OXIDANTS AND ANTIOXIDANTS AS RISK FACTORS IN YOUNG ARABIAN MALE PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

NAJAH AL-MUHTASEB1, ELHAM AL-KAISSI2*, ZUHAIR MUHI-ELDEEN1, TAWFEEQ ARAFAT2, SABAH AL-MUHTASEB2, HANI ATIYAH4

1Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, University of Petra, Amman, Jordan, 2Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Petra, Amman, Jordan, 3Department of Medical Allied, Zarqa University College, Al-Balqa Applied, University, Salt, Jordan

ABSTRACT

Objective: This study aims to investigate the levels of oxidative stress, antioxidants besides uric acid, C-reactive protein (CRP), lipid profile and cardiac biomarker enzymes in young men admitted to the hospital for the first time with acute myocardial infarction (AMI), to investigate any relationship between them.

Methods: 135 young men age < 40 y old, admitted to the cardiology unit with suspected MI and 130 age and sex matched healthy controls were included in this study. Blood samples were collected from the patients and the control group. The blood samples were collected from the patients on the day of admission and on the day of discharge.

Results: The levels of xanthine oxidase (XO), malondialdehyde (MDA), CRP, uric acid, total cholesterol (TC), total triglyceride (TG), low-density lipoprotein (LDL-C), apoprotein-B 100 (Apo B), and cardiac biomarker enzymes were significantly high, whereas catalase, vitamin C, high-density lipoprotein (HDL-C) and apoprotein-A1 (Apo A1) were significantly low on the day of admission (Time A) and slightly higher on the day of discharge (Time B), but both were still lower than the controls. There was a decrease in XO and MDA activity and an increase in catalase activity and vitamin C level.

Conclusion: These results may indicate possible relationships between these parameters and AMI.

Keywords: Acute myocardial infarction, Xanthine oxidase, Malondialdehyde, Antioxidants, Lipid profile

INTRODUCTION

Cardiovascular diseases, particularly acute myocardial infarction (AMI), are the most alarming health predictors of the new millennium and are considered to be the leading cause of death worldwide [1-3]. Nearly half of these deaths are more likely to occur in young and middle-aged individuals [2]. In the younger age population, the rate of AMI is 5% [3]. AMI is commonly known as “heart attack” results from ischemic necrosis of a variable amount of myocardial tissue as a result of an abrupt acute decrease in coronary flow or increase in myocardial demand for oxygen, which cannot be supplied by obstructed coronary artery [4-6].

The assessment of the myocardial injury varies dramatically with the detection of aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase isoenzymes (CK-MB), and troponin (T) which add prognostic information regarding acute short-term or chronic long-term risk and the severity of the injury [6-7].

Growing evidence support the involvement of oxidative stress due to an imbalance between the production of reactive oxygen species (ROS) (including the superoxide anion, hydrogen peroxide, and hydroxyl radical) and endogenous antioxidant defense mechanism [1] which plays an important role in the pathogenesis and progression of heart failure [8-10]. The imbalance can exert profoundly deleterious effects on endothelial function and monocytes migration [8]. An oxygen free radical generation has been shown to be an important mechanism of cellular injury in the ischemic myocardium [11]. Several mechanisms have been proposed to be involved in the generation of oxygen free radicals, but xanthine oxidase has been shown to be a major source of free radical generation under ischemic conditions [12-13]. Xanthine oxidase (XO) is an enzyme that catalyzes the chain reactions of hypoxanthine oxidizing to xanthine and xanthine oxidizing to uric acid and hydrogen peroxide [14]. Under ischemic conditions, XO is produced. It acts on both hypoxanthine and xanthine at the expense of molecular oxygen producing superoxide ion [15]. Therefore, in ischemic conditions of the heart such as MI, XO plays a pathologic role in heart failure [16].

Uric acid production is elevated in association with increased xanthine oxidase activity. Elevated levels of uric acid correlate with impaired hemodynamics [17] and independently predict an adverse prognosis in heart failure [17] and therefore elevated serum uric acid may act as a marker of underlying tissue ischemia [18].

CRP is an emerging risk marker that is recommended to complement the assessment of patients at primary cardiovascular risk and to a more limited extent, stable patients at secondary risk. Several trials have demonstrated the strong predictive value of CRP in stable and unstable angina, independent of troponin and burden of atherosclerosis [19-20].

