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ANEW CORRELATION BETWEEN MYELOPEROXIDASE LEVELS AND LIPID PROFILE IN 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 الميلوبيروكسايديس كدليل سريري لمعرفة التاثيرات عليه وبدقة . بين التحليل الاحصائي ان هناك أختلاف مميز بصورة عالية في المستوى الكلي للميلوبيروكسايديس – لميونوكلوبيولين نوع جي في مصل الدم بين مرضى أمراض القلب التاجية والذين دخلوا الدراسة كمجموعة تحكمية ؛ بالاضافة الى ذلك ،هناك علاقة ايجابية بين الميلوبيروكسايديس – لميونوكلوبيولين نوع جي وعمر المريض

Introduction

The chest pain or pressure occurs when the blood and oxygen supply the heart muscle can't keep up with the needs of the muscle, when coronary arteries are narrowed by more than 50 to 70 percent. The arteries can't increase the supply of blood to the heart muscle during exercise or other periods of high demand for oxygen. An insufficient supply of oxygen to the heart muscle causes angina.[2]

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 vessels 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. [3]

Myeloperoxidase (MPO) (E.G.1.11.1.7) 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 .[4]

Myeloperoxidase is a dimeric molecule. Consisting of a pair of heavy and light - chain promoters and two iron atoms. The enzyme has two covalently linked carbohydrates containing large subunits of MW about 57.000. Each of which is apparently associated with a small subunit of MW. 10.000.[5] The large molecular weight subunits contain active center hemes[6] which do not appear to be equivalent. [7]

MPO is mostly abundant in the granules of neutrophils. Monocytes contain only about third of the

MPO present in 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 oxic to microbes .

During respiratory bursts, granule contents are released into the phagolysosomes and outside the cell. Allowing released contents to come into contact with any microbes present, MPO catalyzes the conversion of hydrogen peroxide and chloride ion (Cl) into hyperchlorous acid . Hyperchlorous acid is 50 imes more potent in microbial killing than hydrogen peroxide[8]

Auto Anti bodies of Myeloperoxidcise(AAb-MPO) are two subsets of autoantibody to human neutrophil, C-Anti eutrophil cytoplasmic antibody and P-Anti neutrophil a cytoplasmic antibody. The first subtype , C-ANCA hows a cytoplasmic staining by IFA and is diagnostic for wegeners Granulomatosis . The second subtype P-ANCA shows a per nuclear a staining by IFA .[9]

The antigen responsible for the majority of the P-ANCA response has been shown to be MPO ; therefore, he assay of MPO has been designed to detect IgG -MPO because it represents P-ANCA[10].

The leukocyte-derived enzyme Myeloperoxidase MPO is abundantly expressed in neutrophils, monocytes, nd tissue-associated macrophage, and it has been implicated as a potential causal factor in atherosclerosis. In act , MPO is highly expressed in human atherosclerosis lesions .[3]

Recent studies have shown that circulation leukocyte levels of MPO are positively associated with the ncreased risk of developing coronary artery disease ,[11]. The most commonly appreciated view of how MPO can lay a damaging role is its production of reactive oxidizing and chlorinating species that damage mportant bio- molecules. Indeed, this hemoprotine can oxidatively modify LDL[12] by catalyzing lipid eroxidation as well as oxidation and chlorination of protein tyrosine residues.[13,14]

Whereas MPO is typically viewed to contribute to inflammatory injury by catalyzing oxidative damage to ritical biomolecules within the vasculature, that is , its interaction with the nitric oxide (NO) pathway . It as been demonstrated that NO can biophysically modulate the catalytic activity of MPO .[15,16]

Lipids such as phospholipids, triacylglycerol, and cholesterol, are sparing. They are transported by the irculation as components of lipoproteins, globular micelles like particles. They consist of a nonpolar care f triacylglycerols and cholesterol esters surrounded by an amphilic coating of protein, phospholipids and holesterol. Lipoproteins have been classified into five broad categories on the bases of their functional nd physical properties :

. Chylomicrons, which transport exogenous triacylglycerols and cholesterol from the intestines to the tissues.
. Very low density lipoproteins (VLDL),
. Intermediate density lipoprotein (IDL)
. Low density Lipoprotein (LDL).

a group of related particles that transport endogenous triacylglycerol and cholesterol from liver to the ssues.

