Occupational Exposure of Tobacco and Its Effect on Total Antioxidant Capacity in Bidi Workers

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ABSTRACT

Aim: The aim of the study was to evaluate the status and diagnostic utility of urinary Cotinine and plasma Total Antioxidant Capacity (TAC) as indicator of antioxidant status in bidi workers.

Material and method: We conducted a case-control study of 90 Bidi workers. Bidi workers were further subdivided on the basis of exposure period (i.e. work experience in years) as, Group-I with experience of 5-9 years (30 subjects), Group-II with experience of 10-14 years (30 subjects), Group-III with experience of 15-19 years (30 subjects) and Healthy Controls – 30 subjects. We measured urinary Cotinine and Plasma Total Antioxidant Capacity (TAC).

Results: Highly significant urine cotinine levels were found in all groups of bidi workers as compared to the control subjects (P<0.001). Urine cotinine
levels in the study group subjects were elevated as duration of exposure to tobacco dust was progressed. Total antioxidant capacity was found to be decreased in all groups of bidi workers when compared with control subjects (p<0.001)

We also found negative correlation between urinary cotinine and total antioxidant capacity in study group subjects (Scatter Diagram No.3) substantiating decline in total antioxidant capacity gradually progresses with exposure to tobacco dust.

**Conclusion:** Our observed results show increase in the urinary cotinine and decrease in Plasma Total Antioxidant Capacity (TAC) levels in bidi workers as per exposure to nicotine.

**Keywords:** Cotinine, Total Antioxidant Capacity.

**INTRODUCTION**
Over the centuries, tobacco use has become widespread in almost every society and tobacco use is deeply rooted in Indian Culture too. Tobacco has been used from Pre-Columbian times to the present for magicoreligious medicinal and recreational purpose.1 The large number of labour force from lower economic group is engaged in this occupation, women are large part of labour force, doing the most menial work and often employed in the occupation. Bidi rollers, especially women, handle 125-450 gm of bidi tobacco per day and inhale approximately 1.31 mg/m³ of tobacco dust and volatile components present in work environment such as nicotine. Occupational exposure of tobacco leads cutaneous absorption of tobacco constituents.2,3 Since bidi is hand rolled, workers employed in bidi industries are at risk of developing various disorders. These includes respiratory ailments, such as bronchitis and asthma (allergic airway inflammation) from tobacco dust and nicotine, burning eyes, conjunctivitis, occupational dermatitis and green tobacco sickness which is caused by the absorption of nicotine via the skin. In addition irregular menstruation, miscarriage and low birth weight offspring are common complaints among these women workers, they may be related to tobacco exposure.4,5 During handling of tobacco most of the tobacco ingredients are absorbed through intact skin or inhale the tobacco dust and have similar effects as in smokers.6

The objective of this article was to review the effects of tobacco on total antioxidant capacity (TAC). The present study was aimed to assess exposure effect of tobacco dust for which following biochemical parameters were measured quantitatively in Healthy controls ( females not exposed to any dust ) and in female bidi workers. 1] Cotinine in urine  2] Plasma Total Antioxidant Capacity (TAC) and to correlate concentration of Total Antioxidant Capacity with the levels of Urinary Cotinine in study group subjects.
MATERIAL AND METHODS
The present study was carried out in the Tertiary Health Care Hospital (both OPD & IPD patients were included).
Selection and Distribution of Subjects (Table No.1). :-
Control Group- Healthy Controls – 30 subjects. Normal healthy age matched female subjects (not exposed to any occupational dust) were selected as controls. The study group of 90 Bidi workers were further subdivided on the basis of exposure period (i.e. work experience in years) as,
Group-I  Bidi workers exposed to tobacco dust
with experience of 5-9 years (30 subjects)
Group-II  Bidi workers exposed to tobacco dust
with experience of 10-14 years (30 subjects)
Group-III  Bidi workers exposed to tobacco dust
with experience of 15-19 years (30 subjects)

The subjects with the clinical history of diseases which may lead to oxidative stress such as diabetes mellitus, CVDs, infectious diseases, pulmonary diseases etc and having history of smoking were excluded from the study.

Collection and preservation of samples :-
After obtaining prior consent, venous blood was collected from the subjects under aseptic condition by venipuncture using 20ml sterile disposable syringe and needle. About 3-4 ml of blood was collected of which 2 ml of blood was poured into sterile heparinised bulb and remaining blood was taken in a sterile plain bulb and was allowed to clot, then serum was separated. Also overnight urine samples from the subjects were collected early in the morning for analysis of cotinine. All the samples were stored at 4°C before analysis and were analysed on the same day of collection.

