Evaluation of new Correlation Between Myeloperoxidase –AB and Trace Elements in Sera of Ischemic Heart disease patients

Rana M.Hameed Perry H. Saifullah *Mufeed J.Ewadh

University Of Baghdad ,College Of Science For Women ,Iraq
***University Of Babylon ,College Of Medicine ,Hilla ,Iraq**

E-mail :mewadh@yahoo.com

Abstract

Ischemic heart disease is one of most common diseases, and it is caused by an imbalance between the myocardial blood flow and the metabolic demand of the myocardium.

Many factors were taken as diagnosis guides and to follow up heart disease patients, few studies took myeloperoxidase levels as an indicator. The present study is an attempt to confirm that serum myeloperoxidase levels are a clinical indicator to know, in advance, the pathology of the heart disease.

The Statistical analysis revealed that there is a highly significant difference in the total serum MPO-IgG between IHD patients and those enrolled in the control group; in addition, there is a positive correlation between MPO-IgG and the patients' age.

الخلاصة

أمراض القلب التاجية هي واحدة من اكثر الامراض شيوعا ،وتحدث بسبب فقدان التوازن بين مجرى الدم للعضلة القلبية والحاجة الايضية لعضلة القلب .ولمتابعة مرضى أمراض القلب أخذت عوامل كثيرة كدليل تشخيصي لهذه الامراض ،وهناك دراسات قليلة أخذت مستوى الميالوبيروكسايديس كدليل سريري لمعرفة التاثيرات عليه وبدقة .

بين التحليل الاحصائي ان هناك أختلاف مميز بصورة عالية في المستوى الكلي للميالوبيروكسايديس –امينوكلوبيولين نوع جي في مصل الدم بين مرضى أمراض القلب التاجية والذين دخلوا الدراسة كمجموعة تحكمية ؛ بالاضافة الى ذلك ،هناك علاقة ايجابية بين الميالوبيروكسايديس –امينوكلوبيولين نوع جي وعمر المريض .

Introduction

The heart acts as two separate pumps operating in the parallel ; the right heart generates the circulation to the lungs and the left heart feeds the rest on the body . The right atrium drains deoxygenated blood from the superior and inferior venae cava and discharges blood to the right ventricle, which in turn pumps it into the pulmonary artery.

Myocardial infraction:

Myocardial infraction (also known as a heart attack) is a death of heart muscle from the sudden blockage of a coronary artery by a blood clot. Coronary arteries are blood vassels that supply the heart muscle with blood and oxygen . Blockage of a coronary artery deprives the heart muscle of blood and oxygen , causing

injury to the heart muscle . Injury to the heart muscle causes a chest pain. (1).

Chemical diagnosis:

A number of laboratory test are available as markers for ischemic heart diseases such as troponins, myoglobin,C-reactive protein,Creatine kinase,Creatine kinase-MB fraction, Lactate dehydrogenase,and Myeloperoxidase (MPO).

Myeloperoxidase MPO:

Myeloperoxidase (E.C.I. 11,1.7) is a human enzyme in the Europhilic granules of neutrophils and in the Lysosomes of monocytes. Its major role is to aid in microbial killing .(2)

Biochemistry of MPO:

Myeloperoxidase is a dimeric molecule. Consisting of a pair of heavy and light - chain protomers and two iron atoms. Its absorbance is at 430 nm.(3).The enzyme has two covalently linked carbohydrates containing large subunits of Mw. about 57.000. Each of which is apperently associated with a small subunit of Mw. 10 KD.(4) The large molecular weight subunits contain active center hemes(5) which do not appear to be equivalant.(6)

MPO is mostly abundant in the granules of neutrophils . When neutrophils become activated , which can happen in conjunction with phagocytosis , they undergo a process referred to as a respiratory burst . This respiratory burst causes production of superoxide , hydrogen peroxidase and other reactive oxygen derivatives , which are all toxic to microbes .

