypertension, in addition to that they are without a risk factor "Conventional" which include smoking, obesity, alcoholism and family history.

The project govern the period from ١٥th of December ٢٠٠٥ till ١st of August ٢٠٠٦. These patients were classified into ٢ groups.

Group I : Acute ischemic heart disease (IHD) consisting of ٥٥ patients (٣٨ male and ١٧ female of average age of ٤٥ year) were introduced at Marjan Teaching Hospital in cardiac care unit (CCU). All samples had been taken within ١st twelve hours of admission.

Group II : Chronic ischemic heart disease (IHD), a group of which consisted of ٣٠ patients (٢٤ male and ٦ female of mean age of ٤٧ year) were admitted to cardiac care unit in Marjan Teaching Hospital.

All patients were proved to have coronary heart disease as diagnosed by history, clinical examination, ECC finding and laboratory investigations.

All patients underwent full history and physical examination including: age, sex, residence, smoking, positive family history of IHD, general examination and cardiac examination.

All patients underwent blood pressure checking, ECG, serum uric acid, plasma fibrinogen , serum ferritin and serum iron .

The Control Group: were ٥٠ patients (٤٤ male and ٦ female) their age range was ٣٧ – ٦٥ years with an average age of ٤٤ year; they were chosen as healthy people i.e. non- smoker, negative family history of coronary artery disease (CAD), not obese and they are not alcoholic.

٢- Chemicals

Table (٥): chemical compounds used in the research

No	*Chemical compound*	*Production*
١-	ACID EURIQUE ENZYMATIQUE Pap ١٥٠ (Au PAP ١٥٠) kit . Enzymatic determination of uric acid in human urine , serum and plasma .	BIOMERIEUX Expiry ١٠ . ٢٠٠٦ Made in France .
٢-	BIO – FIBRI KIT Chronometric determination of fibrinogen reagent for quantitative determination of P. fibrinogen in human plasma.	BIOLABO – SA , ٠٢١٦٠ Maizy , France . Expiry ٧-٢٠٠٧
٣-	HUMAN FERRITIN ENZYME IMMUNOASSAY TEST KIT Catalog number : BC – ١٠٢٥	BIO CHEEK, INC . ٣٢٣ vintage park Dr. Foster city , CA ٩٤٤٠٤

٤-	Iron Ferrozine KIT Reagents for measurement of serum iron .	Cromatest LINEAR CHEMICALS , S.L. Barcelona , spain LOT ١١٦٨xx Expiry. ٢٠٠٧ – ١١
٥-	Sodium citrate	BDH Analar BH ١٥ ITD , ٣٠١٢٨٥D , made in England

٣- Instruments and Materials

Table (٦) : instruments used in the research

No	*Apparatus Or Material*	*Production*
١-	Water Bath	MEMMERT ١٦٩٨ – ٩٩٦ Made In Germany .
٢-	Spectrophotometer (Digital Ultraviolet And Visible)	Spectronic CECIL ٧٢٠٠ ١٧٣ – ٣٥٤ Made In England
٣-	Centrifuge	KOKUSAN ٧١٣٦٢ Made In Japan
٤-	Incubator	FISHER ٥٥ No ٥٧٦ Made In England
٥-	Shaker Vortex	STUART ١٠٠٨٣٣ Made In UK
٦-	Micropipettes Automatic (٠.٢ – ١) ml	SLAMED Made In Germany
٧-	Stop Watch	Made In China

٨-	PCV Centrifuge	KARL KOLB ٧٠١٧٤٠ Netherlands
٩-	Timer Alarm	Made In Korea
١٠-	Bioelisa Reader ELX ٨٠٠	Made In England

Section II / Methods

١. Determination of Plasma Fibrinogen Concentration

Principle:

The methods are based on Von Clauss and al studies, validated by Destaing F. and al . When an excess of thrombin is present, the pre – diluted plasma clotting time is in reverse order proportional to the fibrinogen concentration in the specimen .(١٥٦)

Reagents and Composition

Vial R١ : Thrombin

Freeze-dried calcium thrombin from animal origin ; this reagent contains a slight quantity of kaolin which optimizes the optical detection .

Vial R٢ : Diluting Buffer (to use with plasmas) PH ٧.٣٥

Sodium barbital buffer

Anticoagulant (citrate)

Fibrinolysis inhibitor

Reagent Preparation

* Thrombin

use a non – sharp instrument to remove aluminum cap from the vial .

Transfer the amount of distilled water stated on the label into vial.

* Diluting buffer (ready for use)

Specimen Collection and Handling

Careful Vein Puncture (plasma)

* Blood anticoagulant ratio: ٤.٥ ml of blood for ٠.٥ ml of sodium citrate ٠.١٣ M.

* Avoid blood drawing with a syringe that could result in the formation of micro – clots.

* Centrifuge for ١٠ minutes at ٢٥٠٠ g.

The assay may also be performed on capillary blood micro-collection if condition of collection and determination are respected .

Procedure

Dilute plasma ١/١٠ in diluting buffer (vial R٢) . This dilution corresponding to fibrinogen concentration in the test tubes between ٢٠٠ – ٤٠٠ mg / dl which is the optimal range for measurements .

" Hook " Procedure

Prewar the thrombin in a water bath at ٣٧C° , then dispense in test tubes as follows . Homogenize regularly during use . (١٥٧)

Diluted plasma	٠.٢ ml
Incubate for ٢minutes at ٣٧ C°	
Thrombin	٠.٢ ml
Simultaneously start a timer and record the clotting time	

Calculation

According to the Table Enclosed With Kit :

- Normal plasma , dilution ١/١٠ : values are obtained thanks to the table enclosed with kit .
- Abnormal plasma , requiring a different dilution than ١/١٠ : take into account the dilution factor to calculate the result .

Example : dilution ١/٢٠ , multiply by ٢ the value read on the table .

Dilution ١/٥ , divided by ٢ the value read on the table .

٢. Determination of Serum Uric Acid

Principle :

Determination of serum uric acid using the uricase – peroxidase – chromogen sequence :

$$Uric\,acid + 2H_2O + O_2 \xrightarrow{Uricase} allantoin + CO_2 + H_2O_2$$

The hydrogen peroxide formed is tittered following a Trender type reaction :

$$H_2O_2 + 3.5\ \text{dichloro 2- hydroxylbenzene Sulfonic acid} + 4 - a\ \text{min}o\ antipyrine \xrightarrow{Peroxidase} quinonei\ \text{min}e + Hcl + 2H_2O$$

The intensity of the measured coloration (quinoneimine) is proportional to the quantity of uric acid in the sample . (١٥٨)

Reagent ١ Standard ١ × ٨ ml (liquid)	R١	Uric acid
Reagent ٢ chromogen ٢ × ٩٠ ml (liquid)	R٢	Tris buffer PH ٨.٠ ٣.٥ dichloro – ٢ – hydroxybenzene sulfonic acid (DCHBS) surface – active agent
Reagent ٣ (reconstituted with R٢) Enzymes ٦ × ٢٥ ml (Lyophilized)	R٣	Uricase peroxidase Ascorbate oxidase ٤ – aminoantipyrine Potassium ferrocyanide

Storage Conditions

- Store the kit at ٢ – ٨ C°

- Specimen collection: Serum or plasma collected in tube with sodium heparinate .

Instruction for Use – Manual Method

Stability in the original vial , in dark

- ١ month at ٢ – ٨ C°
- ٦ days at ٢٠ – ٢٥C°

Procedure

Instructions without deduction of sample blank

Wave length ٥٢٠ nm (٥١٠ – ٥٣٠ nm) .

Zero adjustment reagent blank .

	Reagent blank	Standard	Sample
Distilled water	٢٠ µl	-	-
Standard	-	٢٠ µl	-
Sample	-	-	٢٠ µl
Reconstituted reagent ٣	١ml	١ml	١ml

Mix .

Perform photometry after incubation for :

- ١٥ minutes at ٣٧ C° .
- ١٠ minutes at ٢٠ - ٢٥ C° .

Calculation

Without deduction of sample blank

Concentration of sample = (***A*** sample /***A*** standard) x n

n = standard concentration

٣. Enzyme Immunoassay for the Quantitative Determination of Human Ferritin Concentration in Human Serum .

Principle of the Test

The ferritin Quantitative test is based on a solid phase enzyme - linked immunosorbent assay (ELISA) ; the assay system utilizes one rabbit anti – ferritin antibody for solid phase (micro titer wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody – enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with antibodies , resulting in the ferritin molecules being sandwiched between the solid phase and enzyme – linked antibodies .

After a ٤٥ minutes incubation at room temperature , the wells are washed with water to remove unbound – labeled antibodies . A solution of TMB reagent is added and incubated at room temperature for ٢٠ minutes , resulting in the development of a blue color .

The color development is stopped with the addition of stop solution and the color is changed to yellow and measured spectraphotometrically at ٤٥٠ nm . The concentration of ferritin is directly proportional to the color intensity of the test sample. (١٥٩)

Materials and Components

- Antibody coated microtiter plate with ٩٦ wells .
- Enzyme conjugate reagent , ١٣ ml .

- Ferritin reference standards , containing ٠ , ١٥ , ٨٠ , ٢٥٠ , ٥٠٠ and ١٠٠٠ ng / ml , (NIBSC – WHO ٨٠ / ٦٠٢ , human liver standard) liquid , ready to use .
- TMB reagent (one – step) , ١١ ml .
- Stop solution (١N HCl) , ١١ ml .

Specimen Collection and Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques . This kit is for use with serum samples without additives only .

Reagent Preparation

All reagents should be allowed to reach room temperature (١٨ – ٢٥C˚) before use .

Assay Procedure (١٦٠)

١. secure the desired number of coated wells in the holder .
٢. dispense ٢٠ µL of standard , specimens , and controls into appropriate wells.
٣. Dispense ١٠٠ µL of Enzyme conjugate reagent into each well .
٤. Gently mix for ٣٠ seconds ; it is very important to have a complete mixing in this setup .

٥. Incubate at room temperature for ٤٥ minutes .

٦. Remove the incubation mixture by flicking plate contents into sink.

٧. Rinse and flick the microtiter wells ٥ times with distilled or deionized water.

٨. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets .

٩. Dispense ١٠٠ μL of TMB reagent into each well . Gently mix for ١٠ seconds.

١٠. Incubate at room temperature in the dark for ٢٠ minutes .

١١. Stop the reaction by adding ١٠٠ μL of stop solution to each well .

١٢. Gently mix for ٣٠ seconds . It is important to make sure that all the blue color change to yellow color completely .

١٣. Read the optical density at ٤٥٠ nm with a microtiter plate reader within ١٥ minutes .

Calculation of Results

١. Calculate the average absorbance value (٤٥٠ nm) for each set of reference standards , control , and samples .

٢. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper , with absorbance values on the vertical or Y – axis and concentration of ferritin on the horizontal or X – axis .

٣. Using the mean absorbance value for each sample, determine the corresponding concentration of ferritin in ng/ml from the standard curve.

٤. Determination of Serum Iron.

- **Principle:** The iron dissociated from transferring-iron complex by a solution of guanidine acetate and reduced by ascorbic acid reacts with ferrozine to give a pink complex .
- **Sample :** Serum or heparinzed plasma free of hemolysis .

