Detection of Polycyclic Aromatic Hydrocarbon Exposure from Automobile Exhaust Fumes Using Urinary 1-Hydroxypyrene Level as an Index

Suparat Wattana MSc*
Supeecha Wittayalertpanya MSc*,

*Department of Pharmacology, Faculty of Medicine, Chulalongkorn University

Polycyclic aromatic hydrocarbons (PAHs) are hydrocarbon compounds which originate from incomplete combustion. In humans, PAHs are bioactivated to reactive metabolites which can bind covalently to DNA and subsequently initiate mutation and carcinogenesis. The measurement of PAHs exposure may be used as an index to classify a cancer risk group. The purpose of the present study was to measure the level of urinary 1-hydroxypyrene, a metabolite of PAHs, in subjects exposed to automobile exhaust fumes compared to non-exposed subjects. A urine sample was collected from each individual subject after the end of a working day and quantitated for 1-hydroxypyrene and creatinine by HPLC and spectrophotometric method, respectively. The results showed that average urinary 1-hydroxypyrene level in exposed subjects was significantly higher than non-exposed subjects (mean ± SD of 0.0035 ± 0.0032 and 0.0011 ± 0.0010 μmol/l, respectively; P = 0.000). Average urinary creatinine level in exposed subjects was also significantly higher than non-exposed subjects (mean ± SD of 0.01 ± 0.005 and 0.008 ± 0.006 mol/l, respectively; P = 0.040). The ratio of urinary 1-hydroxypyrene/mol creatinine level, of the exposed subjects was significantly higher than that of the non-exposed subjects (mean ± SD of 0.37 ± 0.28 and 0.19 ± 0.22 μmol/mol creatinine, respectively; P = 0.002). Conclusion : Automobile exhaust fume exposed subjects have a higher risk to be exposed to PAHs than the non-exposed subjects. Urinary 1-hydroxypyrene level can be used as an index for an exposure of PAHs which have originated from automobile exhaust fumes and other sources as well.

Keywords : *Polycyclic aromatic hydrocarbons, 1-Hydroxypyrene*

J Med Assoc Thai 2004; 87 (Suppl 2): S233-8
e-Journal: *http://www.medassocthai.org/journal*

Polyclic aromatic hydrocarbons(PAHs) are a large group of organic compounds with two or more fused aromatic rings[1].They originate from incomplete combustion processes or pyrolysis of organic material such as processing of coal, crude oil and natural gas etc[1-3].They are also found contaminated in tobacco smoke, automobile exhaust and dietary sources[4-6]. PAHs generally exist as colorless, white, or pale yellow-green solids. They can have a faint, pleasant odor. The solubility of PAHs in water is low, but PAHs are highly soluble in fat[1,7,8]. PAHs reacts with pollutants such as ozone, nitrogen oxides and sulfur dioxides, yielding diones, nitro- and dinitro-PAHs, and sulfonic acids, respectively. PAHs may also be degraded by some microorganisms in soil [1]. There are more than 100 different PAHs. PAHs generally occur as complex mixtures, not as a single compound[8].

Correspondence to : Wittayalertpanya S. Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

PAHs are present throughout the environment, and humans may be exposed to these substances at home, outside, or at the workplace. If PAHs enter the body, many factors will determine harmful health effects such as dose, duration, route or pathway and individual characteristics such as age, sex, nutritional status, family traits, lifestyle, and state of health[8].

PAHs are highly lipid-soluble and are absorbed from the lung, gut and skin of mammals. Small amounts are stored in the spleen, adrenal glands, and ovaries[1]. PAHs require a multistep metabolic activation by specific enzymes. The enzyme system primarily responsible for PAHs metabolism is the mixed function oxidase system, which requires NADH or NADPH and molecular oxygen to convert the nonpolar PAHs into the polar hydroxy derivatives and arene oxides. The terminal step, PAHs can be changed to diol epoxides which can bind covalently to DNA and subsequently initiate mutation and carcinogenesis[9]. Most metabolites of PAHs are excreted in urine

and feces[1,10,11]. Moreover, PAHs have been well established as an enzyme inducer of cytochrome P450 1A (CYP 1A) family, CYP 1A1 and CYP 1A2[1].