Oxidative stress and inflammation are two processes that appear to have a significant role in the risk of AMI [21-22]. Increased levels of low-density lipoprotein cholesterol (LDL-C) are known as one of the major risk factors in atherosclerotic disease [23]. Epidemiological studies have shown that apolipoprotein B-100 (Apo B), with or without Apo A1, is a better predictor of coronary artery disease than LDL-C and other non-HDL lipoproteins [24-25]. One of the most frequently used biomarkers indicating lipid peroxidation is plasma concentration of malondialdehyde (MDA). This molecule is one of the end products of lipid peroxidation in the cell membrane or in LDL-C [26-27].

Antioxidants are either endogenous or exogenous compound that prevent the generation of harmful free radicals, reduce the generated radicals, inactivate their harmful reactivity, and thereby block the chain reactions of these oxidants [27].
Myocardial antioxidants inhibit or delay the oxidative damage to subcellular proteins, carbohydrates, lipids and DNA. This is achieved mainly by the activities of enzymatic antioxidants such as catalase or non-enzymatic antioxidants such as vitamin C. These have been reported as predictive indices of CAD [28]. Catalase is believed to play a major role in the first line of enzymatic antioxidant defense. Catalase reacts efficiently in peroxisomes with hydrogen peroxide (H₂O₂) to form water and molecular oxygen [29-30]. It was observed that antioxidant vitamin C abolished the malondialdehyde-induced negative contractile response which suggests that MDA may elicit its inotropic effect associated with enhanced oxidative stress in the heart. Antioxidant vitamin C also had been shown to stimulate the immune system; shown to block damaged pathways through the stimulation or maintenance of T cell proliferation in response to infection [31].

The aim of this study is to assess the extent of oxidative stress such as xanthine oxidase (XO) and MDA levels, the levels of enzymatic antioxidants such as catalase and non-enzymatic antioxidants vitamin C, alongside the measurement of CRP, uric acid, lipid profile, and cardiac biomarkers enzymes in young Arabi AMI patients and compare them to healthy controls.

MATERIALS AND METHODS

135 male inpatients, aged 38.2±5.3 y with BMI <25 kg/m² and AMI admitted to the hospital for the first time were investigated. The study included 130 age, sex, height, and body weight-matched group of healthy individuals were set as control. Clinical examinations of all patients were performed to detect any co-existing disorder to be excluded. All patients were on medical therapy and none of them were smokers or on anti-hyperlipidemia, antioxidant or vitamin supplements medication. Consent for all procedures was obtained from the patients and the healthy controls. The study was approved by the hospital’s ethics committee and was in accordance with the ethical standards. Diagnosis of AMI was based on the clinical history, typical chest pain lasting 30 min or longer, electrocardiographic (ECG) signs of infarction and elevated serum enzymes, including CK, AST, LDH, Xo enzymes MB (CK-MB) and troponin T within 12h after the onset of pain. Both patients and healthy controls were subjected to routine investigations.

Preliminary evaluation

10 ml of venous blood samples were collected from patients with AMI and from the controls by vein puncture. For this study, the blood samples of AMI patients were collected on the day of admission (Time A) and on the day of discharge from the hospital (after 5-7d, Time B). The blood samples were centrifuged at 3000 rpm for 10 min after allowing the blood to clot at room temperature. The serum was assayed immediately to detect the enzymatic markers of tissue damage. AST, LDH, CK, CK-MB were measured using the standard technique (Cobas 8000 Analyzer, Roche Diagnostics GmbH, Germany). Troponin T was analyzed using commercially available sandwich ELISA kit (Roch Diagnostics, Germany) and performed as described by the manufacturer. Results were expressed as unit/l (IU/l) of enzyme activity evaluated in serum. CRP levels were determined by a Nephelometric method using Beckman Array protein system, USA.

Biochemical parameters detection

Biochemical parameters were detected in the sera of the patients and the controls. TC was measured by an Enzymatic (CHOD-PAP) colorimetric method [32]. TTR was measured by an Enzymatic (GPO-PAP) method [33]. HDL-C was estimated by a Precipitant method [34], and LDL-C was estimated using Friedewalds formula [35] as has been shown LDL-C=TC–HDL-C–(TG/5), glucose [36], uric acid [37], Serum Apo A1 and Apo B were measured by Immuno nephelometry (Dade Behring, Marburg, Germany).

The estimation of serum lipid peroxidation (MDA), XO and antioxidant (vitamin C and catalase)

Lipid peroxidation product (MDA) was estimated by measurement of thiobarbituric acid reactive substances in plasma by the method of Buege and Aust [38]. The pink chromogen produced by the reaction of the thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated. The absorbance of the clear supernatant was measured against reference blank at 535 nm.