-High density lipoproteins (HDL): which transport endogenous cholesterol from the tissues to the ver.[17]

MPO is an abundant heme protein released by activated neutrophils and monocytes and present in some tissue nacrophages such as these in vascular lesions. [18]

MPO may play a role in monocyte-macrophage oxidation of LDL by a variety of distinct pathways .[19]

MPO can act to amplify the oxidizing potential of H_2O_2 , the dismutation product of O_2 , by using it as a CO-substrate to generate a variety of oxidants , including diffusible radical species [20] reactive halogens dehydes and nitrating agents [19,21].The present study aims at the following:-

. Determination the level of MPO - Ab in the sera of patients with ischemic heart disease and control ubjects as an indicator of the cardiac disease.

Estimating the level of cholesterol and LDL level in the sera of patients and control subjects.

3. Carrying out a correlations between MPO-Ab, LDL, and cholesterol to identify the relation between them .

MATERIALS AND METHODS:

Chemical All chemical used were highly pure and used without any further purification .

Patients Three groups of patients with ischemic disease have been included in this study .Group1: 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 IsG antibodies to Mveloperoxidase 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 Inc., USA).

The quantitative determination of lipid profile in sera patient with IHD.

A group of 20 patients with ischemic heart disease (12 males,8 females) was collected from [Margin Hospital] and [Instructional Hilla Hospital] to determine levels of LDL-cholesterol and levels of total cholesterol. Using kits provided by Griesse diagnostic , Roma .

Results and Discussion :

Plasma Levels ofMPO-Ab IsG:

Serum IgG levels are measured by enzyme linked Immunosorbant Assay (ELISA) system. It is designed to detect IgG class anti bodies to Myeloperoxidase antigen purified MPO-antigen is attached to a solid phase microasay well. Diluted test sera are added to each well if the antibodies are present that recognize the antigen .Antigen-antibodies complexes are formed. After incubation, the wells are washed to remove unbound antibody. An enzyme labeled antihuman IgG is added to each well. If antibody is present, the conjugate will bind to the antigen-antibody complexes. After incubation the wells are washed to remove unbound conjugate. Substrate solution is then added to each well. If enzyme is present, the substrate will undergo a color change.

After an incubation period the reaction is stopped and the color intensity is measured photometrical producing an indirect measurement to specific antibody in the patient's specimen.

The levels of MPO - IgG in the three groups of patients with ischemic heart disease and control are illustrated in table (1):

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*

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. [22]

Recent studies have emphasized the importance of myeloperoxidase for disease. Myeloperoxidase levels are higher in patients with cardiovascular disease and predict future cardiovascular events after risk factors. Myeloperoxidase may play a pathophysiological role in atherogenesis. Another potentially important consequence of myeloperoxidase activity is the consumption of nitric oxide and induction of endothelial dysfunction kinetic studies that first show that myeloperoxidase can serve as a catalytic sink for nitric oxide. Consistent with such an effect , myeloperoxidase impairs nitro oxide - dependent vasodilatation in isolated arterial and tracheal rings and deceases nitric oxide bioavailability in cultured cell by myeloperoxidase is rapidly taken up by endothelial cells by a transcytotic process and accumulates within the sub endothelial space , positioning it anatomically to interfere with the effects of nitric oxide in the vessel wall .[23]

For total (75) ischemic heart disease patients, 46 males (61.3%) and 29 females (38.7 %) were studied, there was a significant different between serum IgG for MPO levels and age, as show in figure (1).

