Biomonitoring of Genotoxicity and Antioxidant Enzymatic Changes in Workers Occupationally Exposed to Asphalt Fumes

Khaled F. El-Said*, Hossam Ghoneim**

Abstract:
Background: Asphalt fumes consist of the gases and vapor emitted from the heated asphalt as well as the aerosols and mists resulting from their condensation after volatilization. Moreover, asphalt workers are exposed to variety of asphalted fumes and dusts, which can exert their toxic effects via production of reactive oxygen species. The spectrum of oxidative DNA damage includes strand breaks. Information on genotoxic risk for bitumen workers is limited. Objective: The aim of the present study was biomonitoring of genotoxicity and different antioxidant enzymes activity of workers occupationally exposed to asphalted fumes. Methods: It was conducted on fifty male workers employed in asphalted and proofing materials manufacturing. Fifty subjects were recruited as a control group matched for age, sex and socio-economic status. Results: revealed that high significant association in the levels of genotoxicity (DNA single strand breaks and urinary thioethers) and slightly significant reduction in the levels of antioxidant enzymes (SOD and CAT) and significant increase in GSH levels in exposed workers in comparison with control group. Moreover, level of Sulphur dioxide and Nitrogen dioxide did not exceed the standards Particulate matter which is exceeded the standard limits of indoor air quality. Conclusion: Present data indicate that asphalted fumes exposed workers exhibit an increase in GSH levels and oxidative stress induced by fumes may partly be attributed to the inhibited activities of SOD and CAT as well as increase in the potentials genotoxic risks by increasing the percentage of DNASSB and increase excretion of urinary thioethers.

INTRODUCTION

There is specific concern about the potential carcinogenicity of occupational exposure to bitumen fumes and aerosols. Bitumen is mainly used in roofing and in a mixture with stone in asphalt for road paving. During hot application of bitumen, complex mixtures of aerosols and vapors are emitted, which contain polycyclic aromatic hydrocarbons (PAH) and their derivatives as well as other compounds.(1) The potential carcinogenicity

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of bitumen has been attributed to the presence of PAH.\(^{1}\) However, some data suggest that PAH are not the sole genotoxic compounds in bitumen fume condensates. Nitrogen-, sulfur- and/or oxygen-containing PAH or their alkyl-substituted analogues may also contribute to genotoxic effects.\(^{2}\)

Condensates of bitumen fume can induce DNA damage both in vitro and in vivo but the mutagenic effect is less strong than for condensates from coal-tar fumes.\(^{3}\)

Carcinogens exert probably their biological effect not only through direct DNA damage, but also through the generation of reactive oxygen species. Bioactivation of PAH and other compounds requires oxidative metabolism by phase I enzymes and in particular the cytochrome P-450 system.\(^{4}\) These result in the formation of radical cations, anti–diol-epoxides and redox-active O-quinones. Reactive oxygen species may lead to the formation of oxidative DNA damage and have been shown to participate in all stages of the carcinogenesis process.\(^{5}\) The interacting pathways for prevention and repair of oxidative DNA damage have been recently reviewed.\(^{6}\) The spectrum of oxidative DNA damage includes strand breaks, apurinic/apyrimidinic sites, and oxidized bases. Information on genotoxic risk for bitumen workers is limited.

Bitumen, a bi-product of oil-refining, is mainly used in paving and roofing. In paving operations, bitumen is heated and mixed with mineral aggregates. The use of recycled materials and industrial bi-products in asphalt mixtures is increasing. Hot bitumen (130–200 °C) emits vapors and aerosols (fumes) that contain various compounds, including polycyclic aromatic hydrocarbons (PAHs) and sulphur-containing organic chemicals.\(^{7}\)

Asphalt fumes consist of the gases and vapor emitted from the heated asphalt as well as the aerosols and mists resulting
from their condensation after volatilization. They are a mixture of saturated straight chain hydrocarbons, monocycloparaffins, alkylbenzenes, branched chain aliphatics, and various aromatic compounds. Within the aromatic fraction, trace levels of polycyclic aromatic hydrocarbons have been detected.(8) Moreover, asphalt makers are exposed to a variety of asphalted fumes and dusts, which can exert their toxic effects via production of reactive oxygen species (ROS).(9) ROS are believed to cause lipid peroxidation that in turn damages the biological membranes. Antioxidants such as enzymatic and non-enzymatic defense systems are necessary to prevent the expected cellular damage. Smokers, are also exposed to significant quantities of ROS.(10)

The concentrations of ROS have to be controlled by several defense mechanisms, which involve also a number of antioxidant and detoxifying enzymes. Their induction reflects a specific response to pollutants. A balance between free radical reactions and antioxidant activities is very important for normal liver functioning. This balance is altered in pathological processes.(11) The aim of the present study was to biomonitoring of genotoxicity and different antioxidant enzymatic activities among workers occupationally exposed to asphalted fumes.