XO catalyzes the oxidation of hypoxanthine to xanthine and then catalyzes the oxidation of xanthine to uric acid. It was estimated by the method of Nishino [39]. The rate of formation of uric acid is determined by measuring increased absorbance at 290 nm. A unit of activity is that forming one µmol of uric acid/min.

Vitamin C was estimated by the method of Roe and Kuether [40] on the basis of the oxidation of ascorbic acid by copper followed by treatment with 2,4-dinitrophenylhydrazine that undergoes rearrangement to form a product with an absorption maximum at 520 nm. Catalase activity was measured according to the Sphoethrometric method of Goth [41]. This assay is based on the ability of hydrogen peroxide to form a stable stained complex with ammonium molybdate measured at 405 nm.

Statistical analysis

Statistical analysis was carried out using Student’s t-test by statistical packages for social science software (SPSS). Values are expressed as mean±SD and values of p<0.05 were considered statistically significant. The relationships between variables were calculated using Pearson Correlation Coefficients.

RESULTS

The levels of the cardiac biomarkers in the serum of young AMI patients and in the controls are shown in table 1.

Table 1: Serum levels of cardiac markers in young male AMI patients and in the controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls n=130</th>
<th>Patients n=135</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Admission day (time A)</td>
<td>Discharge day (time B)</td>
</tr>
<tr>
<td>AST (IU/I)</td>
<td>23.2±3.3</td>
<td>396±59.1*</td>
</tr>
<tr>
<td>LDH (IU/I)</td>
<td>185.3±23.7</td>
<td>2684±39.6*</td>
</tr>
<tr>
<td>CK (IU/I)</td>
<td>68.2±18.7</td>
<td>131.2±21.6*</td>
</tr>
<tr>
<td>CK-MB (IU/I)</td>
<td>13.2±4.7</td>
<td>88.62±13.4*</td>
</tr>
<tr>
<td>Troponin T (ng/ml)</td>
<td>0.031±0.01</td>
<td>1.16±0.27*</td>
</tr>
</tbody>
</table>

Values represent the means±standard deviation, AST, aspartate transaminase; LDH, lactate dehydrogenase; CK, creatine Kinase; CK-MB, isoenzyme, T, troponin.

*P<0.0001 significant differences between AMI patients (Time A & B) and control subjects.
†P<0.0001 significant differences between AMI patients in (time A) and (time B).

No significant differences were seen between the AMI patients in (time B) and control subjects.

There were significant increases in the serum levels of AST, LDH, CK, and CK-MB, and troponin T between AMI patients (time A) when compared with AMI patients at (time B) and the control subjects. There was no difference in the serum levels of AST, LDH,
CK, and troponin T between AMI patients (time B) when compared to the control subjects. The changes in the biochemical parameters in the AMI patients are shown in table 2.

**Table 2: Comparison of biochemical changes in young male AMI patients and in the controls**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls n=130</th>
<th>AMI n=135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time A</td>
<td>Time B</td>
<td>Time A</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.51±0.081</td>
<td>5.88±1.01*</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>98.6±10.41</td>
<td>103.55±12.31</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.91±0.81</td>
<td>7.91±1.91*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>169.6±20.66</td>
<td>249.81±30.32*</td>
</tr>
<tr>
<td>Total triglyceride (mg/dl)</td>
<td>113.18±18.1</td>
<td>250.89±33.89*</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>104.11±13.91</td>
<td>165.71±22.81*</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>56.63±12.31</td>
<td>39.44±9.21*</td>
</tr>
<tr>
<td>Apo A1 (mg/dl)</td>
<td>145.13±13.61</td>
<td>120.6±8.81*</td>
</tr>
<tr>
<td>Apo B (mg/dl)</td>
<td>98.33±14.33</td>
<td>156.91±18.44*</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein, LDL, low-density lipoprotein, HDL, high-density lipoprotein, Apo, apolipoproteins. Values represent the mean±standard deviation.

*|P<0.0001; †P<0.01 significantly different between AMI patients (time A & B) and the Controls. 

|P<0.001; *P<0.01 is significantly different between AMI patients on the day of admission (time A) and the day of discharge (time B)

|P<0.0001; †P<0.01, significantly different between the day of discharge of patients and the controls.

The comparison of biochemical parameters in the controls and in the AMI patients is shown in table 2, and indicate that the patients had a significant increase in the levels of CRP, uric acid, TC, TG, LDL-C, Apo B levels but significant decrease in HDL-C and Apo A1 levels as compared to the controls on the day of admission and at the day of discharge. In this study, serum CRP levels in AMI patients significantly higher when compared to the control on the day of admission and at the day of discharge. As expected in this study, the changes in each parameter had significantly increased at the day of discharge in comparison to the controls.

