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ORIGINAL PAPER

Urinary PAHs metabolites in Karakoram Highway's heavy traffic vehicle (HTV) drivers: evidence of exposure and health risk

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Abstract The current study features PAHs exposure on Karakoram Highway, a route of utmost importance in Pakistan. The drivers of heavy traffic vehicles (HTV) on Karakoram Highway spend long hours amid dense traffic and therefore, inevitably inhale huge amount of PAH carcinogens. The urinary metabolites of PAHs in such drivers (meeting selection criteria $n=48$) and a control group ($n=49$) were comparatively profiled. The higher urinary biomarkers among ninety-six percent HTV drivers were evident of PAHs exposure. We observed elevated concentrations of urinary benzo[a]pyrene metabolites (3-OH-BaP = 3.53 ± 0.62 ng g^{-1} creatinine and 9-OH-BaP = 3.69 ± 0.74 ng g^{-1} creatinine) in HTV driver's samples compared to controls (0.85 ± 0.08 and 0.31 ± 0.03 ng g^{-1} creatinine, respectively). Interestingly, urinary benzo[a]pyrene metabolites were

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detected in almost similar amount among HTV drivers irrespective of their working hours. A distinct smoking effect was manifested with rising urinary levels of 1-hydroxypyrene, 2-hydroxyphenanthrene, and 3-hydroxybenzo[a]pyrene with corresponding increase in driving hours per day. These metabolites exhibited characteristic exposures to low molecular weight volatile PAHs that are commonly found in vehicular exhaust. The elevated PAH body burden was directly linked to the nature of their job and the route-long environmental pollution on Karakoram Highway. Additionally, the poor economic status and smoking also increased HTV driver's health vulnerability and significantly declined their health capacity. There was conclusive evidence that HTV drivers were exposed to PAHs during a ride on Karakoram Highway, back and forth, an aspect not reported earlier.

Keywords Karakoram Highway · Urban PAH exposure · HTV drivers

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are important environmental and food contaminants that are formed when coal, petroleum, wood, tobacco, organic macromolecules, and other organic compounds are not entirely burnt. PAHs are primarily absorbed by three routes: the respiratory tract, the digestive tract, and the skin. They are then converted into hydroxy PAHs in the body by a series of enzymes, and ultimately eliminated in urine (Cheng et al., 2021). There is a strong link between PAH exposure levels in the environment and PAH metabolite concentrations in the urine. As a result, the concentration of PAH metabolites in urine is commonly employed to identify and assess the real degree of PAH exposure in the environment (Hisamuddin & Jalaludin, 2022). Exposure to varying level of PAHs was found to be positively linked with chromosomal DNA damage in several earlier investigations (Fu et al., 2019; Liu et al., 2018). Because mitochondrial DNA (mtDNA) lacks histone protection and the DNA damage repair system is flawed compared to nuclear DNA, it is very vulnerable to external carcinogens and is regarded as a key target of carcinogens (Cheng et al., 2021).

Vehicle emissions are recognized to be a major source of polycyclic aromatic hydrocarbons in the atmosphere (PAHs). Vehicle exhaust emissions account for about 10% of the PAHs in the air. Active air samplers are commonly used to evaluate PAH levels in traffic zones or along roadways, and it is well understood that these samplers provide a snapshot of contamination. Historic changes in PAH emission levels and profiles can be gathered using this approach and repeated measurements, which can then be utilized to reflect differences in fuel usage and whether or not emission control activities are effective (Zhang et al., 2021). Vehicle emission includes elements of incomplete combustion of fossil fuel, comprising of PAHs mainly derived when diesel is used as fuel. These compounds contain hydrogen and carbon atoms that constitute simple to complex benzene rings arranged in two or three structural configurations (Vichi et al., 2005). PAHs often do not carry substitutes or hetero-atoms (Islam et al., 2017; Nguyen et al., 2014). There are two broad categories of PAHs; light PAHs with four or less rings and heavy PAHs having greater than four benzene rings (Kuppusamy et al., 2016).

Respiratory tract, ingestion and dermal contact are the major routes of human exposure to PAHs (Nazmara et al., 2020). As a result, measuring concentrations of film-bound and airborne PAHs in automobiles allows for the assessment of possible human health risks for drivers and passengers. Currently, studies rely on Monte Carlo simulation to carry out a risk assessment that included ambiguities resulting from physiological differences, individual body weight, and variations in inhaling rate (Zhang et al., 2021).