Uptake of PAHs in the body may be monitored by different biomarkers[8,12], for example metabolites in urine, urinary thioethers, urinary mutagenicity, PAH-protein adducts and PAH-DNA adducts. Both urinary mutagenicity and urinary thioethers are non-specific indicators of exposure to mutagenic agents. These methods are not suitable for routine applications[13-15].

A specific metabolite of pyrene, 1-hydroxypyrene, in urine has been suggested as a biomarker of human exposure to PAHs because the abundance of the pyrene, a non-carcinogenic PAHs, is relatively high in PAHs-mixtures and the analytical method is specific and sensitive so 1-hydroxypyrene is the good biomarker for routine application[4,15,16].

The main purpose of the present study was to measure the level of urinary 1-hydroxypyrene in those subjects between automobile exhaust fumes exposure and non-exposure. Measurement in the form of urinary 1-hydroxypyrene/ mol creatinine level was used.

Material and Method

Subjects

The study was approved by the Ethic Committee of the Faculty of Medicine, Chulalongkorn University. Forty-five exhaust exposed and forty-seven non-exposed healthy female volunteers were included in the present study after giving informed consent. Age (mean $\pm$ SD) of the exposed group was 37.25 $\pm$ 6.48 years (range, 21-47 years) and control (non-exposed) group was 33.25 $\pm$ 6.78 years (range, 20-45 years). Each subject was confirmed in good health by clinical laboratory parameters including liver and renal function test. The study group was exposed to automobile exhaust for at least one year while the control group was not exposed. They were not usually exposed to fumes or smoke from other sources and do not often have grilled, smoked or roasted food. Five ml of blood sample and 60 ml at urine were collected from each subject. Urine samples were collected in a container protected from light and stored at -20 °C until analysis.

Chemicals

1-Hydroxypyrene was obtained from Dr.Ehrenstorfor GmbH, Germany. β-Glucuronidase, hydrochloric acid, creatinine anhydrous, picric acid-saturated solution (1.3%) and anthracene as the internal standard were obtained from Sigma Chemical Co.Ltd. Acetonitrile HPLC grade was from Merck. Sodium acetate anhydrous was from Fluka and double distilled with deionized water was used throughout the study.

Apparatus

HPLC from Shimadzu consisted of LC-10 ADVP liquid chromatography for delivering the mobile phase, SIL- 10 ADVP autoinjector for sample injection, SLC-10AVP system controller, DGU-12 A degasser, CTO-10AVP column oven and RF-1AXL fluorescence detector used to monitor 1-hydroxypyrene at excitation 270 nm and emission 387 nm. A ODS-3 C18 stainless steel column (4.6x250 mm, GL Sciences Inc., Japan) was suitable in the condition. A computer system with class-VP version 6.12 SP1 software was used to analyse peak and set the standard system. Creatinine level in urine was determined by spectrophotometer.

Sample preparation

The method for sample separation was modified from Ruchirawat M et al[17]. 5 ml of urine sample, 5 ml 0.2 M sodium acetate buffer (pH 5.0) and 10 µl of 1.36 mg/ml anthracene were mixed. This mixture was incubated overnight (20 h) with 25 µl of β-glucuronidase/aryl sulphatase at 37 °C in a shaking water bath.

A sample enrichment and purification cartridge packed with C_{18} reversed-phase liquid chromatographic material (Sep-pak C_{18} cartridge, Supelco) was used for the extraction of the metabolite. The cartridge was primed with 2.5 ml of acetonitrile, followed by 10 ml of distilled water, and the hydrolysed sample was passed through the cartridge at a rate of approximately 2.5 ml/min. Subsequently the cartridge was washed with 5 ml of sodium acetate buffer (pH 5.0). The retained solutes were eluted with 2 ml of acetonitrile. The solvent was evaporated by speed vacuum and residue was dissolved in 1 ml of acetonitrile. 10 µl of the supernatant was injected into the HPLC system.

Chromatographic condition

The mobile phase for 1-hydroxypyrene assay was the mixture of acetonitrile and water (65: 35) which was delivered through HPLC system at flow rate of 1.0 ml/min. Quantitation was based on peak area integration by computer software. The chromatogram is shown in Fig. 1.