Reagents

Reagent ١	Guanidine , Hcl Acetate buffer PH٥	٤.٥ µmol / L
Reagent ٢	Ascorbic acid	
Reagent ٣	Ferrozine	٤٠ µmol / L
Reagent ٤	Standard	١٠٠ mg / dl – ١mg / L ١٧.٩ µmol / L

Preparation

Reagent A : Add one measure (٢٥٠ mg) of reagent ٢ at ٥٠ ml of reagent ١ .

Reagent B : (chromogen buffer) Mix ١ volume (ex: ٤٠ ml) of reagent ٣ with ٢٥ volumes (ex : ١ml) of reagent A .

Measurement :

Against reagent blank for standard and sample .

Against reagent A for sample blank .

The solutions must be brought at ٢٠ – ٢٥ C˚ before use .

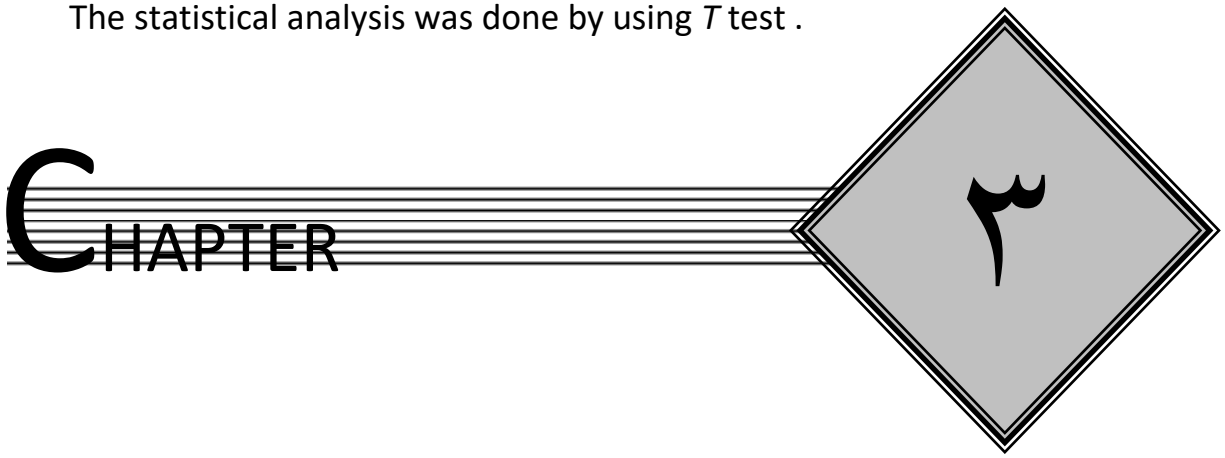
	Reagent blank	Standard	Sample Blank	Sample
Distilled water	٢٠٠ µL	-	-	-
Standard R٤	-	٢٠٠ µL	-	-
Sample	-	-	٢٠٠ µL	٢٠٠ µL
Reagent A	-	-	١ml	-
Reagent B	١ml	١ml	-	١ml
Mix and let stand for ١٠ minutes at +٢٠-٢٥ C°, then read the absorbance, the color of reaction is stable for at least ٣٠ minutes.				

Calculation

Iron = (OD sample – OD sample bank /OD standard) × C_{std}

C_{std} = ١٠٠ mg/dl (١ mg/L - ١٧.٩ µmol/L)

The statistical analysis was done by using *T* test .



Results and Discussion

Plasma fibrinogen Concentrations [mg / dl] , Serum ferrition Concentration [ng / ml] , serum iron Concentrations [µmol /L] and serum uric acid Concentrations [mg /dl] in sera and plasma of acute coronary heart disease , chronic coronary artery disease patients and healthy controls were measured from ١٣٥ samples being collected so hard, because the patients , the study dealt with, had been selected without risk factors . The following results were obtained:

Group A :

We have ٥٥ cases with acute coronary disease, with a mean age of ٤٥ years. ٣٨ of the cases are males and ١٧ cases are females.

١. Plasma fibrinogen: ٣٨ cases result in high level of plasma fibrinogen i.e. ٦٩ % result in a positive test for PF while ١٧ cases show a negative test for plasma fibrinogen i.e. ٣١٪ result in a normal level of plasma fibrinogen.

٢. Serum ferritin: ١٠ cases result in high level of serum ferritin i.e. ١٨ % result in a positive test while ٥ cases result in a reduced level of serum ferritin i.e. ٩٪ of this group while the majority of this group ٤٠ cases show a negative test i.e. ٧٢ % result in a normal level of serum ferritin.

٣. Serum iron: ٧ cases result in a high level of serum iron i.e. ١٢% result in a positive test while ٥ cases result in a reduced level of serum iron i.e. ٩٪ of this group. ٤٣ cases of acute coronary heart disease group result in a normal level of serum iron i.e. ٧٨ % of the patients.

٤. Serum uric acid:١٥ cases show an elevated serum uric acid level i.e. ٢٧ % of patients result in a positive test while ٤٠ cases show a negative test i.e. ٧٢٪ result in a normal level of serum uric acid.

Group B:

We have ٣٠ patients with chronic coronary artery disease, with a mean age of ٤٧ years. ٢٤ cases are males and ٦ cases are females:

١. Plasma fibrinogen: ٦ cases of this group result in a high level of plasma fibrinogen i.e. ٢٠% of patients result in a positive test while ٢٤ cases of this group show a negative test i.e. ٨٠٪ of patient result in a normal level of plasma fibrinogen.

٢. Serum ferritin: ٣ cases result in a high level of serum fertitin i.e., ١٠ % of patients show a positive test while ٤ cases of this group result in a reduced level of serum ferritin i.e. ١٣٪ of patients. The remainder of patients of this group show a negative test for serum ferritin i.e. ٧٦٪ result in a normal level of serum ferritin.

٣. Serum iron: ٤ cases of chronic coronary heart disease group show elevated serum iron i.e. ١٣ % of this group result in a positive test while only one case results in a reduced level of

serum iron i.e.٣٪ of the patients. ٢٥ cases show a negative test result i.e. ٨٣ % result in a normal level of Serum iron.

٤. Serum uric acid: All the patients (٣٠ cases) show a negative test result i.e. ١٠٠ % of result in a normal level of Serum uric acid.

Group C:

We have ٥٠ healthy control cases, with a mean age of ٤٤ years. For all ٥٠ cases studied parameter results in a normal level of plasma fibrinogen, serum ferritin, serum iron and serum uric acid i.e. ١٠٠ % of this group results in a negative test for all parameters.

٣.١ Plasma Fibrinogen in Coronary Heart Diseases Patients:

The means (± S D) of Plasma Fibrinogen levels in male patients with acute Coronary heart diseases were:

٤٧٠.٥٦ (± ١٠٠.٦٥) in acute MI patients.

٤٥٠.٢٣ (± ١٣٨.٦) in acute angina patients.

While in female patients were:

٤٦١.٥٧ (± ٥٣.٨) in acute MI patients.

٤٢١ (± ٧٢.٦) in acute Angina.

The unit of measurement used in the study is mg / dl

Expected Values :

Adult ٢٠٠ – ٤٠٠ mg/dL

A gainst:

٣٠٢ (± ٤١.٥) in male control.

٣٠٩.٨ (± ٧٦.٢) in female control.

The levels of plasma fibrinogen in patients with acute (CHD) group had shown an increased level in comparison to that of the control group and it revealed a significant difference in relation to plasma fibrinogen levels in control group (P< ٠.٠٠٠٧ in male), (P < ٠.٠٠١ in female) .

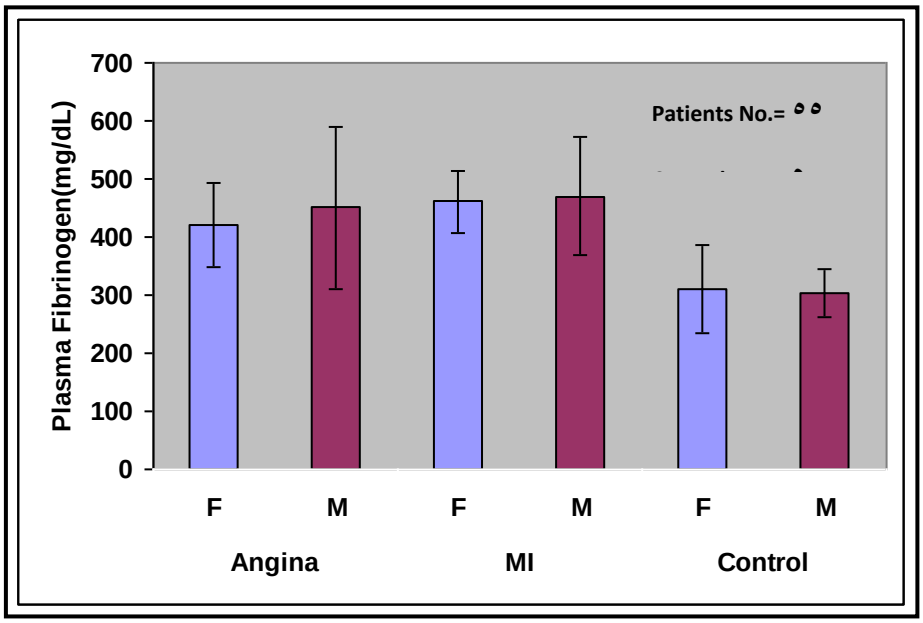


Fig [٣] The means of plasma fibrinogen levels in the Acute coronary heart disease groups.

On other hand the means (±SD) of plasma fibrinogen levels in patients with chronic (CHD) were:

٣٥٣.٦٧ (± ٥٤.١٣) in male patients with MI.

٣٧٩.١٥ (± ٦٦.١١) in male patients with angina.

While the means of plasma fibrinogen levels in female patients were:

٣٩٢.٤٦ (± ٦١.٧٤) chronic MI.

٣٦٦.٩٥ (± ٥٥.٢٨) chronic Angina.

The results showed no difference in mean level of plasma fibrinogen of patients with chronic (CHD) and control group (P> ٠.٠٥),

except that male patients with chronic angina it revealed a significant diffenence with plasma fibrinogen level of control group (P = ٠.٠٠٦).

