Evaluation of Salivary Oxidative Stress Marker (Lipid Peroxidation), and Non-Enzymatic Antioxidants (Vitamin C and Vitamin E) in Patients with Acute Myocardial Infarction

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Abstract
Acute Myocardial Infarction (AMI), is the major cause of mortality and morbidity. There is substantial evidence that oxidative stress plays the major role in the atherosclerotic process. This study was aimed to evaluate the levels of salivary malondialdehyde (MDA), total antioxidant capacity (TAC) and non-enzymatic antioxidants (Vitamin C and Vitamin E) in AMI patients. Materials and methods: Sixty AMI patients (35 Males and 25 Females) with mean age of 54 ± 6.7 years and sixty control subjects with mean age 55 ± 6.2 years were incorporated in this study. Un stimulated saliva were collected from each subject in both groups. Malondialdehyde, and Total Antioxidant Capacity, Vitamin C and Vitamin E levels in saliva were estimated spectrophotometrically. SPSS computer software was used for data analysis. Results: Salivary MDA and TAC was significantly elevated in AMI patients compared with controls, while Vitamin C and Vitamin E were significantly decreased in AMI patients. Conclusions: Oxidative stress was increased in saliva of AMI patients, raised saliva MDA and TAC serve as marker of detecting chronic inflammation or be elevated as a consequence of oxidative stress in AMI patients. It may be taken in consideration that the saliva is so important tool in evaluation of lipid peroxidation and antioxidant agents to confirm the diagnosis of certain diseases.
Introduction:
Myocardial Infarction (MI) or Acute Myocardial Infarction (AMI), commonly known as heart attack leads to changes in size, shape, and functions of the heart. These changes are referred to as cardiac remodeling and encompass a vast array of pathophysiological alterations, including electrophysiological changes, ventricular dilatation, myocyte hypertrophy, and interstitial fibrosis (1). Myocardial ischemia may occur either from increased demand of oxygen by the myocardium, or decreased oxygen supply to the myocardium, or both (2). Oxidative stress is defined as a state in which ROS overproduction in vivo exceeds the buffering capacity of antioxidant enzymes and antioxidants thus resulting in a local imbalance between ROS production and destruction (3). These reactive oxygen species can damage proteins, lipids and DNA, altering the organism’s structure and function which plays a significant role in the pathogenesis of many diseases and mechanisms of complications (4). Oxidative stress has been associated with the development and progression of heart failure. ROS can attack important molecules inside cardiomyocytes, thereby impairing the biochemical function of these molecules, for example, DNA, RNA, and proteins. There is a plasma membrane-associated oxidase, nicotinamide adenine dinucleotide phosphate oxidase or NAD (P) H oxidase, which can be activated by angiotensin II in cardiomyocytes. In neutrophils, NAD (P) H oxidase activation is important for generation of ROS needed to destroy invading microorganisms. In cardiomyocytes, activation of this oxidase results in excessive ROS levels (5). Saliva is an aqueous, hypotonic solution which protects all the tissues of the oral cavity (6). The use of saliva as an alternative diagnostic tool to blood offers certain advantages. Saliva possesses a wide range of antioxidants agents including uric acid, vitamin C, reduced glutathione, oxidized glutathione, and others. Such antioxidants work in concert, and total antioxidant capacity may be the most relevant parameter for assessing the defense capabilities (7).

Materials and Methods:
Subjects:
This study was conducted during the period from November 2018 to March 2019. This study includes sixty patients with Acute Myocardial Infarction (AMI) admitted to Cardiac Care Unite (CCU) at Tikrit Teaching Hospital in Tikrit city. Patients included (35 males and 25 females), with age (39-61) years. Exclusion criteria included those patients with a history of diabetes mellitus or any chronic diseases and any current disease also the menstruating women were excluded. Sixty subjects with matched age (20-60) years, sex and body mass index, were included in this study as control group.