Urine creatinine level determination

Urine creatinine level was determined according to the method of Lusgarten, J.A. and Wenk, R.E[18]. The alkaline picrate was prepared by mixing 0.5 M sodium hydroxide and saturated picric acid (1: 7). 2 ml

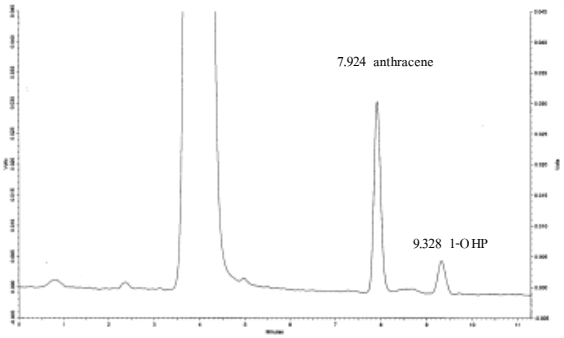


Fig. 1 Chromatogram of 1-hydroxypyrene and anthracene as internal standard with retention time of 9.328 min and 7.924 min,respectively

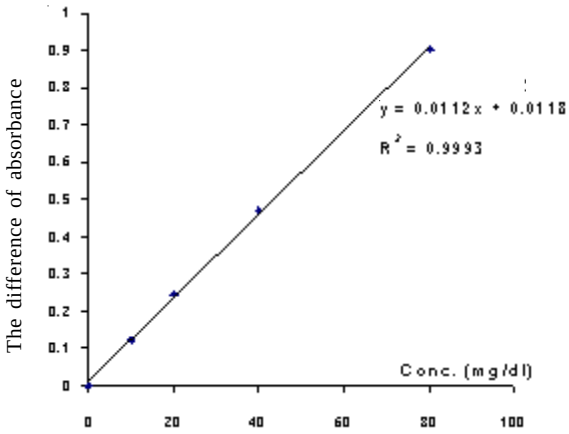


Fig. 2 Standard curve of creatinine

of alkaline picrate was added in cuvette and adjusted to zero at wavelength 515 nm. The solution was spiked 100 µl of urine and mixed. After 20 seconds, the sample was measured for UV absorbance and then 60 seconds later, measured again. The difference of UV absorbance was calculated and calibrated with the standard curve of creatinine, Fig. 2.

Data analysis

Significant differences of urinary 1-hydroxypyrene levels, urinary creatinine levels and urinary 1-hydroxypyrene/mol creatinine ratios between the control group and exposed group were determined by Student's t- test in SPSS program at p-value of 0.05.

Results

In the exposed and non-exposed group, their age and weight were comparable. All subjects were in good health with normal limit of clinical laboratory data (Table 1).

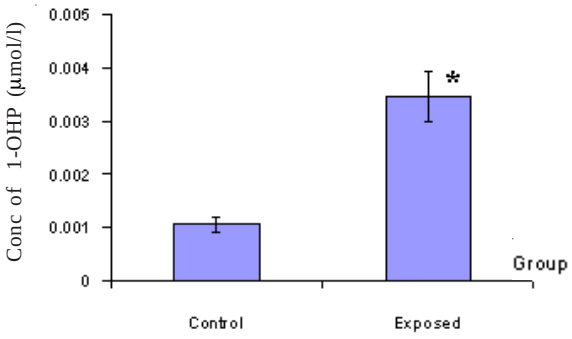
The method for 1-hydroxypyrene and creatinine assay was validated. Accuracy of 1-hydroxypyrene analysis was 100.40±3.07 % and intra-day and inter-day assay variations were 5.08 and 5.19 % CV, respectively. Recovery of 1-hydroxypyrene extraction was 81.40±1.79%. Those were within the acceptable criteria. For creatinine assay, accuracy and precision were also within the acceptable range. Accuracy was 100.88±1.08% and intra-day and inter-day assay variations were 3.15 and 3.12%CV, respectively.