Auto Antibodies of Myeloperoxidase(AAb-MPO):

There are two subsets of autoantibody to human neutrophil, C-Anti neutrophil cytoplasmic antibody and P-Anti neutrophil a cytoplasmic antibody. The first subtype , C-ANCA shows a cytoplasmic staining by IFA and is diagnostic for wegeners Granulomatosis . The second subtype P-ANCA shows a perinuclear a staining by IFA.(7)..The antigen responsible for the majority of the P-ANCA response has been shown to be MPO ; therefore, the assay of MPO has been designed to detect IgG -MPO because it represents P-ANCA. (8)

Clinical significance:

Myeloperoxidase is the most promising cardiac maker at the moment and is a good inflammatory biomarker for autoimmune, inflammatory disease and cancer. By measuring the MPO level in blood, it is possible to predict whether a person is in a risk of heart attack. Previous Cleveland clinic researches have found that an elevated MPO level in white blood cells can signal a person risk for having a heart disease. (9) Previous studies have linked inflammation to increased risk of cardiac vascular events. High MPO levels in people with cardiac vascular disease indicated arterial inflammation.(10)

Trace elements:

The trace elements are called thus because they occur in very small amounts . Advanced analytical techniques like mass spectrograph and paragraphy have made the quantitation of these elements possible with precision and accuracy. Most of these trace elements function in the body as catalysis in a variety of enzyme systems. Two types of roles are recognizable. One, as activation of enzyme in which the metal protein is complex and the other important biological active forms of the trace element are the metalloenzyme.(11)

Aim of work:

The present study aims at the following:-

1. Determination the level of MPO - Ab in the sera of patients with ischemic heart disease and control subjects as an indicator of the cardiac disease.
2. Evaluating the level of trace elements (Zn^{+2} , Cu^{+2} , $Fe^{+2,+3}$) ion in the sera of the patients and the control subjects.

3. Carrying out a correlation between different parameters MPO-Ab and trace elements to identify the relation between them.

Materials and Methods

All chemical used were highly purified and used without any further purification

Patients

Three groups of patients with ischemic heart disease have been included in this study .Group 1: consisted of 24 patients with angina. Group2: consisted of 37 patients with myocardial infraction. Group3: consisted of 14 patients with chest pain.
All patients were viewed and diagnosed the cases were taken from the emergency unit in [special Margin Hospital] and [Instructional Hilla hospital] .

Control

A group of eight healthy subjects (4 males, 4 females) were investigated to serve as a control group; they were randomly selected.

Detection of IgG antibodies to Myeloperoxidase by ELISA system in the sera patients with ischemic heart disease:

Myeloperoxidase IgG ELISA test system was intended for the qualitative and semi-quantitative detection of IgG - class antibody to Myeloperoxidase in human serum. Using kits provided by (Drug) international 1nc.,USA

Determination of trace elements(Cu^{++},Zn^{++},Fe^{+2},$Fe^{+3)}$levels in sera patients with ischemic heart disease (IHD).

Colorimetric test was used to determine the levels of copper , zinc , and iron free and bird using kits provided by (Geiesse diagnostic , Roma).

Results and Discussion

Plasma Levels of MPO-Ab IgG:

Serum IgG levels are measured by enzyme linked Immunosorbant Assay (ELISA) system. It is designed to detect IgG class anti bodies to Myeloperoxidase antigen purifed MPO-antigen is attached to a solid phase microasay well. The levels of MPO - IgG in the three groups of patients with ischemic heart disease and control are illustrated in table (1).
The three groups show a significant increased with ($p < 0.01$) in sera levels of IgG as compared with control group .
These results are in agreement with those obtained by others who reported elevated levels of MPO cardiovascular disease. (12)

These results are in agreement with those obtained by other investigators.

Increased myeloperoxidase with aging may be related to the increased recruitment of inflammatory cells, contributing to protein oxidation accumulation in the aging.(13) The MPO have a differentially affects of the cardiovascular risk based on sex, as shown in table (2) But it was not significant. Plasma levels of MPO tended to be lower females; they showed a tendency towards being a stronger predictor of risk in females than in males. It is of interest to note that estradiol has recently been identified as a potential endogenous substrate for MPO in plasma that is capable of initiating lipid peroxidation. These results are in agreement with those obtained by other investigators.(14)

Correlations between serum MPO-IgG levels and serum trace elements levels:

For total (75) ischemic heart disease patients, there was a positive correlation between serum MPO-IgG levels and serum copper levels, but it was significant ,