**SUBJECT AND METHODS**

The present study included 50 workers selected randomly from asphalt and proofing materials company, and 50 subjects selected from administrative departments and occupationally exposed to asphalt fumes as a control group. Verbal consent, approval of the Ethical committee was obtained. All participants signed individual consents prior to the study, occupational and medical questionnaire was completed with the workers through personal interview. Subjects with history of liver diseases, respiratory illness, or hospitalization for
surgery were excluded from the study.

**Collection and Assay:** Random heparinized and EDTA blood samples were collected from all cases by sterile disposable syringes.

DNA single strand Breaks were measured by elution unwinding assay methods.\(^{(12)}\)

Erythrocyte antioxidant enzymes, superoxide dismutase (SOD), Glutathione reductase (GRH) and Catalase (CAT) were estimated in appropriately diluted hemolysates. SOD was determined according to Beauchamp and Fridovich.\(^{(13)}\) based on inhibition of nitroblue tetrazolium reduction. CT activity was determined by the method of Brannen et al.\(^{(14)}\), which was based on the disappearance of H2O2 in the presence of enzyme source at 26°C and GR activity according to Oldberg and Spooner.\(^{(15)}\)

Spot urine samples were collected from all subjects for analysis of thioether levels in urine. Urinary thioethers were determined after alkaline hydrolysis as described by Vainio et al.\(^{(16)}\).

Environmental monitoring of Sulphur Dioxide (SO2) and Nitric dioxide NO2 in air were measured using direct reading instrument applying infrared analyzer principal.\(^{(17)}\) For PM\(_{10}\) (Particulate Matter) a total of 15 samples were collected during the monitoring program, to cover the fluctuations during different work activities. Before and after sample collection, the filters were conditioned in a dissector for 24 h. Filters were weighed before and after the sampling period and the amount of the collected particulate matter was determined as the gain in sample mass. The concentration of particulate matter was evaluated using the volume of samples and expressed in μg/m3.\(^{(18)}\)

**Statistical Analysis:** The collected data and the laboratory results were computerized. Statistical analysis was done through SPSS version 17.0. The quantitative results were expressed as means ± standard deviation (SD), and Independent t-test and Pearson's
correlation coefficient were used in the analysis of the results.

RESULTS AND DISCUSSION

Table 1: Characterization of the population studied

<table>
<thead>
<tr>
<th></th>
<th>Exposed</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.1±3.7</td>
<td>36.0±4.0</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of Exposure (years)</td>
<td>9.6±3.1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Smoking Habits (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>22(44%)</td>
<td>27(54%)</td>
<td>NS</td>
</tr>
<tr>
<td>Yes</td>
<td>28(56%)</td>
<td>23(46%)</td>
<td></td>
</tr>
</tbody>
</table>

NS: No Significant

In Table 1 no statistical significant association in age and smoking habit between exposed and control group.

Table 2: Comparisons of the Antioxidant activity between the two examined groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exposed</th>
<th>Independent t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>t</td>
</tr>
<tr>
<td>SOD (uU/gHb)</td>
<td>1959.18±37.04</td>
<td>1169.92±183.4</td>
<td>4.3</td>
</tr>
<tr>
<td>Blood GR (Iu/g Hb)</td>
<td>37.37±1.2</td>
<td>25.8±2.8</td>
<td>26.2</td>
</tr>
<tr>
<td>CAT(U/gmHb)</td>
<td>302.4±14.5</td>
<td>258.4±18.6</td>
<td>13.1</td>
</tr>
</tbody>
</table>

In Table 2 there is statistically significantly lower association in the levels of antioxidant activity in exposed workers in comparison with the control group.

Table 3: Comparisons of the Genotoxicity between the two examined groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exposed</th>
<th>Independent t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>t</td>
</tr>
<tr>
<td>DNA SSB %</td>
<td>4.1±2.2</td>
<td>35.4±4.1</td>
<td>47.1</td>
</tr>
<tr>
<td>Urinary Thioether(umol/mmol creatinine)</td>
<td>40.0±3.6</td>
<td>82.6±6.1</td>
<td>42.0</td>
</tr>
</tbody>
</table>

Table 3 showed higher significant levels of asphalt exposed workers relative to control group.
Table 4: Concentrations of measured airborne pollutants in the asphalted fumes manufacturing plant.

