

original article

Urinary 1-hydroxypyrene as a biomarker of carcinogenic polycyclic aromatic hydrocarbons in Iranian carbon anode plant workers

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ABSTRACT

Aims: This study was designed to evaluate the validity of urinary 1-hydroxypyrene as a biomarker in carcinogenic PAHs (cPAHs) exposed Iranian carbon anode plant workers.

Materials and Methods: The study population consisted of 42 workers working in a carbon anode plant and control group consisted of 43 office workers. Personal air sampling was performed to assess workers atmospheric exposure to carcinogenic PAHs. Urine samples were collected for analysis of urinary 1-hydroxypyrene using high performance liquid chromatography (HPLC). Statistical analysis was performed with SPSS version 16 software.

Results: The mean concentration of occupational exposure to cPAHs in the exposed group was $11.42 \pm 7.56 \mu\text{g}/\text{m}^3$. Mean level of urinary 1-hydroxypyrene in the exposed and control groups were 6.32 ± 4.9 and $0.54 \pm 0.48 \mu\text{mole}/\text{mole creatinine}$, respectively. Urinary level of 1-hydroxypyrene in the exposed group was significantly higher than the control group ($P < 0.001$). A strong and significant correlation between total cPAHs exposure and urinary 1-hydroxypyrene ($r = 0.79$, $P < 0.001$) was found.

Conclusion: The results confirm urinary 1-hydroxypyrene level as a good biomarker in cPAHs exposed workers. In addition, considering the level of urinary 1-hydroxypyrene, it can be concluded that studied carbon anode plant workers are exposed to substantial risk of cancer and other genotoxic effects which are the result of cPAHs exposure.

Key words: Biomarker, carcinogenic polycyclic aromatic hydrocarbons, 1-hydroxypyrene

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) refer to a group of chemicals consisting of a few hundred compounds with two or more fused benzene rings.^[1] PAHs can be generated naturally as a result of forest fires and volcanic eruptions or by human activities such as industry, heating, waste incineration,

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and traffic.^[2] These compounds are released during many processes, which involve incomplete combustion of fossil fuels (e.g. heating of coal, pitch, and coal-tar, burning of diesel fuels) and some of them are considered as human carcinogens.^[3] Epidemiological studies have demonstrated an association between PAHs exposure and increases in mortality and/or morbidity from respiratory diseases, cardiovascular diseases and cancer.^[4]

Carcinogenic polycyclic aromatic hydrocarbons (cPAHs) include benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[ah]-anthracene, and indeno[cd]pyrene.^[5]

Some PAHs exposed occupational groups are coke oven workers, aluminum industry workers, roofers, iron and steel founding workers, chimney sweeps, asphalt road builders, and workers in various industries with combustion processes.^[3,6-8]

Among hundreds of PAHs identified, there has been no common international agreement on which compounds should be reported concerning human exposure in environmental or occupational settings, but for practical reasons, benzo[a]pyrene has been used as the surrogate marker of choice for measuring ambient exposure to PAH mixtures and measurement of carcinogenic polycyclic aromatic hydrocarbons (cPAHs) is a common approach in PAHs genotoxicity studies.^[5,9-11]

Monitoring of the PAHs external environmental exposure (e.g. measurement of chemicals in air) can be completed by measuring the biomarkers which reflect internal exposure in the human through different routes of exposure (e.g. inhalation, ingestion and dermal uptake). In addition, biomonitoring takes into account inter-individual variation in absorption, metabolism and elimination of xenobiotics by the body.^[12]

Although, comprehensive information on the metabolism of several PAHs is available, only a limited number of PAH metabolites are commercially available. Therefore, biomonitoring of PAHs is restricted to those for which the metabolites are commercially available for making standards in the analysis process. This limitation may be overcome by using metabolites of markers for total or carcinogenic PAH exposure. Since the relative content of pyrene compared to other PAHs is reasonably constant in air samples,^[13] its metabolite (i.e. 1-hydroxypyrene) in the urine has been proposed as a good biomarker for evaluation of PAHs body burden.^[12]

Since factors like genetic polymorphism of metabolizing enzymes can affect the metabolism of PAHs and thereby the level of their metabolites,^[14-15] it is important to evaluate the validity of a proposed biomarker in different populations. Up to our knowledge, no study has been carried out in Iran to address the validity of urinary 1-hydroxypyrene as a

biomarker in cPAHs exposed workers. Hence, this study was designed to evaluate the validity of urinary 1-hydroxypyrene as a biomarker in cPAHs exposed Iranian carbon anode plant workers.

MATERIALS AND METHODS

Study subjects

The exposed population consisted of 42 workers working in a carbon anode plant in an aluminum production industry. The age, BMI (body mass index), economic status, and smoking habit matched control group consisted of 43 volunteered office workers. The inclusion criteria were not having medical treatment, radiography, or vaccination up to 3 months before sampling. Each participant completed a questionnaire on personal information and lifestyle. All participants signed an informed consent form and could exit from the study at any time during the study. In addition, the study was approved by Tehran University of medical sciences ethical committee.

