Investigation of Malondialdehyde (MDA), C- reactive protein (CRP) and Homocysteine in Sera of Patients with Angina Pectoris

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Abstract

Homocysteine thought to be free indicators for the disease like coronary artery. Oxidative stress and free radicals plays important roles in the progressing of Angina Pectoris. Previously, scientists has proven that Oxidative stress exists in patients with cardiovascular diseases (CVDs), and hyperhomocysteinemia, Aiding in inducing production of oxygen free radicals thus determining level of oxidative stress, could help health professionals to predict these kind of diseases. The purpose of our work was to explore whether blood serum homocysteine Hcy levels in patients with Angina Pectoris (AP) has any impact on rate of their lipid peroxidation. To see the probability effect of homocysteine (Hcy) in the oxidative stress activations of those patients. To find levels of the plasma homocysteine(Hcy), plasma malondialdehyde (MDA) and C- reactive protein (CRP) were measured in 60 unstable Angina Pectoris patients, also 30 healthy volunteers has been examined. Hcy was measured by an enzymatic colorimetric method and MDA, an index of lipid peroxidation, by spectrophotometer. Serum Hcy levels seen to be bigger significantly patients with in angina pectoris (AP) in compare the control group (23.2±8.0 vs 10.76 ± 2.55 micromol/L; P <0.0001), Similarly, total MDA were significantly higher in angina pectoris (AP), patients than the controls (12.19 ± 0.68 vs 5.68 ± 0.45 nmol/L; P <0.0001).Our findings shows that tHcy and HsCRP increased levels could be related with the pathogenesis of angina pectoris (AP).

Keywords: Homocysteine, Malondialdehyde, Angina pectoris, C-reactive protein.

الخلاصة

تم اعتبار الهوموسيستين كعامل خطر مستقل لمرض الشريان التاجي من المعروف أن الإجهاد التأكسدي والجذور الحرة لها أدوار هامة في تطوير الذبحة الصدرية ,الإجهاد ألتأكسدي موجود في الأمراض القلبية الوعائية و إن فرط ارتفاع مستوى الهوموسيستين فى الدم، وهو عامل خطر مستقل لهذه الأمراض، قد تلعب دورا من خلال تحفيز إنتاج الجذور الحرة للأوكسجين.الهدف من هذه الدراسة هو تحديد العلاقة المحتملة بين مستوى الهوموسيستين في مصل الدم والدهون البيروكسيدوية في المرضى الذين يعانون من الذبحة الصدرية ومن أجل تقييم الدور المحتمل للهوموسيستين في إحداث الإجهاد التأكسدي في الذبحة الصدرية، تم قياس مستوى الهوموسستين (البلازما) والمالونديالدهيد البلازما والبروتين التفاعلي C- لدى ٦٠ مريضا مصابا بالذبحة الصدرية الغير مستقرة ، قمنا باختبار ٣٠ متطوعا من الأصحاء، وقد تم قياس مستوى الهوموسيستين بطريقة الأنزيمية اللونية وقد تم قياس مستوى المالونديالدهيد مؤشر البيروكسيد الدهون، بطريقة المطيافية الضوئية. وكانت النتائج: هناك زيادة معنوية في مستوى الهوموسيستين مصل الدم في مرضى الذبحة الصدرية (٢٣,٢ ± ٨,٠ وهى أعلى بكثير بالمقارنة مع مجموعة السيطرة ١٠,٧٦ ± ٢,٥٥ ميكرومول / لتر؛ P <0.0001)، وايضا هناك زيادة معنوية في مستوى المالونديالدهيد مصل الدم مرضى الذبحة الصدرية (١٢,١٩ ± ٠,٦٨ وهى أعلى بكثير بالمقارنة مع مجموعة السيطرة ٥,٦٨ ± ٠,٤٥ نانومول / لتر؛ P <0.0001). الاستنتاج: بينت النتائج التي توصلنا اليها أن المستويات المرتفعة لكل من الهوموسيستين والمالونديالدهيد وكذلك والبروتين التفاعلي C- مرتبطا بحصول مرض الذبحة الصدرية .

Introduction

Among European Union, 40% of deceases are caused by Cardiovascular Disease (CVD). Most known examples of coronary atherosclerosis include stable angina pectoris, myocardial infarction or sudden cardiac death [1]. There are different diseases of cardiac, some exists from an early age or at beginning age like rare malformations of the structure of the heart, and others take a lifetime to acquire such as the Angina Pectoris It mainly develops in male but females also affect it [2]   Angina pectoris caused when heart delivered a minimal dosage of oxygenated blood. Angina pectoris patients are known to be at danger positions to get heart attacks; the most common sign of angina pectoris is chest pain behind the breastbone. The discomfort may feel like pressure, squeezing, burning or tightness [3] .