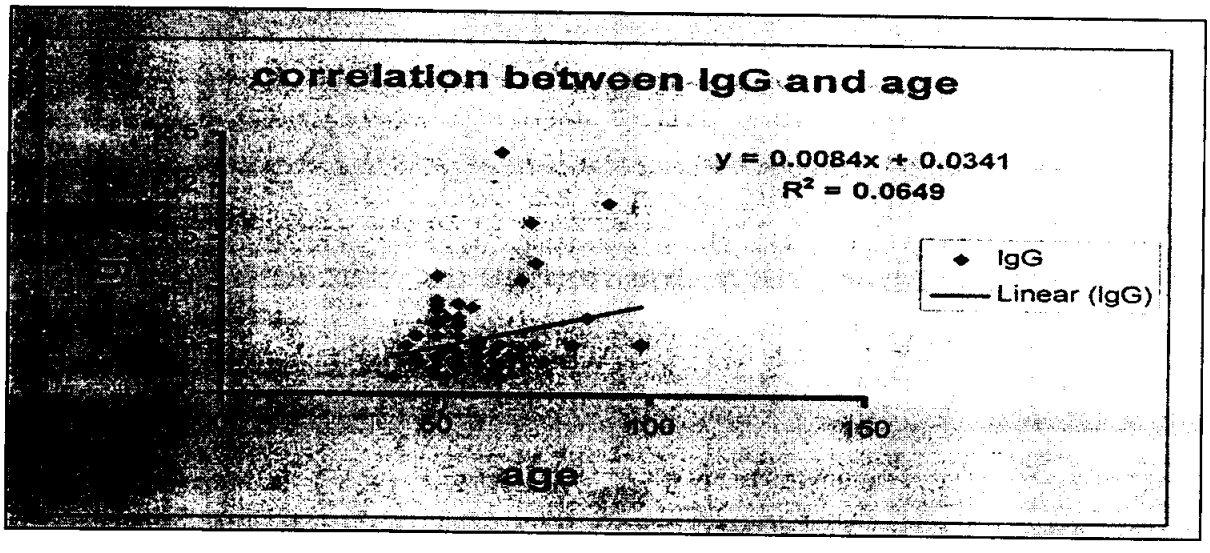

Figure (1) Shows the correlation between IgG levels and age.

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.[24]

The MPO have a differentially affects of the cardiovascular risk based on sex, as shown in table (2) But it was not significant.

Table (2) :The mean and standard deviation of MPO-IgG Levels (AAU\ml) 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

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.[25]

Plasma levels of LDL-cholesterol and plasma levels of total cholesterol:

Serum LDL cholesterol was measured by polyvinyl sulphate method (PVS).

LDL cholesterol can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene -glycolmonomethyl ether. After precipitation reaction they took the supernatant to determine the concentration of the serum total cholesterol according to the enzymatic method (CHOD - PAP). Under the action of cholesterol esterase (CHE) cholesterol esterase resolved in cholesterol and fatty acids. Cholesterol oxides (CHOD) oxidized the above mentioned cholesterol together with the loose cholesterol releasing cholesterol -3-one and hydrogen peroxide.

In the presence of peroxidase (POD), the released hydrogen peroxide reacted with a phenol substitute and 4-amino antipyrine to form a red dye compound. The intensity of the red color produced was directly proportional to the total cholesterol in the simple what read at 500-520 nm. The mean levels of LDL cholesterol and total cholesterol in patients with ischemic heart disease and control are illustrated in table (3).

Table (3): Shows the mean and standard deviation value and Min, Max (A) for LDL cholesterol in patients with IHD and control (B) cholesterol levels in patients and control.

(A)

Bind Iron	Patients	Control
No. of cases	20	8
Mean	83.35	52.08
+ SD	51.56	48.9
Min.	26.49	30.87
Max.	219.73	70.12

(B)

Bind Iron	Patients	Control
No. of cases	20	8
Mean	174.21	139.18
+ SD	67.51	22.9
Min.	103.07	98.96
Max.	324.5	166.4

These result in agreement with those obtained by other investigators.[26,27] LDL-cholesterol a bad cholesterol, because the elevated of it is associated with an increased risk of coronary heart disease and other cardiovascular diseases.

LDL deposits cholesterol on the artery walls which to the formation of a hard, thick substance called cholesterol plaque. Cholesterol plaque causes thickening artery walls and narrowing of the arteries. The ratio of HDL cholesterol to LDL cholesterol is more important than the level of cholesterol to determine the risk for heart disease.[26] Fort total (20) ischemic heart disease patient , 12 male and 8 females were studied, there was a positive correlation between serum LDL cholesterol levels and age , but it was statistic not significant (p>0.439) , as shown in figure (2).

