Investigation of the Relationship between High Sensitivity C Reactive Protein and some Myocardial Enzymes, and Oxidative Stress in Patients with Acute Coronary Syndrome (ACS)

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Abstract

Acute coronary syndrome (ACS) is a group of clinical syndromes characterized by rupture or invasion of coronary atherosclerotic plaque, resulting in complete or incomplete coronary occlusion. The present study was designed to determine and compare the levels of hsCRP, myocardial enzymes (CPK, LDH) and Oxidative stress (MDA, Cp, Tf) among patients with (ACS) & healthy individuals & assess the titer of hsCRP among different types of ACS to predict its role in risk stratifications of Acute coronary syndrome and healthy individuals. Serum high sensitivity C reactive protein, creatine phospho kinase, lactate dehydrogenase, malondialdehyde, ceruloplasmin, and transferrin levels were measured in 100 patients Acute coronary syndrome and 70 supposed healthy subjects. The levels of serum high sensitivity C reactive protein, creatine phosphokinase, lactate dehydrogenase, malondialdehyde, and ceruloplasmin, were revealed significant increase among patients with coronary artery disease as compared to control group whereas the levels of transferrin (Tf) showed a significant decrease in coronary heart disease patients in comparison to control subjects ($P \leq 0.01$). This study was also revealed the correlation between the concentrations of the measured parameters and hsCRP. This study revealed significant in hsCRP among patients with ACS. This study revealed significant in hsCRP among patients with ACS. Lipid peroxidation & oxidative stress is more prominent among patients with ACS comparison to healthy individuals, & this is mostly the leading cause of atherosclerotic processes & resultant complication of coronary occlusion.

Keywords: *Acute coronary syndrome, Coronary Heart Disease, high sensitivity C reactive protein, myocardial enzymes, Oxidative stress.*

Introduction

Acute coronary syndrome (ACS) is a group of clinical syndromes characterized by rupture or invasion of coronary atherosclerotic plaque, resulting in complete or incomplete coronary occlusion. It is a type of coronary heart disease (CHD), which is responsible for one-third of total deaths in people older than 35. Some

forms of CHD can be asymptomatic, but ACS is always symptomatic.⁽¹⁾ The a etiology of CAD is multifactorial, and a number of risk factors are known to predispose to the condition. Some of these-such as age, gender, race and family history-cannot be changed, whereas other major risk factors, such as serum cholesterol, smoking habits, diabetes and hypertension, can be modified⁽²⁾.

Inflammation plays a major role in atherothrombosis, and measurement of inflammatory markers such as hsCRP may provide a novel method for detecting individuals at high risk of plaque rupture. Several large-

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scale prospective studies demonstrate that hsCRP is a strong independent predictor of future myocardial infarction and stroke among apparently healthy men and women. The present data describing CRP within athermatous plaque⁽³⁾.

Creatine Kinase (CK-MB) is the enzyme being used as the definitive serum marker for the diagnosis or exclusion of acute myocardial infarction (AMI)⁽⁴⁻⁶⁾. This enzyme catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP), as shown below:⁽⁷⁾.

Lactate dehydrogenase (LDH, EC 1.1.1.27) is a hydrogentransfer enzyme that catalyses the oxidation of L-lactate to pyruvate with nicotineamide-adenine

dinucleotide(NAD)⁺ as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. The reaction is reversible and the reaction equilibrium strongly favors the reverse reaction, namely the reduction of pyruvate (P) to lactate (L)⁽⁸⁻¹⁰⁾.

Oxidative stress is characterized by an increased concentration of oxygen derived products that provoke critical, even irreversible, cell injury. Oxygen reduction leads to the synthesis of reactive intermediate compounds such as the superoxide anion, hydroxyl radical, hydrogen peroxide and per oxidative derivatives of polyunsaturated fatty acids(PUFA) such as conjugated dienes, lipid hydro peroxides and malonyldialdehyde (MDA)⁽¹¹⁾.

Free radicals and reactive oxygen species are too reactive to be tolerated in living tissues and their removal and control may have had a dominating evolutionary pressure with the first appearance of O₂ in the atmosphere. A hierarchy of mechanisms has been evolved to deal with these reactive intermediates⁽¹²⁻¹⁴⁾.

Figure 1: Effect of Free Radicals⁽¹⁵⁾.

ceruloplasmin is synthesized in the liver, and secreted into plasma⁽¹⁶⁾.

Transferrin is a major plasma protein of biological interest because of its evolutionary history and because of its multi regulatory control⁽¹⁷⁾.

Subjects and Methods

This study was conducted at AL-Hussein Teaching Hospital, especially coronary care unit (CCU) ,Biochemistry Laboratory, and the Hormones and immunes Laboratory. It included (170) subjects, control (70) and patients(100) diagnosed with(Acute Myocardial

Infarction and Un Stable Angina/ Non STEMI).