As homocysteine (Hcy) level gets higher, It could serve as good factor indicating that the person at higher risk for having atherosclerosis. prothrombotic environment, platelet activation and endothelial leukocyte interactions occurred when endothelial function interfered as a result of changing in the Hcy levels.[4]. Inducing of inflammatory responses also can occur by Hcy changing level which can serve as indicators of atherosclerotic disease [5]. Markers of inflammation are explained by many studies [6]. Proposing that could be related to broad fields of the atherothrombotic process, could serve as indicator to anticipate coronary artery disease (CAD).   Inflammatory process in atherosclerotic plaques is represented by cytokines coming out from inflammatory cells. Another biomarker that is been depended on to anticipate CAD Is the C-reactive protein (CRP) .Examining CRP level during presentation or discharge, could play a diagnostic role in the anticipation of acute coronary syndrome (ACS), as these was declared by many previous works. Amino acid called Homocysteine, which is in the methionine metabolism, a process occurring outside the structure of proteins. By a reaction catalyzed by vitamin B-6, Hcy turned into 1 -cystathione and discharged to outside of the body while 2 - methionine catalyzed by folic acid and vitamin B-12.

All tissues have Homocysteine in low dosage but it become accumulated when depletion occur in those catalytic vitamin. Previously has been shown by [7] that another independent factor could serve in anticipation of cardiovascular diseases is hyper. In the most tissues, Oxidative metabolism of normal cells delivers free oxygen radicals have the have potential damages to the cell structures, namely different biomolecules like nucleotides and lipids. Based on this method, Researchers have proven when endogen antioxidants not work properly to detoxify those toxic radicals, deformation will occur in the cell`s morphology and function [8]. Various previous animal researchers declared that the increased levels of Hcy in accordance with reduced activities of antioxidants and increased lipid peroxides [9].

MDA as previously identified as a byproduct of lipid-peroxidation [10] and the MDA level determination by the thiobarbituric acid reaction could serve as a good biomarker to predict whether a lipid peroxidation process has occurred. Recent research also found that rising lipid peroxidation is induces different biological mechanisms like platelet activation and tissue destruction leading to serious disease [11-13].
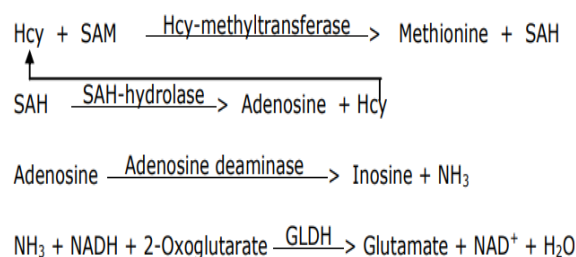
Materials and Methods

Our study was performed on the sixty patients during March 2017 and May 2017, in which patient's gender were (47 males) and (13 females). The patient`s average age were (51.5±7.8 years) where the minimal age was 35 and maximum age was 52 yrs. The work was took place in the cardiac hospital and Nanakaly hospital located in Erbil province, where cases are decided by health professionals based on the clinical criteria, normal electrocardiographic, enzymatic analysis. Samples also have been drawn from 30 healthy volunteers (decided by health professionals) and their analytical data was added to our study as control groups. The gender of control groups were divided into (21 males) and (9 females).

The volunteer`s average age were (40.5±6.7 years). In which the youngest was 33 and oldest was 46 yrs. Randomization were considered in picking the samples. Cubital vein has been depended on to draw a blood sample at different time of the studied period. In order to study the daily evolution of the cases, also to precise the exact momentum, the samples were run on the centrifuge to collect the serum in order to be tested for the MDA and Hcy level.