The results showed that the average urinary 1-hydroxypyrene level in the exposed group was significantly higher than that of the non-exposed group with mean ± SD of 0.0035 ± 0.0032 and 0.0011 ± 0.0010 µmol/l, respectively; P = 0.0000 (Fig. 3). Average urinary creatinine level in the exposed group was

Table 1. Demographic data of non-exposed and exposed group

Parameter	Normal value	Non-exposed (n=47)		exposed (n=45)	
		Range	mean ± SD	Range	mean ± SD
Age (yr)	-	20-45	33.25 ± 6.78	21-47	37.25 ± 6.48
Weight (kg)	-	43-80	56.99 ± 9.63	40-95	60.84 ± 11.40
Height (cm)	-	142-180	155.30 ± 7.03	145-170	156.87 ± 5.60
SBP (mmHg)	90-140	90-140	114.34 ± 11.52	90-150	117.18 ± 11.24
DBP (mmHg)	60-90	60-90	70.77 ± 8.74	50-110	71.31 ± 10.24
BUN (mg/dl)	0-38	4-17	11.21 ± 2.44	7-22	12.11 ± 3.49
Creatinine (mg/dl)	0-38	0.4-1	0.68 ± 0.11	0.5-0.9	0.69 ± 0.08
Albumin (g/dl)	0-1	3.3-4.8	4.22 ± 0.28	3.9-4.7	4.33 ± 0.21
TB (mg/dl)	3.4-5.5	0.06-0.94	0.36 ± 0.19	0.09-1.11	0.41 ± 0.22
AST (U/L)	39-117	13-51	23.42 ± 7.06	15-38	22.66 ± 4.65
ALT (U/L)	10-20	5-55	18.13 ± 10.48	5-34	16.31 ± 6.48
AP (U/L)	0.5-2	42-108	62.55 ± 15.18	31-110	56.16 ± 18.27

Note: SBP = systolic blood pressure, DBP = diastolic blood pressure, BUN = blood urea nitrogen, TB = total billirubin, AST = aspartate aminotransferase, ALT = alanine aminotranferase, AP = alkaline phosphatase



* denotes significant differences, p < 0.05

Fig. 3 1-Hydroxypyrene level of the non-exposed (control) and exposed groups. The figure shows mean ± SE of n = 47 in the control and n = 45 in the exposed group

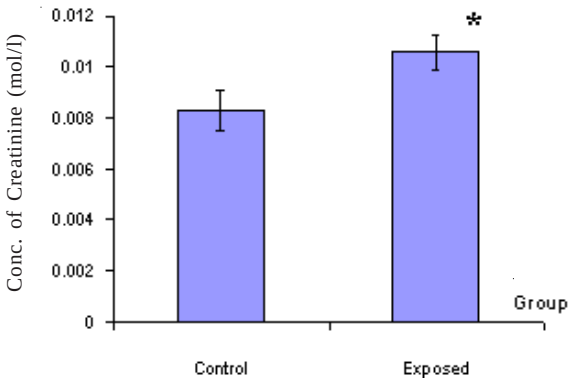
significantly higher than that of the non-exposed group with mean ± SD of 0.01 ± 0.005 and 0.008 ± 0.006 µmol/l, respectively; P = 0.0040 (Fig. 4). With the measurement in the form of urinary 1-hydroxypyrene/ mol creatinine ratio, in the exposed group was significantly higher than that of the non-exposed group with mean ± SD of 0.37 ± 0.28 and 0.19 ± 0.22 µmol/l, respectively; P = 0.002 (Fig. 5).

Discussion and conclusion

There are several methods to measure 1-hydroxypyrene level but some are not suitable for routine assay[13]. However, the procedure for 1-hydroxypyrene determination developed in this experiment was simplified and validity. 1-hydroxypyrene is a major metabolite of pyrene which is relatively high in PAH-mixtures that are found in automobile exhaust fumes, cigarette smoke, waste incinerator fumes, etc[5,16]. It is mainly excreted in urine. Thus, urinary 1-hydroxypyrene level should be a useful biomarker for human exposure to PAHs detection. Serum paraxanthine/caffeine ratio has been used to determine the activity of CYP1A2[19]. CYP1A2 activity is induced by some agents including PAHs[1]. From a previous study, serum paraxanthine/ caffeine ratio was significantly higher in the automobile exhaust exposed group than in the non-exposed group [19]. It implied that higher activity of CYP 1A2 was induced by PAHs, one of the components found in automobile exhaust fumes. Thus, the present study was to directly measure 1-hydroxypyrene, creatinine and 1-hydroxypyrene/ mol creatinine in urine of the same population.