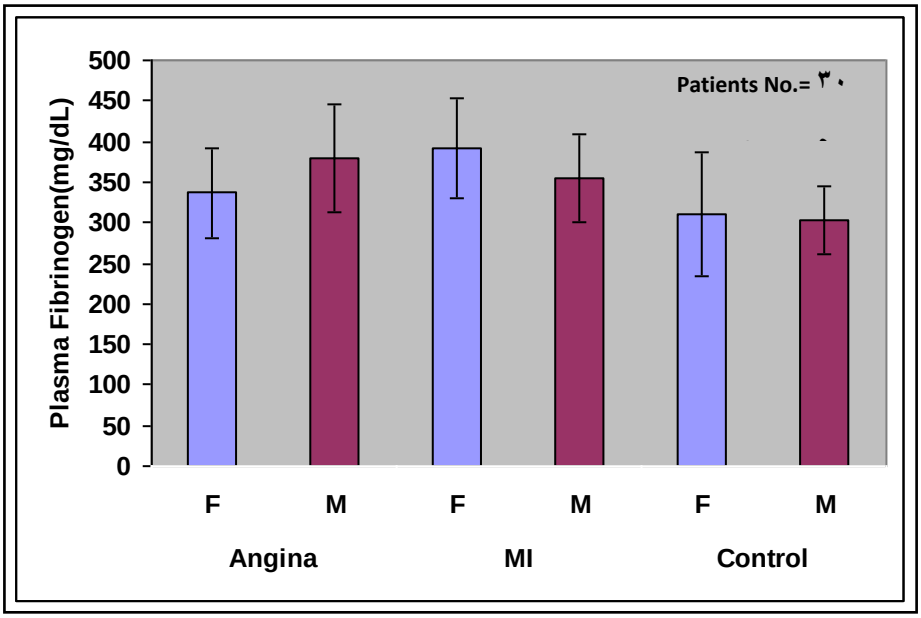


Fig. [٤] The means of plasma fibrinogen levels in the chronic coronary heart disease group and in the control group.

The difference between the mean of plasma fibrinogen level of patients with acute (CHD) and control group can be explained by its behavior (fibrinogen) as an acute phase reactant which is increased after inflammation, tissue necrosis and any condition of Oxidative stress. (٨٣)

Table (١): the mean of plasma fibrinogen in patients with coronary heart disease in contrast to control group.

Group	***No. of cases***	***Mean P. F. (mg / dl)***	***Lower value***	***Higher value***	***S.D. (mg / dl)***	***P. value***
Acute (CHD) group	٥٥	٤٥٠	٢٦٠	٦٩٢.٢	± ١١٩.٦	٠.٠٠٢
Chronic (CHD) group	٣٠	٣٦٥.٥	٢٧٠	٤٨٧	± ٦٠.١	٠.١٦
Control group	٥٠	٣٠٦.٣	٢٠١.٢	٤٤٣.٨	± ٥٨.٨	

Also this increment in plasma fibrinogen levels in male patients with (CHD) can be related to the seasonal variation with peak level in winter season, and also genetic expression of fibrinogen gene and its variation "polymorphism" might be associated in interpretation of this increment of plasma fibrinogen level in male patients with chronic (CHD). (٧٦, ٨٥)

On the other hand, fibrinogen as an inflammation mediator by its inter- action with leukocytes through the surface receptors of the latter termed integrin and its contribution in cell-cell interaction (leukocytes- endothelial cell, platelet-endothelial cell), thus fibrinogen plays its role in atherogenesis and thrombogenesis mechanisms and consequent or resultant cardiovascular events ; fibrinogen can be regarded as a causal agent .(٥١, ٥٤).

٣.٢ Serum Ferritin in Coronary Heart Disease

The means (± SD) of serum ferritin levels in male patients with acute coronary heart disease were:

١٣٦.٦ (± ٦٣. ٣) in acute MI male patients.

١١٠.٦(± ٥٤.٦) in male acute angina male patients.

The unit of measurement used in the study is ng /ml

Expected value :

Male ٢٠ - ٢٠٠ ng/mL

Female ١٥ - ١٥٠ ng/mL

While in female patients the results were:

١١٢.٤ (± ٥٢.٢) in acute MI.

١٤٣ (± ٧٩.٣) in acute Angina.

Against:

٤٥.٣ (± ٢٣.٩) in male control.

٥٣.٩ (± ٢٥.٥) in female control.

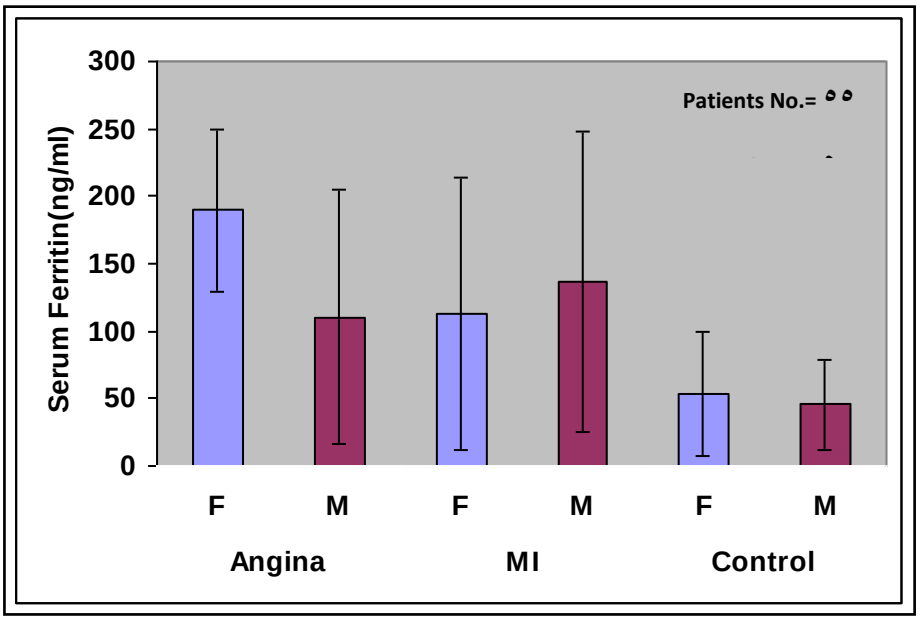


Fig. [٥] The means of serum ferritin levels in the acute coronary heart disease group and in the control group.

Although the result of serum ferritin in patient with acute (CHD) demonstrate in a high level in comparison to that of the control group , but no significant differences had been found in female group

(P > ٠.٠٥) while the mean of serum ferritin concentration in male patients were significantly higher than that of control group (P < ٠.٠٥) .

The means (±SD) of serum ferritin in patients with

chronic (CHD) were:

٨٠.٣ (± ٣٥.٧) in male patients with MI.

٤١.٨ (± ٢٦.٢) in male patients with angina.

While in female patients were:

١٩.١٥ (± ٧.٥) in female with MI.

٥٢.٣ (± ٢٤.٥) in female with Angina.

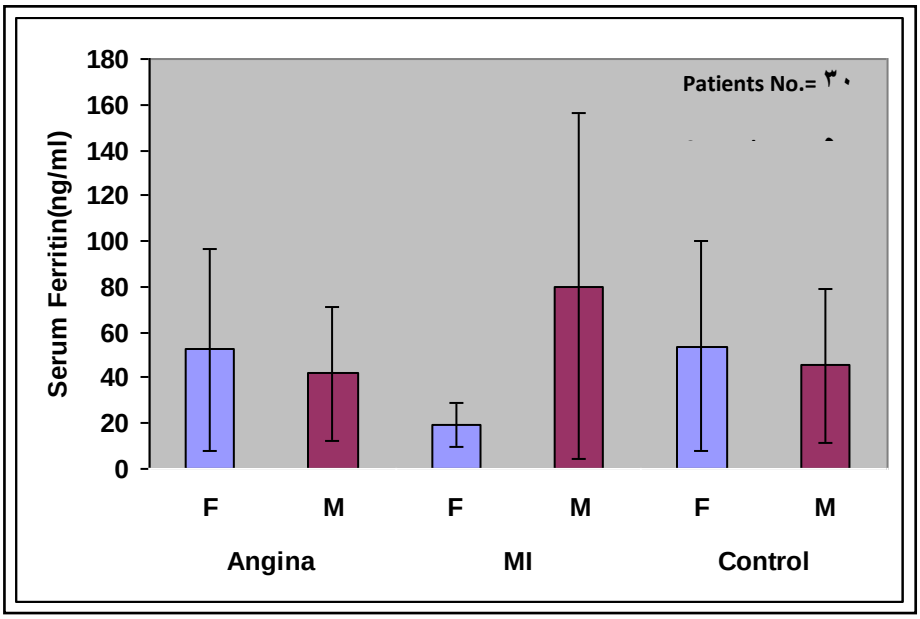


Fig. [٦] The means of serum ferritin levels in the chronic coronary heart disease group and in the control group.

In all patient groups the mean levels of serum ferritin were clearly lower than that of the control subjects , but there has been no significant difference in P value (p > ٠.٠٥) .

Although, male patients with chronic (MI) showed high serum ferritin levels.

Table (٨) : The mean of serum ferritin in patients with coronary heart disease in contrast to control group.

Group	*No. of cases*	*Mean S.F. (ng/ml)*	*Lower value*	*Higher value*	*S.D. (ng / ml)*	*P. value*
Acute (CHD) group	٥٥	١٢٦.٦	٧.٧	٦٥٠	± ٥٨.٩	٠.٠٠٢
Chronic (CHD) group	٣٠	٤٨.٣	١١.٢	٥١٥	± ٣٠.٩	٠.٢
Control group	٥٠	٤٩.٢	١٠.٨	١٣٨.٦	± ٢٤.٧	

٣.٣ Serum Iron in Coronary Heart Disease:

The means (± SD) of serum Iron level in acute coronary heart disease group in male patients were:

٣٣ (± ١٩.٥) in those with MI .

٢٥.٧ (± ١١.٣) in those with angina.

The unit of measurement used in the study is µmol /L

Expected value :

Men ١١.٥ – ٢٨.٣ µmol /L

Women ١٠.٧ – ٢٦ µmol /L

While the means (± SD) of serum Iron in female patients were:

٣١.٨ (± ١٤.١) in those with MI .

٢٩.٤ (± ١٢.٣) in those with angina.

Against:

١٨.٥ (± ٥.٩) in male control subjects.

١٩.٢ (± ٧.٤) in female control subjects.

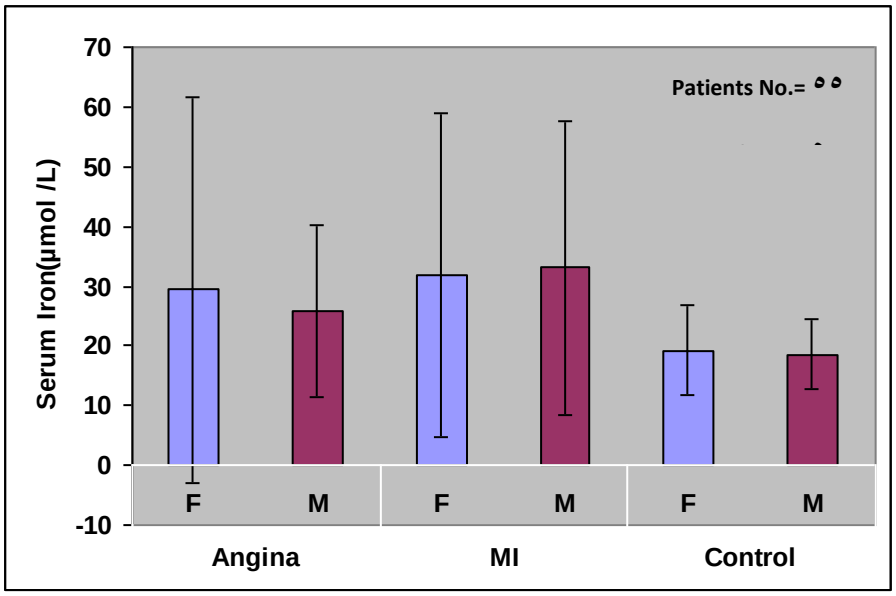


Fig [٧] The means of serum iron levels in the acute coronary heart disease group and in the control group.

