Lipid Peroxidation and Caeruloplasmin Levels in Serum and Saliva of Acute Myocardial Infarction patients

ABSTRACT

Background: Coronary artery disease is the major cause of mortality and morbidity. There is substantial evidence that oxidative stress plays the major role in the atherosclerotic process.

Objectives: This study was aimed to evaluate the levels of serum and saliva lipid peroxidation product malondialdehyde (MDA) and caeruloplasmin in acute myocardial infarction (AMI) patients.

Materials and methods: Fifty AMI patients with mean age of 54 ± 6.7 years and fifty control subjects with mean age 55 ± 6.2 years were incorporated in this study. Blood and unstimulated saliva were collected from each subject. Serum lipid profile was analyzed enzymatically. Malondialdehyde (MDA) and caeruloplasmin levels in serum and saliva were estimated spectrophotometrically.

Results: Serum total cholesterol, triglycerides and LDL-cholesterol were significantly elevated in AMI patients compared with controls, while HDL-cholesterol was significantly decreased. The levels of MDA and caeruloplasmin in serum and saliva were significantly raised (p<0.001) in those patients.

Serum MDA and caeruloplasmin were significantly correlated with saliva MDA and caeruloplasmin (p<0.001).
Conclusions:- Oxidative stress was increased in AMI patient, in both serum and saliva, raised serum and saliva caeruloplasmin serve as a marker of chronic inflammation or it might be elevated as a consequence of oxidative stress in AMI patients. Saliva is important for the evaluation of lipid peroxidation and antioxidants.

Keywords:- AMI patients, lipid peroxidation, caeruloplasmin.

INTRODUCTION
Oxidative stress induced by reactive oxygen species (ROS) is implicated in the pathogenesis of a variety of vascular diseases, including atherosclerosis, hypertension and coronary artery disease (1). Oxidative stress ensues when ROS evade or overwhelm antioxidants (2). Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids of membranes and are capable of further lipid peroxidation by a free radical chain reaction (3).

Malondialdehyde (MDA) is a break down product of peroxidation of long chain fatty acids which accumulates when lipid peroxidation increases (4). The effect of lipid peroxides i.e. endothelial cell damage, uncontrolled lipid uptake; decreased prostaglandin synthesis and associated thrombogenicity are strongly implicated in the pathogenesis of atherosclerosis (5).

Caeruloplasmin, the major serum copper containing glycoprotein, can perform its antioxidant function as ferroxidase and superoxide scavenger (6). It is a minor long lasting a cute phase protein. The liver is the major source of serum caeruloplasmin in adult (7).

Hypercholesterolemia is associated with high plasma levels of inflammation sensitive proteins (ISP) such as caeruloplasmin, the presence of which may predict ischemic stroke (8). It has been proposed that serum caeruloplasmin can serve as independent marker or risk factor for cardiovascular diseases (9,10). The increased risk has been attributed to the pro-oxidant activity of caeruloplasmin and recent experimental studies demonstrating the ability of caeruloplasmin oxidatively to modify low density lipoprotein (LDL) (11,12).

The preset study was undertaken to evaluate the levels of lipid peroxidation by estimation of serum and saliva malodialdehyde (MDA) in AMI patients, Serum and saliva caeruloplasmin in those patients, and compare them with controls. Also this study was aimed to see if there is a correlation between serum and saliva parameters.

MATERIALS AND METHODS:-
The study consisted of 50 patients (29 men and 21 women) with a cute myocardial infarction (AMI), admitted to the intensive care unit AL-Kadhumia hospital. The age range was (45 – 67) years with mean age of 54 ± 6.7 years. The diagnosis of AMI was established according to diagnostic criteria: ischemic type of chest pain, changes on serial ECG tracings (ST elevation of 2 mm or more) and elevation of serum cardiac markers serum creatinine phosphokinase (cpk-MB) and aspartate amino transferase enzyme.

The control group consisted of 50 age-sex matched healthy volunteers, 29 men and 21 women. Their mean age was 55 ± 6.2 years.

Patients with diabetes mellitus, renal insufficiency, hepatic disease, lung disease, thyroid disease, or patients taking lipid lowering drugs were excluded from the study.
Sample collection: -

Blood and saliva were collected for each subject at the same time after 12 hour fasting at 8-10 A.m.

Blood sample: - About 8mls of venous blood were aspirated from anticubital vein of each individual, using plastic disposable syringes. Serum was obtained by centrifugation at 3000 rpm for 10 minutes; transferred immediately into another tube and frozen at (-20°C) for subsequent analysis.

Saliva: - About 5mls of unstimulated (resting) whole saliva was collected by spitting saliva into plastic polyethylene tubes. The collected saliva was centrifuged at 3000 rpm for 10 minutes; the clear supernatants were separated and stored frozen at (-20°C) until assayed.

Biochemical assay: - Lipid profile, total serum cholesterol (TC), triglycerides (TG) and high and low density lipoprotein cholesterol (HDL-C & LDL-C respectively) were analyzed enzymatically using kit obtained from (Randox Laboratories limited, Crumlin, UK).