Tobacco smoking and high-temperature cooking are the other common sources of PAH exposure in the general population. PAHs are still a major source of exposure in the workplace, and numerous sectors that require high levels of exposure to PAH mixtures are recognized as suspected carcinogens in humans (Barul et al., 2021). Occupations such as the coke production, driving trucks/motor vehicles, oil refining, coal gasification, bituminous products roofing, etc., are a few examples. Some other ways by which humans are exposed to PAHs include ingestion of contaminated food, passive or active smoking, breathing polluted indoor air, and ambient air pollution, etc. (Zhang et al., 2019). However, the most unprecedented exposure accounts to

occupational nature where drivers of heavy vehicles are on top owing to their job characteristics.

PAHs exposure from aforementioned sources impacts occupational workers to a large extent. These high levels of PAHs pollutants may be associated with a variety of symptoms amongst the occupational workers, such as nausea, eye irritation, diarrhea, inflammation, skin irritation and vomiting. Environmental and occupational exposure to air pollutants is often mixture of chemicals found in ambient air. In such cases naphthalene, anthracene and benzo[a] pyrene are revealed as direct skin irritants; however, benzo[a]pyrene and anthracene cause allergic skin reactions and are known as skin sensitizers (Lawal, 2017).

In Pakistan's northern areas, vehicle trafficking is a significant environmental issue. The Karakoram Highway, upon completion of road networks under China–Pakistan Economic Corridor, is planned to carry up to 7000 trucks each day, releasing up to 36.5 million tonnes of CO_2 along the way to Gwadar. By deteriorating air quality, CO_2 emissions and particulate matter will alter the local climate and environment (Kouser et al., 2020). Air pollution is a persistent issue around the world, and it has resulted in a variety of health issues. Traffic is the most important contributor to outdoor air pollution because it is associated with adverse effects on human health. Incomplete combustion of organic materials is the principal source of PAHs in the environment; however, they are also generated through vehicle exhaust during incomplete combustion of diesel and petrol. Because of their persistence and carcinogenic qualities, PAHs have attracted the attention of scientists (Kamal et al., 2015).

Truck and heavy vehicle driving is among many poor's occupation in Pakistan. People have to work on long routes, overburdened with prolonged work hours to make ends meet. One of such route is Karakoram Highway, where there is limited evidence of exposure available so far in literature studies. Epidemiological studies have discerned the association between PAHs exposure and hypertension and/or cardiovascular disease in the general population (Mirzababaei et al., 2022). Under these considerations (long routes, overburdened with prolonged work hours), this pilot project aimed to quantify the PAH-exposure while driving truck/bus on this well-known route. We further examined the relationship between urinary biomarkers and the effect of PAH exposure on apparent symptom development among heavy vehicle drivers.

Material and methods

Description of study area and hotspot selection

The Karakoram Highway (KKH) with a total length of 1300 km connects the Pakistani provinces of Punjab, Khyber Pakhtunkhwa and Gilgit-Baltistan with China's western Xinjiang Uyghur Autonomous Region. It was built by the governments of Pakistan and China in 1959 and opened in 1979. The KKH passes through some of the world's most difficult and rugged terrain. Since its completion in 1979, the 840-km-long KKH's stability has been questioned. Its distinct geological, seismological, and atmospheric settings make it a fascinating subject to research (Ali et al., 2021). The KKH, which spans between Rawalpindi and Gilgit, is known as Pakistan's most trafficked trade route. This road is associated with many different environmental problems including pollution from occupational and transport sectors. Jumbled mass of transportation system is running on this road which is responsible for environmental pollution, especially air pollution. Release of PAHs from heavy vehicles is an unsought feature of air pollution in this area. Due to heavy traffic, and a huge number of vehicles, recurrent traffic jams are common throughout this route. The described route, due to its growing traffic swarm, was selected for the sampling of exposed subjects. Map of the study area is given in Fig. 1.