On the other hand, the means (± SD) of serum iron level, in chronic coronary heart disease group in male patients were:

٢٠.٨ (± ١٠.٠) in those with MI .

٢٧(± ١١.١) in those with angina.

While the means (± SD) of serum iron in female patients were:

١٣.٥ (± ٧.٧) in those with MI .

٢٠.٨(± ٩.٤١) in those with angina.

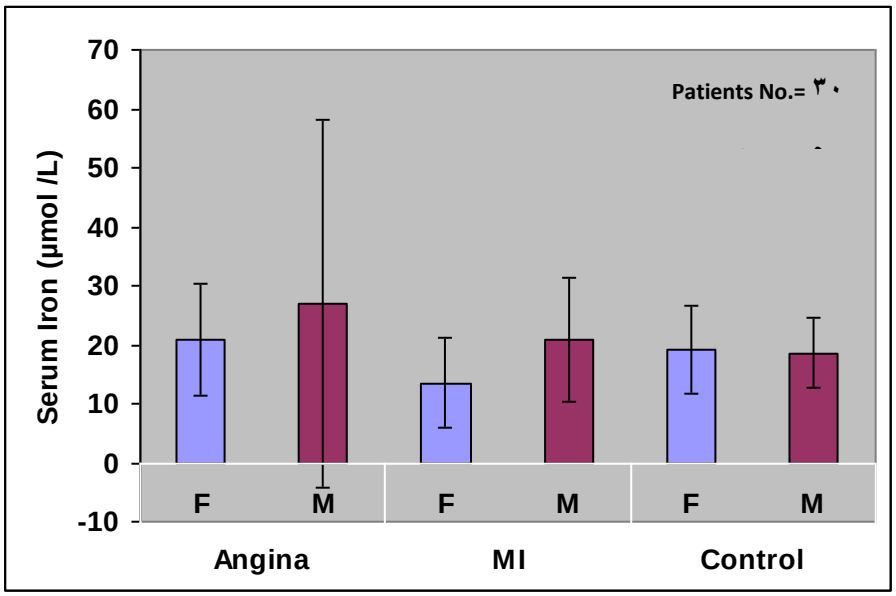


Fig [٨] The means of serum iron levels in the chronic coronary heart disease group and in the control group.

In all patients , the mean levels of serum iron show no significant differences in comparison to that of the control subject (P > ٠.٠٥) except that in male patients with acute (CHD) they revealed a significant difference with mean level of serum iron of the control group (P < ٠.٠٥).

Table (٩) : the mean of serum Iron coronary heart disease patients in contrast to control group.

Group	*No. of cases*	*Mean S.I (µmol /L)*	*Lower value*	*Higher value*	*S.D (µmol /L)*	*P. value*
Acute (CHD) group	٥٥	٣٠	٥	٩٥	± ١٩.٥	٠.٠٢
Chronic (CHD) group	٣٠	٢٠.٥	٧	٤١	± ١٠.٠	٠.٢٦
Control group	٥٠	١٨.٨	٨.٥	٢٩	± ٧.٦	

Regarding serum ferritin and serum iron, the study results in: ١٣ cases of both acute and chronic coronary heart disease groups which show elevated serum ferritin concentrations.

And ٩ cases show a reduced level of serum ferritin. On the other hand, we have ١١ cases of acute and chronic (CHD) groups which result in a high serum iron level, and ٦ cases show reduced serum iron concentrations.

This elevation in serum ferritin and serum iron could play a role in the process of atherosclerosis by catalyzing the formation of free radicals and thus enhancing peroxidation of lipoproteins , both lipid and protein moiety , and formation of oxidized LDL .

Iron - catalyzed generation of free radicals also contributes reperfusion damage.

This increment in both serum iron and serum ferritin mainly in male patients, can be explained by sex type (male), excessive iron intake (over supplementation with iron table, repeated blood transfusions and iron utensils), and excessive absorption of iron from intestinal lumen. (٨٧)

While low serum iron and serum ferritin levels are detected mainly in female cases can be related for a lesser extent by chronic blood loss and low iron intake (dietary cause). (٩٥)

٣.٤ Serum Uric Acid in Coronary Heart Disease

The means (± SD) of Serum uric acid in male patients with acute coronary heart disease were:

٦.٣ (± ١.١) in those with MI .

٥(± ١.٤) in those with angina.

The unit of measurement used in the study is mg/dl

Expected value :

Men ٣.٣ – ٧ mg/dl

Women ٢.٥ – ٦ mg/dl

While in female patients the means (± SD) of serum uric acid were:

٤.٨ (± ١.٨) in those with MI .

٤.٩(± ١.٩) in those with angina.

Against:

٣.٧ (± ٠.٩) in male control.

٣.٣ (± ١.٠٧) in female control.

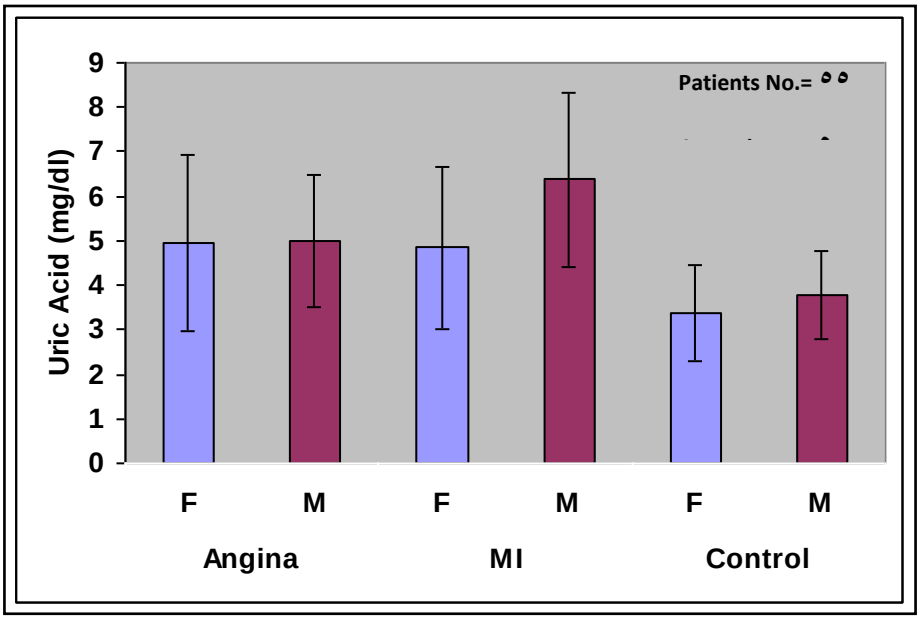


Fig [٩] The means of serum uric acid levels in the acute coronary heart disease groups and in the control group.

On the other hand, the results of means (± SD) of serum uric acid in patients with chronic coronary heart disease in male groups were:

٤.٣٣ (± ١.٥) in those with MI

٤.٢ (± ٠.٩) in those with Angina

While in female patients the results were :

٣.٢ (± ٠.٦) in those with MI

٣.٣ (± ٠.٥) in those Angina

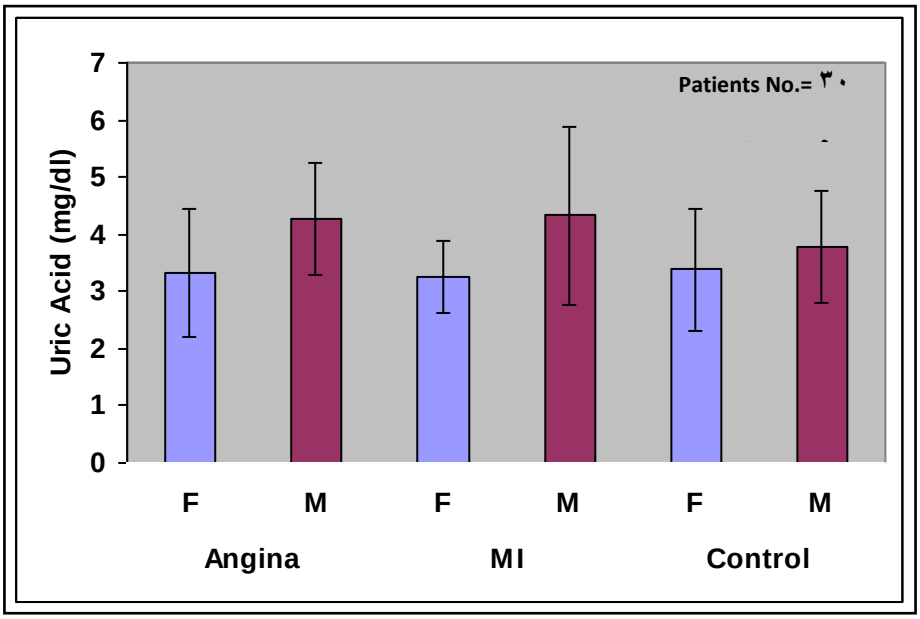


Fig [١٠] The means of serum uric acid levels in the chronic coronary heart disease groups and in the control group.

So it is clear that, the mean levels of serum uric acid in acute coronary heart disease group revealed a positive significant difference from that of the control group ($p < ٠.٠٥$) . In contrast, the mean level of SUA in chronic CHD group had shown no significant difference in relation to the serum uric acid in the control group ($p > ٠.٠٥$).

Table (١٠) : The mean serum uric acid in coronary heart disease patients in contrast to control group .

Group	No. of cases	Mean SUA mg/dl	Lower value	Higher value	S.D (mg/dl)	P. value
Acute CHD group	٥٥	٥.٢٥	٢.٧	٩.٥	± ١.٢	٠.٠٢
chronic CHD group	٣٠	٣.٧٥	٢.٤	٦.٥	± ١.٨	٠.٢٧
control group	٥٠	٣.٥٨	٢.٣	٦.٢	± ١.٧	

This difference between the mean level of (SUA) for patients in acute (CHD) group and control subjects can be related to adenosine synthesis and release which are upregulated, under conditions of hypoxia and tissue ischemia. (١٥١, ١٥٢)

On the other hand the uric acid appears to make a significant contribution to serum anti – oxidant capacity; it could also lead directly or indirectly to vascular injury. (١٥٥)

Ischemia also promotes the conversion of xanthenes dehydrogenises (XDH) to xanthenes oxides (XO), as the likely result of increased intracellular calcium, and activation of proteases.

Whereas (XDH) activity does not produce active oxygen species, the (XO) reaction is a major source of free radicals during ischemia / reperfusion injury. (١٦٠)

So raised serum uric acid concentrations are a powerful predictor of cardiovascular risk and poor outcome, although the underlying mechanisms remain unclear.

By observing the results of this study and after making the correlation between these ٤ parameters in patients and control group we found the following :

٣.٥ The Plotting of Plasma Fibrinogen Against Serum Ferritin in Control and Patients Group Revealed:

١- In control group, there is a mild positive correlation in both (male and female) cases with correlation coefficient (r) = ٠.٠٨, (r) = ٠.١٦ respectively, as shown in figures (١١), (١٢).

