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Research Article

Urinary PAH Metabolites as Potential Biomarkers for PAH Exposure - A Pilot Study in Three Cities of Southern India

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Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) are the large group of organic compounds with benzene rings that are mainly emitted during incomplete combustion of organic materials such as fossil fuels. Many PAHs are linked to carcinogenicity and mutagenicity and their presence in ambient air is an increasing day by day particularly in developing countries. In urban regions, more number of people are exposed to the PAHs since the emission sources of PAHs are many including vehicular emissions. Understanding the health impacts of PAHs in human beings is one of the challenging tasks which include laborious experiments. As an easy approach, biomarkers of PAH exposure offers an immediate insight into exposed population by simple methods. In the present study, we analyzed urine samples of 30 volunteers in three different cities of Southern India for PAH metabolites that are considered as potential biomarkers for PAH exposure. The presence of 2-hydroxynaphthalene (2-NAP) and 1-hydroxypyrene (1-OHP) in majority of samples indicated that a substantial population might be exposed to PAHs.

Keywords: PAH; Biomarkers; Urinary Metabolites

Introduction

Biomarkers have been increasingly recognized worldwide for exposure assessment of hazardous chemicals. Particularly in exposure assessment the exogenous substance or first metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured as an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance [1]. In many cases, biomarkers can also serve as indicators of preclinical conditions, and demand the need for a detailed assessment. Biomarker assessment is also considered as advantageous and quick technique over the laborious dose-effect studies. Recent attention in epidemiology focuses on understanding the genetic basis for individual susceptibility to the development of chronic disease and interaction with the biomarkers of exposure [2]. Genetic susceptibility markers are very useful for addressing gene–exposure (i.e., gene–environment) interactions.

In evaluating the health effects and risks of PAH, great advances have been achieved in the development of various types of biomarkers, including chemical-specific biomarkers of exposure and characterization of genetic variation in many genes of relevance to carcinogenesis [3]. The most commonly used biomarkers of PAH exposure includes hydroxylated PAH metabolites in urine (OH-PAHs, 2-naphthol, 2-hydroxyfluorene, 3-hydroxyphenanthrene, 1-hydroxypyrene, 6-hydroxychrysene and 3-hydroxybenzo[a] pyrene) [4].

The assay for the glucuronide conjugate of 1-OHP (1- OHPG) has been developed and successfully applied to population with various PAH exposures. 1-OHPG is more sensitive than 1-OHP, since 1-OHPG is 3–5 times more fluorescent than 1-OHP. On the other hand, the 1-OHPG assay requires more urine than the 1-OHP assay. Urinary 2-naphthol, a stable PAH metabolite, reflects more specifically ambient PAH exposure, whereas 1-OHP levels can be affected by diet and smoking. Thus, urinary 2-naphthol is suggested as a specific marker for exposure to airborne particulates whereas urinary 1-OHP has been used as a marker for exposure to PAHs by non-specific exposure routes [2].

While evaluating the occupational exposure of PAHs, [5] observed that waste incineration workers showed higher level of 1-OHP and 2-naphthol than the automobile emission inspection workers in Korea. The levels of 1-OHP in automobile emission inspectors, waste incineration workers and control subjects were 0.298 ± 0.212, 0.531 ± 0.427 and 0.061 ± 0.094 μmol/mol creatinine, respectively. The mean values of 2-naphthol were 5.894 ± 4.683, 8.947 ± 5.931 while compared to control 1.924 ± 3.441 mol/mol creatinine, which ascertained that biomarker is a potential tool for exposure studies. In contrary to the belief that inhalation as a major route of exposure to PAHs, [6] in a study at Japan identified dietary exposure was significantly correlated with urinary 1-OHP excretion than inhalation. Biomarker experiments also revealed that the average concentrations of 1-OHP in the urine samples were higher smokers and passive smoker groups than that of the non-smoking group [7].

Zheng et al (2013) suggested hydroxylated PAH metabolites (OH-PAHs) as suitable biomarkers for wood smoke exposure. In an experiment involving nine volunteers, urinary OH-PAHs, except 1-hydroxypyrene (1-PYR), correlated with those of PM 2.5, levoglucosan and PAHs in personal PM 2.5 samples. In the children subjects also urine samples appeared to be the best biomarkers; a

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Table 1: The optimal excitation and emission wavelengths for two PAH metabolites.