Determination of serum and saliva MDA: - MDA levels were estimated by thiobarbituric acid (TBA) reaction using trichloroacetic acid (TCA) according to shah and walker (13). The end products of lipid peroxidation particularly malondialdehyde (MDA) react with thiobarbituric acid under acidic condition and heating to give a pink color that measured spectrophotometricaly at 532 nm.

Determination of serum and saliva ceruloplasmin: - Ceruloplasmin was measured according to the method of Menden etal, (14). Ceruloplasmin catalyzed oxidation of p-phenylene-diamine to give blue-violet color that measured spectrophotometricaly at 525 nm.

Statistical analysis: - All statistical analysis was performed using statistical package for the social sciences (spss). Data were expressed as mean ± S.D . Results were evaluated using the student t-test for paired data, correlation coefficient (r) to find the correlation between two parameters.

A p-value less than 0.05 are considered significant and more than 0.05 is non-significant.

RESULTS: -

The present study showed a significantly higher serum total cholesterol levels in AMI patients (p<0.01) than that in the controls. Also a significantly higher serum triglyceride and LDL-cholesterol levels (p<0.001) in those patients as compared to control with a significant reduction in HDL-cholesterol (p<0.01) (table-1).

Table-2 shows a marked significant increase (p<0.001) in the levels of serum MDA in AMI patients (mean ± S.D,4.9 ± 1.9 µmol / L) as compared to controls ( 2.7 ± 0.8 µmol / L). The same thing was found in the levels of saliva MDA which was significantly higher (p<0.001) in those patients than in the controls , the mean ± S.D for AMI patient was 2.5 ± 1.1 µmol / L while for the controls was 1.2 ± 0.6 µmol / L.

Serum caeruloplasmin was significantly higher (p<0.001) in AMI Patients than controls, the mean ± S.D was 785.8 ± 256 mg / L and for the controls was 280 ± 106.4 mg / L, the same thing was in saliva caeruloplasmin which was also significantly higher in AMI patients than in the control subjects( p<0.001).
The mean ± S.D of saliva caeruloplasmin in AMI patients was 45.5 ± 8.1 mg / L and in the controls was 25.6 ± 6.5 mg / L (table-2).

Table-3 shows a significant positive linear correlation between serum MDA of AMI patients and each of TG (p<0.001) and LDL-cholesterol (p<0.05), but a significant negative correlation with serum HDL- cholesterol (p<0.05). Also saliva MDA of AMI patient showed a significant positive correlation with TG (p<0.001) and with LDL-cholesterol (p<0.05), but a significant negative correlation with serum HDL-cholesterol (p<0.05).

Serum caeruloplasmin has a significant positive linear correlation with serum total cholesterol (p<0.001) while saliva caeruloplasmin has a non- significant correlation.

Serum MDA has a positive significant correlation with saliva MDA, also serum caeruloplasmin has a significant positive correlation with saliva caeruloplasmin (p<0.001) (tab-4).

Table (1):- Comparison of serum lipid profile of AMI patients and control subjects (using t-test).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control subjects (n=50) mean ± S.D</th>
<th>AMI patients (n=50) mean ± S.D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>4.9 ± 0.7</td>
<td>5.2 ± 1.02*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.2 ± 0.4</td>
<td>2.6 ± 1.4**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL - C</td>
<td>1.15 ± 0.27</td>
<td>0.93 ± 0.2*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL - C</td>
<td>2.5 ± 0.5</td>
<td>3.76 ± 0.7**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

** p<0.001
*p<0.01
Table (2):- Comparison of serum and saliva MDA and caeruloplasmin in AMI patients and control subjects (using t-test).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control subjects (n=50) mean ± S.D</th>
<th>AMI patients (n=50) mean ± S.D</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum M DA μmol / L</td>
<td>2.7 ± 0.8</td>
<td>4.9 ± 1.9**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Saliva M DA μmol / L</td>
<td>1.2 ± 0.6</td>
<td>2.5 ± 1.1**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum caeruloplasmin mg / L</td>
<td>280 ± 106.4</td>
<td>785.8 ± 256**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Saliva caeruloplasmin mg / L</td>
<td>25.6 ± 6.5</td>
<td>45.5 ± 8.1**</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table (3):- The correlation coefficient (r) between serum & saliva MDA, caeruloplasmin with lipid profile of AMI patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total Cholest.</th>
<th>T.G.</th>
<th>HDL - C</th>
<th>LDL - C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum M DA</td>
<td>0.01 NS</td>
<td>0.42**</td>
<td>-0.31*</td>
<td>0.33*</td>
</tr>
<tr>
<td>Saliva M DA</td>
<td>0.13 NS</td>
<td>0.46**</td>
<td>-0.29*</td>
<td>0.31*</td>
</tr>
<tr>
<td>Serum caeruloplasmin</td>
<td>0.37**</td>
<td>0.08 NS</td>
<td>0.02 NS</td>
<td>0.03 NS</td>
</tr>
<tr>
<td>Saliva caeruloplasmin</td>
<td>0.03 NS</td>
<td>0.1  NS</td>
<td>0.001 NS</td>
<td>0.07 NS</td>
</tr>
</tbody>
</table>

*Correlation Significant at the level 0.05  
**Correlation Significant at the level 0.001
Table-4:- Correlation coefficient (r) between serum and saliva MDA & caeruloplasmin of AMI patients.

