Original Research Article

Oxidative stress in cigarette smoker and smokeless tobacco user among ethnic group north-eastern population of Uttar Pradesh, India

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ABSTRACT

Background: Cigarette smoking and other form of tobacco abusing habits are high prevalence in India at present which can be compare the oxidative level among them. This study aimed to measure the oxidative level among different cigarette smoke and other form of users in north-eastern, Uttar Pradesh of India.

Methods: Total of 934 male and female subjects were selected in which 387 were controls (Group I), 140 were active smokers (Group II), 105 were passive smokers (group III), 182 were tobacco users (Group IV) and 120 were active smokers plus tobacco users (group V). Cigarette smoker and tobacco user prevalence <10/day, >10/day for 5 years’ duration were collected. MDA, SOD, GR and CAT were measured.

Results: Cigarette smoker and tobacco user prevalence is high in >10 cigarette/day for 5 years, Mean and SD value in oxidative stress in cigarette smokers and tobacco users MDA level 1314±330.1µmol/mg is increased, whereas SOD, GR and CAT level 2.229±0.248 units/ml, 0.0152±0.0071mg/ml and 0.345±0.046mg/ml respectively were reduced in active smoker plus tobacco users.

Conclusions: Present study concluded that cigarette smoker and tobacco user showed their increased MDA and decreased SOD, GR and CAT which represented the significantly increased oxidative stress in north-eastern of Uttar Pradesh, India.

Keywords: Catalase, Malondialdehyde, Reduced glutathione, Superoxide dismutase

INTRODUCTION

Cigarette smoking and tobacco chewing has more common in throughout the world. In India 86% is used to smoke cigarette and 14% is used to chewing tobacco form.1 Tobacco consumption is more dangerous to human health and still highly consumed in throughout the world.2 Tobacco consumption cause more than 5 million people death every year.3 Smokeless tobacco is consumed in un-burnt forms through chewing or snuffing and contains several carcinogenic compounds. Smokeless tobacco has been associated with oral cancer, hypertension, heart disease and other conditions.4 Smokeless tobacco may also refer to tobacco dipping, snuff, snus, creamy snuff or tobacco toothpaste, tobacco gum, dissolvable tobacco, topical tobacco paste, tobacco water, herbal smokeless tobacco, etc. Smokeless tobacco use is more prevalent in countries of Asia, Africa and the Middle East than in Europe and the Americas.5

Cigarette smoke (CS) contains over 4000 chemical compounds such as nicotine, nitrogen oxides, carbon monoxide, hydrogen cyanide and free radicals, leading to generation of more toxic and carcinogenic compounds harmful to the health.6 Nicotine is a major addictive agent in tobacco which acts as a psychoactive drug.

CS causes metabolic activation leads to formation of reactive intermediates and potentially damaging metabolites that can promote cell injury and elicit toxic
effects.7 Tobacco consumption causes oral cancers, 30-40% of cancer cases accounted in India.8 It is also generates increased oxidative stress and lipid peroxidation (LPO). The gas phase of CS contains free radicals such as superoxide radicals, hydroxyl radicals and hydrogen peroxide (H₂O₂) turn to causes oxidative stress.9,10 Tobacco consumption produces free radicals; it also increased oxidative stress and LPO.8 Oxidative effects via free radical generation in smokers’ cause LPO, oxidation of proteins and damage to tissues mainly that of lung. The antioxidant enzymes superoxide dismutase (SOD) catalyses the dismutation of superoxide anions (O₂⁻) to hydrogen peroxide (H₂O₂), and glutathione peroxidase (GPx), catalase (CAT) catalyzes the degradation affected by CS resulting in deleterious effects.11

The aim of the present study was to correlate the oxidative stress among users of various form of cigarette smoker, cigarette smoke exposure and tobacco user in population of North-Eastern Uttar Pradesh, India.

METHODS

Study population

The present study was conducted in the Department of Psychiatry and Anatomy, Institute of medical science, Banaras Hindu University, Varanasi, Uttar Pradesh, India. Total of 934 male and female subjects were selected in which 387 were controls (Group I), 140 were active smokers (AS) (Group II), 105 were passive smokers (PS) (group III), 182 were tobacco users (TU) (Group IV) and 120 were active smokers plus tobacco users (AS and TU) (group V).

All participated subjects were personally interviewed and collected history include age, sex, details of tobacco consumption (>10/day and <10/day 5 years’ duration), smoking cigarettes (>10/day and <10/day 5 years duration). Exclusion criteria were Alcohol consumption, mental illness, systemic diseases such as Diabetes mellitus, Hypertension which may also influence the antioxidant status. The age group of participants were ranged from 18-55 years.