Time (min)	Excitation wavelength (nm)	Emission wavelength (nm)	PAH metabolites
0-14	227	355	2-hydroxynaphthalene (2-NAP)
17-22	242	388	1-hydroxypyrene (1-OHP)

Table 2: Urinary PAH metabolite levels of 10 individuals in age group 30-40 and their background in Chennai.

Height (cm)	Weight (kg)	Occupation	mode of travel	Smoking habits	2- Naphthol ng/L levels	1-OHP ng/L levels
168	54	Student	walk	No	16.69	2.976
172	70	Student	walk	No	BDL	2.878
165	64	Student	walk	No	26.41	11.74
195	99	Student	Two Wheeler	Yes	34.85	22.11
160	55	Student	Train	No	BDL	BDL
180	78	Foundry	Train	No	76.08	2.288
155	60	Student	walk	Yes	15.82	BDL
156	55	IT Professional	Train	Yes	24.05	1.13
164	60	Press	Train	Yes	28.06	0.756
174	71	Student	walk	Yes	BDL	BDL
BDL: Below detectable levels						

Table 3: Urinary PAH metabolite levels of 10 individuals in Coimbatore of age group 26-52 and their background.

Height (cm)	Weight (kg)	Occupation	mode of travel	Smoking habits	2- NAP ng/L	1-OHP ng/L
154	51	Homemaker (Female)	Bus	No	BDL	BDL
172	75	Clerk (Male)	Two wheeler	Yes	12.55	0.71
172	75	Police (Male)	Four wheeler	Yes	93.07	1.08
174	62	Farmer (Male)	Bus	Yes	30.03	BDL
171	75	Engineer(Male)	Two wheeler	No	9.47	BDL
160	45	Merchant (Male)	Bus	Yes	BDL	10.45
158	51	Clerk (Male)	Two wheeler	No	15.3	BDL
150	48	Homemaker (Female)	Bus	No	BDL	1.88
155	70	Homemaker (Female)	Bus	No	41.84	BDL
162	51	Student (Male)	Bus	Yes	BDL	8.92

study in Ohio at 126 homes and 16 daycares observed associations between selected sociodemographic/lifestyle factors and urinary 1-OHPyr levels [8,9]. The median urinary 1-OHPyr level was 0.33 ng/mL. Therefore, large number of studies on biomarkers have widely recognized PAH urinary metabolites as potential biomarkers for human exposure studies.

Materials and Methods

Urine samples (early morning) from 30 volunteers (10 each from 3 cities: Chennai, Coimbatore and Tiruchirappalli; Figure 1) were collected and analyzed for urinary PAH metabolites. During the sample collection, volunteers were requested information on health and living habits, such as age, height, weight, disease prevalence and smoking habits. Early morning urine samples were collected in bottles pre-cleaned with deionized water and 0.1 M Hydrochloric acid (HCl). The samples were taken immediately to the laboratory. Volunteer's age were between 23 to 45 years, of which 80% are males and 20% females. All of them resided in urban environments

Urinary metabolite was analyzed according to the method of [10]. In brief 10 ml of urine was buffered with 20 ml of acetate buffer and the metabolite was deconjugated by 15 μl of β-glucuronidase/arylsulfatase (type H-2, Sigma, USA) at 37°C. The hydrolyzed urine sample was then loaded on to the SEP-Pack C18-E cartridge (Phenomenex-USA) at a flow rate of <1 ml/min. The column was washed with 10 ml water and 10 ml 30% methanol to remove the matrix interferences. The two PAHs were eluted with 4ml of methanol. Elute was concentrated almost to dryness under a gentle stream of nitrogen, and then dissolved in 1 ml of methanol. The solution was filtered through 0.2 μm filter, and then stored at -20°C until analysis.

Two PAH metabolites [2-hydroxynaphthalene (2-NAP) and 1-hydroxypyrene (1-OHP)] standards obtained from SUPELCO were used to generate standard chromatogram and the optimum wavelengths were standardized. Analysis was carried out with a HPLC-FLD (Make: Waters) with PAH C18 column (5 μm 4.6 x 250 mm). Elution was performed using a water–methanol gradient: 0-15 min a linear gradient from 50% to 70% methanol and 15-20 min a linear gradient from 70% to 90% methanol. The flow rate was 0.80 ml/min. The volume of sample injection was 20 μl.

