**Regular article**

**Biomonitoring of Oxidative DNA Damage in Traffic Policemen Exposed to Urban Air Pollution**

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The urinary excretion of 8-hydroxy-2′-deoxyguanosine (8-OHdG) has been used as a biomarker of oxidative DNA damage in both the clinical and occupational setting. The urinary 8-OHdG in traffic policemen posted at busy traffic junctions were estimated along with the control population away from the busy traffic junctions those doing administrative job. A total of 105 urinary samples (60 samples of traffic policemen and 45 samples of control population) were collected for estimation of 8-OHdG and analyzed using enzyme linked immunosorbent assay (ELISA). The mean 8-OHdG was significantly higher (13.42 ± 1.61 μg/g creatinine) than those of control group (9.34 ± 1.36 μg/g creatinine) (p < 0.05). The study showed that urinary 8-OHdG is associated with occupational exposure and other lifestyle factors.

Key words: urinary 8-hydroxy-2′-deoxyguanosine, traffic policemen, ELISA

**Introduction**

Vehicular exhaust pollution is a major concern in urban cities of India. Because it is considered as causative factor for increased incidences of diseases such as asthma, lung cancer, morbidity of rhinitis, pharyngitis, trachoma syndrome, neurasthenia, joint pains and disorders of digestive system (1). More than 3000 environmental chemical compounds have been identified in the ambient atmosphere including a variety of mutagenic and/or carcinogenic agents, such as polycyclic aromatic hydrocarbons (PAH) (2). Exposure to chemicals with carcinogenic properties occurring in the human environment viz., ambient air, food, or lifestyle-related factors such as cigarette smoke promotes and/initiates carcinogenesis (3–6).

Traffic related air pollution is a growing concern today because of its constituent’s viz., PAHs, volatile organic compounds (VOCs) along with diesel particles reported to be strong mutagens and carcinogens (7). An increased risk of cancer has been reported in occupations with heavy exposure to traffic related pollution (8). Chronic occupational exposure to urban pollutants increases the risk of harmful cardiovascular, respiratory and exercise performance (9). Earlier studies have demonstrated that automobile exhaust can induce gene mutations, chromosomal aberrations, sister chromatid exchanges, micronuclei and cell transformation (10,11).

Since some mutagens and carcinogens are found in automobile exhaust pollutants monitoring of biomarkers of early genetic effect will be a useful tool on exposed group personnel. The biomarkers are considered that make possible human risk assessment before noticeable indicative health effects appear. A wide range of methods are presently used for the detection of early biological effects of DNA damaging agents in environmental and occupational settings (12). Several markers of early biological effects have been developed and applied over the last decades (13).

Evaluation of urinary 8-OHdG has been used as a good biomarker in both the clinical and occupational setting (14–19), because it is non invasive method compared to collect tissue or leukocyte samples. 8-OHdG is known to represent one of the major forms of oxidative DNA damage, many researchers have measured 8-OHdG in tissues or urine as a marker of useful oxidative stress (20). 8-OHdG is premutagenic because it can mispair with adenine instead of cytosine during DNA replication and if this occurs, a G→T transversion mutation develops. Measurement of urinary 8-OHdG has recently become more popular as a means of evaluating the level of oxidative stress in the human body, because it probably reflects the level of oxidative DNA damage in the body as a whole (21). Exposure to various environmental pollutants such as polycyclic hydrocarbons, fly ash and fine particulate matter 2.5 μm containing metal fumes of vanadium, chromium, manganese, nickel,
copper and lead increased the level of urinary 8-OHdG (22–24). Because particles smaller than 2.5 microns in diameter can get into the deepest portion of the lungs where the gas exchange occurs between the air and blood stream. These are the dangerous particles because the body has no efficient mechanisms for removing them. Also a dose response relationship was found between personal exposure to benzene and urinary 8-OHdG concentrations (16).

Our recent study on occupational exposure (personal) to urban air pollutants in traffic policemen showed benzene, toluene and m-xylene concentration from 4.35–55.10, 8.73–103.61 and 1.53–135.67 µg/m³, respectively, during their work. The trans, trans-muconic acid, a known biomarker of benzene concentration was estimated along with the control population away from the control subjects not working in the traffic zones were compared to the same age group of people in control subjects (9.39 ± 3.30 µg/g creatinine). In the 36–45 age group of traffic policemen and administrative staff is also shown in the table. The statistical analysis of the data among each variables and comparative analysis of the data of traffic policemen and administrative staff is also shown in the table.