Selection of subjects and documentation of self-reported health status

The exposed vehicular driver's group consisting of 48 males, with age ranging between 22 and 45 were recruited. These drivers worked in high traffic i.e., the most congested road of Pakistan. The control group consisted of 40 males and 9 females. The control subjects were selected from the areas, considered relatively less polluted, and located away from the heavy traffic areas. The inclusion criterion of sampling procedure was daily occupational exposure to the heavy traffic exhausts (at least 6 h/

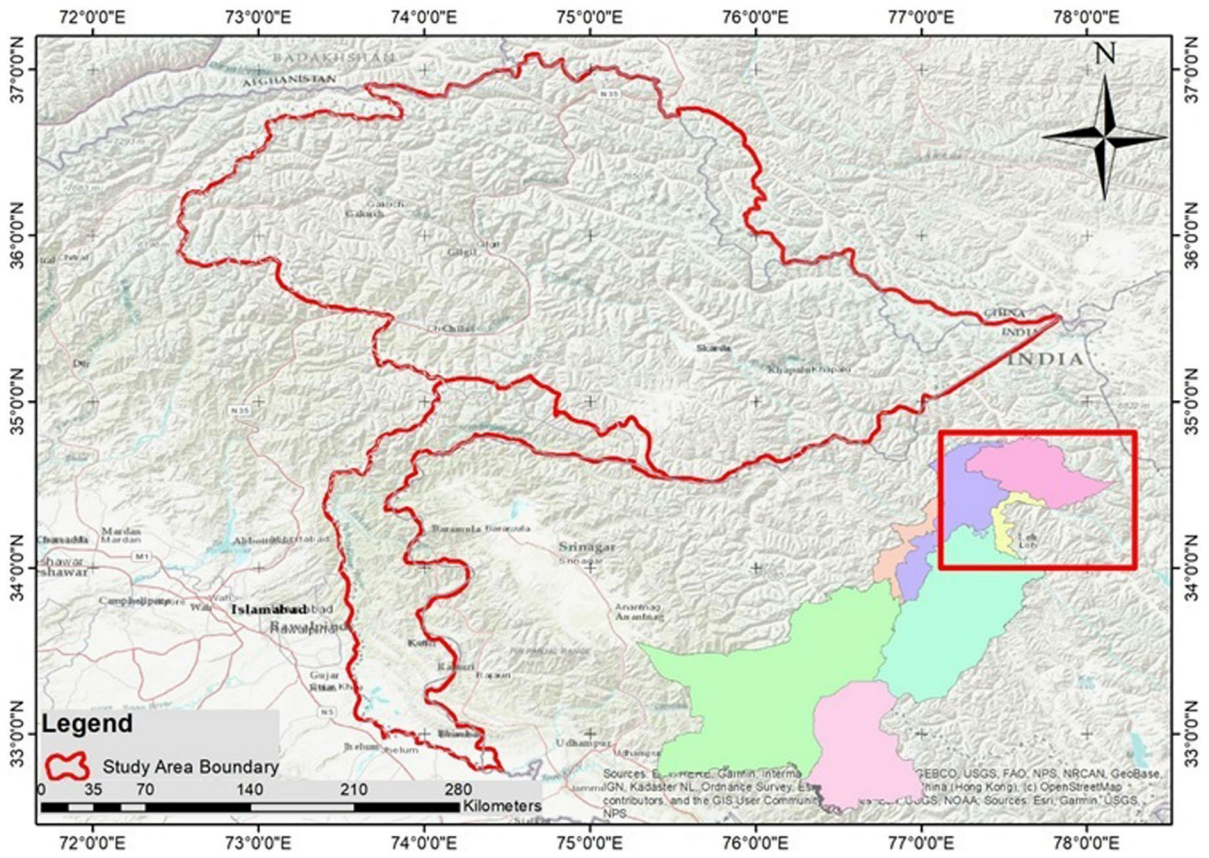

Fig. 1 Map of the study area

day). The biological sampling was accompanied by an interview to fill in the questionnaire. The simple multiple choice questionnaire was designed to promptly document information of subjects on their health status and demographics such as age, education, marital status and smoking habits. We also documented common health issues related to air pollution, i.e., whether they had any cough symptoms or not, suffered from nausea during job duration, and self-reported episodes of any headache symptoms.

Ethics This study was approved by the ethical review board of the department of environmental sciences, of PMAS Arid Agriculture University Rawalpindi. Prior to biological sampling, the participants were asked for their willingness to participate in this study, and therefore had to sign the consent form to document their willingness.

Laboratory analysis

Sample collection and preparation

The samples (urine) of heavy traffic vehicle drivers were collected at the end of shift. These were also obtained from the control subjects at the same time. The spot urine samples of the subject meeting selection criteria were collected in sterile, pre-labeled containers, and were completely sealed and labeled in a proper way. The samples were immediately transferred to the laboratory for pre-treatment, and stored at –20 °C before further analysis.