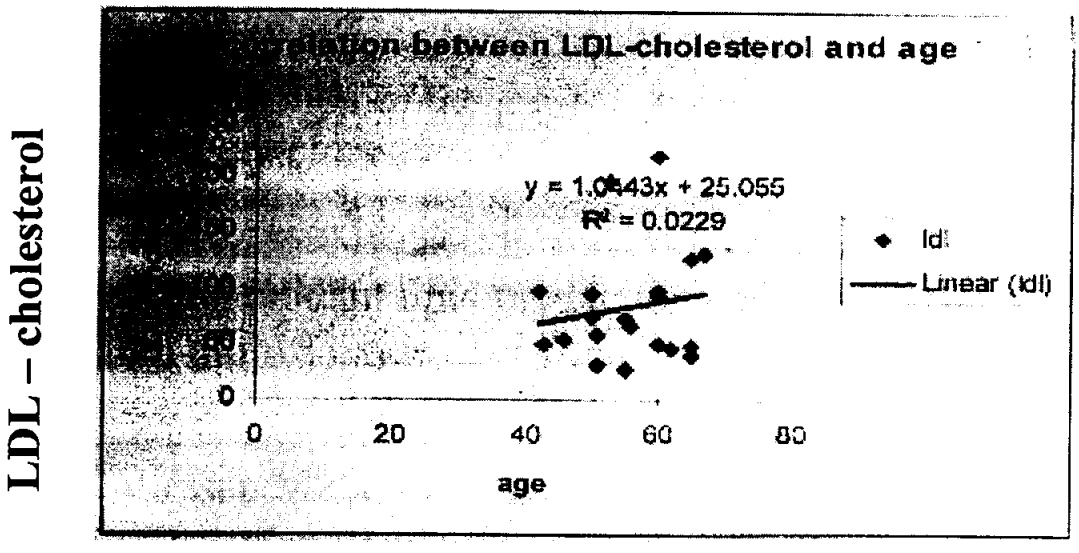

Figure (2) Correlation between LDL – cholesterol and age .

For all (20) patients with ischemic disease there was no different change between serum LDL cholesterol levels in male and serum LDL cholesterol levels in female patients with mean levels 86.2 for male , 78.8for female .

For the same patients, there was a positive correlation between serum total cholesterol levels and age, but it was statistic not significant (p>0.452), as shown in figure (3) And there was no correlation between serum total cholesterol levels and sex.

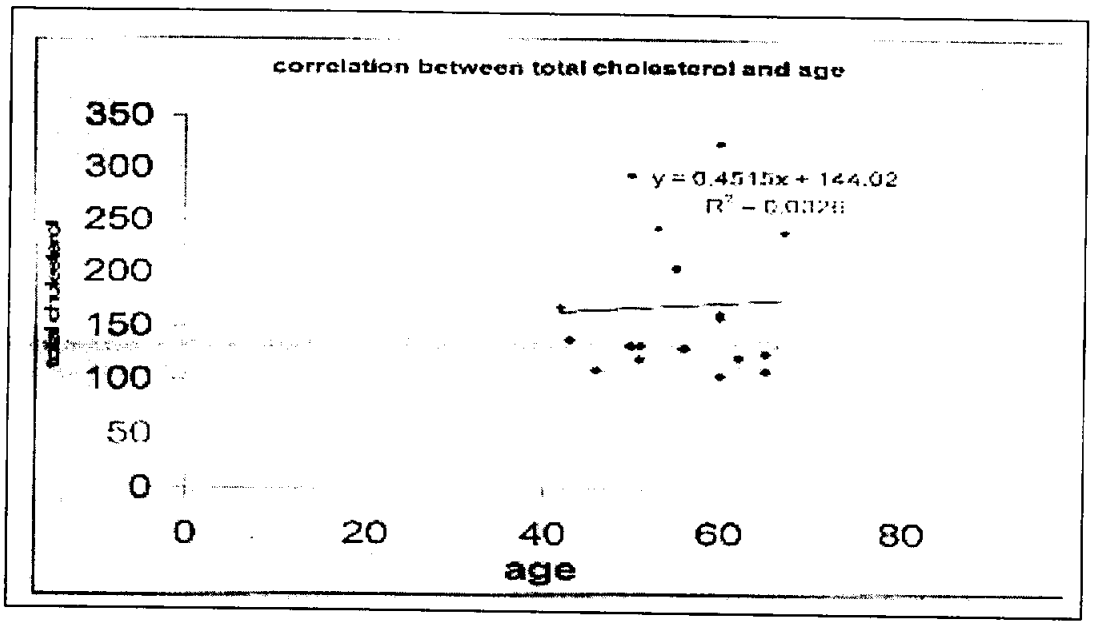

Figure (3): Correlation between total cholesterol and age.

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

*Myeloperoxidase is leukocyte enzymes that generates reactant oxidant lipid production and vasoconstriction from nitrous oxide depletion.

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

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

*On analysis, a significant difference between serum MPO-IgG levels with aging is found .

*High levels of LDL-cholesterol have consistently been shown to be associated with ischemic heart disease.

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