All the biochemical parameters measured in study group subjects were statistically compared with those estimated in controls. Biochemical parameters were expressed as mean±SD for each group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Work Experience</th>
<th>No of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>--</td>
<td>30</td>
</tr>
<tr>
<td>Bidi Workers :</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Group-I</td>
<td>5-9 years</td>
<td>30</td>
</tr>
<tr>
<td>Group-II</td>
<td>10-14 years</td>
<td>30</td>
</tr>
<tr>
<td>Group-III</td>
<td>15-19 years</td>
<td>30</td>
</tr>
</tbody>
</table>
RESULTS
Highly significant urine cotinine levels were found in all groups of bidi workers as compared to the control subjects \((P<0.001)\) (Table No.2, Graph No.1). Urine cotinine levels in the study group subjects were elevated as duration of exposure to tobacco dust was progressed. Total antioxidant capacity was found to be decreased in all groups of bidi workers when compared with control subjects \((p<0.001)\) (Table No.3, Graph No.2). We also found negative correlation between urinary cotinine and total antioxidant capacity in study group subjects (Scatter Diagram No.3) indicating decline in total antioxidant capacity gradually progresses with exposure to tobacco dust.

Table 2: Levels of Urinary Cotinine in healthy controls and in different groups of Bidi Workers.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. Of Subjects</th>
<th>Urinary Cotinine (mmol/mol of creatinine)</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Controls</td>
<td>30</td>
<td>0.00</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Bidi workers :-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group-I</td>
<td>30</td>
<td>2.00±0.27</td>
<td>39.89</td>
<td>(p&lt;0.001)</td>
</tr>
<tr>
<td>Group-II</td>
<td>30</td>
<td>2.50±0.32</td>
<td>42.07</td>
<td>(p&lt;0.001)</td>
</tr>
<tr>
<td>Group-III</td>
<td>30</td>
<td>2.67±0.27</td>
<td>53.25</td>
<td>(p&lt;0.001)</td>
</tr>
</tbody>
</table>

Values expressed in Mean ± SD.

Statistical Group Comparison :-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gr-I with Gr-II</th>
<th>Gr-II with Gr-III</th>
<th>Gr-I with Gr-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Cotinine</td>
<td>t=6.43</td>
<td>t=2.18</td>
<td>t=9.44</td>
</tr>
<tr>
<td>(mmol/mol of creatinine)</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

\(p<0.05\) = Significant
\(p<0.001\) = Highly Significant
Graph: 1 Levels of Urinary Cotinine in healthy controls and in different groups of Bidi Workers.

Table No.3 Levels of Total Antioxidant Capacity in healthy controls and in different groups of Bidi Workers.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. Of Subjects</th>
<th>Total Capacity (μmol/L)</th>
<th>Antioxidant</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Controls</td>
<td>30</td>
<td>949.13±31.71</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Bidi workers :-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group-I</td>
<td>30</td>
<td>432.93±76.06</td>
<td>33.78</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Group-II</td>
<td>30</td>
<td>391.53±42.59</td>
<td>56.59</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Group-III</td>
<td>30</td>
<td>385.50±10.95</td>
<td>90.53</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed in Mean ± SD.

Statistical Group Comparison :-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gr-I with Gr-II</th>
<th>Gr-II with Gr-III</th>
<th>Gr-I with Gr-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Capacity</td>
<td>t=2.54</td>
<td>t=0.74</td>
<td>t=3.30</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>p&lt;0.01</td>
<td>p&gt;0.05</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

p<0.05  = Significant
p<0.001= Highly Significant
Graph: 2 Levels of Total Antioxidant Capacity in healthy controls and in different groups of Bidi Workers.

Scatter Diagram: 3a) Correlation between Urinary Cotinine and Total Antioxidant Capacity in Group-I Subjects

$r = -0.90$
Scatter Diagram: 3b) Correlation between Urinary Cotinine and Total Antioxidant Capacity in Group-II Subjects

\[ r = -0.89 \]

Scatter Diagram: 3c) Correlation between Urinary Cotinine and Total Antioxidant Capacity in Group-III Subjects

\[ r = -0.91 \]
DISCUSSION
Present study comprises tobacco dust exposure and its effects on the status of antioxidants in Bidi workers. To achieve this we studied the levels of Urinary Cotinine, and Total Antioxidant Capacity (TAC)

Nicotine / Cotinine :-
Nicotine absorbed is detoxified by the liver to convert it into cotinine. Cotinine has half life of 16 hrs (as compared to nicotine which is having only 2 hrs) & therefore it is measured as a biomarker of nicotine exposure / absorption.$^{(9)}$

In this study, the urinary cotinine concentration was measured in various groups of bidi workers. Highly significant urinary cotinine levels were found in all groups of bidi workers (highest levels of urinary cotinine in group-III bidi workers) as compared to the control subjects ($p<0.001$) (Table No.2). Marked augmented increase in urinary cotinine levels were seen in study group subjects as duration of exposure to tobacco dust was progressed.