Table 3 shows circulating oxidants and antioxidants in young AMI patients and in the controls. As shown in table 3, serum xanthine oxidase and malondialdehyde (MDA) activity were significantly higher in AMI patients (time A and slightly less in time B) but yet the levels were higher than in the controls.

**Table 3: The levels of the circulating oxidants and antioxidants in young male AMI patients and in the controls**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls n=130</th>
<th>AMI n=135</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time A</td>
<td>Time B</td>
<td>Time A</td>
</tr>
<tr>
<td>X0 units/mg protein</td>
<td>0.011±0.006</td>
<td>0.071±0.008*</td>
</tr>
<tr>
<td>Serum MDA (μmol/L)</td>
<td>0.98±0.071</td>
<td>1.64±0.60*</td>
</tr>
<tr>
<td>Serum catalase (μmol of H2O2 consumed/min/mg protein)</td>
<td>59.1±9.31</td>
<td>41.34±6.21*</td>
</tr>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>1.31±0.23</td>
<td>0.54±0.31*</td>
</tr>
</tbody>
</table>

Values represent the mean±standard deviation, X0; xanthine oxidase, MDA; malonaldehyde.

*P<0.0001; †P<0.01 significantly different between AMI patients (time A & B) compared to the control subjects

|P<0.001; *P<0.01 is significantly different between AMI patients on the day of admission (time A) and the day of discharge (time B)

|P<0.0001; †P<0.01 significantly different between the day of discharge of patients and the controls.

On the other hand, when comparing the levels in the AMI patients on discharge time (time B) to the levels at the admission time (time A), the AMI patients in time A had significantly lower levels of uric acid, TC and LDL-C and apo B, but no change in apo A1 and significantly higher levels of HDL-C.

Table 3 shows circulating oxidants and antioxidants in young AMI patients and in the controls. As shown in table 3, serum xanthine oxidase and malondialdehyde (MDA) activity were significantly higher in AMI patients (time A and slightly less in time B) but yet the levels were higher than in the controls.

The activity of antioxidant catalase and vitamin C were significantly low in AMI patients at time A and slightly higher at time B but significantly lower than the controls.

**DISCUSSION**

Myocardial infarction or heart attack results from the interruption of blood supply to a part of the heart, causing heart cells to die after about 30 min of anoxia. As a result of cell death, the acute inflammatory response cause leukocytosis, increased levels of acute phase reactant proteins in the circulation, particularly C-reactive protein [14, 42], elevated markers of inflammation as CRP which are associated with increased risk of further cardiovascular disease as AMI [19], and CRP appears to provide predictive value for short-term prognosis in AMI [18]. In this study, serum CRP levels in AMI patients are significantly higher when compared to the control subjects on the day of admission and at the day of discharge. In addition, due to the increase in the levels of CRP, liberation of intracellular contents and their appearance in the circulation was detected, particularly enzymes measured for the purpose of diagnosing AMI including CK, CK-MB, AST, LDH and troponin T. Elevated levels of these enzymes, especially CK-MB, have been regarded as biochemical markers of myocyte necrosis, and still have a place in defining AMI [43]. In this study, increase CK-MB levels were found in patients with AMI as compared to the controls. The peak is usually on the day of admission and returns to baseline level at the day of discharge. As expected in this study, the changes in each of the serum enzyme activities after AMI, measured on the day of admission and on the day of discharge, exhibit a specific time course as shown in table I. Our results are in accordance with other reports [6, 42, 44, 45]. The troponins are highly sensitive and specific markers of myocardial damage [43]. In accordance with this, our results indicate high levels of serum troponin in patients with AMI in the day of admission followed by a decline not reaching the baseline at the day of discharge in comparison to the controls.

Several studies have identified the importance of serum uric acid concentration in young populations in predicting the risk of cardiovascular disease, such as AMI [46]. This study shows that serum uric acid levels are higher in younger patients with AMI on the day of admission and the day of discharge when compared to the control. Serum uric acid has antioxidant properties and contributes to free radical scavenging activity in human serum [47]. Thus, uric acid can be protective against oxidative stresses, but it can also lead directly or indirectly to vascular injury [47]. High levels of serum uric acid may induce endothelial dysfunction by decreasing the production of nitric oxide, which is a potent vasodilator in the...
vascular endothelial cells [47]. In this study, there is a significant increase in serum uric acid concentration. Our results agree with most of the other studies that suggest that elevated serum uric acid may act as a marker for tissue ischemia, cardiac failure and myocardial infarction [18, 19].