However there was a positive correlation between serum MPO-IgG levels and Zn\Cu ratio ,but it was statistically not Significant as shown figure(3) (12,15)
And there was no correlation between serum MPO-IgµG levels and serum Zn levels ,serum free iron levels ,serum bind iron levels ,as shown in figures (4) (5) (6)

Serum Levels of Zinc and Copper:

The distribution of the mean and standard deviation values of zinc and copper in the three groups of ischemic heart disease and control are shown in table (3). For total (75) ischemic heart disease patients, there was no significant difference between total serum zinc and copper with sex, as shown in tables (5) (6).
These results are in agreement with those obtained by other investigators. (16) These trace elements are related to the degree of myocardial damage and thus may play a role in pathogenesis of ischemic heart disease .(17)One study suggested that one risk factor in the development and vascular disease may be abnormal copper and zinc metabolism . This study hypothesized that alteration in copper and zinc metabolism may be one factor underlying tissue damage in those patients .(18) Another experiment hypothesized that an alteration of the amount of metallic elements would produce a change in the concentration of cholesterol in the plasma of the animals . the increased ratio of zinc to copper (with SD 1.276 for patients with IHD and SD ~0.351 for healthy control) caused an increased concentration of cholesterol in plasma , and presumably , resulted is an increased risk of coronary heart disease . Such increased risk may add to genetic , deity , and other factors that influence the atherogentic process .(19)
These results explain the strong significant statistical . between total serum zinc levels and Zn/Cu Ratio (r = 0.603**) (p = O.0001), as shown in figure (7)And between total serum copper levels and Zn/Cu ratio with (r= - 0.514**) (p < 0.0001), as shown in figure (8).(20)

Correlation between serum Zn levels A serum Cu levels:
For all patients with ischemic heart disease work included, there was a positive correlation between serum Zn levels and serum Cu levels, as shown in figure (9).
At present, there is no explanation for the value of zinc and copper levels in serum patients with ischemic heart disease.(19)

Plasma levels of iron Ferene , in sera of patients with IHD:
The spectrophotometer technique was used to determine the levels of iron ferene in patients with IHD .Iron is present in the buffer system at pH 4.8,it is first liberated from trasferrine, his vector protein and after reduced at ferrous state. Ferrous ions join with ferene forming a stable complex which intensity of blue color is proportional to the quantity of iron in the sample. The distribution of the individual values of iron ferene level in the three groups of IHD and control is shown
In table(7) and it appears that free iron had the tendency to but not significant .(19)
The levels of Iron were related to the degree of myocardial damage and thus may play a role in the pathogenises of Ischemic heart disease (IHD).(15)
In the presence of O_2, Fe(III) and an appropriate electron donor, a number of enzymic and nonenzymic oxygen free radical generating systems were able to catalyze the oxidative modification of proteins. Little peptide bond cleavage occurred when proteins were exposed to metal catalyzed

oxidation (MCO) system. The MCO systems catalyzed the reduction of Fe (III) to Fe (II) and of O_2 to H_2O_2 and that these products reacted at metal - binding sites on the protein to produce active oxygen (free radical), (OH , ferry 1 ion).(20)

For all three groups of patients and control group there was no significant difference between total serum Iron ferene levels with sex , (p > 0831) as shown in table (8).

Plasma levels of Iron bind in sera patients with IHD:

The colorimetric method (chromazurol B) was used to the quantitative determination of iron in serum. Transferrin bound Iron was released at an acidic

PH in serum Fe+3 and Fe+2 , reacted with chromazurol B (2,6- Dichloro- 4- hydroxyl- 3-dimethylfuchsone-5,5-dicarboxylic acid) And the cetyltrimethyl ammonium bromide (CTMA) , forming a ternary blue colorcomplex . The intensity of the color was proportional to the sample iron concentration. The mean and standard deviation values of serum iron levels for three groups of patients with ischemic heart disease and control group are shown in table (9).

The result shows a highly significance between the Iron bind and ischemic heart disease patients with (PO.132)

Iron can also be a toxic substance, when too much iron is available for transferring to carry, problems occur. Transferring heavily saturated lose the ability to tightly bind iron, Unbound or free iron was dangerous since it triggered free radical activity. Free iron provided nourishemeia pathogens as listeria and vibrio bacteria. These bacteria were harmless normal levels, but when transferring was highly saturated with iron these bacteria could be deadly.