<table>
<thead>
<tr>
<th></th>
<th>SO2 (μg/m^3)</th>
<th>NO2 (μg/m^3)</th>
<th>PM10 (μg/m^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>0.210±0.36</td>
<td>0.035±0.05</td>
<td>4.18±21</td>
</tr>
<tr>
<td>Range</td>
<td>3.0</td>
<td>0.2</td>
<td>8.21</td>
</tr>
<tr>
<td>Egyptian Standard (μg/m^3)</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4 All measured airborne pollutants except for PM10 which exceeds the Egyptian standards.

Table 5: Correlation Coefficient of air Particulate (PM10) and biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>Air Particulate(PM10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA SSB %</td>
<td>r = 0.907</td>
</tr>
<tr>
<td>Urinary Thioether(umol/mmol creatinine)</td>
<td>r = 0.903</td>
</tr>
<tr>
<td>SOD (ugU/gHb)</td>
<td>r = -0.273</td>
</tr>
<tr>
<td>Blood GR (lu/g Hb)</td>
<td>r = -0.881</td>
</tr>
<tr>
<td>CAT (U/gm Hb)</td>
<td>r = -0.781</td>
</tr>
</tbody>
</table>

Table 5 showed direct positive correlation in the levels of DNA SSB and thioethers in exposed workers in comparison with control subjects. There is indirect negative correlation between antioxidant enzymatic activities in exposed workers and such changes might be due to exposure to asphalted fumes.

Table 6: Comparison between smokers and non-smokers exposed workers

<table>
<thead>
<tr>
<th></th>
<th>Smokers</th>
<th>Non-Smokers</th>
<th>Independent t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=28</td>
<td>N=22</td>
<td>t</td>
</tr>
<tr>
<td>DNA SSB %</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td>6.8</td>
</tr>
<tr>
<td>Urinary Thioether(umol/mmol creatinine)</td>
<td>73.9±2.7</td>
<td>32.1±3.1</td>
<td>6.8</td>
</tr>
<tr>
<td>SOD (ugU/gHb)</td>
<td>86.5±4.5</td>
<td>77.7±4.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Blood GR (lu/g Hb)</td>
<td>934.1±66.0</td>
<td>1470±413.0</td>
<td>1.46</td>
</tr>
<tr>
<td>CAT (U/gm Hb)</td>
<td>23.7±1.8</td>
<td>28.0±0.8</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>243.5±5.5</td>
<td>277.4±10.0</td>
<td>15.3</td>
</tr>
</tbody>
</table>

*NS: Not significant
Table 6 & Figure (1) showed that there is higher significant association in the level of genotoxicity parameters and antioxidant enzymatic activities and smoking habits.

Exposure to asphalt fumes is a well known factor that affects antioxidant status and increases free oxygen radical generation.

The anti-oxidative systems include non-enzymes antioxidants such as glutathione (GSH), vitamins A, C and E; enzymes antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase. SOD, the first defense against oxygen-derived free radicals catalyzes the dismutation of O2⁻⁻ into H2O2, which is further metabolized into H2O and O2 by catalase and GSH-Px via the oxidation of GSH. Glutathione reductase catalyzes the reduction of oxidized glutathione (GSSG) to GSH\(^{16}\)

Environmental pollutants, such as asphalt fumes and others reactive chemicals have been shown to contribute to the production of oxidative stress both in vitro and in vivo.\(^{(17-19)}\)

Results showed that the erythrocyte SOD activity of exposed workers was higher than that at the control subjects, and that smoking status and the number of cigarettes smoked, but not exercise, affected SOD levels. The latter findings are consistent with those of recent studies.\(^{(20,21)}\), who reported that exercise, working conditions, meat intake, body mass index and smoking influence oxidative DNA damage.

The erythrocyte GSH levels in exposed workers were higher than those of control subjects. The relatively large variations of the SOD activity and the GSH levels at these two groups may partly be explained by smoking and duration of employment. It was found that smokers had significantly higher blood SOD activities and lower GSH levels than the nonsmokers.

Detailed analysis of the frequency of
DNA strand breaks in lymphocytes of the workers revealed significantly more DNA strand breaks with increasing internal exposure result from exposure to asphalt fumes exposure. Table (6), Figure (1) Moreover, elevated DNA damage was shown when compared with the control group at high levels of urinary thioethers.