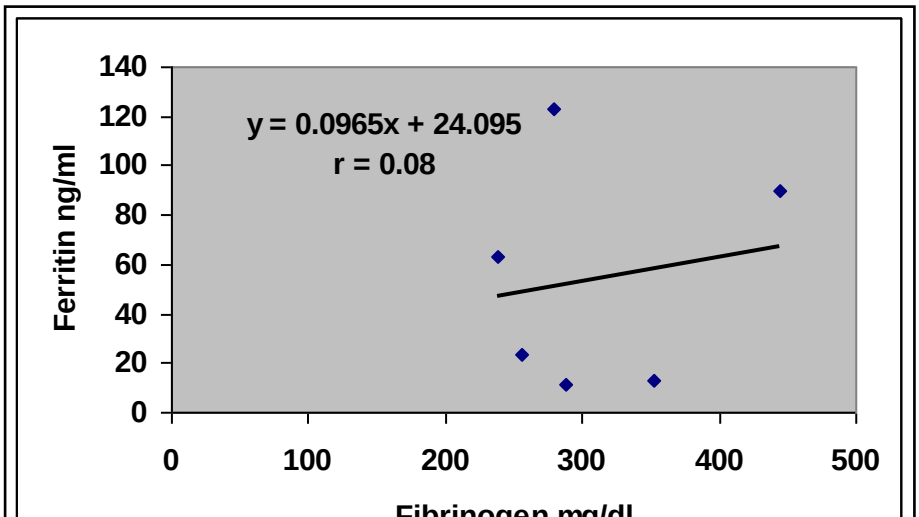


Fig. [١١] The correlation between plasma fibrinogen and serum ferritin in healthy males

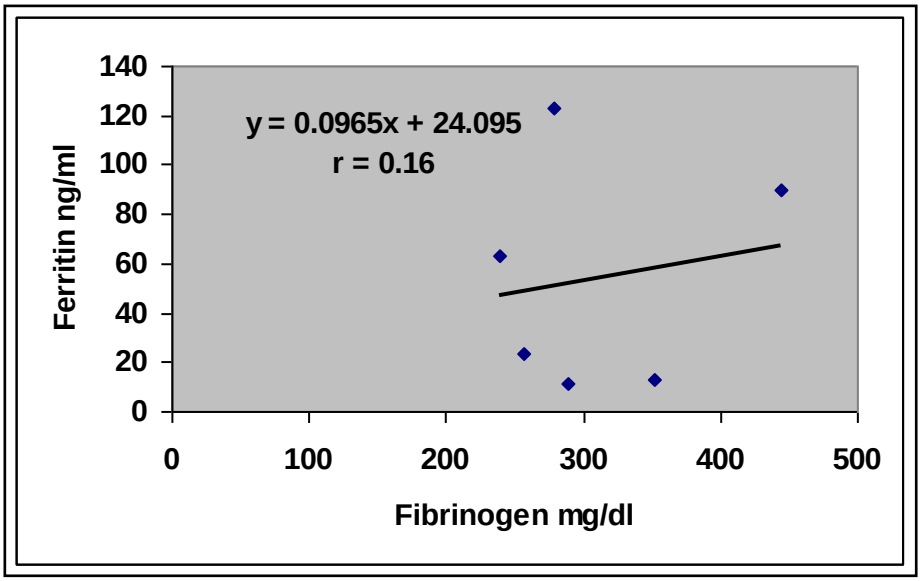


Fig. [١٢] The correlation between plasma fibrinogen and serum ferritin in healthy females

٢- In acute CHD, the male patients revealed a significant positive correlation with correlation coefficient (r) ٠.٥ in those with Angina, while those with MI showed a mild positive correlation with correlation coefficient (r) ٠.٠٧ respectively as shown in figures (١٣),(١٤)

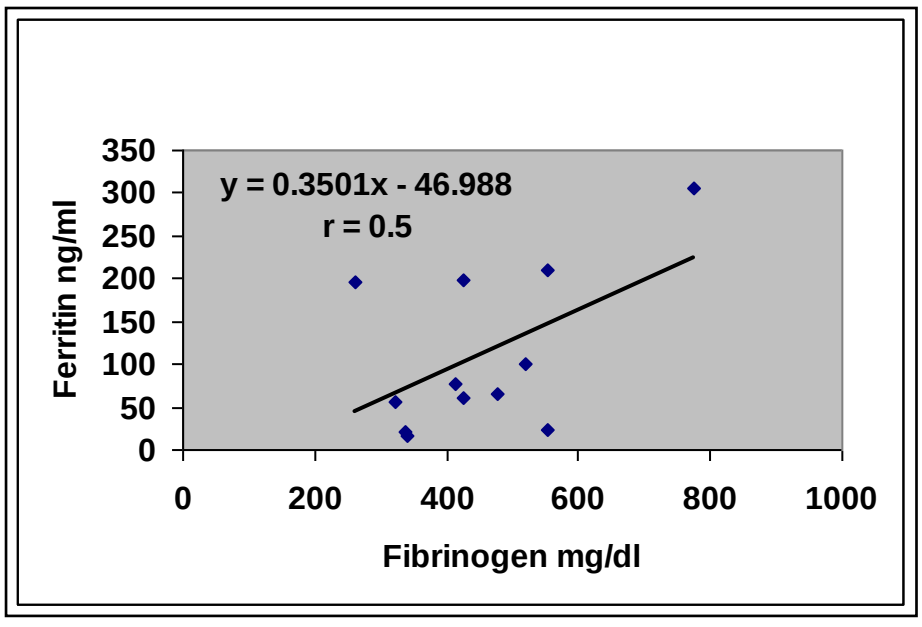


Fig. [١٣] The correlation between plasma fibrinogen and serum ferritin in male patients with Acute Anginas

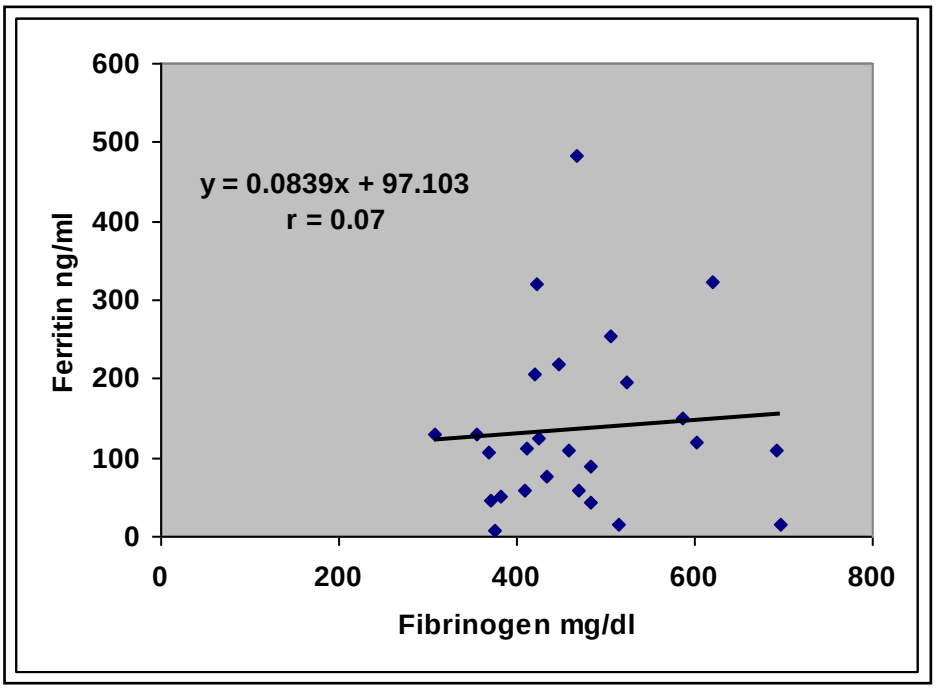


Fig. [١٤] The correlation between plasma fibrinogen and serum ferritin in males patients with AMI

While in female patients with acute coronary heart disease, the result showed a negative correlation with correlation coefficient (r) ٠.٣٣ in those with angina, and (r) ٠.٠٤ in those with acute MI, as shown in figure (١٥) , (١٦).

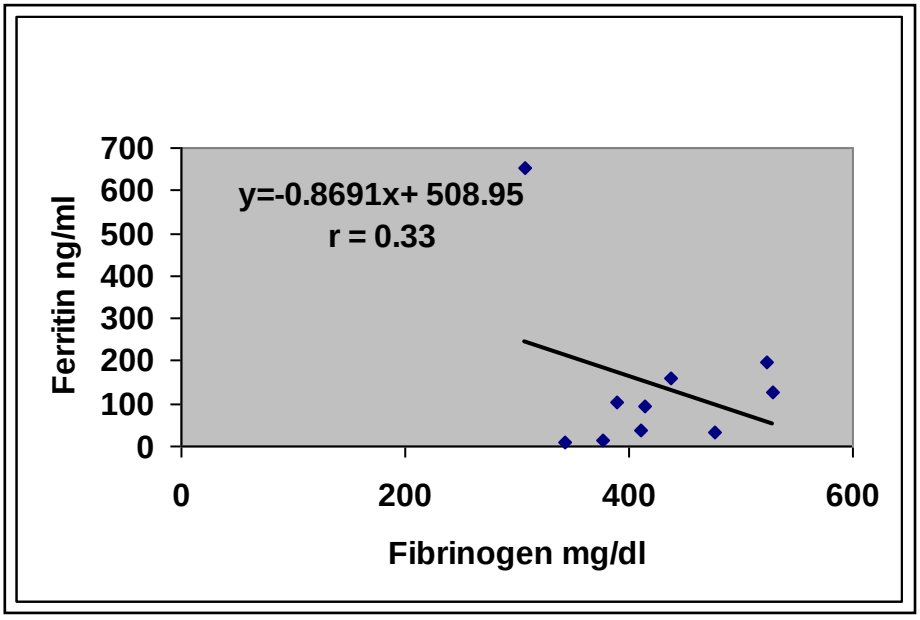


Fig. [١٥] The correlation between plasma fibrinogen and serum ferritin in females patients with acute angina

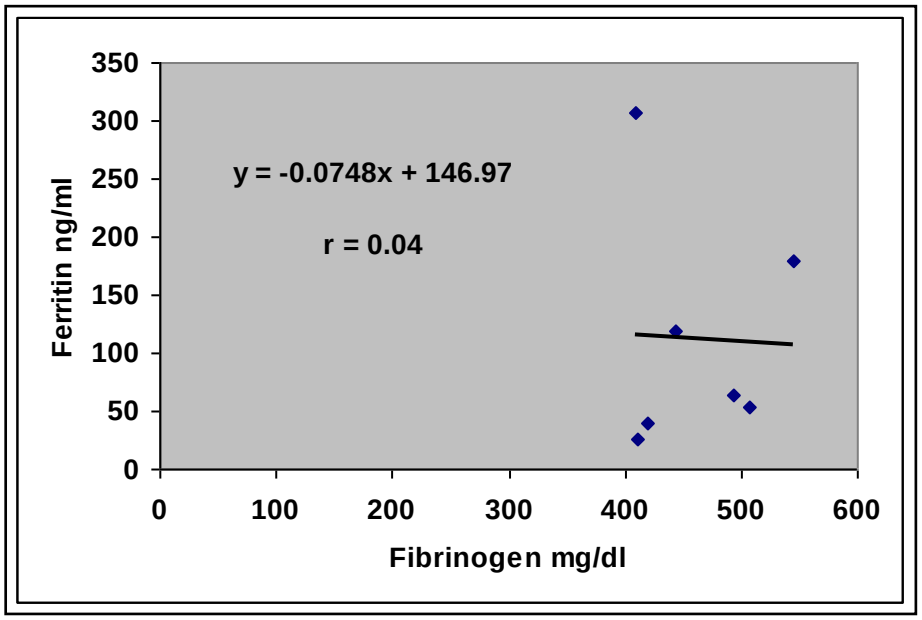


Fig. [١٦] The correlation between plasma fibrinogen and serum ferritin in females patients with AMI

٣- In chronic coronary heart disease groups, the male patients with chronic angina showed a significant negative correlation with correlation coefficient (r) ٠.٥٩, while those with chronic MI revealed a positive correlation with correlation coefficient (r) ٠.٢٣ as shown in figures (١٧) , (١٨).