These bacteria grew best inside macrophages that were iron loaded. This macrophages were white blood cells that protected against disease, but when such microorganism rendered them unless, our defense system began to break down. (21).There was no effect of Iron bind levels and sex of patients with IHD as shown in table (10).

Correlation between serum free iron levels and serum bind iron levels:

For all (75) patients with ischemic heart disease, there was no correlation between serum free iron levels and serum iron bind levels, as shown in figure (10).

In the light of the present study, the following points can be concluded:

1- Elevate MPO levels predicts an increased risk of cardiac events and it may be used as an early marker in the ischemic heart disease.

2- Plasma levels of MPO are a strong predictor of cardiovascular disease risk in females than in males.

3- On analysis ,a significant difference between serum MPO-IgG levels with aging is found and the trace elements levels are related to the degree of myocardial

damage and thus ,may play a role in the pathogenesis of ischemic heart disease.

Further study needed to clarify the correlation between the MPO-level and the

lipid profile that concentrate with cholesterol and low density lipoprotein.

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Table (1) Levels of MPO- IgG in the three groups of patients with IHD and control

	No. of Cases	Mean Age	Mean Serum IgG Level	T - Test	P - Value
Group 1	14	59.28	0.385	135.82**	< 0.01
Group 2	24	62.5	0.684	59.40**	< 0.01
Group 3	37	58.18	0.453	80.45**	< 0.01
Control	8	50.62	0.244	-----	< 0.05*

Table (2) The mean and standard deviation of MPO-IgG Levels for three groups of patients with IHD according to sex.

IgG	Patients	
	Male	Female
No.of cases	46	29
Mean	0.548	0.457
± SD	± 0.450	± 0.249
P- Value	> 0.218	> 0.076

Table (3) Mean and standard deviation of Copper for three groups of IHD and control.

µg/dl	Group1	Group2	Group3	Group4
No.of cases	14	24	37	8
Mean	232.23	344.84	197.35	114.28
± SD	195.41	272.74	135.8	35.25

Table (4) The mean and standard deviation of zinc in the three groups of IHD and control.

Zn µg/dl	Group1	Group2	Group3	Group4
No.of cases	14	24	37	8
Mean	228.56	162.11	209.91	110.78
± SD	115.48	58.85	125.18	8.732

Table (5) Shows the determination of zinc (Ug/dl) according to sex for patients.

Zn µg/dl	Patients	
	Male	Female
No.of cases	46	29
Mean	216.73	173.43
± SD	106.76	71.30
P- Value	> 0.066	

Table (6) Shows the determination of zinc (Ug/dl) according to sex for control.

Zn µg/dl	Patients	
	Male	Female
No.of cases	4	4
Mean	115.15	106.37
± SD	± 9.29	5.03
P- Value	> 0.147	

Table (7) The mean and stndard deviation values for patients and control included in the study.

Iron µg/dl	Group1	Group2	Group3	Group4
No.of cases	14	24	37	8
Mean	94.480	117.005	110.41	95.57
± SD	33.72	30.65	59.813	24.08

Table (8) Shows the mean and standard deviation (A) for patient and (B) control included in the study according to sex .

(A)

Fe Free µg/dl	Patients	
	Male	Female
No.of cases	46	29
Mean	107.51	109.96
± SD	± 55.97	±31.30
P- Value	> 0.831	

(B)

Fe Free µg/dl	Patients	
	Male	Female
No.of cases	4	4
Mean	107.31	83.83
± SD	6.456	30.72
P- Value	> 0.185	

Table (9) Mean and standard deviation and P values of iron levels in patients groups and control.

Iron µg/dl	Group1	Group2	Group3	Group4
No.of cases	14	24	37	8
Mean	82.76	86.85	85.92	83.50
± SD	5.649	9.926	8.040	18.70
P- Value	0.05*	0.05*	0.05*	0.529

Table (10) Shows the mean and standard deviation values (A) for patients and (B) for controls included the study according to sex.