The aim of this study was to investigate urinary levels of the oxidative injury biomarker, 8-OHdG in traffic policemen working in Bangalore city, Karnataka state, India.

Material and Methods

Analysis of urinary 8-OHdG: The urinary 8-OHdG in traffic policemen posted at busy traffic junctions were estimated along with the control population away from the busy traffic junctions those doing administrative job. A total of 105 urinary samples (60 samples of traffic policemen and 45 samples of control population) were collected for estimation of 8-OHdG. Urinary 8-OHdG levels were determined using a competitive ELISA immunoassay (Japan Institute for the Control of Ageing, Fukuroi, and Shizuoka, Japan). The collected urine samples were centrifuged at 2000 rpm for 10 min and the supernatants were used for the determination of 8-OHdG. 50-µL of urine samples and standards were added to precoated 8-OHdG protein conjugate microtitre plates followed by 50 µL of the primary antibody, anti 8-OHdG monoclonal antibody solution. After incubation for 1 h at 37°C, the plates were washed and the enzyme-labeled secondary antibody (100 µL) was applied for 1 h at 37°C. After washing, 100 µL of the chromatic substrate, 3,3’,5,5’-tetramethylbenzidine, were added to the plate and allowed to react at room temperature for 15 min. The intensity of color produced for each sample was measured at an absorbance of 490 nm. All the values of urinary 8-OHdG levels were subsequently adjusted by urinary creatinine levels. The data pertaining to personal habits like smoking, alcohol consumption, nutritional data and other potential confounding factors were collected using a questionnaire survey. The ethical committee of the centre has approved the study. Before initiating the study all the participants were informed about the study and their consent was obtained.

Statistical analysis: Statistical analyses were performed using SPSS statistical software version 10. Mean and standard error were determined for all subjects. Due to positively skewed distribution of the data, Wilcoxon rank-sum test were performed to compare two groups.

Results and Discussion

The urinary 8-OHdG levels among the study and control subjects according to their age groups, experience, habits of smoking, chewing, alcohol consumption, tea/coffee drinking, eating of vegetarian and non-vegetarian food and exercise habits are given in Table 1. The statistical analysis of the data among each variables and comparative analysis of the data of traffic policemen and administrative staff is also shown in the table.

It was observed that the traffic policemen between the age group of 25–35 showed similar levels of 8-OHdG (16.82 ± 3.26 µg/g creatinine) compared to the same age group of people in control subjects (9.39 ± 3.30 µg/g creatinine). In the 36–45 age group of traffic policemen the levels were 12.77 ± 2.71 µg/g creatinine whereas in the control group it was 8.77 ± 1.52 µg/g creatinine. Similarly in the higher age group of traffic policemen i.e ≥ 46, the levels were 10.23 ± 1.82 µg/g creatinine and in the control subjects of the same group it was 9.64 ± 2.19 µg/g creatinine. The mean 8-OHdG levels of the total study group were significantly higher (13.42 ± 1.61 µg/g creatinine) than the total control group (9.34 ± 1.36 µg/g creatinine) (p < 0.05).

The traffic policemen those working less than 10 years showed the 8-OHdG levels of 15.18 ± 2.35 µg/g creatinine and those working more than 11 years and below 20 years 10.96 ± 1.97 µg/g creatinine. These values in the control subjects not working in the traffic zones were
8.95 ± 1.85 μg/g creatinine among those below 10 years of experience. Even when we look into the control group working between 11–20 and ≥30 years of experience, the levels were 10.00 ± 2.09 μg/g creatinine and 7.30 ± 2.79 μg/g creatinine, respectively. Studies on urinary 8-OHdG as a biomarker of oxidative DNA damage in workers exposed to fine particulates were carried out by Kim et al. (24), and they concluded that occupational exposure to fine particulate matter. Chaung et al. (8) studied the urinary 8-OHdG on taxi drivers from traffic exhaust and/or smoking in exposed and non-exposed individuals, and found the level significantly higher in drivers than in community men (13.4 ± 4.7 vs. 11.5 ± 4.7 μg/g creatinine).

