ABSTRACT
Evidence suggests that free radicals are the central pathogenic factor in the atherosclerotic process. Biochemical marker of oxidative stress malondialdehyde and antioxidants viz., serum vitamin E, glutathione (GSH) and magnesium were measured in MI patients (n=47) on admission and monitored over 96 h and compared with healthy controls (n=28). MI subjects were grouped according to the treatment – thrombolyzed (T) and non-thrombolyzed (NT). Mean age of MI onset was 40 y, majority of the subjects had a lower protein status and higher waist-hip ratio. An atherogenic lipid profile was observed in >50% of the subjects. Serum Malondialdehyde (MDA) were significantly (p<0.05) elevated on day one of admission (T - 10.6 ± 0.49, NT-11.83 ± 0.18 nmol/l), which decreased at 96h in both MI groups, however the levels were higher in the thrombolyzed group. Serum vitamin E, GSH and magnesium levels were also markedly lower (p<0.05) compared to normal subjects. Serum vitamin E levels increased after 24 h, as result of vitamin supplementation. Antioxidant rich foods were used less frequently. The observations suggest that increased generation of free radicals in the MI event maybe mediated through the oxidation of LDL as majority had markedly elevated LDL levels (> 140mg%). The findings indicate a persistent oxidative stress that might be associated with intravascular inflammation.

Keywords: Oxidative stress, Serum vitamin E, myocardial infarction, thrombolysis, serum magnesium.

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INTRODUCTION

Oxidative stress has been implicated in a number of disorders including cardiovascular malfunction, cataracts, cancers, rheumatism and auto-immune disease besides aging. Current concepts in the etiology of cardiovascular disease have been focused on free radicals as the central pathogenetic factor of the disease.\(^2\) Oxidative modification of low-density lipoproteins (LDL) is recognized as an important step in the initiation of atherogenetic plaque formation, which is a primary determinant for development of CVD and coronary thrombosis.\(^3\) This hypothesis is supported by biochemical and animal studies and also from epidemiological studies. The antioxidant status of biological tissues can be influenced by dietary antioxidants. Biochemical evidence indicates that nutrient antioxidants such as vitamin A, C, and E can stabilize free radicals by donating an electron, thus inhibiting lipid peroxidation.\(^4,5\)

Epidemiological studies in western countries have shown that low plasma levels of essential antioxidants are associated with increased risks; in particular, low levels of carotene and vitamin E, with the risk of cancer and IHD, respectively.\(^2,6,7\) A cross-cultural epidemiological study suggests that individuals with low plasma vitamin E levels had increased mortality from IHD.\(^8\) A high inverse correlation between age specific mortality from IHD and plasma vitamin, E levels has been observed in different European populations.\(^8\) Also, a highly significant negative association between intake of fruits and vegetables and CVD mortality is reported. \(^9,10\) The protection that fruits and vegetables provide against diseases, has been attributed to the presence of antioxidant vitamins, including ascorbic acid, vitamin E or β-carotene.\(^2,5\)

Evidence suggests that populations consuming less magnesium (Mg) obtained either from water or diet are prone to CVD states. Clinical studies also indicate a link between Mg depletion and myocardial infarction (MI).\(^11\) The problem of coronary mortality is not confined to industrialized countries only, but is also seen in developing countries. Although there are differences in the prevalence of cardiovascular risk factors across different regions, CVD has emerged as the leading cause of death in all parts of India, including poorer states and rural areas.\(^12\) India is in a state of epidemiological transition with an increase in chronic life style-related diseases.\(^13\) Indians, both immigrant and native, are predisposed to develop a cluster of risk factors and consequently are more prone to manifest CVD as compared to other ethnic groups.\(^14\) Factors presumed to be responsible for increased propensity to CVD in Indians are abdominal obesity, smoking, glucose intolerance and peculiar dyslipidemic profile.\(^13-16\) Studies in India reveal a striking urban-rural gradient in conventional risk factors such as plasma-cholesterol, blood pressure and obesity.\(^13\) In addition, significantly higher
plasma lipid peroxide levels were observed in urban men in South India compared to their rural counterparts. There are reports of significant reduction in antioxidant vitamins and enzymes and increased free radical stress in older individuals leading to oxidative stress and disease. Studies in India have reported enhanced free radical activity in MI. However, the potential interdependence of oxidants, antioxidants i.e., vitamin and enzyme, Mg status with diet and other lifestyle habits have not been reported. Hence, the present study was planned to study the changes in lipid peroxides and antioxidant status following MI.