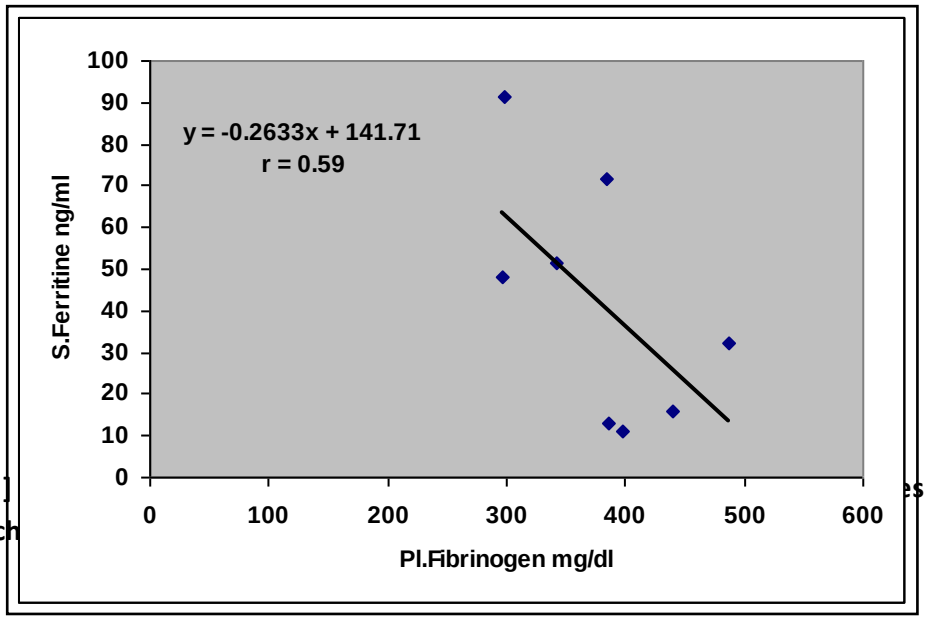


Fig. [١٧] ...es patients with ch

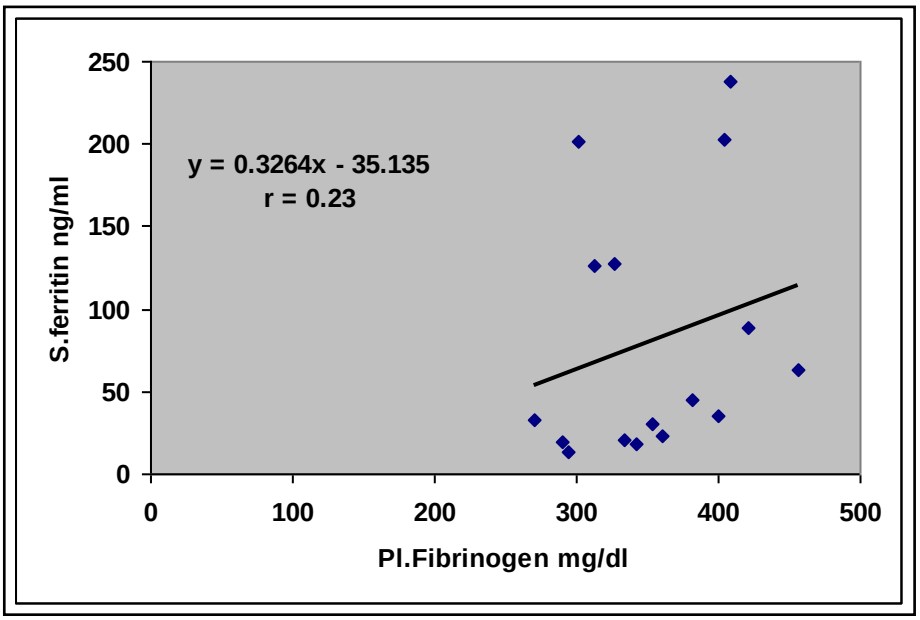


Fig. [١٨] The correlation between plasma fibrinogen and serum ferritin in males patients with chronic MI

The female patients with chronic coronary heart disease, revealed the same results as in the male patients with correlation coefficient (r) ٠.٥٩ in those with chronic angina and (r) ١ in those with chronic MI as shown in figures (١٩) , (٢٠) .

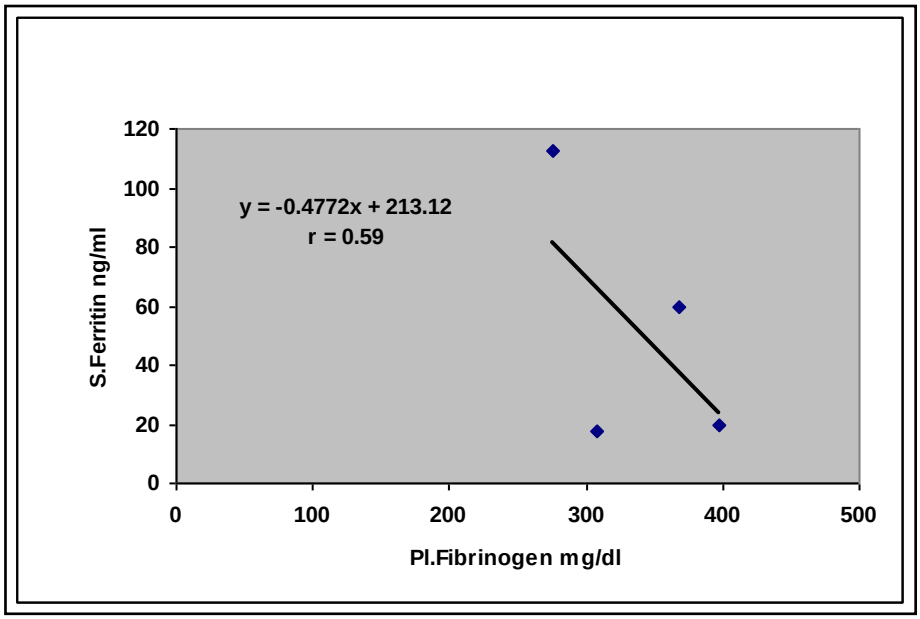


Fig. [١٩] The correlation between plasma fibrinogen and serum ferritin in females patients with chronic angina

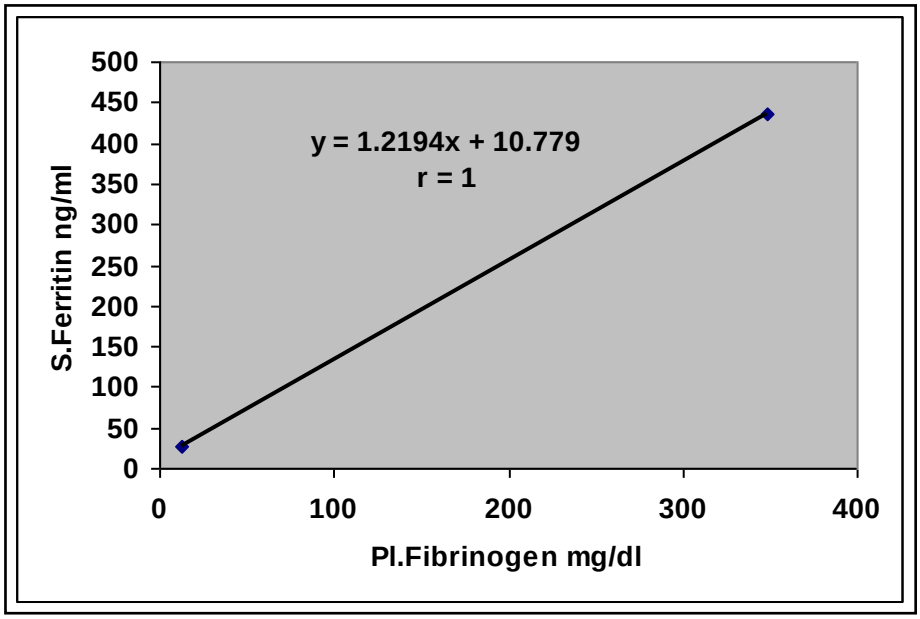


Fig. [٢٠] The correlation between plasma fibrinogen and serum ferritin in females patients with chronic MI.

٣.٦ The plotting of serum ferritin concentration against serum iron concentrations in control and patients group, the results were:

١. In control group, both male and female cases show a significant positive correlation, with correlation coefficient (r) ٠.٨٩, (r) ٠.٩٢ respectively as shown in fingers (٢١), (٢٢).