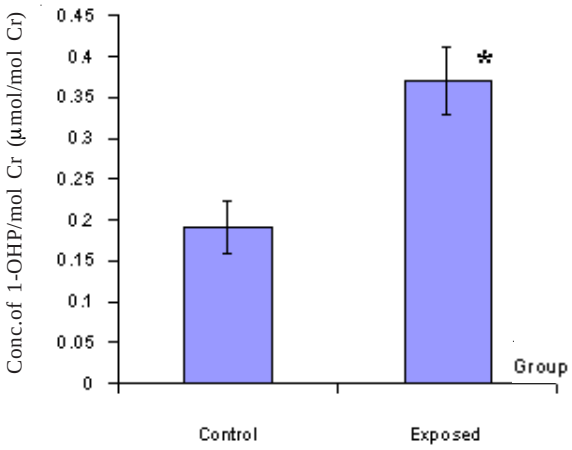
The criteria for subject recruitment should definitely screen healthy subjects. In the present study, subjects were adults aged from 20-47 years and had normal liver and renal function. Whole factors that influence PAHs metabolism or might be sources of PAHs were excluded. Although male subjects should be used to represent the population, many of them usually smoke cigarettes and have an alcohol habit. Alcohol has been confirmed as an important agent interfering with CYP450 activity[5]. Accordingly, female subjects were good samples for the study. Subjects who often smoked, grilled and roasted food or other sources of PAHs were also excluded. Other disease factors that might affect hepatic drug metabolizing enzyme were screened out by clinical laboratory data.

Due to the average elimination half life of 1-hydroxypyrene of 18 hours, urine samples should be



* denotes significant differences, p < 0.05

Fig. 4 Creatinine level of the non-exposed (control) and exposed groups. The figure shows mean ± SE of n = 47 in the control and n = 45 in the exposed group



* denotes significant differences, p < 0.05

Fig. 5 1-Hydroxypyrene/mol creatinine ratio of the non-exposed (control) and exposed groups. The figure shows mean ± SE of n = 47 in the control and n = 45 in the exposed group

collected within 24 hours after exposure[20]. Besides, urinary 1-hydroxypyrene monitoring, urine creatinine level could be assayed for measurement in the form of 1-hydroxypyrene/mol creatinine. As that is due to the difference in drinking water in each subject, dilution of urine will differ[21].

In the present study, the authors found that urinary 1-hydroxypyrene and creatinine in the automobile exhaust exposed group was significantly higher than that of the non-exposed group. In addition urinary 1-hydroxypyrene/mol creatinine ratio was significantly higher in the automobile exhaust exposed group. It can be concluded that the population exposed to automobile exhaust fumes has more exposed PAHs than the controls and might have a higher risk of chemical carciongenesis. Urinary 1-hydroxypyrene level can be used as an index for PAHs exposure which is caused by automobile exhaust fumes and can be applied to populations that are exposed to PAHs from other sources.

Acknowledgement

This investigation was supported by the Rachadapiseksompoj China Medical Board Research Fund and a Ministry of University Affairs (MUA)-CU Thesis Grant.