Diagnostic criteria:
World Health Organization (WHO) formulation in 1979 have classically been used to diagnose MI. A patient is diagnosed with myocardial infarction if two (probable) or three (definite) of the following criteria are satisfied.
1- Clinical history of ischemic type chest pain lasting for more than 20 minutes.
2- Changes in serial ECG tracings.
3- Rise and fall of serum cardiac biomarkers such as creatine kinase-MB fraction and troponin (8).

Samples collection:
From each patient as well as control. Saliva were collected at period between (8-9 A.M.) after 10 hours fasting. Following the washing of mouth with distilled water for 5 minutes under relaxed conditions and the patients asked to spit un-stimulated saliva for 5 minutes in a previously labelled sterile tube, centrifuged 5 minutes at 4000 rpm. The supernatant was separated by micropipette then stored in plane tubes at (-20 °C) till being assessed. Serum SOD, GSH, MDA, G-Px, and catalase levels were measured by spectrophotometric kit, Measurement of Vitamin E and C were performed.

according to the method described by Tietz\(^9\).

**Statistical Analysis:**
Statistical analysis was performed using SPSS-21 Unpaired t test was done to evaluate significant variance between means. P < 0.05 was considered statistically significant.

**Results:**
Descriptive statistics for both sex are illustrated in Table (1), the study conducted on 60 patients 42% female and 58% male, and control with 52 % female and 48% male, the mean age in years was (44.40±8.13) and (40.00 ±9.83) respectively . In this study the mean of salivary pH was (6.98±0.35), and flow rate was (0.52±0.02), were significantly lower in comparison with control group (7.3±0.29: P=0.000), (0.68±0.07: p<0.002). Regarding the mean values of biochemical parameters of groups, the control and the AMI patients which shown in Table (2). The analysis revealed highly significant decrease in the concentration of both non-enzymatic antioxidant agents, Vit. E (0.741± 0.205 vs 1.339 ± 0.222 mg/L) as in Fig.(1), and Vitamin C (0.646 ± 0.162 vs 1.258 ± 0.178 mg/ dl) as in Fig.(2) in saliva of AMI patients compared with that in healthy control group. On other hand MDA, and TAC concentration were highly significantly increased in saliva of patients with AMI (4.990 ± 0.840 vs 2.270 ± 0.706 μmol/L) as in Fig.(3), and (17.30 ± 2.12 vs 12.45 ± 2.03 mmol/L) as in Fig.(4) respectively. Finally, Pearson correlation revealed that there was negative correlations between salivary MDA with both vitamin E (r=-0.123), and vitamin C (r=-0.094) respectively.

**Discussion:**
Reactive oxygen species can be formed in the heart by a variety of mechanisms, including generation during oxidative phosphorylation in the mitochondria as a product of normal cellular aerobic metabolism. Oxygen is a major determinant of cardiac gene expression, and a critical participant in the formation of ROS. Increased levels of MDA in this study are an indication of increased oxidative stress. The oxidation of LDL is a free radical driven lipid peroxidation process and the aldehyde products of lipid hydroperoxide breakdown are responsible for the modification of the LDL apoprotein. Aldehyde modified Apo B protein has altered receptor affinity, causing it to be scavenged by macrophages in an uncontrolled manner with the development of foam cells and the initiation of the atherosclerotic lesion\(^10\). Malondialdehyde 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 \(^11\). Increased level of salivary MDA in AMI patients was highly significant as compared to controls. Similar findings were observed in other studies \(^12\) whom proposed that rise in MDA could be due to increased generation of ROS which in turn oxidizes many important biomolecules including membrane lipids. Total antioxidant capacity, is an indicator of oxidative stress, reflecting the redox balance between oxidation and antioxidation\(^13\). We measured the salivary total antioxidant capacity (TAC) in the present study which combines the concentrations of individual antioxidants and reflects the overall antioxidant capacity known as ferric reducing-antioxidant power which is found to be increased significantly when compared to control levels. The increased TAC levels could paradoxically reflect a high OS evidenced by increased MDA levels in patient group that has stimulated the compensatory up-regulation of antioxidants\(^14\). Vitamin C is a physiological antioxidant of major importance for protection against diseases and degenerative processes caused by oxidative stress and is associated with