Chemicals and reagents

1-Hydroxypyrene was purchased from Sigma-Aldrich (Germany); indeno (1,2,3 cd) pyrene from Supelco (USA); all other c-PAHs from Dr. Ehrenstorfer GmbH (Germany); β -glucuronidase-arylsulphatase from Roche (Germany); acetonitrile, benzene, cyclohexane, methanol, methylene chloride and toluene from Merck (Germany), C18 cartridge from Macherey-Nagel (Germany); PTFE Filters and washed XAD-2 sorbent tube from SKC (USA).

Personal exposure monitoring

Air sampling and analysis was performed to assess workers' atmospheric exposure to cPAHs including benzo[a]pyrene (B[a]P), benz[a]anthracene (B[a]A), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[ghi]perylene (B[ghi]Pe), chrysene (CHRY), dibenz[ah]anthracene (DB[ah]A), and indeno[cd]pyrene (I[cd]P) according to the NIOSH 5515.^[16] In this regard, air samples were taken with a flow rate of 2 L/min using a universal XR personal air sampling pump (SKC, USA), PTFE Filter (2- μ m pore size, 37-mm diameter) and washed XAD-2 sorbent tube. Analysis of the air samples was performed using a GC-FID (Waters, USA) with a capillary column (30 m x 0.32-mm ID, fused silica, 1- μ m DB-5).

Hydroxypyrene analysis

Urine samples were collected after the work shift on the last working day and stored at -20°C until analysis. Urinary 1-hydroxypyrene was measured according to the Jongeneelen *et al.* method.^[17] In brief, 10 ml of urine of each participant was adjusted to a pH of 5 using 1 N HCl. Then, 0.1 M acetate buffer (pH 5) was added to urine to a final volume of 30 ml. This solution was incubated overnight (16 h) with 15 μ l glucuronidase-arylsulphatase in a shaking bath at 37°C. A C18 reversed-phase cartridge was used for the

extraction of the metabolites. The cartridge was activated with 5 ml of methanol followed by 10 ml of distilled water. The urine sample was passed through the cartridge at a flow rate of 10 ml/min. The cartridge was washed with 3 ml of distilled water followed by 3 ml of 50% methanol in water. Thereafter, 1-hydroxypyrene was eluted with 8 ml methanol. The solution was completely evaporated and reconstituted with 1 ml of methanol. The concentration of 1-hydroxypyrene in this extract was determined by high performance liquid chromatography (HPLC) with a C18 reversed phase column and fluorescence detector (Agilent, Germany).

Creatinine concentration of urine was determined by Shahid Mohammadi Hospital laboratory and urinary 1-hydroxypyrene was calculated in $\mu\text{mol}/\text{mol}$ creatinine.

Analysis of urinary cotinine

Cotinine is a metabolite of nicotine and it is commonly used for determining the smoking status of human subjects.^[4] For determination of urinary cotinine level, a commercially available direct ELISA kit (from Abnova, Taiwan) was used. The concentration of cotinine was measured according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS version 16 software. Kolmogorov-Smirnov test was used to test the normality of data. An independent samples *t*-test was used for comparison of variables between two groups and Pearson correlation test was used to evaluate the relationship between quantitative variables.

RESULTS

Characteristics of the studied population

General characteristics of the studied population are shown in Table 1. Subjects with urinary cotinine level of more than 500 ng/mg creatinine have been considered as smokers.^[4]

Exposure monitoring and urinary 1-hydroxypyrene levels

The mean concentration of occupational exposure to cPAHs in the exposed group was $11.42 \mu\text{g}/\text{m}^3$ ranging from 3.6 to $31.5 \mu\text{g}/\text{m}^3$ and the mean level of occupational exposure to benzo(a)pyrene in this group was $1.41 \mu\text{g}/\text{m}^3$ ranging from 0.5 to $5.2 \mu\text{g}/\text{m}^3$. Internal exposure dose to cPAHs was evaluated by measuring urinary 1-hydroxypyrene. The results of urinary

1-hydroxypyrene analysis are presented in Table 2. According to these results, the mean level of urinary 1-hydroxypyrene in the exposed group is $6.32 \pm 4.9 \mu\text{mol}/\text{mol}$ creatinine, ranging from 0.56 to $19.7 \mu\text{mole}/\text{mole}$ creatinine and in the control group it is $0.54 \pm 0.48 \mu\text{mole}/\text{mole}$ creatinine, ranging from 0.04 to $2.1 \mu\text{mole}/\text{mole}$ creatinine. According to the statistical analysis the level of 1-hydroxypyrene in the exposed group was significantly higher than the control group ($P < 0.001$). In addition, there was a significant difference in urinary 1-hydroxypyrene between smokers and non-smokers in the both exposed ($P < 0.001$) and control ($P = 0.027$) groups.