Urinary metabolite extraction and analysis

The urine samples were properly treated and prepared in laboratory to prevent from contamination.

Each urine sample of 10 ml volume was mixed with 1 M acetate buffer, the pH was adjusted to 5.0. Urine samples were then transferred to test tubes and were spiked with predeuterated surrogate standards (i.e., 40 ng of PHE-D10 and PYR-D10 per sample) as Internal Standards (IS). After the addition of 20 µl IS, the samples were again buffered with acetic acid at pH 5.0, followed by hydrolysis with β-glucuronidase/sulfatase (CNW, Shanghai, China). The mixture were then shaken and incubated overnight at 37 °C.

The urine samples were treated with 3 ml hexane, vortexed the samples at maximum speed and few drops of absolute ethyl alcohol was added before centrifugation. Supernatant was transferred to next batch of test tubes. Samples were dried under nitrogen evaporator and 1 ml acetonitrile was added to re-dissolve the residue. Residue was transferred to Eppendorf tubes followed by 12,000 rpm centrifugation for 5 min. After that supernatant was transferred to the salinization bottles and 50 µl IS was added. Supernatant was concentrated under gentle nitrogen blow for derivatization.

Derivatization

Derivatization of urine samples is the most important step as for appropriate GC–MS analysis; desired volatilization level needs to be achieved. This was carried out by recommended derivatization grade solvents which includes Bis-(tri-methylsilyl)tri-fluoro-acetamide. BSTFA was added to the samples in salinization bottles. As BSFTA reagent is very sensitive towards moisture and air so to replace air from salinized bottles 2–3 s nitrogen blow was enough for dryness of air and moisture. At 60 °C, the samples were incubated at 60 °C for 30 min. About 150 µl of each sample was transferred to the 250-µl linear glass pipette and sealed in GC vials. Creatinine-based adjustments for urinary OH-PAH determination were carried out with reference to previously reported protocol (Campo et al., 2010; Zhou et al., 2016). For each participant, the matrix effect in urine was resolved using clean urine samples to obtain an experimental standard curve. Hence the target OH-PAHs were determined accurately as concerned contaminants in clean urine samples were extremely low. The measurements were carried out in a blind fashion, and the concentrations of OH-PAHs were calibrated after adjustment with urinary creatinine and presented as nanograms per gram creatinine.

Analytical procedure of GC/MS analysis

The urinary monohydroxy (OH-PAHs) were quantified using gas chromatography (Agilent 7890B, USA) coupled with a triple quadruple tandem mass spectrometer (MS/Ms, Agilent 7010 B) equipped with HP5MS capillary column (30 m × 0.25 mm × 0.25 µm) negative chemical ionization (NCI) and electron impact (EI) ionization model. For PAH, temperature programming was set as oven temperature initial 60 °C, held for 1 min, increased from 60 to 120 °C with 40/minute, held for zero, finally increased 120 to 310 °C with 5/minute. The temperature of transfer line was 290. The identification of the PAHs contained in samples was carried out by comparing their retention times with PAHs in a standard commercial mixture. The individual OH-PAH identified and quantified were: 1-hydroxypyrene (1-OH-PYR), 1-hydroxyphenanthrene (1-OH-PHE), 2-hydroxyphenanthrene (2-OH-PHE), 3-hydroxyphenanthrene (3-OH-PHE), 4-hydroxyphenanthrene (4-OH-PHE), 9-hydroxyphenanthrene (9-OH-PHE), 3-hydroxybenzo[a]pyrene (3-OH-BaP), 9-hydroxybenzo[a]pyrene (9-OH-BaP), and 6-hydroxychrysene (6-OH-CHR). The OH-PAHs analysis information for limits of quantification and qualification (LOQs) is presented in supplementary material—see Table S1.

Statistical analysis

The mean concentrations of the nine OH-PAHs were compared using ANOVA. Pearson correlation was performed to calculate coefficient between nine metabolites of PAHs and duty hours. Likelihood of disease prediction among exposed workers was performed through logistic regression analysis. A p value of less than 0.05 was considered significant for the interpretation of the results. SPSS 16 was used for statistical analysis. A flowchart with an experimental procedure for the present study is presented in Fig. S1.