Typically, reactive oxygen species (ROS) are formed in cells through the reduction of oxygen by biological reducing agents, with the catalytic assistance of electron transfer enzymes and redox active chemical species such as redox active organic chemicals and metals. \(^{(22)}\) In the area of endogenous antioxidant defenses, excess amount of ROS leads to the depletion of the protective antioxidants, superoxide dismutase (SOD) and glutathione (GSH). \(^{(23)}\)

Superoxide dismutase (SOD) is an enzyme extensively used as a biochemical indicator of pathological states associated with oxidative stress. It is the only enzymes dismounting superoxide radicals. There are numerous mechanisms by which cells defend themselves against oxidants. While, glutathione reductase (GR) is the key enzyme of glutathione metabolism and is widespread in all tissues and blood cells. This enzyme catalyses reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of NADPH. \(^{(23)}\)

In the current work, the antioxidants (SOD and GR) in the asphalt workers were significantly decreased compared to their controls, and this decrease increases significantly with the increase in the duration of exposure, as there were inverse correlations of the levels of SOD with the duration of exposure.

Compatible to the present result, serum SOD activities were significantly lower in house painters exposed to organic solvents. \(^{(23)}\)

Asphalt fumes might manifest their toxicity through elevation in lipid
peroxidation and reduction in the antioxidant levels. These findings suggest that the decrease in SOD might be due to the major damage on the human body as a result of exposure to asphalt fumes, such as bitumen, and that detection of the low levels of SOD might provide an earlier sign for the bitumen damage. Contrary to the current results, in others occupational exposures\(^{(24)}\), there was an increase in the SOD levels of people working with paint thinner compared with controls. However, others study found that workers exposed to a mixture of hydrocarbons, the SOD activities showed no significant changes.\(^{(25,26)}\)

![Graph showing mean levels of genotoxicity and antioxidant activity in exposed workers in comparison with control.](image)

**Figure 1** illustrate mean levels of genotoxicity and antioxidant activity in exposed workers in comparison with control where some parameters showed high significant increase in its levels and others showed reduced effects.

In this study, asphalt fume exposure was also associated with significant increases in DNA strand breaks. DNA strand breaks and DNA crosslinks have a counteracting effect due to a masking of the effect of DNA strand breaks by simultaneously existent DNA crosslinks. Particularly, changes in DNA repair capacity, cell
turnover, and apoptosis may influence level of DNA damage measured at different time points.

The present study and previous reports are consistent and show changes in DNA strand break frequency in workers occupationally exposed to bitumen-based products.\(^{(27)}\)

Recent in vitro study on DNA adduct formation indicated that bitumen fume condensates induced significant amounts of DNA adducts and that these levels are higher than would be expected based on the PAH content ROS are believed to cause lipid peroxidation, which in turn damages biological membranes. Antioxidants such as enzymatic and nonenzymatic defense systems are necessary to prevent cellular damage.\(^{(28)}\)

For degradation of ROS are responsible the following antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). SOD catalyses dismutation of the superoxide anion (O\(_2^-\)) into H\(_2\)O\(_2\), which is then deactivated to H\(_2\)O by CAT and/or GPx.\(^{(28)}\)

Figure 2 illustrate mean levels of genotoxicity parameters and antioxidant activity in relation to smoking habits.
Activity of SOD was significantly higher in smoking workers in comparison to non-smoking subjects exposed to asphalt fumes might due to heavier exposure conditions. (Fig. 2) Non-smoking workers revealed lower activity of SOD and GPx in relation to the non-smoking control. Activity of GPx in smoking workers was significantly decreased in comparison to the smoking control. The same tendency was observed in respect to GPx and CAT values of smoking workers and the non-smoking control. In cigarette smokers an increase of antioxidant activity was also observed. (29,30)

An insignificant decline in CAT activity in the exposed work relation to SOD and CAT was observed. The significant decrease in SOD and CAT activity between nonsmoking workers and non-smoking control was also observed. These findings indicate reduced antioxidant activity in erythrocytes of workers these enzymes belong to enzymatic antioxidant defense and prevent oxidant stress. The observed decrease of parameters of the antioxidant system indicates that an increase in oxidative stress caused by asphalt fumes workers may overwhelm this enzymatic system. (31-33)

CONCLUSION

Present data indicate that asphalted fumes exposed workers exhibit an increase in GSH levels most likely as a compensatory response to asphalted fumes exposure. However oxidative stress induced by fumes may partly be attributed to the inhibited activities of SOD and CT as well as increase in the potentials genotoxic risks by increasing the percentage of DNAssb and increase excretion of urinary thioethers.

Thus the study provides further evidence to dysregulation of antioxidant/oxidant balance and risks of tumors formation among workers exposed to asphalt fumes vapors.

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