Alteration of Oxidative Stress Biomarkers Resulting from Environmental Exposure to PAHs

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Abstract

The aim of this work was to investigate the effect of environmental polycyclic aromatic hydrocarbons (PAH) exposure on biomarkers related to antioxidant function and oxidative stress. The lipid peroxidation (as malondialdehyde, MDA), activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) as well as levels of glutathione, vitamin E and C were investigated in 100 male bus drivers and 100 normal controls. Urinary 1-hydroxypyrene (1-OHP) was measured as biomarker of PAH exposure in both groups. The means of 1-OHP in controls and bus drivers were 0.032 and 0.132 µmol/mol Cr, respectively (p < 0.001). MDA level in bus drivers (2.052 µM) was statistically higher than in the control subjects (1.219 µM, p < 0.001). Important findings were the significant increases in the activities of SOD and catalase in bus drivers when compared to control (2,330.15 vs 1,527.85 U/gHb and 42,496 vs 36,239 U/gHb, respectively). The concentration of GSH, vitamin C and vitamin E significantly increased compared to the control group. These results suggested that environmental exposure to PAHs was associated with oxidative stress. These also supported the importance of biomarker evaluation related to possible health risks as described in this study. Additional studies with biomarkers of PAHs effect and susceptibility are necessary for better understanding and health prevention.

Keywords: Antioxidants, 1-hydroxypyrene, Malondialdehyde, Oxidative stress, Polycyclic aromatic hydrocarbons

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**Abstract**

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**Background**

The present study was designed to determine the effect of environmental polycyclic aromatic hydrocarbons (PAH) exposure on biomarkers related to antioxidant function and oxidative stress. These results suggested that environmental exposure to PAHs was associated with oxidative stress. These also supported the importance of biomarker evaluation related to health risks as described in this study. Additional studies with biomarkers of PAHs are necessary.
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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced by incomplete combustion of both solid and liquid fuels, wood, garbage or other organic substances, such as tobacco and charbroiled meat. The primary sources of exposure to PAHs for most of the general population are inhalation of the compounds in ambient air (resulting from vehicle exhausts, and asphalt roads, etc.), tobacco smoke, wood smoke and consumption of PAHs in foods. There is an increasing health concern with respect to cancer and other organ dysfunctions, especially in the mechanism of reactive oxygen species (ROS) production. Several factors also determine the severity of those health effects. These factors include the dose, duration, route, other exposed chemicals and individual characteristics such as age, sex, nutritional status, family traits, life style and health status.

Uptake of PAH in the human body could be monitored by assess internal exposure (the amount absorbed by the body and distributed to various organs and tissues) to PAHs. Most of these methods are based on determining metabolites in urine of exposed people. The most widely used method is the determination of 1-hydroxypyrene (1-OHP) in urine, and this has been demonstrated to provide useful evaluation of recent exposure to PAHs\(^1\). Recommended analytical method for 1-OHP is HPLC with fluorescence detector, after the process of enzymatic hydrolysis to release the conjugated part of 1-hydroxypyrene. Then the analyses are separated from the matrix and enriched by reversed phase column extraction. The results are corrected for urinary creatinine content\(^2\).

Metabolic pathway of PAH via CYP1A1 could generate both electrophilic metabolites and ROS. Moreover, PAH o-quinones also undergo non-enzymatic reduction back to catechols. This event establishes futile redox cycles, which amplify the generation of ROS and may lead to a pro-oxidant cellular state\(^3\). Production of ROS leads to oxidative stress and induction of detoxification systems like antioxidant defense system. This system includes enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), antioxidant vitamins (A, C, E and other carotenoids)\(^4\) and low molecular weight antioxidant molecule such as glutathione\(^5\). SOD, CAT and GPx within cells play an important role in removing superoxide and peroxides before they react with other metals to form more reactive species. The non-enzymatic
antioxidants such as glutathione, α-tocopherol and ascorbic acid are important in reaction with activated oxygen species, thereby preventing the propagation of free radical chain reaction. When antioxidant and free radical scavenging systems are overwhelmed, the changes at cellular and molecular level occur. Among several biomarkers of oxidative stress, malondialdehyde (MDA) is most frequently studied because of its biological importance and the sensitivity of the assay method. This work is a contribution to a crucial evaluation of environmental PAH exposure on biomarkers related to antioxidant function and oxidative stress.