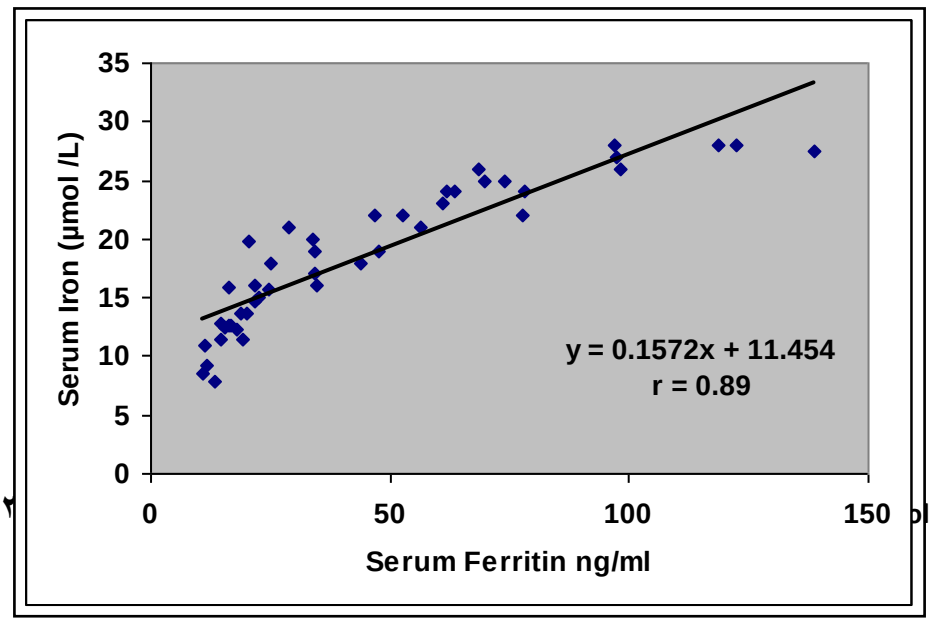


Fig. [٢

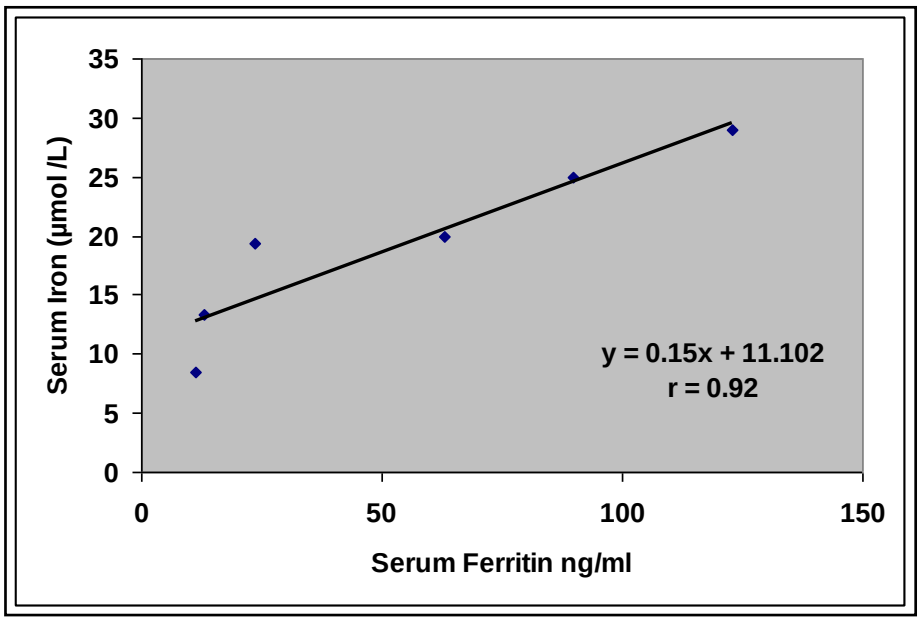


Fig. [٢٢] The correlation between serum ferritin and serum iron in females control

٢. In acute coronary artery disease group, the male patients revealed a significant positive correlation in both, those with acute angina and those with acute MI, with correlation coefficient (r) ٠.٩٣ , (r) ٠.٥ respectively, as shown in figures (٢٣) , (٢٤) .

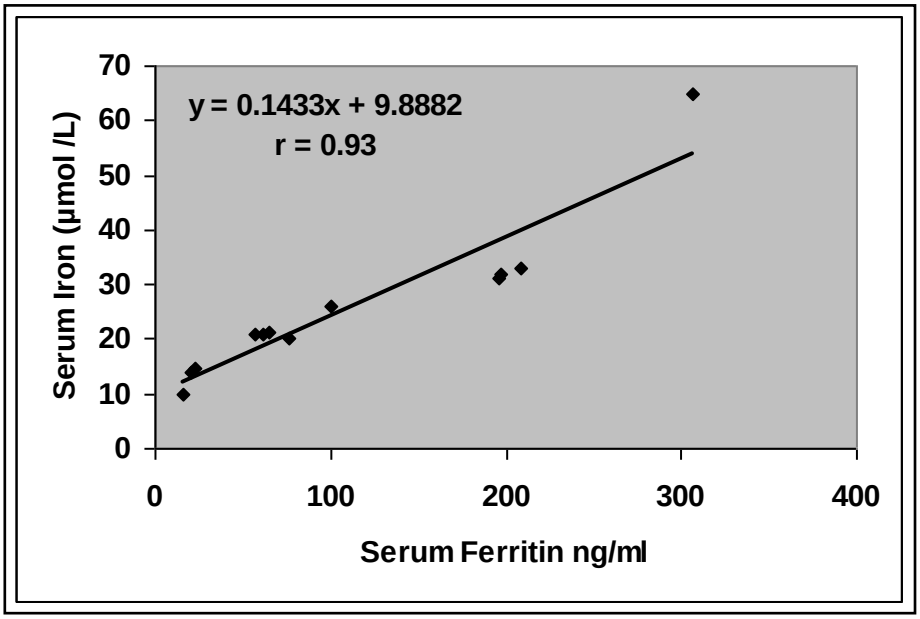


Fig. [٢٣] The correlation between serum ferritin and serum iron in males patients with acute angina

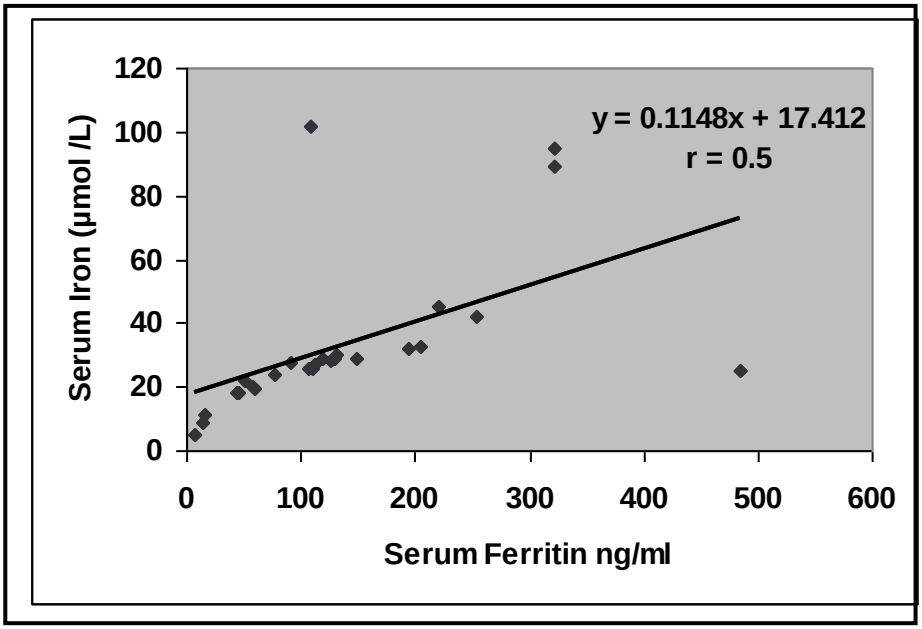


Fig. [٢٤] The correlation between serum ferritin and serum iron in males patients with acute MI.

Nearly, the same correlations resulted in the female patients with acute coronary heart disease, with correlation coefficient (r) ٠.٩٩ in those with a cute angina and (r) ٠.٩٣ in those with MI, as shown in figures (٢٥) , (٢٦).

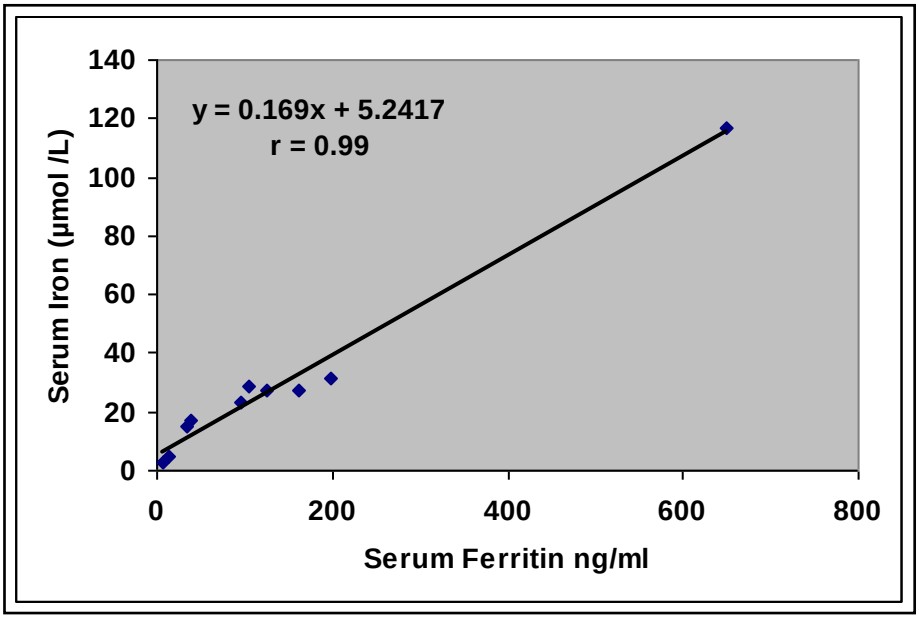


Fig. [٢٥] The correlation between serum ferritin and serum iron in females patients with acute angina

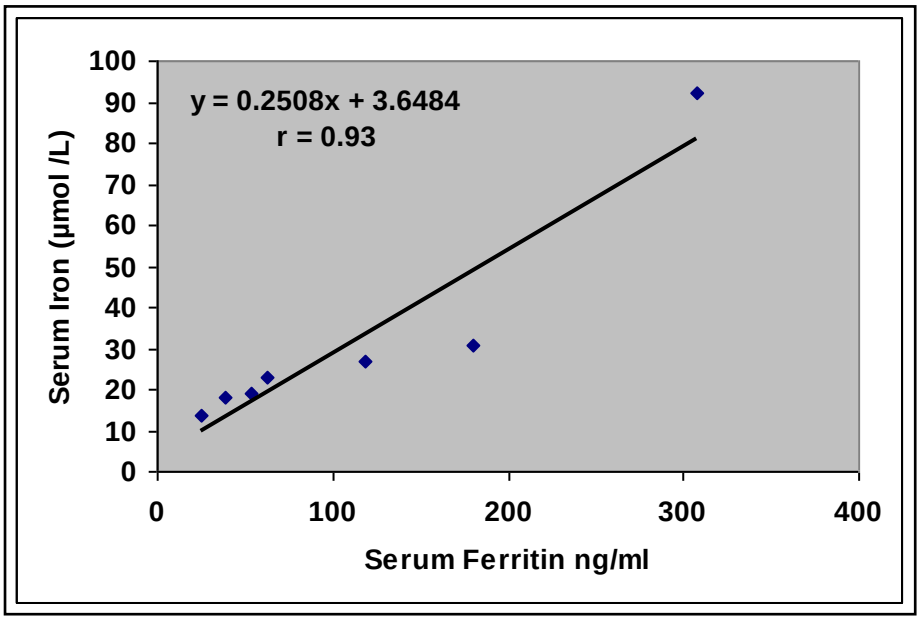


Fig. [٢٦] The correlation between serum ferritin and serum iron in females patients with acute MI

٣. In chronic artery disease groups, the male patients show a positive correlation in both, those with angina and those with MI, with correlation coefficient (r) ٠.٣٣, (r) ٠.٨٥ respectively, as shown in figures (٢٧) , (٢٨) .