(A)

Bind Iron µg/dl	Patients	
	Male	Female
No.of cases	46	29
Mean	84.26	87.31
± SD	6.519	10.86
P- Value	> 0.132	

(B)

Bind Iron µg/dl	Patients	
	Male	Female
No.of cases	4	4
Mean	84.08	82.93
± SD	5.087	4.109
P- Value	> 0.738	

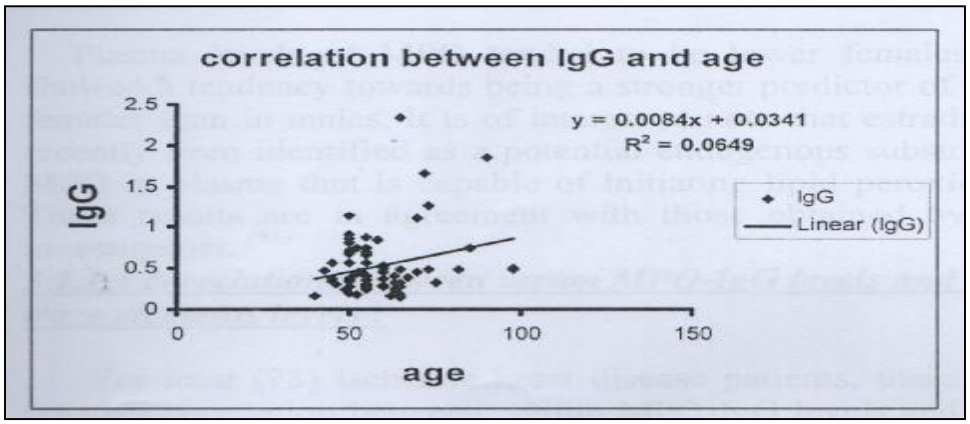

Figure (1) Shows the correlation between IgG levels and age.

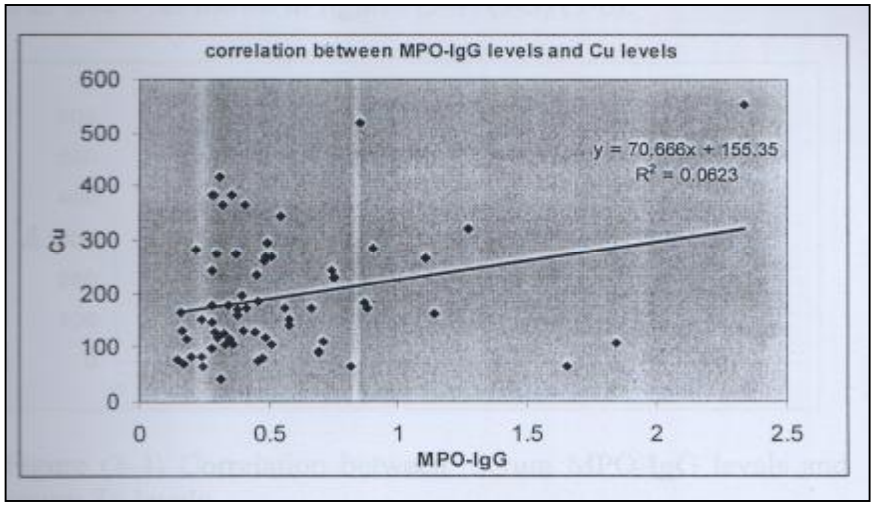

Figure (2) Correlation between serum MPO-IgG levels and serum copper levels.