METHOD

38 patients (27 males, 11 males) in the age range of 40-66 y, admitted at the CSI Holdsworth Memorial hospital, Mysore with acute MI presenting within 6 h of onset of typical cardiac chest pain were included in the study by random selection, with the help of attending cardiologist, after obtaining permission from the hospital authorities and consent from the patient attendant’s. All patients had their first episode of MI with diagnostic criteria; typical chest pain, specific abnormalities for MI on the ECG, elevated cardiac enzymes. Patients older than 70 y and with clinical evidence of chronic disease (rheumatic or congenital heart disease) chronic renal failure were excluded from the study. None of the patients were taking antioxidants. Streptokinase was given only to those patients who could afford it. The group not receiving thrombolytic treatment (n = 16) served as control. Venous blood was collected soon after admission (before administration of streptokinase in experimental group) and at 24, 48 and 96 h. Blood was centrifuged at 3000g for 15 min, serum separated and stored at -70°C for biochemical analysis. Serum vitamin E and magnesium were estimated by colorimetric methods. MDA was estimated by thiobarbituric acid reaction, GSH levels were analyzed by the method of Beutler et al. Other biochemical tests viz., lipid profile, urea, creatinine were estimated using standard diagnostic kits. Results were analyzed by one-way ANOVA followed by Duncan’s new multiple range test.

RESULTS AND DISCUSSION

A total of 38 patients were studied, their age ranged from 40-66 y, majority were men. The disease occurred at 60 y in majority of the subjects (81%). The onset of MI was sudden (within minutes) in 50% and gradual over hours in the rest of the subjects. The symptoms at onset as reported by the subject and their family members were chest pain in 37 (97%), vomiting in 16 (42%), loss of consciousness in 5 (13%), sweating in 24 (63%), radiating pain in the left arm 13 (34%), respectively.

The occurrence of MI was interpreted by the attending cardiologist on admission by using routine confirmatory diagnostic tests such as cardiac enzyme (CK-MB) and ECG. The ECG
pattern reveals a positive cardio graphic abnormality. It was observed that 37% of the subjects did not suffer from any other associated health problems before the onset of IHD. The rest of the subjects were known diabetics and hypertensives (13%). 34% of them reported to be previously affected with IHD, while 10 patients had family history of incidence of heart disease.

**Somatic status**

Mean anthropometric measurements of the subjects are presented in Table 1. Body mass index (BMI) indicates energy status of the individual, while mid upper arm circumference (MUAC) and mid upper arm muscle circumference (MUAMC) indicate the protein status of an individual. TSF thickness and WHR indicates body fat status of the individual. Majority of the subjects (56% M, 81% F) had BMI within the normal range of 18.5 - 25, only 44% males, and 19% of the female subjects were found to be overweight. Comparisons of MUAC, MUAMC and TSF thickness with standards showed more number of subjects having low protein and fat status in both groups.

**Table 1: Somatic status of the subjects [mean ± SD]**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Subjects</th>
<th>MI Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n=16)</td>
<td>Female (n=12)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.1±8.8</td>
<td>55.8±5.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.66±0.02</td>
<td>1.57±0.04</td>
</tr>
<tr>
<td>BMI</td>
<td>24±3.2</td>
<td>22±2.07</td>
</tr>
<tr>
<td>WHR</td>
<td>0.9±2.8</td>
<td>0.9±0.9</td>
</tr>
<tr>
<td>MUAC (cm)</td>
<td>27±2.06</td>
<td>27±2.6</td>
</tr>
<tr>
<td>TSF (mm)</td>
<td>7.5±1.3</td>
<td>8.1±1.2</td>
</tr>
<tr>
<td>MUAMC (cm)</td>
<td>18.1±4.7</td>
<td>20.2±4.5</td>
</tr>
</tbody>
</table>