References

1. Polycyclic aromatic hydrocarbons (PAHs). Chapter 5.9. In: Air Quality Guidelines [online]. 2nded. Copenhagen: World Health Organization Regional Office for Europe, 2000. Available from: URL: http://www.who.dk/document/aiq/5_9pah.pdf.
2. Boogaard PJ, Sittert NJ van. Exposure to polycyclic aromatic hydrocarbons in petrochemical industries by measurement of urinary 1-hydroxypyrene. Occup Environ Med 1994; 51: 250-8.
3. Sherson D, Sabro P, Sigsgaard T, Johansen F, Autrup H. Biological monitoring of foundry workers exposed to polycyclic aromatic hydrocabons. Br J Ind Med 1990; 47: 448-53.
4. Jongeneelan F J. Benchmark quideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. Ann Occup Hyg 2001; 45: 3-13.
5. Hu L, Robert IK. Improved HPLC method for analysis of 1-hydroxypyrene in human urine specimens of cigarette smokers. Sci Total Environ 2000; 257: 147-153.
6. Strickland P, Kang D. Urinary 1-hydroxypyrene and other PAH metabolites as biomarkers of exposure to environmental PAH in air particulate matter. Toxicology Letters 1999; 108: 191-9.
7. Jongeneelan FJ. Chapter 4.2: Biological monitoring of polycyclic aromatic hydrocarbons: 1-hydroxypyrene in urine. In: Biological monitoring of chemical exposure in the workplace -volume 2 [online]: WHO-Geneve, 1996. Available from: URL: http://www.industox.nl/ARTHPWHO.htm.
8. ATSDR. Toxicological profile for polycyclic aromatic hydrocarbons (PAHs) [online]. Agency for Toxic Substances and Disease Registry. Atlanta, 1995. Available from: URL: http://www.atsdr.cdc.gov/toxprofiles/tp69-c1.pdf.
9. ATSDR. Polycyclic aromatic hydrocarbons: toxicology [online]. U.S. Department of health & human service, Agency for Toxic Substances and Disease Registry: U.S. Government printing office, 1995. Available From: URL: http://cdfc.rug.ac.be/HealthRisk/PAHs/toxicology.htm.
10. Lafontaine M, Payan JP, Delsaut P, Morele Y. Polycyclic aromatic hydrocarbon exposure in an artificial shooting target factory: Assessment of 1-hydroxypyrene urinary excretion as a biological indicator of exposure. Ann Occup Hyg 2000; 44: 89-100.
11. Breznicki S, Jakubowski M, Czerski B. Elimination of 1-hydroxypyrene after human volunteer exposure to polycyclic aromatic hydrocarbons. Int Arch Occup Environ Health 1997; 70: 257-60.
12. Sherson D, Siggaurd T, Overgaard ES, Poulsen HE, Jongeneclan FJ. Interaction of smoking, uptake of polycyclic aromatic hydrocabons, and cytochrome P450 1A2 activity among foundry workers. Br J Ind Med 1992; 49: 197-202.
13. Scherer G, Frank S, Riedel K, Kossien IM, Renner T. Biomonitoring of exoposure to polycyclic aromatic Hydrocarbons of nonoccupationally exposed persons. Cancer Epidemiol Biomark 2000; 9: 373-80.
14. Moen BE, Nilsson R, Nordlinder R, Ovrebo S, Bleie K, Skorve AH, et al. Assessment of exposure to polycyclic aromatic hydrocarbons in engine rooms by measurement of urinary 1-hydroxypyrene. Occup Environ Med 1996; 53: 692-6.
15. Nerurkar PV, Okinaka L, Aoki C, Seifried A, Lum-Jones A, Wilkens LR, et al. CYP 1A1, GSTM 1, and GSTP 1. Genetic Polymorphisms and urinary 1- hydroxypyrene excretion in non-occupationally exposed individuals. Cancer Epidemiol Biomarkers Prev 2000; 9: 1119-22.
16. Boogaard PJ, Sittert NJ van. Urinary 1-hydroxypyrene as biomarker of exposure to polycyclic aromatic hydrocarbons in workers in petrochemical industries: baseline values and dermal uptake. Sci Total Environ 1995; 163: 203-9.
17. Ruchirawat M, Mahidol C, Tangjarukij C, Pui-ock S, Jensen O, Kampeerawipakorn 0, et al. Exposure to genotoxins present in ambient air in Bangkok, Thailand - particle associated polycyclic aromatic hydrocarbons and biomarker. Sci Total Environ 2002; 287: 121-32.
18. Lusgarten JA, Wenk RE. Simple, Rapid kinetic method for creatinine measurement. Clin Chem 1972; 18: 1491-22.
19. Wittayalertpanya S, Hinsui Y, Lawanprasert S. Paraxanthine/Caffeine Ratio: as an index for CYP 1A2 activity in polycyclic aromatic hydrocarbons exposed subjects. J Med Assoc Thai 2003; 86(suppl2): S310-7.
20. Buchet JP, Gennart JP, Mercado-Calderon F, Delavignette JP, Cupers L, Lauwerys R. Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: assessment of urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. Br J Ind Med 1992; 49: 761-8.
21. Drug Testing Quarterly [online] 2000. Available from: URL: http://www.norchemlab.com/reference/dtg/0004dtq01.htm.