**Materials and Methods**

**Subjects**

The study protocol was approved by the Ethical Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University and all subjects gave written informed consent. The studied group included 100 (male) bus drivers in Bangkok (capital of Thailand), with at least 2 work-years and 100 (male) subjects living in rural area and non-occupationally exposure to PAHs, classified as control group. The mean age of bus drivers was 43.2 years and 33.8 years for control group. Response to a questionnaire related to this study along with personal details about occupation, clinical history and number of cigarettes smoked per day was obtained.

**Analysis of urinary 1-OHP**

The determination of 1-OHP in urine was carried with the HPLC-method developed by Jongeneelen et al. Urine sample (10 ml) was adjusted to pH 5.0 with 1.0 M HCl, then buffered with 5 ml of a 0.1 M acetate buffer (pH 5.0), and incubated overnight with 1,250 U β-glucuronidase-arylsulfatase in a shaking bath at 37°C. The reaction mixture was subjected to a solid phase extraction on a C-18, 100 mg cartridge. The cartridge was sequentially washed with 10 mL of 40% methanol in water. The trapped metabolite was eluted with 10 ml of pure methanol and the eluate was evaporated to dryness. The analyst was carried out on a C-18 column which was eluted with acetonitrile-water gradient at 1.0 ml/min flow rate. The fluorimetric detector was operated at 242 nm and 388 nm, excitation and emission wavelengths, respectively. Creatinine in urine was determined by commercial assay kit. The level of 1-OHP was expressed as µmol/mol creatinine.
Analysis of antioxidants

**GSH Level**

The Spectrophotometric/ microplate reader assay method for glutathione (GSH) involved oxidation of GSH by the sulfhydryl reagent 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm. 0.2 ml of whole blood was assayed for total GSH content in erythrocytes by the method of Beutler and GSH level was expressed in terms of mg/100 ml.

**GPx activity**

GPx activity was assessed, based on the principle that oxidized glutathione (GSSG) produced upon reduction of an organic peroxide by GPx. It was immediately recycled to its reduced form (GSH) with concomitant oxidation of NADPH to NADP+. The oxidation of NADPH was monitored spectrophotometrically as a decrease in absorbance at 340 nm. One GPx unit is defined as 1 µmol of NADH oxidized per minute under the assay conditions.

**SOD activity**

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion (O$_2^-$) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. In order to determine the SOD activity, several direct and indirect methods have been developed. Among these methods, an indirect method using nitroblue tetrazolium (NBT) is commonly used due to its convenience and ease of use. This method uses NBT as indicator, and riboflavin as a superoxide-generating system. Illumination of riboflavin will generate superoxide, which then reduces NBT to formanza. Formaza was measured at 505 nm on a Shimadzu spectrophotometer (UV-160 A) on hemolysates of washed erythrocytes.

**Catalase activity**

The assay was performed on hemolysates of washed erythrocytes. Catalase activity was measured by the Aebi method. The principle of this method is based on the hydrolyzation of H$_2$O$_2$ and decreasing absorbance at 240 nm. The conversion of H$_2$O$_2$ into H$_2$O and 1/2 O$_2$ in 1 min under standard condition was considered to be the enzyme reaction velocity. CAT activity is expressed as U CAT/g hemoglobin. One CAT unit was defined as the enzyme activity necessary to convert 1 µmol H$_2$O$_2$ and molecular oxygen at
25°C and pH 7 in one minute.