BMI = Body mass index: Normal range - > 18.5 – 22.9

WHR - Waist to Hip Ratio, Male- 0.85 Female-0.80

MUAC (cm) = Mid upper Arm circumference Normal Range - Male: 29.3, Female: 28.5

TSF (mm) = Triceps skin fold Normal Range - Male: 12.5, Female: 16.5

MUAMC (cm) = Mid upper arm circumference Normal Range - Male: 25.3, Female: 23.2

**Clinical data**

The biochemical data of the MI subjects is given in table 2. The mean total cholesterol was 190.8 mg% (range 150 - 260 mg%). The mean HDL-C was also low; however the mean LDL-C and triglycerides were higher than the optimal range. The mean fasting blood glucose was also higher, indicating that majority of subjects were diabetics. Blood urea, creatinine and serum sodium were within the normal range. The mean serum potassium levels were
found to be lower (3.71 mmol/L). The levels of enzyme CK-MB, a sensitive measure of MI, was found to be elevated in all the patients, ranging from 10 to 62 IU/L.

The changes in parameters of oxidative stress i.e., lipid peroxides, vitamin E, Glutathione and serum magnesium in both thromboloyzed and non-thromboloyzed subjects are given in table 3. Base line values of MDA were comparable in both thromboloyzed (T) and non thrombolysed group (NT), also the levels did not differ significantly between the two groups on day 2 and day 3, however a significant difference (p<0.05) was observed between the two groups. The MDA levels in healthy controls were significantly lower than the MI group (p<0.05).

**Table 2: Mean (± SD) of biochemical parameters of MI subjects**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>190.8</td>
<td>±27.3</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>36.8</td>
<td>±6.5</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>116.4</td>
<td>±25</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>191.3</td>
<td>±19.8</td>
</tr>
<tr>
<td>Haemoglobin (g%)</td>
<td>10.5</td>
<td>±1.33</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mg%)</td>
<td>193.7</td>
<td>±86</td>
</tr>
<tr>
<td>Urea (mg%)</td>
<td>35.3</td>
<td>±11.4</td>
</tr>
<tr>
<td>Creatinine (mg%)</td>
<td>1.17</td>
<td>±0.22</td>
</tr>
<tr>
<td>CK-MB (IU/L)</td>
<td>25.12</td>
<td>±14.2</td>
</tr>
<tr>
<td>S.Soduim (mmol/L)</td>
<td>135</td>
<td>±3.49</td>
</tr>
<tr>
<td>S.Potassium (mmol/L)</td>
<td>3.71</td>
<td>±0.94</td>
</tr>
</tbody>
</table>

As part of the treatment given post-MI in the intensive care unit (ICU), both groups of patients i.e., N and NT receive α-tocopherol (400 mg/day) from 2nd or 3rd day of treatment. In both groups, a progressive increase in serum vitamin E levels was observed after 24 hrs. The vitamin E levels did not differ between the two groups on day 1, 3 & 5. However, a significant difference was observed between the two groups on day 2 ( p< 0.05).

The total glutathione (GSH) was significantly (p<0.05) lower compared to the healthy controls indicating reduced levels of endogenous antioxidant defense in MI patients.

Due to practical limitations serum magnesium and conjugated dienes (CD) were not analyzed on day 2 and 3. The CD level in both groups of MI patients were significantly higher on day 1 compared to day 5. Higher CD levels are associated with increased generation of free radicals. In the present study, it was found that lipid peroxide levels were significantly (p<0.05) higher on day 1.

The antioxidant data of normal subjects, according to gender is given in table 4. No differences were observed with regard to MDA, magnesium and glutathione. However, vitamin E levels were higher in healthy male subjects.
Table 3: Changes in parameters of oxidative stress in MI patients