In AMI, dyslipidemia has been reported to be the most threatening risk. Most of the previous lipid profile studies in AMI patients found higher TC, TG, LDL-C apo B and lower HDL-C and apo A1 [48-49], which agrees with our finding in young AMI patients. Numerous researchers have confirmed the association between a low HDL and increased risk of coronary heart disease. The levels of LDL correlates positively, whereas the levels of HDL correlates negatively [50]. This is in accordance with our results. HDL is considered the most important protective factor, which participates in the reverse transport of cholesterol [51]. Increased oxidative stress and the generation of free oxygen radicals result in the modification of LDL to oxidized LDL that could lead to atherosclerosis lesions [51-52].

Oxygen free radicals which are responsible for elevated actions of oxidative stress has been considered as one of the most important mechanism of cellular injury and progression of atherosclerosis in ischemic myocardial infarction [11]. Oxidative stress is associated with increased formation of reactive oxygen species (ROS) that modifies phospholipids and proteins leading to peroxidation and oxidation of thiol group [53]. It is also associated with abnormalities in the myocyte function [53-54]. These effects correlate with an increase in MDA in sarcolemma [53]. Increased oxidative stress is recognized to be important in the pathogenesis of cardiovascular diseases [18, 54]. The study of the interaction of free radicals with lipids can be readily carried out to assess the free radical-mediated damage. Lipids, when reacted with free radicals, undergo peroxidation to form lipid peroxides. Lipid peroxides decompose to form numerous products including malondialdehyde. Our data show that MDA increased significantly in AMI on the day of admission when compared to the levels on the day of discharge. The decrease in levels did not reach the control levels. The increase in the levels of MDA in the sarcolemma of cells with AMI indicates an increase in lipid peroxidation and is one of the significant markers that indicate oxidative stress associated with AMI and also agrees with previous studies [48, 55]. The changes in the levels of plasma lipids as shown in our study are complications frequently observed in AMI patients [50].

XO catalysis of the reaction that generates oxygen free radicals is considered one of the most significant. The enzyme acts in the metabolism of purine, converting both hypoxanthine and xanthine to uric acid at the expense of molecular oxygen to produce superoxide ions, which oxidize cellular proteins and membranes resulting in myocardial cellular injury [52, 56]. In the present study, the finding of high levels of XO activity in the blood of patients with AMI compared to the levels in the control subjects indicate that myocardial ischemia has a definite correlation with XO activity, suggesting that XO is specifically involved in the mechanism of peripheral endothelial dysfunction, and it could play a crucial role in the generation of ROS in the body [16]. XO can be used as a biochemical indicator of AMI, along with Electrocardiography observations [56]. Under normal physiological conditions, ROS production is balanced by an efficient system of antioxidants which are molecules that are capable of scavenging ROS and thereby preventing oxidative damage. At the cellular level, these are naturally occurring enzymatic antioxidants such as catalase and non-enzymatic antioxidant such as water soluble antioxidant vitamin C. In this study catalase and vitamin C levels were significantly lower in AMI patients than in the control subjects, suggesting that AMI is able to increase oxidative stress to the point of severe damage to the antioxidant system, which is unable to prevent oxidative stress and inflammation [57].

CONCLUSION

This study shows a significant increase in lipid peroxidation, in total oxidant status and a significant decrease in antioxidant status in young male patients with AMI. This indicates an imbalance between oxidant and antioxidant molecules requiring rectification as this can cause other comorbidities. The association between oxidant stress parameters, antioxidant markers, the inflammatory index and the lipid status parameters suggest their involvement in AMI development.

We conclude that apart from the enzymatic markers of tissue damage and lipid profile, other factors may increase the risk of further myocardial events. An example is inflammatory markers like CRP which may need to be monitored at regular intervals. Future prospective studies in larger data include different populations are needed to confirm our data. Nevertheless, this finding should be supported by research that is more extensive in order to put it into practice.

ACKNOWLEDGEMENT

The authors would like to thank the University of Petra/Faculty of Pharmacy, for providing the necessary facilities to carry out this work.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

REFERENCES

30. Roe HJ, Kuiter-CMA. Inhibition of ascorbic acid in whole blood and urine through the 2,4-dinitrophenyl-hydeazine derivative of dehydroascorbic acid. J Biol Chem 1943;147:399-407.