Sample collection and bio chemical assay

Blood samples of subjects were collected in 10% EDTA vials for determining the oxidative stress. The samples were kept in ice box to maintain 4°C. Centrifugation was done at 1,000 rpm for 5 min, and serum was carefully separated. The separated supernatant was long term stored at -20 °C for determination of enzyme activities. All chemicals and reagents were obtained from Sigma Chemical Co. (St Louis, MO, USA). Malondialdehyde (MDA) was estimated by thiobarbituric acid reactive substances (TBARS) test protocol from Satoh K at 532 nm by ELICO- SL-104 double beam UL-UV Spectrophotometer.12 Superoxide dismutase (SOD) activity was estimated on the inhibition of the formation of NADH-phenazine methosulphate-nitro blue tetrazolium formazan from modified protocol of Nandi A et al.13 The colour formed at the end of the reaction and measured at 560 nm by ELICO- SL-104 double beam UL-UV Spectrophotometer. Reduced glutathione (GR) level was estimated on the development of yellow colour when 5’5’ dithiobis (2-nitrobenzioc acid) was added to sulphhydryl compound test protocol from Butler E et al.14 This reaction was read at 420 nm by ELICO- SL-104 double beam UL-UV Spectrophotometer.

Catalase (CAT) activity was measured in the serum by the method of Luck Het al.15 The colour formed at the end of the reaction and measured at 560 nm by ELICO- SL-104 double beam UL-UV Spectrophotometer.

Statistical analysis

Obtained data were entered in excel sheet of MS-2007. Statistical analysis was carried out using Graph pad Prism (version 6) for windows. The results were reported as means±SD (SE). Statistically analysis by using one way ANOVA and Bonferroni’s multiple comparison test were used to compare between control and smoker and smokeless tobacco user groups. A value p<0.05 was considered statistically significant.

RESULTS

Subjects characteristics

Hospital based, cohort study was done in OPD of psychiatry, Institute of Medical Sciences, Banaras Hindu University, from March 2015 to December 2016. Total of 547 subjects (171 females and 376 males). It was observed that prevalence of active smoker (AS), tobacco user (TU) and AS plus TU was more in male and exceeded 55.49% (Table 1). The prevalence of passive smoke in female was observed to be 85.71%. Female prevalence showed nil in AS and AS plus TU (Table 1). On age wise prevalence of all aforementioned habits were more under age of 18-30 yrs as compare to other age groups whereas the prevalence of 51 yrs and above subject’s all habits were least (Table 1). Prevalence of TU was 44.5% under 31-40 years which was higher than the other age group (Table 1). Subjects were categorized according to their cigarette and tobacco use frequency per day for 5 years and above (Table 1). It was obtained that AS habit found to be highest percentile under <10 frequency/day whereas AS plus TU found to be highest under >10 frequency/day (Table 1).
Table 1: Subject’s characteristic data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (N=387)</th>
<th>Active smoker (N=140)</th>
<th>Passive smoker (N=105)</th>
<th>Tobacco user (N=182)</th>
<th>Active smoker plus tobacco user (N=120)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>192</td>
<td>140</td>
<td>15</td>
<td>101</td>
<td>120</td>
</tr>
<tr>
<td>Female</td>
<td>195</td>
<td>0</td>
<td>90</td>
<td>81</td>
<td>0</td>
</tr>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-30</td>
<td>221</td>
<td>67</td>
<td>59</td>
<td>71</td>
<td>61</td>
</tr>
<tr>
<td>31-40</td>
<td>130</td>
<td>45</td>
<td>33</td>
<td>81</td>
<td>42</td>
</tr>
<tr>
<td>41-55</td>
<td>25</td>
<td>20</td>
<td>10</td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td>51-55</td>
<td>11</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Habitat (frequency/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>0</td>
<td>126</td>
<td>34</td>
<td>104</td>
<td>34</td>
</tr>
<tr>
<td>&gt;10</td>
<td>0</td>
<td>14</td>
<td>71</td>
<td>78</td>
<td>86</td>
</tr>
</tbody>
</table>