better scavenging properties in vivo than the other antioxidants, because of its presence both in intracellular and the extracellular fluid. Plasma Ascorbic acid is the only endogenous antioxidant that can completely protect the lipids from the peroxidative damage induced by aqueous peroxyl radicals. Vitamin C also acts as a co-antioxidant by regenerating alpha-tocopherol from alpha-tocopheroxyl radical produced during scavenging of oxygen free radicals\(^{(15,16)}\). In our study, the salivary vitamin C levels were significantly lower in AMI patients observed in the present study may be linked with the increased consumption of ascorbic acid due to increased oxidative stress, as evident from higher MDA levels. This study consistent with the results of a study by Entedhar R. Sarhat, et al.\(^{(17)}\) indicates that the decreasing levels of Vitamin C due to their free radical scavenging action and to preserve the body antioxidant reserve and in normalization of vascular superoxide formation. Our study showed a significant decrease in the salivary levels vitamin E in AMI patients compared to healthy controls group. This indicates severe damage to antioxidant system which is unable to combat oxidative stress. Vitamin E is an important membrane constituent of cardiac muscle which haults lipid peroxidation by trapping the peroxyl radical and stabilizes lipid bilayer of cell membranes, where it interacts with phospholipases to reduce membrane rearrangements. It may provide protection against ischaemic myocardial damage by eliminating pro-oxidants and scavenging free radicals\(^{(18,19)}\). Furthermore, the cardio-protective potential of vitamin E has been attributed to its potent antioxidant action. This contention is supported by the fact that \(\alpha\)-tocopherol shows antioxidant potential by donating hydrogen radical to remove the free radicals reacting with it to form non-radical products or trapping of lipid radicals\(^{(20)}\).

Fig.(1): Salivary vitamin E in control and AMI group.
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Fig.(2): Salivary vitamin C in control and AMI group.

Fig.(3): Salivary MDA in control and AMI group.
Table (1): Demographic characteristic of study samples (mean ±SD).

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AMI patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years± S.D)</td>
<td>40.00±9.83 (20-60)</td>
<td>44.40±8.13 (39-61)</td>
</tr>
<tr>
<td>Gender</td>
<td>Female 31(52%)</td>
<td>25 (42 %)</td>
</tr>
<tr>
<td></td>
<td>Male 29 (48 %)</td>
<td>35 (58 %)</td>
</tr>
</tbody>
</table>

Table (2): Descriptive statistics of salivary parameters for control and study group.

<table>
<thead>
<tr>
<th>Salivary parameters</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.3±0.29</td>
<td>6.98±0.35**</td>
</tr>
<tr>
<td>Flow rate ml/min</td>
<td>0.68±0.07</td>
<td>0.52±0.02**</td>
</tr>
<tr>
<td>Vitamin E (mg/L)</td>
<td>1.339 ± 0.222</td>
<td>0.741 ± 0.205*</td>
</tr>
<tr>
<td>Vitamin C (mg/ dl)</td>
<td>1.258 ± 0.178</td>
<td>0.646 ± 0.162 **</td>
</tr>
<tr>
<td>MDA (μmol/L)</td>
<td>2.270 ± 0.706</td>
<td>4.990 ± 0.840**</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>12.45 ± 2.03</td>
<td>17.30 ± 2.12**</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.0001

Fig.(4): Salivary TAC in control and AMI group.
References


3-Yosuke Kayama, Uwe Raaz, Ann Jagger , Matti Adam, Isabel N. Schelling, Masaya Sakamoto, Hiromuni Suzuki , Kenseki Toyama,Joshua M. Spin, and Philip S. Tsao. Diabetic Cardiovascular Disease Induced by Oxidative Stress. Int. J. Mol. Sci. 2015; 16(1):, 25234-25263