<table>
<thead>
<tr>
<th>Groups</th>
<th>Base Line</th>
<th>24 h Day 1</th>
<th>48h Day 2</th>
<th>96h Day 3</th>
<th>Healthy Control (n=23)</th>
<th>± SE (df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10.6 ±f</td>
<td>8.37 ±e</td>
<td>5.15 ±d</td>
<td>2.46 ±ab</td>
<td>3.37 ±bc</td>
<td>0.49</td>
</tr>
<tr>
<td>II</td>
<td>11.83 ±f</td>
<td>7.86 ±e</td>
<td>4.66 ±cd</td>
<td>1.60 ±a</td>
<td>-</td>
<td>(110)</td>
</tr>
<tr>
<td>Vitamin E (µg %)</td>
<td>I 0.87 ±a</td>
<td>1.83 ±bc</td>
<td>3.16 ±d</td>
<td>5.0 ±e</td>
<td>2.05 ±c</td>
<td>0.13</td>
</tr>
<tr>
<td>II 0.73 ±a</td>
<td>1.60 ±b</td>
<td>2.86 ±d</td>
<td>4.96 ±e</td>
<td>-</td>
<td>(110)</td>
<td></td>
</tr>
<tr>
<td>Glutathione (mmol/ml)</td>
<td>I 0.012 ±a</td>
<td>0.033 ±bc</td>
<td>0.051 ±cd</td>
<td>0.074 ±e</td>
<td>0.417 ±f</td>
<td>0.006</td>
</tr>
<tr>
<td>II 0.013 ±a</td>
<td>0.028 ±ab</td>
<td>0.045 ±bc</td>
<td>0.0683 ±de</td>
<td>-</td>
<td>(110)</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>I 1.76 ±a</td>
<td>NA</td>
<td>NA</td>
<td>2.05 ±b</td>
<td>2.01 ±c</td>
<td>0.23</td>
</tr>
<tr>
<td>II 1.76 ±a</td>
<td>NA</td>
<td>NA</td>
<td>2.08 ±b</td>
<td>-</td>
<td>(44)</td>
<td></td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>I 1.76 ±a</td>
<td>NA</td>
<td>NA</td>
<td>1.98 ±a</td>
<td>NA</td>
<td>0.04</td>
</tr>
<tr>
<td>II 2.98 ±b</td>
<td>NA</td>
<td>NA</td>
<td>1.73 ±a</td>
<td>-</td>
<td>(66)</td>
<td></td>
</tr>
</tbody>
</table>

I – Thrombolysed Group (n=18)
II – Non-Thrombolysed Group (n=6) NA – Not Analyzed

Any two mean values carrying different superscript a,b,c.. differ significantly (p < 0.05)

Linear correlations were calculated between free radical LDL and vitamin E. The correlations did not attain statistical significance between free radicals and LDL (r=0.04). A negative relationship was observed between free radicals and vitamin E levels, which were not significant (r=0.01), probably due to lower number of subjects.

Majority of subjects were not in the habit of using fruits, the reasons, as quoted by the subjects, being diabetic and cost of food item being high. Consumption of fruits and vegetables, which are rich sources of antioxidants, was low and less frequent in majority of the subjects, which reflected in a low anti oxidant status.