Results and discussion

Socio-demographic and health parameters

Table 1 shows the socio-demographic and health characteristics of the respondents. When compared to the unexposed group, the exposed group had a lower level of education. In terms of income, a substantial difference between the two groups appears, with the exposed group having less monthly income than the control group ($p < 0.001$). Both the groups differ significantly ($p = 0.003$) with regards to their smoking status as higher number of active smokers were reported among exposed group. Sole earning members were also observed in a significantly higher proportion among exposed category ($p < 0.001$) as compared to control group (Table 1). Drivers' working conditions imply that they are frequently exposed

Table 1 Relationship between works related parameters, demographic and exposure characteristics of exposed and control group

Variable	Job duration (hours day^{-1})			Chi-sq (p value)	All exposed ($n=48$)	Control ($n=49$)	Chi-sq (p value)
	<4 ($n=16$)	4 to 6 ($n=16$)	>6 ($n=16$)				
Gender							
Male	15	17	16	n.a	48	40	n.a
Female	–	–	–		0	9	
Age (years)							
<18				<0.001			<0.001
18 – 35	10	10	7		27	40	
>35	6	6	9		21	9	
Marital status							
Single/unmarried	6	1	4	0.040	11	3	0.006
Married	10	15	12		37	46	
Education							
Illiterate	6	2	4	0.029	12		<0.001
Primary school	10	11	12		33	3	
High school	0	3	0		3	12	
College or above	0	0	0		0	34	
Monthly income (Rs.)							
$<30,000$	14	13	12	<0.001	39	3	<0.001
30,000 to 60,000	2	3	4		9	24	
$>60,000$	0	0	0		0	22	
Smoking							
Active smoker	10	12	14	0.054	45	34	0.003
Non-smoker	6	4	2		3	15	
Are you only earning member in family							
No	7	4	6	0.385	17	37	<0.001
Yes	8	13	10		31	12	
Frequently having (cough)							
No	9	5	6	0.009	20	44	0.003
Yes	7	11	10		28	5	
Nausea							
No	12	9	7	<0.001	28	45	0.063
Yes	4	7	9		20	4	
Headache							
No	10	7	4	<0.001	21	46	<0.001
Yes	6	9	12		27	3	

to hydrocarbon-containing tools and clothes, resulting in brittle nails and skin dryness. Very serious consequences, such as dermal skin cancer, could occur under such hydrocarbon exposure conditions (Xia et al., 2010). Lee and Dong (2010) highlighted that workers/drivers exposed to diesel exhausts for longer duration are more at risk as symptoms mentioned above from diesel exposure (Table 1). Headache was determined to be an important epidemiological marker for distinguishing exposed and unexposed individuals, as more than 50% of exposed workers complained of headache. However, in the unexposed group, only a few people had headaches ($p < 0.001$; Table 1).

Our study also indicates that the exposed population, i.e., HTV drivers experienced other health problems like headache and other respiratory problems during regular driving hours but these problems were more frequently experienced in those drivers who are exposed to diesel exhaust for longer duration. In addition, the nausea and headache were reported higher in all exposed subjects than control. In accordance with the self-evaluation of exposed subjects these health symptoms are related with the traffic pollution or diesel exhaust (Li et al., 2016).

Comparative analysis of urinary 1-hydroxypyrene

In this study, comparative analysis among exposed subjects (HTV drivers) showed an increasing trend of urinary level of 1-OH-PYR in those drivers whose duty hours were > 6 h/day. Comparatively the mean concentration of 1-OH-PYR was higher in all exposed populations as compared to control population (Table 2). In the exposed population i.e., humans' significant amount of PAHs entered in body via respiration, ingestion and skin. These results are evidence of the high concentration of 1-OH-PYR in HTV drivers who worked for > 6 h/day (Table 2). The high level of urinary 1-OH-PYR in exposed population showed that on Karakoram Highway, HTV drivers are exposed from diesel exhaust or traffic pollution (Table 2). It is noteworthy that 1-OH-PYR can be used to indirectly assess the carcinogenic risk of working people (Zajac et al., 2018). According to Apostoli et al. (2000), 4.4 mg of 1-OH-PYR per gram of creatinine is associated with a relative risk of 1.3 for lung cancer mortality, and this quantity is used as a benchmark by some authors. Prevalence of renal, cardiovascular and neurasthenic diseases among HTV drivers therefore have certain health implications who worked for > 6 h/day with increased 1-OH-PYR in their urine.