About(5mL)of blood samples of the patients with acute myocardial infarction(AMI),un stable angina (UA) /Non STEMI patients and controls were taken and allowed to clot at room temperature in empty disposable tubes centrifuge to separate it in the centrifuge at 3000 xg for 10min,the serum samples were separated and stored at (-20°C)until analyzed for High Sensitivity C Reactive Protein (hsCRP),creatinephosphokinase, lactate dehydrogenase malondialdehyde , ceruloplasmin , and transferrin . Serum(**hsCRP**) was estimated by enzyme linked immunoassay method byELISA Reader,USA using kit supplied by De meditec, Germany.

Methods

The following criteria are commonly found in the literature for the relation between the **hsCRP** values and the risk for developing CVD.

CRP values<1.0mg/L=Low risk for CVD.

hsCRP values 1.0-2.9mg/L=Intermediate risk for CVD.

hsCRP values >3.0mg/L=High risk for CVD.

Serum Creatine phospho kinase (**CPK**) was analyzed by enzymatic colorimetric method by UV/VIS spectrophotometer, Japan using kits supplied by Biolabo, France.

Serum Lactate dehydrogenase (**LDH**) was analyzed by enzymatic colorimetric method by UV/VIS spectrophotometer, Japan using kits supplied by Biomagrab, Tunisia.

Serum Malondialdehyde (**MDA**) was measured as thiobarbituric acid (TBA) activity by using the colorimetric method recommended, MDA level of the plasma was measured according to a modified method of Fong *et al*⁽¹⁸⁾.

Serum Ceruloplasmin (**Cp**) was measured by Menden *et al*⁽¹⁹⁾.It was based on the ceruloplasmin-catalyzed oxidation of colorless para-phenylene diamine (PPD) to blue-violet oxidize form. The reaction was followed photometrically and the blank value was determined after inhibition of the enzyme with sodium azide at (0°C).

Serum Transferrin (**Tf**) was analyzed by enzymatic colorimetric method by UV/VIS spectrophotometer, Japanusing kits supplied byBiolabo, France.

The results were expressed as mean $\pm$ standard deviations (mean $\pm$ SD). tow way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.01$) were considered statistically significant.

Person correlation coefficient (r) was used to test the correlation relationship among the different parameters in each patients group.

Findings

In this study we measured the level of **hsCRP**, **CPK**,**LDH** , **MDA**, **Cp**, and **Tf** among patients with different ACS(AMI,UA/Non STEMI , and healthy individuals).also the correlation between the concentration of **hsCRP** and(**CPK**,**LDH** , **MDA**, **Cp**, and **Tf**) were evaluated in this study.

The levels of serum **hsCRP** , **CPK**,**LDH** , **MDA**, and **Cp**, were showed significant increase among patients coronary artery disease as compared to control group whereas the levels of **Tf** showed a significant decrease in coronary artery disease patients in comparison to control subjects.

Also the titer of hsCRP was significantly elevated among patients with acute coronary syndrome (AMI,UA/ Non STEMI).

Table(1):-Serum high sensitivity C reactive protein concentrations of (control),(AMI) and(UA) groups

Group	n	hsCRP concentration (mg/L) mean± SD
control	70	1.33±0.33b
AMI	55	8.11±2.48a
UA	45	7.17±2.65a

* Each value represents mean ± SD values with non-identical superscript (a , b or c ...etc.) were considered significantly differences ($P \leq 0.01$).

n:number of subjects.

AMI: Acute Myocardial Infarction.

UA: Unstable Angina.

SD: Standard Deviation.

Table(2):-Serum CreatinePhospho Kinase and Lactate Dehydrogenase concentrations of (control),(AMI) and(UA) groups

Group	n	CPK concentration (U/L) mean± SD	LDH concentration (IU/L) mean± SD
control	70	215.44±49.06c	220.59±47.65b
AMI	55	674.78±134.76b	1694±541.98a
UA	45	834.24±276.38a	1584.72±509.67a

- Legend as in table (1)

Table(3):-Serum Malondialdehyde, Ceruloplasmin, and Transferrin concentrations of(control),(AMI) and(UA) groups

Group	n	MDA concentration (nmol/mL) mean± SD	Cp concentration (g/L) mean± SD	Tf concentration (g/L) mean± SD
control	70	59.33±16.21b	2.82±0.69b	10.43±2.57a
AMI	55	124.69±33.20a	3.68±0.78a	2.57±0.80b
UA	45	124.51±22.22a	3.93±0.76a	2.71±0.89b

- Legend as in table (1)

***Correlation relationship between hsCRP and all parameters in patients groups (AMI),(UA).

Figure(1) shows the positive correlation relationship between hsCRP and CPK in (AMI)patients group with coefficient correlation ($r = 0.48$) while in the (UA) patients group, negative correlation relationship between hsCRP and CPK with coefficient correlation ($r = -0.15$).