**Analysis of vitamin E and C**

Vitamin E was determined by method of Urbanek et al.\textsuperscript{11}. Serum 500 µl was deproteinized by cool ethanol denatured with 5% methanol. Then 2,500 µl of \textit{n}-hexane was added to this mixture and extracted for 5 min by a vortex apparatus. After centrifugation the aliquot (2,000 µl) of the clean extract was separated and evaporated under nitrogen (60°C). The residue was dissolved in 400 µl methanol and analyzed by reversed-phase HPLC. For vitamin C analysis, we modified the procedure from the previous method\textsuperscript{12}. Principle of this technique is based on ascorbic acid which is oxidized by copper to form dehydroascorbic acid and diketogulonnic acid. These products are treated with 2,4-dinitrophenylhydrazine to form the derivative bis-2,4-dinitrophenyhydrazzone. This compound undergoes a rearrangement to form a product with an absorption band that is measured at 520 nm.

**Analysis of plasma malondialdehyde**

Plasma malondialdehyde levels were determined by Khoschsorur’s method\textsuperscript{13}. Briefly, plasma was mixed with H\textsubscript{3}PO\textsubscript{4}, aqueous thiobarbituric acid, H\textsubscript{2}O and the mixture was heated in a boiling-water bath. After cooling, alkaline methanol was added. The samples were centrifuged at 2,500g for 3 min. The neutralized reaction mixture was then chromatographed on HPLC. Fluorometric detection was performed with excitation at 527 nm and emission at 551 nm.

**Statistical analysis**

All statistical analyses of relationship between biomarkers of both study group were performed by the statistical package SPSS for Window, version 11 (SPSS, Chicago, IL, USA). Correlations were calculated as Pearson correlation coefficient. For comparison of means, we used Student’s \textit{t} test. \textit{p}-values < 0.05 were considered significant. The values were expressed as mean ± SD.

**Results**

Table 1 shows the demographic and clinical characteristics of the study groups. Men in the bus drivers group were on average older than (43.2 ± 9.11 years) those in the control group (33.8 ±10.5 years). In addition, high prevalence of smokers and number of cigarette smoked per day were found in bus drivers (74% and 15.1 cigarettes/day.
Table 1  Characteristics of the study population (Mean and SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Bus drivers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>((n = 100))</td>
<td>((n = 100))</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>33.8 ± 10.5</td>
<td>43.2 ± 9.11</td>
</tr>
<tr>
<td>Working duration (y)</td>
<td>11.1 ± 9.8</td>
<td>9.66 ± 8.55</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>22.8 ± 3.1</td>
<td>22.9 ± 3.1</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>133.1 ± 16.16</td>
<td>132.1 ± 19.2</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74.2 ± 11.7</td>
<td>80.5 ± 13.6</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondrinkers (%)</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>Drinkers (%)</td>
<td>75</td>
<td>76</td>
</tr>
<tr>
<td>Smoking habit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers (%)</td>
<td>66</td>
<td>26</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>34</td>
<td>74</td>
</tr>
<tr>
<td>Current smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. cigarettes per day</td>
<td>10.1 ± 6.4</td>
<td>15.1 ± 9.3</td>
</tr>
<tr>
<td>Time of smoking (y)</td>
<td>8.84 ± 7.8</td>
<td>18.83 ± 11.3</td>
</tr>
</tbody>
</table>

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure

There were statistically significant differences between mean levels of urinary 1-OHP and MDA in both study groups. The means of 1-OHP in controls and bus drivers were 0.032 and 0.132 µmol/mol Cr, respectively \((p < 0.001)\). MDA level in bus drivers (2.052 µM) was statistically higher than that in the control subjects (1.219 µM, \(p < 0.001\)). Levels of non-enzymatic and enzymatic antioxidants are given in Table 3. Important findings were the significant increases in the activities of

for bus drivers and 34% and 10.1 cigarettes/day for the control group.

