Study of serum malondialdehyde and vitamin C in smokers

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Abstract

Smoking is associated with high incidence of morbidity and mortality. The free radicals released during smoking thought to play an important role in forming imbalance between oxidants and antioxidants. A total number of 200 subjects comprising of 50 healthy controls and 150 cases of smokers divided into mild, moderate and heavy smokers studied. In all the subjects, serum levels of malondialdehyde (MDA) as a biomarker of lipid peroxidation and antioxidant serum vitamin C were estimated. Serum MDA was significantly increased in smokers when compared to controls. The oxidative stress level was elevated in accordance with the intensity of smoking. The antioxidant serum vitamin C was significantly decreased in smokers when compared to controls. The presence of increased systemic oxidative stress in smokers seems to be associated with current active smoking and systemic inflammation. The decrease in antioxidant levels appears to be mainly a consequence of increased oxidative stress. Hence by advising cessation of smoking and taking diet rich in antioxidants may prevent oxidative damage and prevent oxidative stress related diseases.

Keywords: Smoking, Oxidative stress, Antioxidants, Serum MDA, Vitamin C.

Introduction

Tobacco smoking is a practice of burning tobacco and inhaling the smoke (consisting of gaseous phases and particles). A more broad definition may include taking tobacco smoke into the mouth, and then releasing it, as is done by some with tobacco pipes and cigars. The practice may have begun as early as 5000-3000 BC. Smoking plays an important role in disturbing the antioxidant balance. Normally blood contains a healthy complement of antioxidants that keep oxidative damage to a minimum. Tobacco smoke contains abundant reactive oxygen species and also activated neutrophils released due to smoking also add to the pool of reactive oxygen species which deplete these antioxidant mechanisms leading to tissue damage.

Malondialdehyde is a organic compound with the formula CH2 (CHO) 2. This reactive species occurs naturally and is a marker for oxidative stress. Reactive oxygen species degrade polyunsaturated lipids present on cell membrane forming malondialdehyde. This aldehyde product is used as a biomarker to measure the level of oxidative stress in an organism.

Antioxidants depletion or deficiency may contribute to oxidative stress. Antioxidants not only protect against the direct injurious effects of oxidants, but also alter the inflammatory events that play an important role in the pathogenesis of oxidative stress related diseases. Vitamin C is a water soluble free radical scavenger, can directly scavenge O2 and OH- radicals and help to neutralize physiological oxidant burden created by both exogenous and endogenous sources.

Present study is undertaken to evaluate serum malondialdehyde as indicator of oxidative stress and serum vitamin C as indicator of antioxidant level in smokers and non smokers.
MATERIAL AND METHODS

This was a prospective study conducted from April 2009 to April 2010. Controls and cases were selected from a tertiary care hospital in South India. Each participant gave an informed consent and this study was approved by the ethical committee. The subjects were selected based on the following criteria.

Inclusion criteria:

Male subjects aged between 20-50 years, non smokers and smokers (cigarette and beedi smoking) of >2 years duration. The smokers were divided into three groups. Mild smokers (Group I) = 1-10 cigarettes/bidis per day. Moderate smokers (Group II) = 11-20 cigarettes/bidis per day. Heavy smokers (Group III) = >20 cigarettes/bidis per day.

Exclusion criteria:

1) Subjects with history of medical disorders such as diabetes, hypertension, hepatic, renal and cardiac disorders

2) Ex smokers, alcoholics, obese (BMI >30), family history of dyslipidemia

3) Subjects on medications such as beta blockers, steroids, vitamin supplementation and herbal medications.

Collection of blood sample

About 4ml of blood collected from large peripheral vein under aseptic precaution after overnight fasting in a plain bulb for estimation of serum MDA and serum vitamin C.

Estimation of Serum Malondialdehyde

Serum malondialdehyde estimated by Kei Satoh Method. It is based on the principle of auto-oxidation of unsaturated fatty acids, involves the formation of semistable peroxides, which then undergo a series of reactions to form malondialdehyde (MDA). MDA reacts with thiobarbituric acid (TBA) to form pink colored chromogen. The resulting chromogen is extracted with 4.0ml of n-butyl alcohol and the absorbance of which is measured at 530 nm.

Estimation of Serum Vitamin C

Serum vitamin C was estimated by 2, 4 – dinitrophenyl hydrazine method. This method is based on the principle that ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products are treated with 2,4-dinitrophenyl hydrazine (DNPH) to form the derivative bis-2,4-dinitrophenyl hydrazone. This compound in strong sulfuric acid, undergoes rearrangement to form a colored product which is measured at 520nm. The reaction is run in the presence of thiourea to provide a mildly reducing medium, which helps to prevent interference from non-ascorbic acid chromogen.