Instrument and Chemicals

-Spectrophotometer
- Evacuated tubes containing heparin
- Thiobarbituric acid (TBA) 99% pure was purchased
- malondialdehyde tetrabutylammonium salt (MDA salt)
- Methanol 99.8% pure
- Glacial acetic acid (99–101% pure)
- Ultrapure deionized double distilled water
- L-Homocystine, D, L-DTT
- Formic acid, ammonium salt

To determine the peroxidation level of the Lipid, we used a technique which is based on the estimation of thiobarbituric acid reactive substances (TBARS). A byproduct of reaction of MDA with thiobarbituric acid was put into the spectrophotometer at speed of 532 nm, in which the tetrametoxypropane was bank on as a standard solution. The measured parameter were expressed as nmol/ml. Serum total homocysteine was determined by enzymatic colorimetric method for the quantitative determination of total homocysteine [14], using Globe diagnostics kit, Italy. The Principle The analysis of enzymes like homocysteine usually depends on the chains of reactions between compounds resulting in the decrease of absorbance value because of the oxidation of NADH to NAD+. In the equation, the density of Hcy is proportional directly with amount of transformed NADH to NAD+. As Explained bellow:

$$Hcy + SAM \xrightarrow{Hcy\text{-}methyltransferase} Methionine + SAH$$

$$SAH \xrightarrow{SAH\text{-}hydrolase} Adenosine + Hcy$$

$$Adenosine \xrightarrow{Adenosine\ deaminase} Inosine + NH_3$$

$$NH_3 + NADH + 2\text{-}Oxoglutarate \xrightarrow{GLDH} Glutamate + NAD^+ + H_2O$$

- Hcy: Homocysteine
- SAM: S-adenosylmethionine
- SAH: S-adenosylhomocysteine
- GLDH: Glutamate dehydrogenase

Statistical Analysis

The data in our research were presented in means ± SD. The SPSS program was depended to evaluate all the parameters in our research. The considered p-value was <0.05 to be the edge of the significance level. To explore the variety among different groups, a normal T-test hypothesis has been calculated on. Along with that, our result also bank on chi square and ANOVA test to explore the distinct group from other groups.

Results and Discussion

The final data of our work has been expressed in the table below, in which the Table (1) & Figure (1) shows the average serum homocysteine levels of the study population. There was a significant increase (p<0.0001) in the mean level of homocysteine in AP patients compared to controls (23.2±8.0 vs. 10.76 ± 2.55μmol/L). Similarly Multiple past studies have reported Significant raised total serum homocysteine level in angina Pectoris [3,5,6].

Table1: Shows Serum (Hcy) levels, (MDA) levels and CRP in patients plus control group.

| Serum parameters	SAP patients (n=60)	Controls (n=30)	P-value
Hcy (μmol/L)	23.2±8.0	10.76±2.55	P< 0.001
MDA (nmol/mL)	12.19±0.68	5.68±0.45	P< 0.001
CRP (mg/L)	6.8634±1.901	1.16±2.03	P< 0.001

Mean±SD

Serum MDA levels of the study population

The MDA level of the cases represented as mean ± S.D (12.19±0.68 nmol/L), whereas in control group it was (5.68±0.45 nmol/L) There were a significant differences between the two groups (p<0.0001) as shown in (Figure 2) & Table 1. This study is in agreement with another study done by researchers in 2004 that showed the levels of Malondialdehyde Serum were significantly elevated in patients suffering from angina pectoris [10]. The level of CRP was significantly higher in Angina Pectoris (AP) patients (6.8634±1.901mg/L) as compared to control subjects (2.03±1.16mg/L) as shown in Table1.
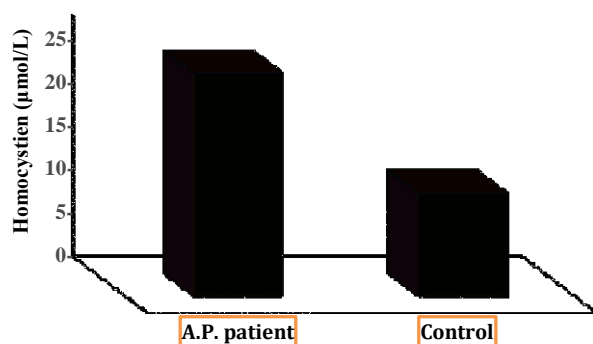
Figure 1: Serum homocystein (Hcy) levels in the patients group with angina pectoris and control group.
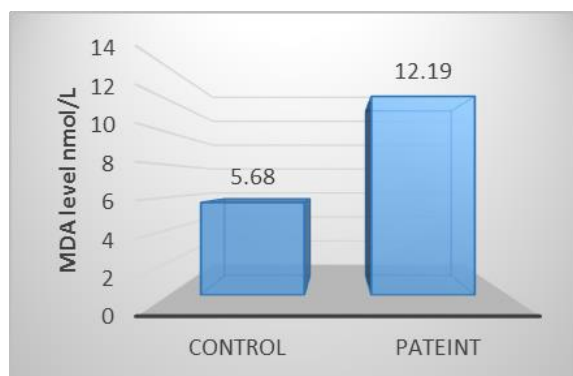
Figure 2: Serum Malondialdehyde levels in the patients with angina pectoris and control groups.