Statistical analysis of atmospheric exposure to cPAHs and excretion of 1-hydroxypyrene in the urine using Pearson correlation test showed that, there is a strong and significant correlation between total cPAHs exposure and urinary 1-hydroxypyrene ($r = 0.79$, $P < 0.001$). In addition, urinary 1-hydroxypyrene was significantly correlated with cPAHs exposure in the cPAHs exposed smokers ($r = 0.65$, $P = 0.021$) and non-smokers ($r = 0.77$, $P < 0.001$). Moreover, a direct positive and significant correlation was found between benzo(a)pyrene exposure and urinary 1-hydroxypyrene ($r = 0.69$, $P < 0.001$).

DISCUSSION

Aluminum industry workers may be exposed to high levels of PAHs, especially during the anode production. In this study, the anode plant workers' exposure to cPAHs was evaluated and the results showed that, the mean level of environmental exposure to cPAHs in the exposed subjects is $11.42 \pm 7.56 \mu\text{g}/\text{m}^3$. According to the previous studies this level of exposure to cPAHs is high enough to have a significant increase in urinary 1-hydroxypyrene level^[8,18] and our results also showed a significant increase in urinary 1-hydroxypyrene level of exposed subjects in comparison to controls.

Since the results of this study revealed a significant correlation between urinary 1-hydroxypyrene and personal exposure to cPAHs ($r = 0.79$) it can be concluded that, urinary 1-hydroxypyrene can serve as a good biomarker in cPAHs exposed workers. This conclusion is in agreement with Petchpoung *et al.* study which underlined the importance of urinary 1-hydroxypyrene as a good biomarker of PAHs exposure.^[13] In addition, our results are in agreement with Bosso *et al.* findings that, showed occupational exposure to fume and particulate matter arisen from burning the sugarcane foliage results in significantly higher levels of urinary 1-hydroxypyrene in sugarcane workers in

Table 1: General characteristics of the studied population

Factors	Exposed workers	Controls
Number of subjects	42	43
Age ^a (years)	30.4 ± 4.5	32.5 ± 5.7
Number of smoker subjects	12	12
Number of non-smoker subjects	30	31

^aMean \pm SD

Table 2: Comparison of urinary 1-hydroxypyrene levels^a ($\mu\text{mole}/\text{mole}$ creatinine) in the exposed and control groups

Parameter	Smokers	Non-smokers	Total	P (t-test)
Exposed group	2.91 ± 1.74	7.68 ± 5.12	6.32 ± 4.91	< 0.001
Controls	0.80 ± 0.59	0.44 ± 0.40	0.54 ± 0.48	0.027

^aMean \pm SD

comparison to control group.^[19] Our results are also in line with Kato *et al.* study which reported higher urinary 1-hydroxypyrene levels in charcoal workers exposed to wood smoke compared to that of control subjects. In their study it was also revealed that urinary 1-hydroxypyrene increases monotonically with the level of exposure to wood smoke.^[20] Comparing to Petchpoung *et al.* who reported urinary 1-hydroxypyrene to be $0.124 \pm 0.007 \mu\text{mole/mole creatinine}$ in the PAHs exposed Thai bus drivers;^[15] we found higher levels of urinary 1-hydroxypyrene in the exposed subjects ($6.32 \pm 4.9 \mu\text{mol/mol creatinine}$). This high level of difference in the concentration of excreted 1-hydroxypyrene can be attributed to the higher levels of PAHs release in the aluminum anode plant compared to that of city traffic. The level of urinary 1-hydroxypyrene in our control subjects was also higher than that of Petchpoung *et al.* (0.54 ± 0.48 versus 0.032 ± 0.003). This difference may be due to different nutritional habits and different status of metabolizing enzymes polymorphism.^[12]

It can be inferred from Jongeneelen *et al.* studies that, in a urinary 1-hydroxypyrene level of more than $1.4 \mu\text{mol/mol creatinine}$ genotoxic effects should be observable.^[8] In addition, according to the results of Siwinska *et al.* study which conducted to evaluate the association between urinary 1-hydroxypyrene and genotoxic effects in coke oven workers, a urinary 1-hydroxypyrene limit of $1 \mu\text{mol/mol creatinine}$ should be established to prevent genotoxic effects in PAHs exposed workers.^[21] In our study, the mean level of urinary 1-hydroxypyrene in the exposed group was found to be $6.32 \pm 4.9 \mu\text{mol/mol creatinine}$. Regarding the results of Siwinska and Jongeneelen studies it is revealed that the level of urinary 1-hydroxypyrene in our cPAHs exposed subjects is several folds higher than the concentration at which observable genotoxic effects are expected. Therefore, it can be concluded that carbon anode plant workers in the studied aluminum industry are exposed to substantial risk of cancer and other genotoxic effects which may arise from exposure to carcinogenic polycyclic aromatic hydrocarbons.

The findings of our study emphasizes on the need for implementing preventive measures for reduction of carcinogenic polycyclic aromatic hydrocarbons exposure in the carbon anode plant in the studied aluminum industry.

CONCLUSION

This study results confirm urinary 1-hydroxypyrene level as a good biomarker in cPAHs exposed workers. In addition, considering the level of urinary 1-hydroxypyrene, it can be concluded that carbon anode plant workers in the studied aluminum industry are exposed to substantial risk of cancer and other genotoxic effects which may arise from exposure to carcinogenic polycyclic aromatic hydrocarbons.

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