Table 4: Antioxidant status of the normal subjects

<table>
<thead>
<tr>
<th>SL No</th>
<th>MDA (nmol/ml)</th>
<th>Vitamin E (mg/dl)</th>
<th>Magnesium (mg/dl)</th>
<th>Glutathione (mmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Male (n=16)</td>
<td>3.40 ±1.4</td>
<td>2.68 ±0.12</td>
<td>2.0 ±0.19</td>
<td>0.33 ±0.12</td>
</tr>
<tr>
<td>Female (n=12)</td>
<td>3.20 ±0.9</td>
<td>1.8 ±0.69</td>
<td>2.0 ±0.11</td>
<td>0.51 ±0.27</td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study, we monitored dynamic changes in markers of oxidative stress viz., serum malondialdehyde (MDA) vitamin E, glutathione (GSH) during the convalescence period of patients with acute myocardial infarction (MI) treated by thrombolysis. Blood samples were taken at admittance and at day one, two and four following admission. Myocardial damage is the known cause for pathological condition associated with oxidative stress, the results indicate persistent oxidative stress during convalescence in patients with
MI. Oxidative stress has been reported after MI treated with percutaneous coronary intervention. Disturbances in oxidants and antioxidant metabolism irrespective of the gender are reported in Acute myocardial infarction. Our observations are in good agreement with the findings of other authors. Very few studies report the dynamic changes in oxidative stress following MI. A short-term follow up study reported that patients with diabetes are subject to significant oxidative stress 2 weeks after AMI implying that hyperglycemia exacerbates oxidative stress after MI. The markedly elevated MDA levels observed in both groups indicates a state of enhanced lipid per oxidation in MI. It is currently believed that lipid per oxidation is involved in the oxidative modification of LDL and this ultimately results in the initiation of and progression of atherosclerosis process. The present study results of increased MDA and decreased GSH in AMI patients had a fair correlation with findings of many workers. Reperfusion injury is defined as the damage that occurs to a tissue during the resumption of blood flow following ischaemia. It is generally agreed by clinicians and researches that the re-oxygenation after a period of ischaemia / hypoxia, is associated with further tissue damage, which is likely to be mediated by free radical induced oxidation. Treatment with thrombolytic drugs plays a crucial role in salvaging the myocardium in MI patients. The effect of thrombolytic therapy on lipid per oxidation is not clearly understood. In the present study, no significant difference was seen in free radical levels between the two groups of MI patients (i.e. thrombolysed vs. non thrombolysed). This maybe due to the fact that, both groups received antioxidants (vitamin E and Flavedon). A similar study has reported increased free radical production after thrombolysis. A fall in serum magnesium following acute myocardial infarction has been observed by many workers. Serum magnesium levels were significantly lower (p<0.05) in MI subjects compared to healthy controls. Magnesium is an obligatory co factor in the enzyme reactions of GSH synthesis and in all biosynthetic enzyme reactions involving ATP and magnesium deficiency has been reported to inhibit biosynthesis of GSH. Serum magnesium levels are in turn influenced by the dietary intake of magnesium. Evidence suggests that population consuming magnesium- deficient diets are prone to CVD and magnesium deficiency promotes oxidative injury. Dietary anti oxidants are indicated to offer protection against chronic disease. Vitamin E is a major chain-breaking anti oxidant and is considered the first line of defense against lipid per oxidation. In the present study, the MI patients had significantly (p<0.05) lower levels of Vitamin E initially (on admission). A gradual increase was observed thereafter, which can be attributed to supplementation of Vitamin E. Animal and clinical studies suggest antioxidant
supplementation as a practical approach to attenuate oxidative attack of vital organs post MI.

GSH is another excellent parameter of oxidative stress. After obtaining an electron it gets oxidized to GSSH, hence, its level falls in oxidative stress. In the present study, decreased levels of GSH were observed in both groups of MI patients than in healthy controls. Post- MI, a significant gradual increase was seen in both groups. These observations are in agreement with the results of other coworkers. Radicals and CD are produced even after the onset of myocardial infarction.

In recent years, the concept that diet plays an important role in oxidative stress has been emphasized by several research studies. The biologic action of natural antioxidants viz. vitamins A, C and E and minerals (Se, Fe, Cu, Zn) has been related to oxidative stress and resultant disease. These nutrients presumably play important roles in the balance between pro-oxidant and antioxidant systems in humans. Fruits, vegetables and certain spices are good sources of protective nutrients. In the present study, the frequency of consumption of vitamins and mineral antioxidant foods by the MI subjects was studied. The dietaries were essential that of vegetarian types, with occasional use of animal foods. Cereals were the predominant items of the daily meals. Consumption of green leafy vegetables, fruits, milk and milk products was on the lower range. The frequency of use of foods, which are rich sources of vitamin antioxidants viz., vitamin E, A, C and β-carotene ranged widely among the MI group, majority of subjects opined to consume them once a week. These observations are similar to earlier studies on the dietary habits of diabetics and MI patients.

The results indicate increased production of free radicals as seen after MI episode, which is also accompanied with decreases in serum levels of Magnesium, Vitamin E and GSH enzyme. These observations suggest that lipid per oxidation involved in the MI event may be through the formation of oxidized LDL, as majority of the subjects had markedly higher LDL levels. In addition, their antioxidant status was lower as a consequence of inadequate consumption of protective foods. Hence, it may be concluded that dietary and lifestyle habits, play an important role in the occurrence and progression of degenerative diseases. The role of nutrient components in oxidative stress should be a continuing and important field of investigation. The high prevalence of CVD among Indians indicates a need to adopt a highly aggressive strategy towards life style modification.

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