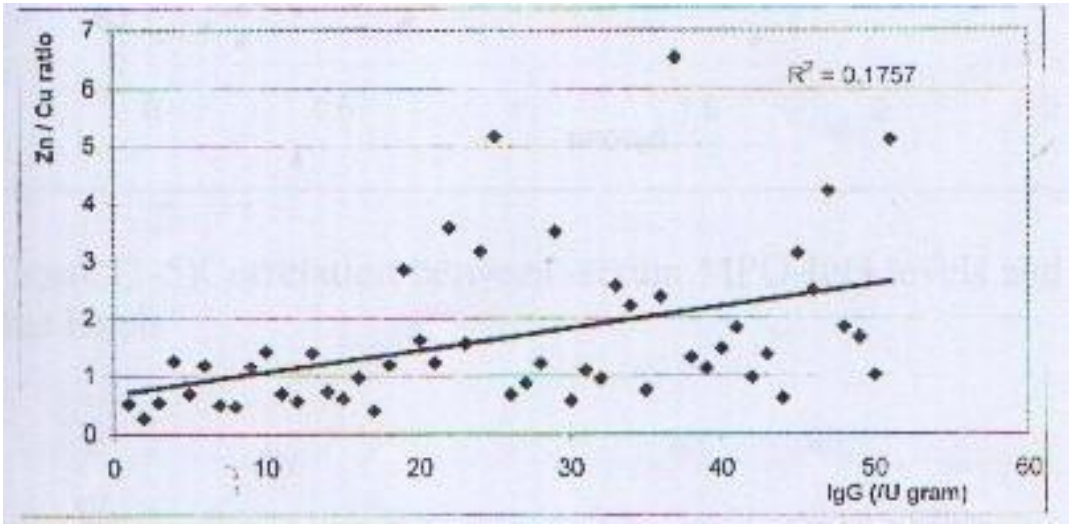

Figure (3) Correlation between MPO-IgG and Zn\Cu ratio.

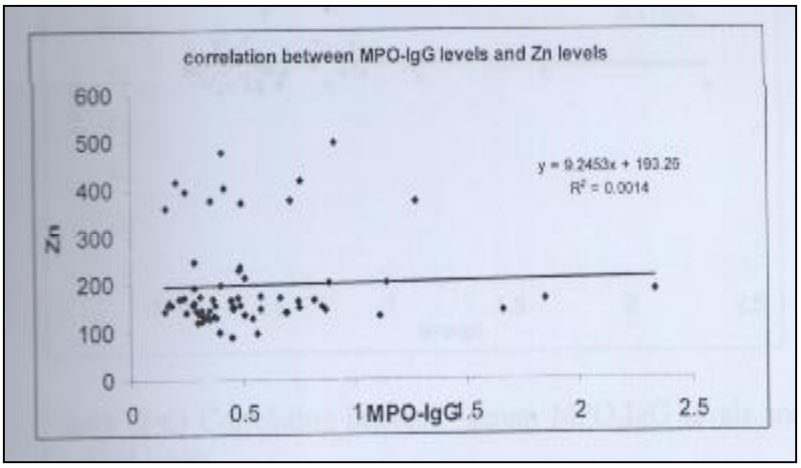

Figure (4) Correlation between serum MPO-IgG levels and serum Zn levels.

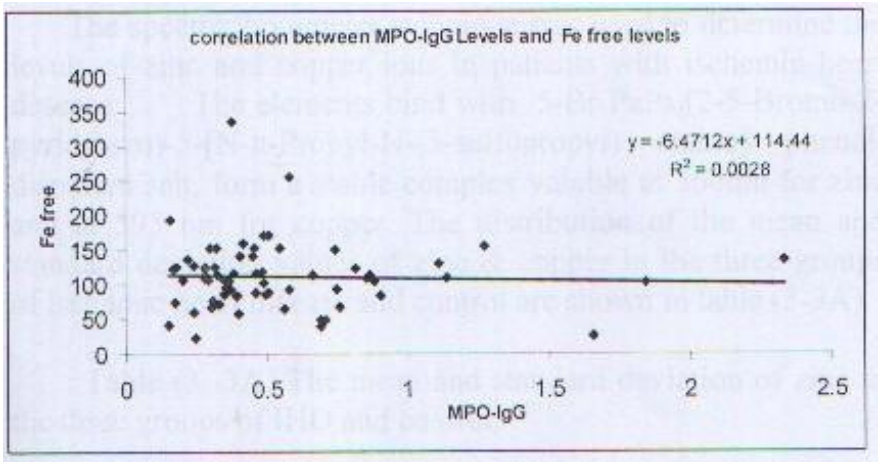

Figure (5) Correlation between serum MPO-IgG levels and Fe free levels.