การตรวจวัดการได้รับสารโพลีไซคลิคอะโรมาติคไฮโดรคาร์บอนจากควันท่อไอเสียรถยนต์โดยใช้ระดับ1-ไฮดรอกซีไพรีนในปัสสาวะเป็นตัวชี้วัด

สุภารัตน์ วัฒนา, สุพีชา วิทยเลิศปัญญา

โพลีไซคลิคอะโรมาติคไฮโดรคาร์บอน (พีเอเอช) เป็นสารประกอบไฮโดรคาร์บอนที่เกิดขึ้น จากกระบวนการเผาไหม้ไม่สมบูรณ์ของเชื้อเพลิง เมื่อสารพีเอเอชเข้าสู่ร่างกาย จะถูกเปลี่ยนแปลงได้เมแทบอไลท์ที่มีฤทธิ์ก่อมะเร็ง โดยเมื่อจับกับ DNA มีผลก่อการกลายพันธุ์และก่อให้เกิดมะเร็งได้ การตรวจวัดการได้รับสารพีเอเอชในร่างกายจึงน่าจะมีประโยชน์ในการบ่งบอกถึงกลุ่มคนที่เสี่ยงต่อการเป็นโรคมะเร็ง การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อทำการตรวจวัดระดับ 1-ไฮดรอกซีไพรีน ซึ่งเป็นเมแทบอไลต์ของสารพีเอเอช โดยตรวจวัดในปัสสาวะของกลุ่มคนที่มีโอกาสสูงต่อการสัมผัสกับสารพีเอเอชที่มีแหล่งกำเนิดจากควันท่อไอเสียรถยนต์ เปรียบเทียบกับกลุ่มคนที่มีโอกาสสัมผัสกับควันท่อไอเสียรถยนต์น้อยกว่า โดยเก็บปัสสาวะหลังจากอาสาสมัครเสร็จสิ้นการทำงานที่ต้องสัมผัสกับควันท่อไอเสียรถยนต์ ทำการวิเคราะห์หาความเข้มข้นของ 1-ไฮดรอกซีไพรีนในปัสสาวะโดยวิธี HPLC และวัดระดับครีเอตินีนในปัสสาวะโดยวิธีสเปกโทรโฟโตเมทรี พบว่า ระดับของ 1-ไฮดรอกซีไพรีนในปัสสาวะของกลุ่มคนที่ได้รับควันจากท่อไอเสียรถยนต์ มีค่าสูงกว่ากลุ่มคนที่มีโอกาสสัมผัสกับควันท่อไอเสียรถยนต์น้อยกว่า โดยมีค่าเฉลี่ยเท่ากับ 0.0035 $\pm$ 0.0032 และ 0.0011 $\pm$ 0.0010 ไมโครโมล/ลิตร ตามลำดับ (P = 0.000) และระดับของครีเอตินีน ในปัสสาวะของกลุ่มคนที่ได้รับควันจากท่อไอเสียรถยนต์ มีค่าสูงกว่ากลุ่มมีโอกาสสัมผัสกับควันท่อไอเสียรถยนต์น้อยกว่า โดยมีค่าเฉลี่ยเท่ากับ 0.01 $\pm$ 0.005 และ 0.008 $\pm$ 0.006 โมล/ลิตร ตามลำดับ (P = 0.040) และเมื่อคำนวณในรูปของ 1-ไฮดรอกซีไพรีน/โมลครีเอตินีน พบว่า ปัสสาวะของกลุ่มคนที่ได้รับควันจากท่อไอเสียรถยนต์ มีค่าสูงกว่ากลุ่มคนที่มีโอกาสสัมผัสกับควันท่อไอเสียรถยนต์น้อยกว่า โดยมีค่าเฉลี่ยเท่ากับ 0.37 $\pm$ 0.28 และ 0.19 $\pm$ 0.22 ตามลำดับ (P = 0.002) การศึกษานี้แสดงให้เห็นว่า กลุ่มคนที่ได้รับควันจากท่อไอเสียรถยนต์ ได้รับสารประกอบพีเอเอชในปริมาณสูง และระดับของ 1-ไฮดรอกซีไพรีน/โมลครีเอตินีน สามารถใช้เป็นตัวชี้วัดการได้รับสารพีเอเอชที่มีแหล่งกำเนิดจากควันท่อไอเสียรถยนต์ อีกทั้งสามารถนำไปประยุกต์ใช้ในกลุ่มคนที่ได้รับสารพีเอเอชจากแหล่งอื่น ๆ ต่อไป