Table 2 Hourly profiles of PAHs metabolites in urine (ng $g.^{-1}$ creatinine) of heavy vehicle drivers

PAH metabolite	Work hours per day		
	< 4 h	4-6 h	> 6 h
1-hydroxypyrene (1-OH-PYR)	1.22	2.95	9.32
1-hydroxy-phenanthrene (1-OH-PHE)	0.05	1.25	4.17
2-hydroxy-phenanthrene (2-OH-PHE)	2.14	2.93	5.30
3-hydroxy-phenanthrene (3-OH-PHE)	1.53	3.23	14.78
4-hydroxy-phenanthrene (4-OH-PHE)	0.04	0.33	1.03
9-hydroxy-phenanthrene (9-OH-PHE)	3.53	1.57	1.74
3-hydroxybenzo[a]pyrene (3-OH-BaP)	3.43	3.54	3.62
9-hydroxybenzo[a]pyrene (9-OH-BaP)	3.58	3.71	3.78
6-hydroxy-chrysene (6-OH-CHR)	0.49	0.53	0.57

Urinary 1-OH-PYR is detected in the same amount in the bus drivers and people who traveled in heavy traffic and it is also found in air pollution. Higher level of 1-OH-PYR in drivers suggested that diesel or petroleum emission is an important source of exposure to PAH such as pyrene (Hansen et al., (2004). Pyrene is not carcinogenic but the presence of 1-OH-PYR in the urine of an individual indicates that these subjects must be exposed to PAHs. The concentration of 1-OH-PYR in urine of occupationally exposed population might also depend on the metabolic activity at the point of entry into the organism (skin and respiratory tract) and its distribution in the human body (Chuang & Chang, 2007).

Hourly concentration of individual PAH metabolites in HTV drivers' urine

Urinary metabolites of HTV drivers were explained on the basis of their driving duty hours (Fig. 2). The duty hours were categorized as < 4, 4–6 and > 6 h/day. The results as depicted in Fig. 2 show that nine compounds belong to OH-PAHs were found in the samples and they were 1-OH-PYR, 4-OH-PHE, 1-OH-PHE, 3-OH-PHE, 2-OH-PHE, 9-OH-PHE, 3-OH-BaP, 6-OH-CHR, and 9-OH-BaP. It is evident from results that the amounts of all nine OH-PAHs levels were higher in the HTV drivers who were exposed to diesel exhaust for longer duration. The