Table 2: Serum levels in MDA, SOD, CAT and GR in smokers, smokeless tobacco users and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MDA (Mean±SD)</th>
<th>SOD (Mean±SD)</th>
<th>GR (Mean±SD)</th>
<th>Catalase (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>205.7±53.40</td>
<td>7.490±2.096</td>
<td>0.0852±0.011</td>
<td>0.804±0.674</td>
</tr>
<tr>
<td>Active smokers</td>
<td>659.7±52.93</td>
<td>2.761±0.696</td>
<td>0.0317±0.008</td>
<td>0.384±0.052</td>
</tr>
<tr>
<td>Passive smokers</td>
<td>240.4±7.26</td>
<td>6.900±1.262</td>
<td>0.0564±0.0169</td>
<td>0.759±0.126</td>
</tr>
<tr>
<td>Tobacco users</td>
<td>344.6±59.21</td>
<td>6.494±1.360</td>
<td>0.0558±0.0197</td>
<td>0.737±0.103</td>
</tr>
<tr>
<td>Active smoker plus tobacco users</td>
<td>1314±330.1</td>
<td>2.229±0.248</td>
<td>0.0152±0.0071</td>
<td>0.345±0.046</td>
</tr>
</tbody>
</table>

Oxidative level of subject

The ratio of serum MDA level was observed to be significantly higher (P<0.0001) in AS and TU (1314±330.1) as compared to aforementioned habitat (Table 2), whereas group IV tobacco use habit is less significant with P<0.01 (Figure 1 and Table 2).

Figure 1: Serum MDA level in different habits group of control, cigarette smoking, cigarette smoke exposure and tobacco user (shows significance difference *P<0.01 and ***P<0.0001).

Figure 2: Serum SOD level in different habits group of control, cigarette smoking, cigarette smoke exposure and tobacco user (shows significance difference *P<0.01 and ***P<0.0001).
Serum SOD level were observed to be significantly reduced (P<0.0001) in Group II, active smoking habit and group V, active smoking plus tobacco use habit in compared to control (Figure 2 and Table 2), whereas group IV tobacco use habit is less significant with P<0.01 (Figure 2 and Table 2).

**DISCUSSION**

Present study involved the demographic study and estimation of different parameters such as MDA, SOD, GR and CAT to measure the oxidative status in cigarette smoker, cigarette smoke exposure, and tobacco user came to treatment in Sir Sundarlal Hospital, Banaras Hindu University, Varanasi, Uttar Pradesh, India.

In the present study, Male habitat of cigarette smoking showed dominancy over female by 100%. Similar studies cigarette smoking habitat was done in other state of India.  

Present study, on age range distribution of the habits showed the maximum prevalence in range of 18-30-year group followed to 31-40, 41-50, 51-60 year group in contrast the age group 31-40 showed the maximum percentile prevalence in tobacco use. The least prevalence of all habits was found to be in ethnic group 51-60 years. Active smoking habit showed 47.85% prevalence in ethnic group 18-30 years. In other part of India by research also reported most prevalence in this group aged over 18 year. 17,18

In present study, those habits were recategorized on the basis of consumption frequency per day less than 10 and more than 10 at least for 5 year. Active smoking was found to be the highest prevalence in less than 10 frequencies per day. Tobacco user and cigarette smoking was the least prevalence.

Biochemical parameters to assess the oxidative status in present study showed that MDA is the biomarker as end product of lipid peroxidation was found to be the highest in active smokers and active smoker plus tobacco users in compared to control. Other antioxidants such as SOD, GR and CAT were found to be the lowest in active smokers and active smoker plus tobacco user in compared to control. Similar findings have been reported in cigarette smokers and tobacco user in. 19,20,24

The present study showed that MDA was found the highest in Group V, active smoker plus tobacco user and Group II, active smoker in compared to control, however the Group IV was less significant in compared to control, which suggested the MDA level increased due to the consumption cigarette smoke and tobacco since tobacco constituents is responsible to lipid peroxidation.  

SOD is an enzymatic antioxidant marker for oxidative stress, SOD level was highly significantly reduced in Group V, active smoker plus tobacco user and Group II, active smoker in compared to control, however the Group IV was significantly reduced in compared to control. Similar study was found in cigarette smoking. 22

GR is an enzymatic marker to estimate oxidative level, GR is highly significantly reduced to all habits in
compared to control. Similar study was found in tobacco smoking.\textsuperscript{23,24}

CAT is an enzymatic marker to estimate oxidative level, CAT level was highly significantly reduced in Group V, active smoker plus tobacco user and Group II, active smoker in compared to control, however the Group IV was significantly reduced in compared to control. Similar finding was found in cigarette smoker and tobacco users.\textsuperscript{25,26}

CONCLUSION

Present study concluded that cigarette smoker, cigarette smoke exposure, and tobacco user showed their increased MDA and decreased SOD, GR and CAT which represented the significantly increased oxidative stress.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Central Ethical Committee of Banaras Hindu University, Varanasi, Uttar Pradesh, India (reference-Dean/2015/EC/256)

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