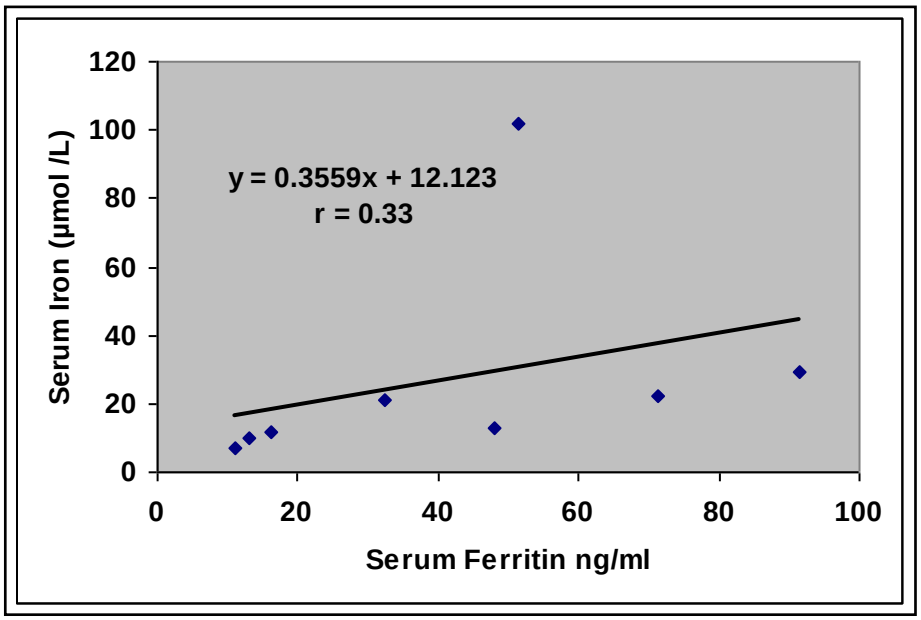


Fig. [٢٧] The correlation between serum ferritin and serum iron in males patients with chronic angina

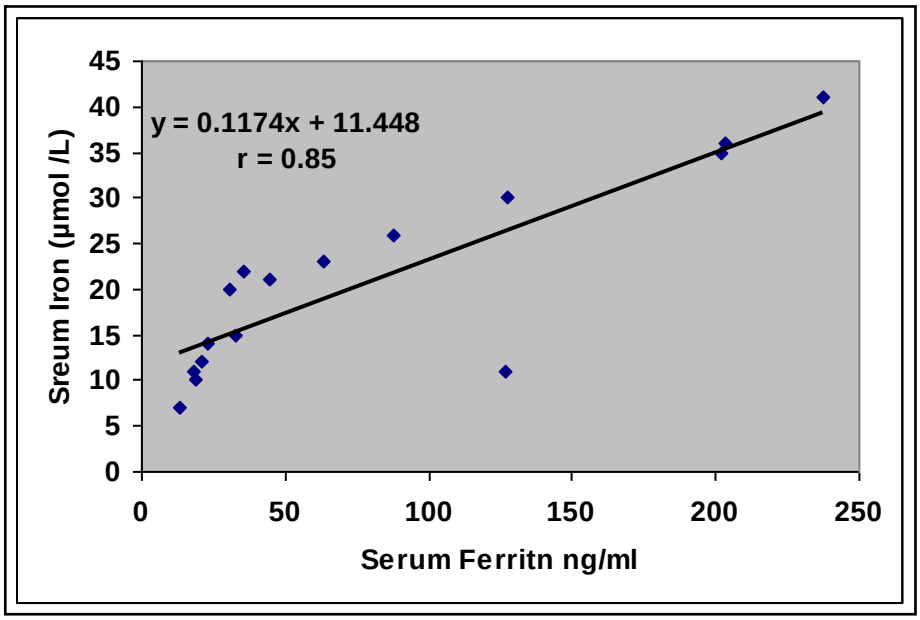


Fig. [٢٨] The correlation between serum ferritin and serum iron in males patients with chronic MI.

The results of correlation obtained from female patients with chronic coronary heart disease reflect a significant positive correlation in both, those with angina and those with MI with correlation coefficient (r) ٠.٨٨ , (r) ١ respectively as shown in figures (٢٩) , (٣٠) .

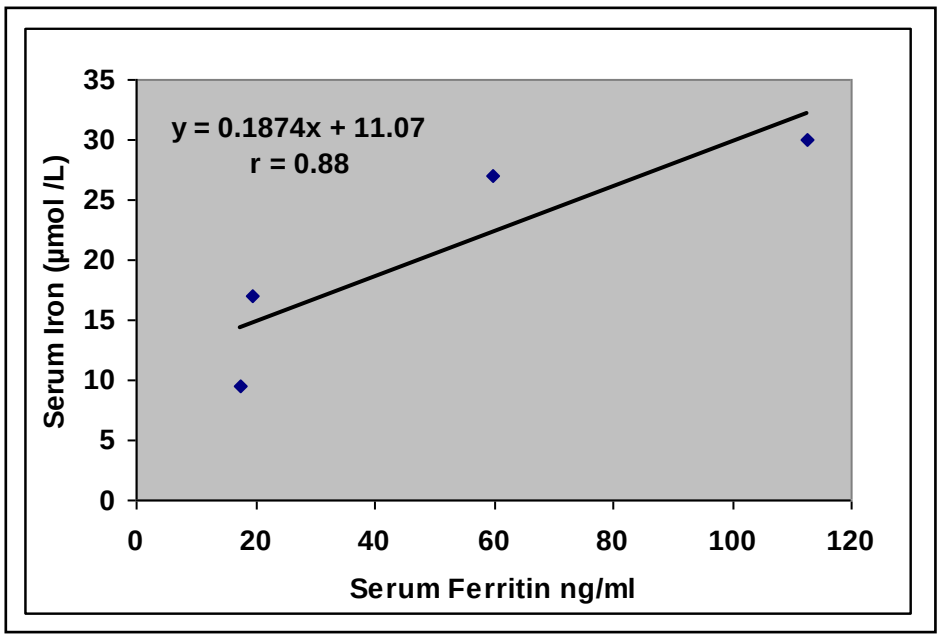


Fig. [٢٩] The correlation between serum ferritin and serum iron in females patients with chronic angina

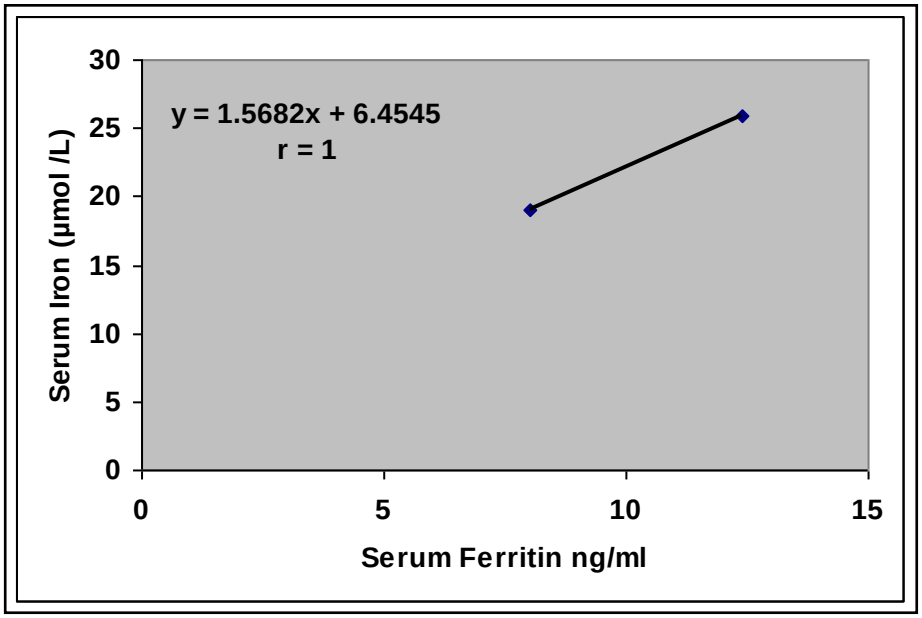


Fig. [٣٠] The correlation between serum ferritin and serum iron in females patients with chronic MI.

Conclusions and Recommendations

Conclusions:

١. Plasma fibrinogen level increased nearly in all patients with acute CHD and in few cases with chronic CHD. Furthermore, plasma fibrinogen concentrations are positively correlated with nearly all other parameters, and may be a common mechanism by which these parameters predispose to cardiovascular events.

٢. The study revealed that the highest number of cases with elevated or reduced serum ferritin and serum iron levels occurred in acute CHD group in comparison with chronic CHD group ; most of the cases with elevated level were males , while most of cases with reduced level were females.

٣. Serum uric acid had shown mild increment in only a quarter of the patients number of CHD group, while the remainder showed a normal serum uric acid concentration so that serum uric acid levels are unlikely to be a major determinant of CHD.

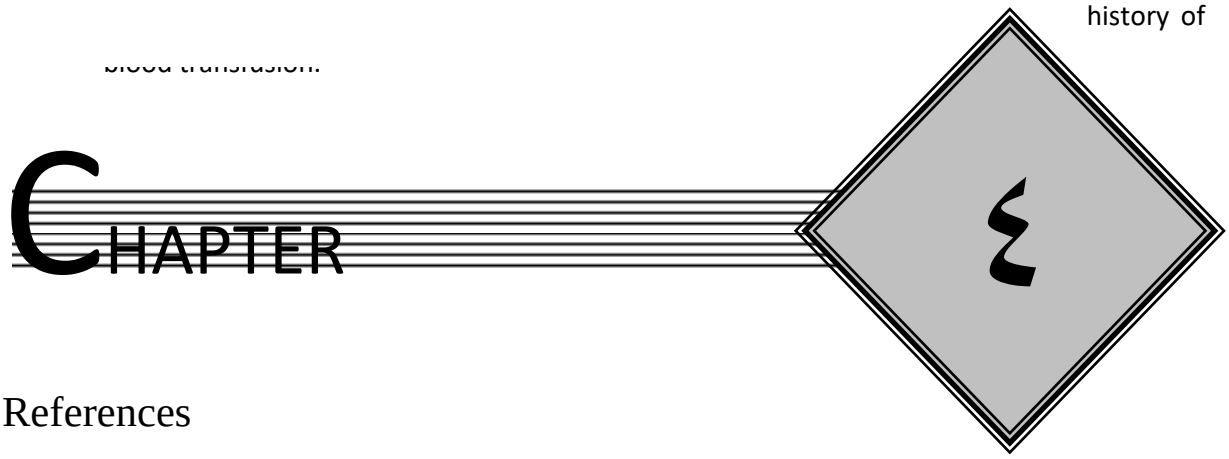
Recommendations:

١. Further studies dealing with lipoprotein (a) , high sensitive c-reactive protein and Troponin T or I, should be carried out in the field of cardiovascular system.

٢. Specified cardiac centers must be created and should have clear, informative and true recording in order to help planning correctly and successfully for the future in this field.

٣. Advanced lab equipments like HPLC and PCR are needed in order to determine the Troponin isoenzyme, lipoprotein (a) and Homocysteine and other new predictive risk factors for cardiovascular disease.

٤. Introduction of chelating therapy with desferrioxamine in those with elevated serum iron history of blood transfusion.



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