The characteristics of bus engine without air conditioning are presented in Table 2. More than 70% of bus engine were in moderate condition and 68% used diesel fuel. For the performance of vehicle emission checking, bus drivers reported as occasional checking and more than 60% of buses caused air pollution (black smoke). Use of personal protective equipment as cloth mask in bus drivers was slightly low (14%).
**Table 2** Characteristics of bus engine (without air conditioning, in Bangkok) and use of personal protective equipment in bus drivers.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Machine status</td>
<td></td>
</tr>
<tr>
<td>- Moderate</td>
<td>72</td>
</tr>
<tr>
<td>- Good</td>
<td>28</td>
</tr>
<tr>
<td>Fuel</td>
<td></td>
</tr>
<tr>
<td>- Diesel</td>
<td>68</td>
</tr>
<tr>
<td>- LPG (liquifed petroleum gas)</td>
<td>32</td>
</tr>
<tr>
<td>Performance of vehicle emission checking</td>
<td></td>
</tr>
<tr>
<td>- Checking only problems</td>
<td>5</td>
</tr>
<tr>
<td>- Occasional checking</td>
<td>95</td>
</tr>
<tr>
<td>Assessment for black smoke</td>
<td></td>
</tr>
<tr>
<td>- Never</td>
<td>12</td>
</tr>
<tr>
<td>- Yes</td>
<td>26</td>
</tr>
<tr>
<td>- No</td>
<td>62</td>
</tr>
<tr>
<td>Use of personal protective equipment (cloth mask)</td>
<td></td>
</tr>
<tr>
<td>- Never</td>
<td>57</td>
</tr>
<tr>
<td>- Occasional</td>
<td>29</td>
</tr>
<tr>
<td>- Frequent</td>
<td>14</td>
</tr>
</tbody>
</table>

**Table 3** Means of non-enzymatic and enzymatic antioxidants, malondialdehyde, urinary 1-OHP in the control group and bus drivers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Bus drivers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$(n = 100)$</td>
<td>$(n = 100)$</td>
</tr>
<tr>
<td>CAT (U/gHb)</td>
<td>$36,239 \pm 15,990$</td>
<td>$42,496 \pm 18,974^b$</td>
</tr>
<tr>
<td>GPx (U/gHb)</td>
<td>$35.55 \pm 12.00$</td>
<td>$34.56 \pm 10.88$</td>
</tr>
<tr>
<td>GSH (mg/dL)</td>
<td>$39.20 \pm 7.27$</td>
<td>$36.22 \pm 9.09^b$</td>
</tr>
<tr>
<td>SOD (U/gHb)</td>
<td>$1,527.85 \pm 410$</td>
<td>$2,330.15 \pm 1120^b$</td>
</tr>
<tr>
<td>Vitamin C (mg/dL)</td>
<td>$0.912 \pm 0.295$</td>
<td>$0.756 \pm 0.323^b$</td>
</tr>
<tr>
<td>Vitamin E (mg/dL)</td>
<td>$0.982 \pm 0.271$</td>
<td>$0.795 \pm 0.261^a$</td>
</tr>
<tr>
<td>MDA (µM)</td>
<td>$1.219 \pm 0.430$</td>
<td>$2.052 \pm 0.797^b$</td>
</tr>
<tr>
<td>Urinary 1-OHP (µmol/mol Cr)</td>
<td>$0.032 \pm 0.003$</td>
<td>$0.132 \pm 0.007^b$</td>
</tr>
</tbody>
</table>

$^a p < 0.01$ different from control group

$^b p < 0.001$ different from control group
SOD and catalase in bus drivers when compared to control (2,330.15 U/gHb vs 1,527.85 U/gHb and 42,496 U/gHb vs 36,239 U/gHb, respectively). The concentration of GSH, vitamin C and vitamin E increased significantly compared to the control group (Table 3).