The comparison of 8-OHdG levels within the study group according to the smoking habit, it was clarified that smoking habit decreased the excretion level significantly, although no effect was observed in the control group. 8-OHdG levels in the study and control groups with smoking habits were similar: 6.47 ± 1.24 μg/g creatinine and 7.74 ± 1.57 μg/g creatinine, respectively. In contrast, in the non-smoking subjects, the 8-OHdG levels were significantly different (p < 0.01) between the study group (15.74 ± 1.20) and control group (9.79 ± 1.69). The data analysis among the smokers and non-smokers of the present study contradicts the earlier studies, as regard to excretion of 8-OHdG. The earlier studies showed that the smoking habit had effect on 8-OHdG excretion. Several studies showed an increase in urinary 8-OHdG concentrations in smokers compared with nonsmokers (7,21). Smoking can have a considerable effect on the concentration of 8-OHdG. However, studies by Nilsson et al. (22) failed to show the effect of smoking on the urinary excretion of 8-OHdG. Whereas Kim et al., (24) reported that mean baseline 8-OHdG concentrations were not significantly different between smokers and nonsmokers. Previous occupational studies, in which workers were exposed to various carcinogens, including PAHs and asbestos, found that smoking status was not a significant predictor of urinary 8-OHdG levels (18,19,26). However, Nia et al. (27) reported that 8-OHdG was consistently increased among smokers. Heavy exposure to air pollution in occupational settings in terms of diesel exhaust, polynuclear aromatic hydrocarbons, and benzene has been associated with increased 8-OHdG excretion, whereas non-occupational exposure to ambient air pollution did not significantly lower the urinary 8-OHdG level in smokers as compared with non-smokers. They concluded that ox-

<table>
<thead>
<tr>
<th>Variables</th>
<th>Traffic policemen</th>
<th>Administrative staff</th>
<th>p-value*</th>
<th>p-value*</th>
<th>p-value †</th>
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<tr>
<td>Age (yrs)</td>
<td></td>
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<td>25–35</td>
<td>16.82 ± 3.26 (24)</td>
<td>9.39 ± 3.30 (6)</td>
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<td>36–45</td>
<td>12.77 ± 2.71 (13)</td>
<td>8.77 ± 1.52 (14)</td>
<td>0.458</td>
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<td>≥ 46</td>
<td>10.23 ± 1.82 (23)</td>
<td>9.64 ± 2.19 (25)</td>
<td>0.502</td>
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</tr>
<tr>
<td>Total</td>
<td>13.42 ± 1.61 (60)</td>
<td>9.34 ± 1.36 (45)</td>
<td>0.031</td>
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<td></td>
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<td>Experience (yrs)</td>
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<tr>
<td>≤ 10</td>
<td>15.18 ± 2.35 (37)</td>
<td>8.95 ± 1.85 (13)</td>
<td>0.825</td>
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<td>11–20</td>
<td>10.96 ± 1.97 (20)</td>
<td>10.00 ± 2.09 (26)</td>
<td>0.406</td>
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<td>≥ 31</td>
<td>8.06 ± 3.66 (3)</td>
<td>7.30 ± 2.79 (6)</td>
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<td>Smoking habit</td>
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<td>Yes</td>
<td>6.47 ± 1.24 (15)</td>
<td>7.74 ± 1.57 (10)</td>
<td>0.861</td>
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<td>No</td>
<td>15.74 ± 1.20 (45)</td>
<td>9.79 ± 1.69 (35)</td>
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<td>Yes</td>
<td>14.61 ± 1.68 (4)</td>
<td>12.35 (1)</td>
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<tr>
<td>No</td>
<td>13.33 ± 1.68 (56)</td>
<td>9.27 ± 1.38 (44)</td>
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<td>Alcohol Consumption</td>
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<td>Yes</td>
<td>14.63 ± 2.05 (27)</td>
<td>10.70 ± 3.07 (16)</td>
<td>0.602</td>
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<td>No</td>
<td>12.43 ± 2.42 (33)</td>
<td>8.58 ± 1.28 (29)</td>
<td></td>
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</tr>
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<td>Tea/Coffee</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>14.16 ± 1.71 (55)</td>
<td>10.01 ± 1.48 (40)</td>
<td>0.050</td>
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<td>No</td>
<td>5.26 ± 2.37 (5)</td>
<td>3.99 ± 1.44 (5)</td>
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<td>Vegetarian</td>
<td>11.07 ± 2.31 (14)</td>
<td>6.65 ± 0.96 (17)</td>
<td>0.215</td>
<td>0.186</td>
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<td>Nonvegetarian</td>
<td>14.14 ± 1.98 (46)</td>
<td>10.97 ± 2.05 (28)</td>
<td>0.176</td>
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<td>Exercise</td>
<td></td>
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<tr>
<td>Yes</td>
<td>9.23 ± 1.45 (26)</td>
<td>9.21 ± 1.85 (30)</td>
<td>0.455</td>
<td>0.485</td>
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</tr>
<tr>
<td>No</td>
<td>16.62 ± 2.50 (34)</td>
<td>9.60 ± 1.79 (15)</td>
<td></td>
<td>0.059</td>
<td></td>
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</tbody>
</table>