Figure(2) shows the negative correlation relationship between hsCRP and LDH in patients groups with coefficient correlation ($r = 0.23$) in group (AMI)

with coefficient correlation ($r = -0.34$) in group (AMI) and ($r = -0.31$) in (UA) group.

and ($r = 0.12$) in (UA) group.

Figure(3) shows the positive correlation relationship between hsCRP and MDA in patients groups with coefficient correlation ($r=0.26$) in group (AMI) and ($r = 0.31$) in (UA) group.

Figure(4) shows the positive correlation relationship between hsCRP and Cp in patients groups with coefficient correlation ($r=0.23$) in group (AMI) and ($r = 0.58$) in (UA) group.

Figure(5) shows the negative correlation relationship between hsCRP and Tf in patients groups

Figure (1): Correlation relationship between hsCRP and CPK in patients groups (AMI), (UA)

Figure (2): Correlation relationship between hsCRP and LDH in patients groups (AMI), (UA)

Figure (3): Correlation relationship between hsCRP and MDA in patients groups (AMI), (UA)

Figure (4): Correlation relationship between hsCRP and Cp in patients groups (AMI), (UA)

Figure (5): Correlation relationship between hsCRP and Tf in patients groups (AMI), (UA)

Discussion

Coronary Heart disease is one of the leading causes of death in most industrialized countries of the world and it is now also considered as a prominent health problem in developing countries⁽²⁰⁾.

This study shows an increased level of hs-CRP among patients of CAD .

There is increasing evidence that inflammation plays an important role in pathogenesis of atherosclerosis and its complications.

This finding is matched with the result of Ridker P. (2003)⁽²¹⁾, Liuzzo et al. (1994)⁽²²⁾ and Ferreiros et al. (1999)⁽²³⁾. The levels of hs-CRP were above 3µg/ml in all the patients, which is considered abnormally high .However, in all the control group had hs-CRP level <3µg/ml.

This results confers with the result of study conducted by Lansky et al.(2010)⁽²⁴⁾. Measurement of CK-MB is currently the test of choice to confirm the diagnosis of an AMI ,reported that increases in plasma levels usually occur between 6 to 10 hours after the onset of infarction (in the

absence of thrombolysis). The measurement total CK activity is due to myocardial or skeletal muscle injury (Adams *et al*, 1994)⁽²⁵⁾.

Lactate dehydrogenase activities has been observed in previous studies Burtis et al.(1994)⁽²⁶⁾.

LDH used for the diagnosis of acute myocardial infarction Wu et al.(1999)⁽²⁷⁾.

We observed that increased concentrations of MDA in the circulation of total AMI patients indicating increased lipid peroxidation. Our results are in accordance with previous reports (Senthil *et al.*, 2004)⁽²⁸⁾.

This finding is matched with the result of (Engstrom *et al.*, 2003)⁽²⁹⁾ found an increased level of serum ceruloplasmin in AMI patients, suggests that this molecule may act as an oxidative stress indicator, though mechanism remains unclear. It is an inflammation-sensitive protein and an acute phase reactant and (Hickman and Potter, 2003)⁽³⁰⁾. ceruloplasmin may have possible role in inflammation. In AMI group, All these results indicate that ceruloplasmin may be considered as

an inflammatory molecule.

In humans, increased plasma transferrin levels are found in iron deficiency anemia whereas decreased plasma transferrin occurs in conditions resulting in increased iron stores (Morgan, 1983)⁽³¹⁾. The low plasma transferrin concentration found in humans with increased iron stores may be due to a negative feedback of storage iron levels on transferrin synthesis (Aisen, 1984)⁽³²⁾.

Conclusion

From the data presented in this study, we can conclude that serum hsCRP may have a significant diagnostic role in Acute coronary syndrome (ACS) (AMI and UA). In both studied diseases, the diagnostic role of myocardial enzymes (LDH, CPK) was confirmed. Lipid peroxidation can be reflected by hsCRP according to the positive correlation between it and MDA. There is a disorder in antioxidant system in patients with (AMI and UA) according to the levels of ceruloplasmin and transferrin. Finally the serum levels of hsCRP may be used for risk stratification patients with (ACS) (AMI and UA) and its possible complication.

Conflict of Interest: Nil

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Ethical Clearance

Finally the ethical approval for this study was issued by the ethical committee of college of science of Thi-Qar university

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