The effects of cigarette smoking on biomarkers are presented in Table 4. Compared with non-smokers in bus driver group, there was a significantly low CAT activity in smokers (46,098 U/gHb for non-smoker and 41,231 U/gHb for smoker, \( p < 0.01 \)). Moreover, in exposed group significantly low levels of GSH and vitamin C were found in smokers when compared to non-smokers (35.04 vs 69.58 mg/dl and 0.635 vs 0.810 mg/dl, respectively). However, there were no differences in GPx and SOD activities as well as vitamin E level between the two groups. In this study, we considered the role of antioxidants together based on synergistic function, and further calculated the ratio of antioxidants. Smokers and non-smokers showed no statistical difference in ratios of CAT/SOD, GSH/GPX, Vitamin E/C, GSH/vitamin E and GSH/vitamin C (Table 4). For the non-smoking subjects in controls and bus drivers, mean 1-OHP levels were 0.025 and 0.069 \( \mu \text{mol/mol Cr} \), respectively, and significantly lower than smokers (0.045 and 0.152 \( \mu \text{mol/mol Cr} \), respectively). Plasma MDA level in bus drivers with cigarette smoking remained significantly higher than those in non-smokers (2.11 vs 1.86 \( \mu \text{M} \), respectively).

Figure 1 demonstrates the association between urinary 1-OHP and ratio of CAT/SOD as well as urinary 1-OHP and MDA level. Urinary 1-OHP (\( r = -0.223, p = 0.025 \)) was significantly correlated with ratio of CAT/SOD but showed positive correlation with MDA level (\( r = 0.202, p = 0.044 \)) only in bus drivers.

Analysis of our results in bus drivers showed a correlation between the GPx activity and GSH concentration (\( r = -0.371, p = 0.001 \)) (Figure 2). A positive correlation (\( r = 0.028, p = 0.022 \)) was found between vitamin C and GSH levels. However, these results were not statistically found in the control group. The activities of CAT and SOD were also correlated (\( r = 0.558, p = 0.0001 \) for bus drivers and \( r = 0.288, p = 0.004 \) for the controls).

**Discussion**

Increasing concern exists over the several adverse health effects of PAHs exposure related to urban air pollution. Long term exposure to high concentrations of PAHs increases the risks of lung cancer, respiratory disease,
Table 4 Influence of cigarette smoking on non-enzymatic and enzymatic antioxidants, malondialdehyde, urinary 1-OHP in the control group and bus drivers

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Bus drivers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-smokers</td>
<td>Smokers</td>
</tr>
<tr>
<td></td>
<td>(n = 66)</td>
<td>(n = 34)</td>
</tr>
<tr>
<td>CAT (U/gHb)</td>
<td>36.59 ± 10.52</td>
<td>35.61 ± 10.87</td>
</tr>
<tr>
<td>GPx (U/gHb)</td>
<td>35.35 ± 10.71</td>
<td>35.94 ± 14.35</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>38.71 ± 7.56</td>
<td>40.15 ± 6.69</td>
</tr>
<tr>
<td>SOD (U/gHb)</td>
<td>1,562.8 ± 436.8</td>
<td>1,459.8 ± 348.7</td>
</tr>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>0.945 ± 0.263</td>
<td>0.743 ± 0.344</td>
</tr>
<tr>
<td>Vitamin E (mg/dl)</td>
<td>0.922 ± 0.255</td>
<td>0.842 ± 0.268</td>
</tr>
<tr>
<td>Ratio of CAT/SOD</td>
<td>24.65 ± 8.27</td>
<td>24.83 ± 6.55</td>
</tr>
<tr>
<td>Ratio of GSH/GPx</td>
<td>1.17 ± 0.36</td>
<td>1.21 ± 0.34</td>
</tr>
<tr>
<td>Ratio of Vitamin E/C</td>
<td>1.11 ± 0.26</td>
<td>1.41 ± 0.23</td>
</tr>
<tr>
<td>Ratio of GSH/Vitamin E</td>
<td>46.99 ± 22.94</td>
<td>52.81 ± 18.69</td>
</tr>
<tr>
<td>Ratio of GSH/Vitamin C</td>
<td>46.03 ± 27.58</td>
<td>53.84 ± 25.59</td>
</tr>
<tr>
<td>MDA (µM)</td>
<td>1.20 ± 0.414</td>
<td>1.24 ± 0.46</td>
</tr>
<tr>
<td>Urinary 1-OHP (µmol/mol Cr)</td>
<td>0.025 ± 0.019</td>
<td>0.045 ± 0.028a</td>
</tr>
</tbody>
</table>