S. Swami et al. $^{(9)}$ observed that there was significantly rise in urinary cotinine level in bidi workers as compared to healthy controls. R.B.Govekar et al. $^{(10)}$ also reported that handling of tobacco is related with elevated urinary cotinine level among bidi rollers ($p<0.01$).

No comparative study based on work experience in Bidi workers was found but Bagwe AN et al. $^{(2,11,12)}$ showed significant amount of excretion of urinary cotinine
among bidi workers, indicating nicotine enters through various ways in blood circulation.

Nicotine is readily absorbed from the respiratory tract, buccal mucous membrane and intact skin. Later two ways of absorption are common in bidi workers. While preparing bidis, ingredients of tobacco is likely to be absorbed through intact skin of the hands and inhaled as fine dust. The pH is important in absorption of nicotine across cell membranes. In acidic environment nicotine is in its ionized state and does not readily cross the membrane, at physiological pH (7.4) about 31% of nicotine is non-ionized such that it rapidly crosses the membrane.(1)

Tobacco dust is inhaled by bidi workers as they are exposed to large amount of dust. Inhalation is required to allow nicotine to be absorbed by the huge surface of alveolar epithelium. In the lungs nicotine is rapidly absorbed into systemic circulation, absorption is facilitated because pulmonary capillary blood flow is high representing passage of the entire blood volume through the lung every minute. At work environment, bidi workers sweating may dissolve some nicotine ingredient which can be absorbed through skin. (9)

Total Antioxidant Capacity (TAC):
We found negative correlation between urinary cotinine and total antioxidant capacity in study group subjects

[Scatter Diagram No.3 : a) Group-I (r = -0.90), b) Group-II (r = -0.89), c) Group-III (r = -0.91) & d) All Groups of Bidi Workers (r = -0.93)] substantiating decline in total antioxidant capacity gradually progresses with exposure to tobacco dust.

S. Swami et al. (9) noted significant decline in the levels of total antioxidant capacity in bidi workers whereas, A.N.Suryakar et al. (15) observed significant decrease in the activities of glutathione peroxidase, glutathione reductase and catalase as well as in total antioxidant capacity in bidi workers (p<0.001). We also perceived similar observations i.e. significant decline in total antioxidant capacity with the progression of duration of tobacco dust exposure in all groups of bidi workers.

This diminution in the levels of antioxidant capacity might be the cause of reduced ability of plasma to withstand ROS which is obtrusive as significantly reduced total antioxidant capacity was observed among bidi industry workers.(16). Another interesting study by Chelchowska et al. reported that nicotine from smoking was associated with increased lipid peroxidation levels in mothers and their fetuses and decreased TAC in both groups. (17)

A decrease in antioxidant capacity is the condition when the production of Reactive Oxygen Species (ROS) increases. A single measurement of total antioxidant capacity in plasma or serum is not going to be sufficient but a ‘battery’ or measurements which should include measurement of oxidative damage is necessary to adequately assess
oxidative stress in vivo. A highly significant decrease in levels of total antioxidant capacity was found in each groups of bidi workers (highest decline seen in group-III bidi workers) when compared with controls (p<0.001) (Table No.3), the substantial decrease in total antioxidant capacity is associated with increased duration of exposure to tobacco dust. The linear correlation was perceived between the duration of occupational dust exposure and more pronounced prooxidant and antioxidant imbalance. Various health problems associated with these workers may arise through an upsurge of free radical generation and lowered antioxidant capacity.

The present study had several limitations. First, in this the total antioxidant content was measured and not enzyme activity. The second limitation of this study was that it was a cross-sectional type and may indicate the temporary relationship between tobacco exposure and Total Antioxidant Capacity.

CONCLUSION
Occupational exposure to tobacco can decrease level of Total Antioxidant capacity (TAC), rendering subjects less resistant to oxidative and nitrosative injuries and subsequent diseases. More research is needed to address the role of antioxidant supplementation in tobacco exposure and disease prevention.

REFERENCES
Occupational Exposure of Tobacco and Its Effect on Total Antioxidant Capacity