*Comparison within traffic policemen or administrative staff. †Comparison between traffic policemen and administrative staff.
Oxidative stress imposed by cigarette smoking has a low impact upon certain pathways involved in DNA damage and the antioxidative defense system. Hong et al. (28) concluded that numerous carcinogens present in cigarette smoke generate reactive oxygen species and induce oxidative damages, such as 8-OHdG in isolated DNA's as well as in cell cultures.

It has been reported that smokers had a 50% higher concentration of urinary 8-OHdG than nonsmokers (21). This correlates with the study by Hong et al. (28), which showed that smokers who smoke less that 20 cigarettes a day showed a 48% increase in the excretion of 8-OHdG, compared with non-smokers.

We studied effects of the habit of chewing areca nut, pan masala, tobacco etc. We could not find any effects. The consumption of alcohol did not show any difference in 8-OHdG levels among the study and control groups. Effects of tea/coffee drinks on the urinary 8-OHdG levels were examined. In the study group and also in the control group, the drinking habit increased the 8-OHdG levels. Further, 8-OHdG levels in the study group with drinking habits were significantly higher than those in the control group with the habit.

Nilsson et al. (22) reported that dietary factors can probably also influence the excretion of 8-OHdG. Kasai et al. (29) indicated that low meat intake (less than once/week) induces an increase of 8-OHdG. The attributing factor being low consumption of meat may be associated with the serious modulation of other dietary factors, which might induce oxidative stress. Another possibility was that components in meat may be required to scavenge oxygen radicals or to repair DNA damage efficiently. In our present study no difference could be observed between vegetarians and non-vegetarians.

In general, exercise is recognized as promoting good health and well being, but excessive exercise is associated with oxidative stress, reflected by higher levels of oxidative DNA damage (8-OHdG) and lipid peroxidation (30–32). It is controversial whether or not the indication of oxidative stress is related to an increased risk of disease. Exercise habits were registered and analyzed in this study too. Among study group the levels were significantly higher (p<0.05) in those not doing exercise compared to those having habit of doing moderate exercise. Kasai et al. (29) studied the effects of exercise, working conditions, meat intake, body mass index and smoking habit and other life styles on 8-OHdG excretion. It was found that moderate physical exercise, and high body mass index reduced the 8-OHdG excretion, while physical labour, smoking and low meat intake (less than once/week) increased the excretion levels.

Conclusion
The study demonstrated that the level of urinary 8-

OHdG is associated with occupational and other lifestyle factors. Differences between the results of our study and other investigators might explain variations in sample size, sample composition, and methods of measurement of urinary 8-OHdG. Inclusion of more number of participation from traffic policemen might highlight the association of 8-OHdG excretion and their exposure to air pollutants. We recommend the use of personal protective equipments such as respiratory masks during working hours and also advised to avoid smoking during working hours. Further studies are necessary to elucidate the implications of the 8-OHdG marker in terms of health risk to this occupational group.

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