The current work provides additional data proving that the mean level of homocystein was a significantly higher in AP cases compared to controls. This means that high homocysteine levels are linked to CVD. This finding is in agreement with that demonstrated by [15; 7]. In addition, [16] reported that a rising of 5 μmol/L in the homocysteine level increased cardiovascular mortality by 50%. To determine the linkage between homocysteine and atherosclerosis, various theories has been established, ideas like Growth stimulation of smooth muscles, reduced growth of the endothelial cells, loosening of endothelial cells and dropping in the High DL production [10; 6].

Our data results are in a accordance with previous works done by many researchers from different countries showing a significant raised total serum homocysteine level in ischemic heart disease (unstable angina)[16-22] .It might be due to deficiency of vitamins B6, B12 and folic acid deficiency. Previous works also explained that homocysteine compound undergoes metabolism by transsulfuration and remethylation pathway. In transsulfuration pathway, homocysteine by the aid of serine give rise to cystathionine. This reaction is catalyzed by vitamin B6 which is based on the cystathionine beta-synthase. Cystathionine readily broken down to produce cysteine, the later turned into sulfate by another metabolism process which finally dropped into urine. Due to deficiency of vitamin B6, this transsulfuration pathway is hampered which causes the homocysteine in the total serum to be increased (17; 18). In remethylation pathway, the cobalamine (vitamin B12) catalyzes a reaction in which homocysteine is retained back to methionine by transfer of a methyl group from 5 -Methyltetrahydrofolate. Folic acid also needed by pathway of Remethylation. Deficiency of vitamin B12 and folic acid results in deactivating of the remethylation of homocysteine which causes rising in the total serum levels of homocysteine. Raised serum homocysteine induces vascular dysfunction, platelet generation of thromboxane A2 and platelet aggregation through oxidative stress and also by inhibiting nitric oxide synthesis. Thromboxane A2 released from platelet causes coronary vasoconstriction, platelet aggregation and plate let plug formation. Reduced nitric oxide affects vasodilation of endothelials which requires nitric oxide. These events may lead to formation of unstable angina[3; 23-29] .The presented data shows that the level of MDA, which serve as an indicator of oxidative

stress has been risen significantly in AP patients in compare to MDA level of the control group (Figure 2).

The reason why MDA level is high is still obscure, but some believe that prostaglandin`s activity is the cause [30]. As previously has been concluded by many researchers [31, 32, 33] that conditions like hypoxia, myocardial ischemia and plate let aggregation leads to rising in the releasing rate of the prostaglandin. Despite the resultant of lipid peroxidation which has maximum effect on the rising of MDA level. [34; 35]. And Because of during atherosclerotic reaction the MDA which is transported by low-density lipoproteins (LDL) and attached to it, prefers to combine with the cholesterol esters that are present in those cells, resulting in maximum damage to contents of that cells and tissues [36; 37]. Scientist also declared that prostacyclin biosynthesis becomes reduced by the peroxidation of the lipids [38]. In the light of the previous findings, scientists has come to fact that the increased level of MDA in AP patients could Aid as indicator to announce that the lipid peroxides probably embodiment into the arterial as a result vascular anti aggregate defense may be decreased and atherosclerotic lesion possibly formed.

Another parameter which we considered in those patients is Serum C-reactive protein (CRP) values, which were greater significantly in the patients than the control group (6.8634± 1. 901mg/L) vs. (2.03± 1.16mg/L). Previously many studies [39; 40; 41] has been shown that CRP, which serves as an indicator of inflammation, becomes risen in the Angina pectoris patients. Scientists also established that pro-inflammatory reactions could be enhanced by the ischemia lasting about 15 minutes pursed by perfusion. The outcome would be the production of large amount of free radicals. At that time when the immune system will be stimulated to produce the specific proteins to fight the toxic materials before damaging the cardiac cells and tissues. That's why it`s very necessary to check for CRP in those patients to prevent or delay further complications which may occur in the AP patients.

Conclusions

The present data shows that there was a significant variables in the Homocysteine (Hcy) and malondialdehyde (MDA) levels inside the serum of the cases with Angina Pectoris in compare to randomized healthy controls. Based on our data, we come to the fact that the Homocysteine (Hcy) and malondialdehyde (MDA) levels in the blood serum may play critical role in the analysis and predictions of likely Angina Pectoris occurrence. Also, detections of mentioned parameters in AP patients, may assist healthcare for better management and may be control further organ damages that AP patients could face as these disease continued.

Acknowledgement

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