The data was recorded and analyzed using SPSS software (version 15). Differences between mean values were evaluated by student ‘t’ test. Significance is assessed at 5% level of significance.

Results

The demographic characteristics of the subjects is shown in table

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-smokers</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Age</td>
<td>37.5±5.12</td>
<td>35.1±4.22</td>
<td>36.4±2.92</td>
<td>39.4±4.23</td>
</tr>
<tr>
<td>BMI</td>
<td>25.9±3.12</td>
<td>22.7±2.32</td>
<td>26.4±3.14</td>
<td>24.9±3.01</td>
</tr>
</tbody>
</table>

BMI = Body mass index

No significant difference was seen between the groups

<table>
<thead>
<tr>
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<th>Non-smokers</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (n mol/ml)</td>
<td>2.62±0.52</td>
<td>4.83±0.51*</td>
<td>5.36±0.74*</td>
<td>5.89±0.52*</td>
</tr>
<tr>
<td>VITAMIN C (mg/dl)</td>
<td>1.10±0.16</td>
<td>0.65±0.09*</td>
<td>0.56±0.12*</td>
<td>0.47±0.08*</td>
</tr>
</tbody>
</table>

*P≤0.01 = Strongly significant

Discussion

Cigarette smoking is probably the most addictive and dependence producing form of object-specific, self-administered gratification known to man. According to present estimates, tobacco is responsible for causing more than 5 million deaths every year (World Health Organization, 2008). About 19% of tobacco consumption in India is in the form of cigarettes, while 53% is smoked as bidis. Regular smoking doubles the chances of stroke in men.

It has been estimated that 1016 radicals are present in one puff of cigarette smoke. Free radicals can oxidize lipid, protein and carbohydrate molecules, damaging cell membranes and DNA, thereby altering cellular structure and function. Cell membranes are rich sources of polyunsaturated fatty acids, which are more prone to be attacked by oxidizing radicals causing lipid peroxidation.
Cigarette smoking is an important and independent risk factor for atherosclerosis, coronary artery disease and peripheral vascular disorders. In the present study, the demographic characteristics were comparable between groups (table 1). The oxidative stress marker MDA level was increased in all three groups of smokers compared to non-smokers and was more increased in heavy smokers (Group III) compared to moderate smokers and mild smokers and was statistically significant. The above findings are in accordance with the studies of Birgul Isik et al. and Bulent Ozbay et al.

Antioxidant level vitamin C decreased in all the three groups of smokers compared to non-smokers and more decreased in heavy smokers compared to moderate and mild smokers and was statistically significant. These findings are in accordance with the study of Mukuddar colikoglu et al.

In another study contradictory to these results found conducted by Balkan A and co-workers they observed that antioxidant level increased in healthy smokers indicating body inhibits oxidants by activating antioxidant mechanisms.

Vitamin C is a water soluble free radical scavenger, can directly scavenge super oxide and hydroxyl radicals and helps to neutralize physiologic oxidant burden created by both exogenous and endogenous sources. Vitamin C functions as antioxidant by preventing others substances from being oxidized by donating its electrons. However in this process vitamin C oxidized itself. The compound formed after loss of electron is ascorbyl radical which is relatively stable with half life of 10–5 seconds and is fairly unreactive which explains the antioxidant function of ascorbic acid. The mechanism involved in the reduction of vitamin C level in smokers may be due rapid oxidation of ascorbic acid by free radicals. The negative relationship between vitamin C and MDA may be due to the depletion of vitamin C when the oxidant burden is increased.

MDA is a sensitive marker of lipid peroxidation. Free radicals released from smoking degrade polyunsaturated lipids present mainly in cell membranes forming malondialdehyde (MDA). This compound is a reactive aldehyde and is one among the many reactive electrophilic species that cause toxic stress in cells and form covalent protein adducts which are referred as advanced lipoxidation end products.

Malondialdehyde also reacts with deoxyadenosine and deoxyguanosine in DNA forming DNA adducts, primarily M1G1 which is mutagenic. Increased MDA concentration in smokers may be due to increased production of reactive oxygen species and hence more lipoxidation products.

**Conclusion**

Present study demonstrates there is increased oxidative stress in smokers compared to non-smokers. The oxidative stress level was elevated in accordance with the intensity of smoking. This study also demonstrates the decreased antioxidant level in smokers compared to non-smokers. This study emphasizes that tobacco smoke causes disturbance in balance between oxidative stress and antioxidant level. Hence by advising cessation of smoking and taking diet rich in antioxidants may prevent oxidative damage and prevent oxidative stress related diseases.

**References**