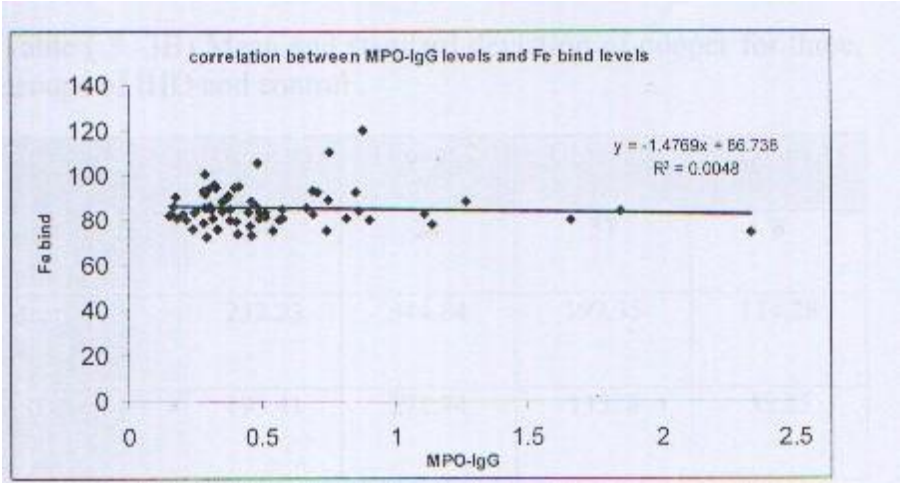

Figure (6) Crrelation between serum MPO-IgG levels and Fe bind levels.

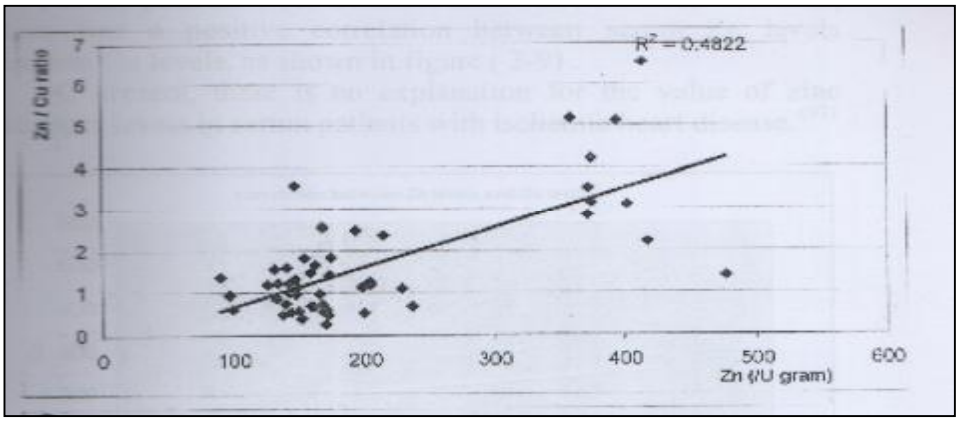

Figure (7) Correlation between zinc levels and Zn\Cu ratio.

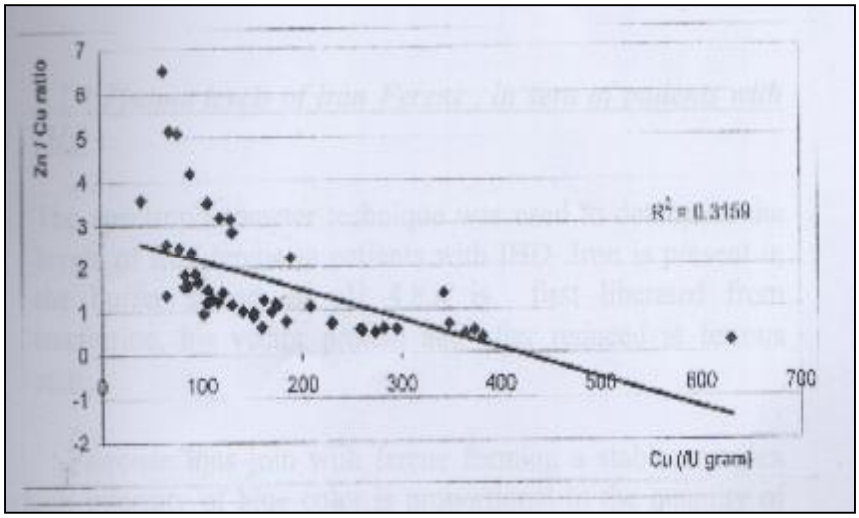

Figure (8) Correlation between copper levels and Zn\Cu ratio.