*p < 0.01 different from non-smokers in each group

Cardiovascular morbidity, and other forms of diseases associated with inflammatory process. Diesel vehicles are a major source of air pollutants and widely known as an important health impact. Two important organizations like NIOSH and IARC have suggested that chronic exposure to relatively low levels of diesel exhaust may be a risk factor to human cancer14,15. The diesel emissions are of high concern because of its air toxic content which includes aromatic compounds (such as benzene, toluene, 1–3 butadiene, PAH), various aldehydes, alkanes, alkenes and ketones. Many PAHs are considered potent carcinogens. A study in Delhi found that the concentration of PAHs ranged between 41.49–56.03 mg/g in the exhaust of buses. The levels of PAHs in the engines were very high in the older vehicles (> 10 y) as compared to 10-year vehicles16. For assessment of environmental PAH exposure, a useful biomarker is urinary 1-OHP, a major metabolite of pyrene. In the present study, the urinary 1-OHP concentration of bus drivers was 0.132 µmol/mol.
creatinine, and that of controls was 0.032 µmol/mol creatinine (Table 3). These results indicated high degree of PAH exposure in bus drivers, which was in good accordance with the data reported by other authors. Hansen et al.\textsuperscript{17} found that mean of 1-OHP of male bus drivers in Denmark was 0.19 µmol/mol creatinine. In addition, the urinary 1-OHP concentration in the bus drivers was higher than the reference interval for Danish smoking and non-smoking men (0.02-0.16 µmol/mol creatinine)\textsuperscript{18}.

Animal study has suggested that oxidative stress was induced by air pollution\textsuperscript{19}. An epidemiologic study conducted in urban areas has also demonstrated that oxidative stress was associated with environmental air pollutants in bus drivers\textsuperscript{20}. Since PAHs are potentially toxic, they are partly detoxified by antioxidants before they undergo processing damage to lipids, proteins or DNA. Antioxidant enzymes such as SOD, CAT and GPx play vital roles in protecting the body against the toxic effect of intermediate-PAH toxicants. Activities of SOD and CAT were found significantly increased in bus drivers but GSH level was significantly lower than the control group (Table 3). These changes can be explained by induction of SOD and CAT by oxidative stress, mainly hydrogen peroxide. Alteration of CAT activity is closely related to the level of SOD because superoxides are converted to H$_2$O$_2$ by SOD or by spontaneous dismutation reduction\textsuperscript{21}. However, antioxidant systems interact in a complex fashion, so that changes in activity or concentration in one component can affect the whole system. Considering two antioxidant-markers as CAT/SOD ratio may be more indicative of responsiveness of antioxidant function than individual enzyme activities. In this study, CAT/SOD ratio showed negative correlation with 1-OHP level only in bus drivers (Figure 1) that could represent the high exposure to PAHs affecting the imbalance of CAT and SOD activities. Positive correlations between SOD and CAT activities were found both in controls and bus drivers groups ($r = 0.558$ and $r = 0.288$, respectively) (Figure 2), which showed possible protection of catalase by SOD from inactivation and/or decreased H$_2$O$_2$ formation in the cells.

In addition to CAT, GPx is another enzyme that is considerably activated by the addition of H$_2$O$_2$. However, there was no difference in the level of GPx activity in both study groups. This was in agreement with the fact that even though GPx and CAT share the same substrate (H$_2$O$_2$), CAT
was more significant at protecting against severe oxidative stress, whereas the glutathione redox cycle was a major source of protection against low levels of oxidants stress.\textsuperscript{22}

For non-antioxidant enzyme, GSH is a non-specific free radical scavenger able to donate protons to unpaired electrons, thereby quenching the free radical.\textsuperscript{23} In the present study, GSH concentration and GPx activity in bus drivers were negatively correlated ($p = 0.0001$, Figure 2). Base on the reasons that both GPx and GSH play together role in the protection against ROS and GPx catalyses the formation of GSSH from GSH during the reduction of free radical, the association between their levels was explained.