Result and Discussion

Urinary 1-OHP and 2-NAP, the biological markers used for

Table 4: Urinary PAH metabolite levels of 10 individuals in Coimbatore of age group 26-52 and their background.

Height (cm)	Weight (kg)	Occupation	Mode of travel	Smoking habits	2- Naphthol (ng/L)	1-OHP (ng/L)
174	64	Business	Two Wheeler	No	67.17	1.98
162	55	Student	Two Wheeler	Yes	BDL	3.46
160	72	Student	walk	Yes	96.93	0.42
175	78	Student	Two Wheeler	No	BDL	0.42
160	58	Student	Bus	Yes	55.89	6.76
180	78	Sales man	Two Wheeler	Yes	36.8	1.67
158	65	Student	Walk	No	BDL	BDL
164	54	Sales man	walk	Yes	BDL	0.3
165	78	Business	Two Wheeler	Yes	BDL	2.65
170	71	Student	walk	No	12.34	2.16

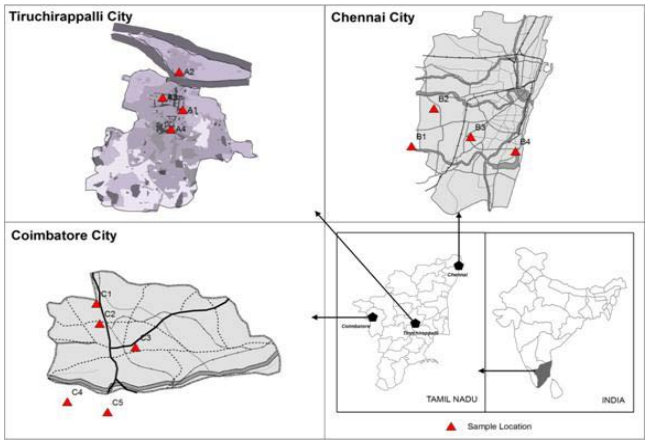

Figure 1: Study Area.

analyzed of PAH exposure analysis is shown in Figure 2.

Ten volunteers selected from Chennai city were in the age group 20-30. Majority of them were students while three people were employed in different occupation Table 2. Two urinary PAH metabolites viz. 2-NAP and 1-OHP were observed with maximum values of 76.08 ng/l and 22.11 ng/l, respectively. The level of 1-OHP and 2-NAP in general was observed higher in smokers. Although no conclusion about PAH exposure can be arrived with limited number samples, these results hint that a substantial number of population might be exposed to higher levels of PAH.

In Coimbatore city, 10 respondents in the age group 26-52 were examined. Urinary metabolites such as 2-NAP and 1-OHP concentration were observed up to the level of 93.07 and 10.45 ng/l respectively Table 3. Sample of police man recorded highest concentration of 2-NAP (93.07 ng/l) indicating clearly is his occupational exposure to traffic emissions. In general, level of urinary metabolites was high in male, particularly smokers. Variation in biomarker levels among the respondents may be due to extent of exposure and other influencing factors such as smoking, body mass index and diet.

In Tiruchirappalli, among the 10 Urine samples analyzed for 1-OHP and 2-Naphthol, Higher levels of 2-NAP concentrations ranging between 36-96 ng/l was recorded in half the number of samples. 1-OHP levels recorded up to 6.76 ng/l in smokers Table 4.

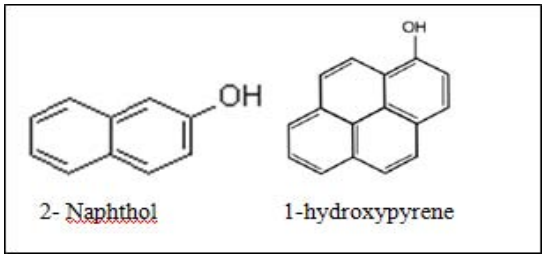

Figure 2: Chemical structure of PAH metabolites.

Conclusion

The excess levels of two urinary PAH metabolites (2-NAP and 1-OHP) observed in majority of respondent hints that possibility of higher population being exposed to PAHs. With the current trend in urbanization and vehicular growth more population will be at risk of PAH exposure. Future urban growth should be inclusive facilities for environmentally benign garbage disposal, traffic decongestion plans and eco-friendly fuel driven public transport systems. Rewarding experiences from western countries such as introduction congestion tax, technology retrofit, and stringent emission norms can also be explored in Indian cities.

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