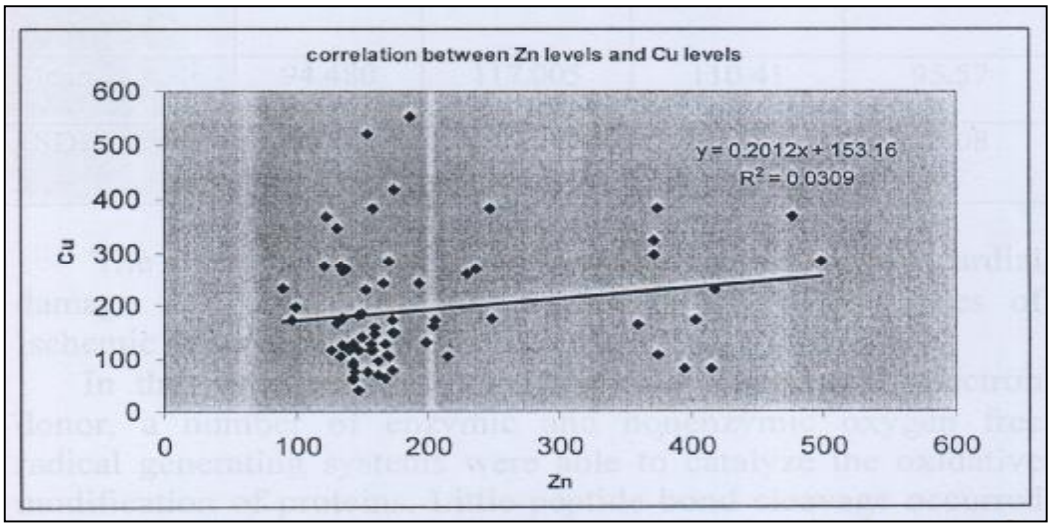

Figure (9) Correlation between serum Zn levels and serum Cu levels.

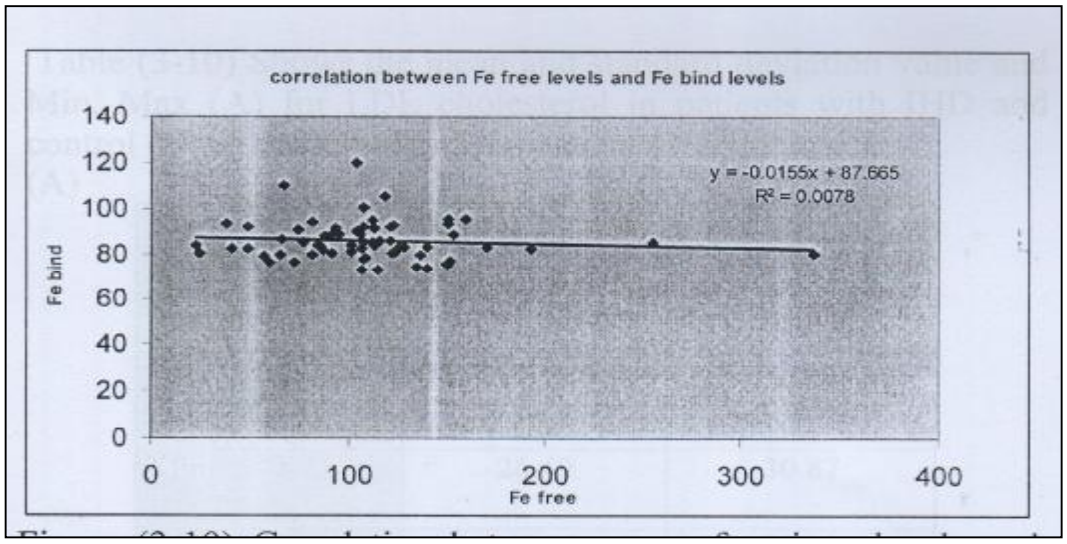

Figure (10) Correlation between serum free iron levels and serum iron bind levels.