Lipid peroxidation is one of indicators for oxidative stress and can be defined as the oxidative deterioration of lipids containing carbon-carbon double bonds. Plasma MDA is a biological marker of lipid peroxidation resulting from oxidative stress.\textsuperscript{24} In the present study, a significant increase in the MDA level was observed in the bus drivers. This result appeared to be associated with the positive correlation with urinary

**Figure 1** Correlations between urinary 1-OHP and ratio of CAT/SOD and MDA level in control and bus drivers groups.
Figure 2 Correlations between antioxidant enzymes and GSH level in control and bus drivers groups.

1-OHP levels ($p=0.044$, Figure 1). Similar to the findings from Pan et al.\textsuperscript{25}, MDA levels were significantly associated with urinary 1-OHP ($p < 0.001$) which reflected exposure to PAHs and oxidative stress in male restaurant workers exposed to PAHs from cooking oil fumes. Our results suggested that ROS generated during the redox cycling of PAH-quinones might...
interact with membrane lipids and consequently induce lipid peroxidation, which was related to the level of PAH-biomarker of exposure, 1-OHP.

Among vitamins, vitamin E plays a key role in protecting membrane lipids from peroxidative injury, commonly by its ability to reduce peroxyl radicals. In addition, Vitamin C has been shown to protect all classes of lipids from oxidation under a number of relevant types of oxidative stress, while other non-enzymatic antioxidants such as vitamin A, vitamin E, glutathione, bilirubin, and urate merely lower the rate of oxidation or act in a more restricted, local environment. Both enzymes have synergistic effects and oxidized vitamin E is presumably regenerated by reaction with vitamin C. In this study, bus drivers with more exposure to environmental PAHs showed significantly decreased vitamin C and E levels compared to those in control group (Table 3). Positive correlation between GSH and vitamin C levels in bus drivers were found (Figure 2) and could indicate an important role of GSH with the cellular recycling of vitamin C and the maintenance of the vitamin in its reduced state.

Tobacco smoke is a major source of human exposure to carcinogenic pollutants, especially PAHs and it has been recognized as a well-known environmental oxidant. A correlation between tobacco smoke and 1-OHP excretion has been reported by other authors: the urinary levels of 1-OHP were higher for smokers than non-smokers (0.408 vs 0.233 µg/g creatinine). Kawamoto, et al. also reported that the urinary 1-OHP of smokers was about 2 times higher than that of non-smokers (p < 0.001). This study confirmed the influence of smoking on 1-OHP concentration, as seen in controls (0.025 µmol/mol creatinine for non-smoker and 0.045 µmol/mol creatinine for smokers) and bus drivers (0.069 µmol/mol creatinine for non-smoker and 0.152 µmol/mol creatinine for smokers) (Table 4). As an indicator of increased lipid peroxidation, we found increased levels of MDA in smokers, observing more significance in bus drivers. The decrease in GSH and vitamin C levels and the increase of MDA level (Table 4) provided the evidence of a significant alteration of pro-oxidant and antioxidant in PAHs exposure.

In conclusion, the present study highlights the imbalance of pro-oxidant and antioxidant which may be one of the major states responsible for the exposure to PAHs. The generation of free radicals as reflected by increased SOD and CAT activities and the oxidative damage
caused by increase MDA levels in bus drivers could be one of the important lines of evidence resulting in the development of adverse health effect in PAH exposed population. The observed CAT/SOD ratio and correlation between MDA and 1-OHP levels could be used to predict prognosis in individual exposed to PAHs compounds. There were also significantly negative correlations at present changes among the MDA levels and vitamin E, and C levels in exposed groups. These findings demonstrate that exposure at low levels of PAHs decreased vitamin E, and C levels and this stage can lead to lipid peroxidation. Therefore, maintaining an adequate level of antioxidant vitamins may be necessary for the prevention or protection of long-term PAH exposure. Further studies, multiple biomarkers of exposure, effects and susceptibility should provide better insight to understanding the continuum between exposure and various diseases.

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