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>Significant level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum MDA &amp; Saliva MDA</td>
<td>0.43</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum caeruloplasmin &amp; Saliva caeruloplasmin</td>
<td>0.51</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DISCUSSION:-
Atherosclerosis is the root cause of acute myocardial infarction. It is a chronic inflammatory condition that is converted to an acute clinical event by the induction of plaque rupture which in turn leads to thrombosis. Hence inflammation occupies a very important central position in all phases of atherosclerosis (15).

Myocardial ischemia occurs when oxygen demand exceeds the oxygen supply and if this condition is not reversed, myocardial infarction precipitates. Reperfusion of the ischemic myocardium can restore oxygen supply but sudden massive increase in oxygen supply causes a burst of oxygen consumption with the consequent generation of free radicals, resulting in an imbalance of oxidative-antioxidative processes. The excess production of reaction oxygen species (ROS) may initiate lipid peroxidation in cell membrane. These processes may result in a loss of contractile function of the heart and lead to severe myocardial cell damage (16).

The significant decrease in endogenous antioxidant in the patients could be due to overwhelming production and accumulation of superoxide anion causing inhibition of antioxidant activity. Antioxidant depletion has relevant impact to the precipitation of myocardial infarction (17).

The present study observed significant increase in serum total cholesterol, triglyceride, and LDL – cholesterol in AMI patients than that in the control subjects, while HDL – cholesterol was significantly decreased in those patients, which was agreed with the results of Kaur et al. (18).

It has been found that serum and saliva MDA were significantly higher in AMI patients than in the controls. The findings of the present study are similar to the observations of studies conducted by other authors who found increased MDA levels in those patients (19, 20, 21, 22).

MDA is a decomposition product of auto oxidation of polyunsaturated fatty acids which is used as an index of oxidative damage. The high concentration of MDA in those patients indicates increased membrane lipid peroxidation. Enhanced lipid peroxidation may occur as a result of the fact that naturally occurring scavenging mechanisms are suppressed and the free radical generation processes are enhanced (23).

It has also been suggested that hyperlipidemia, especially hypercholesterolemia, can cause an increase in lipid peroxidation (24).

A statistically significant increase in the level of caeruloplasmin in AMI patients than in the healthy controls was found. Our results were agreed with those reported by other authors (18, 20, 25).

Caeruloplasmin is an acute phase reactant and increased levels may possibly be due to stress induced by angina or AMI (25).
The liver is the major source of serum caeruloplasmin in adults. Proinflammatory agonists of the acute phase reaction such as certain cytokines and tissue necrotic factor alpha (TNF-α) are known to enhance the gene expression of caeruloplasmin in hepatocytes (7). Also hypercholesteremia is associated with high plasma levels of ISP; one of these proteins is caeruloplasmin. These proteins increase the cholesterol-related incidence of cardiovascular disease (26).

Studies have shown that caeruloplasmin can be considered an important risk factor predicting AMI and cardiovascular diseases. Evidence suggests that LDL can be oxidized to an atherogenic form (oxidized LDL) within arterial wall by macrophages and other cells. This oxidation may be mediated by copper ions released from caeruloplasmin in atherosclerotic lesions (27). An increase of caeruloplasmin can be mediated many unspecific factors causing tissue injury or inflammation. Endothelial injury and inflammatory processes are thought to be involved in the pathogenesis of atherosclerosis, and markers of inflammation and infection, such as leukocytes count, fibrinogen, and c – reactive protein have been shown to be independent risk factors for the CHD (28). This suggests that substantial part of the increased risk associated with high levels of serum caeruloplasmin may be attributed to inflammation processes. The remaining elevated risk may be due to other properties of caeruloplasmin, like its pro – oxidant activity.

The results also showed that serum and saliva parameters were significantly correlated. Saliva's many components provide clues to local and or systemic diseases and disorders of the human body, for example saliva is used in the diagnosis of oral and systemic viral diseases such as measles, mumps, hepatitis A, B, C and HIV, also used to monitor the level of polypeptides and hormones like estrogen, testosterone, progesterone and antibodies (29).

Saliva contains various antioxidants including uric acid which is the major aqueous antioxidant component of the whole saliva and with lesser contribution from ascorbic acid and albumin (30), salivary proteins binds to redox active metal ions, rendering them non active in their capacity for free radical production. The capacity of saliva suppressing redox activity was found to be correlated well with the protein content, so saliva has a profound capacity for reducing redox activity rendered by transition metal ions, correlation well with its protein content (31). So many systemic diseases affect the capacity of saliva suppressing redox activity by affecting the protein content of saliva.

REFERENCES:-