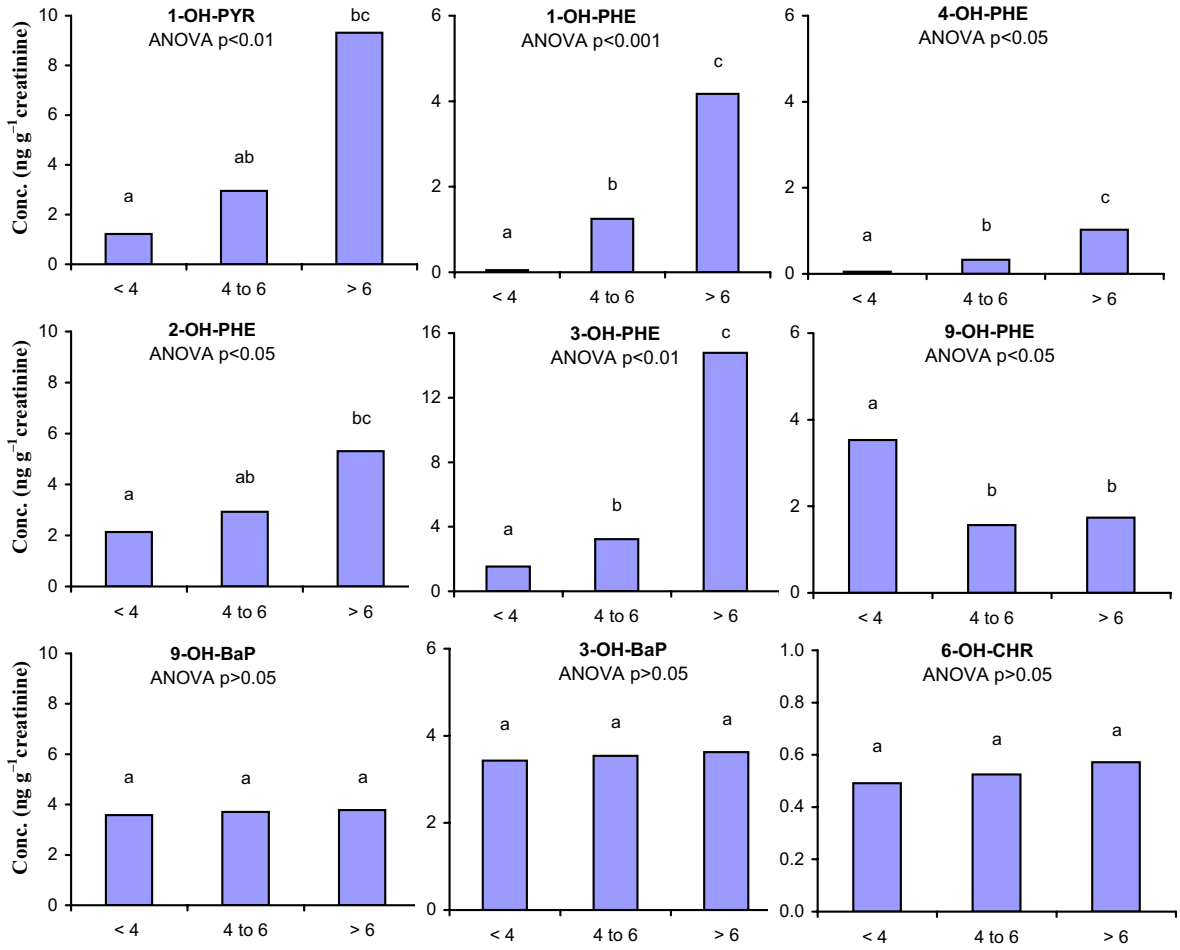

Fig. 2 Working hours-based comparison of urinary metabolites values among vehicular drivers exposed to diesel exhaust. Different letters on bars are indicating significant differences ($p < 0.05$) among mean values compared using ANOVA. Work hours are given in three categories on *x*-axis

more exposed (> 6 h/day) occupational population is shown to have the highest levels for 1-OH-PYR, 2-OH-PHE, 3-OH-PHE, 1-OH-PHE, and 3-OH-BaP, whereas 9-OH-PHE and 6-OH-CHR in lesser quantity and 4-OH-PHE being the minimum of all in concentration (Fig. 2). Findings presented here point out that drivers who were exposed to polluted environment for a longer period of time may absorb PAH metabolites via their bodies (through ingestion, absorption, or respiration).

We observed in our study that high concentration of urinary 1-OH-PYR was found in the urine of HTV drivers whose duty hours were > 6 h/day (Fig. 2). Comparatively, 1-OH-PYR was much higher in > 6 h/day HTV drivers (mean 9.32 ng g^{-1}) as compared to 4–6 h/day (mean 2.95 ng g^{-1}) and < 4 h/day (mean 1.22 ng g^{-1} and $p < 0.05$). Similarly, we have detected five metabolites of urinary Phenanthrene (3-OH-PHE, 1-OH-PHE, 2-OH-PHE, 4-OH-PHE and 9-OH-PHE) in HTV drivers' urine. The concentration of 3-OH-PHE was higher in HTV drivers who were exposed for > 6 h/day (mean 14.3 ng g^{-1}) followed by 4–6 h/day (mean 5.21 ng g^{-1}) < 4 h/day (mean 2.53 ng g^{-1}). These results confirm that exposure to Phenanthrene vary according to the job category with significantly high concentrations in workers confronting long job hours hence are inevitably exposed high diesel-emission environment (Waidyanatha et al., 2003).

Presence of Phenanthrene metabolites in urine of exposed population is a reflection of airborne exposures to these compounds (Sobus et al., 2009). One metabolites of Phenanthrene was found in negligible amount in the urine of drivers (Fig. 2: 4-OH-PHE) and one metabolite (Fig. 2: 9-OH-PHE) was found higher in drivers who drive for < 4 h/day. On the other hand, the concentration of 1-OH-PHE was significantly higher in HTV drivers who were exposed for longer duration (mean 4.17 ng g^{-1}, $p < 0.001$) than those HTV drivers with less exposed categories i.e., 4–6 h/day and < 4 h/day (Fig. 2). The concentration of 2-OH-PHE was also higher in drivers whose job duration was > 6 h/day. The concentration of 4-OH-PHE was found in negligible amount in all HTV drivers irrespective of their exposure duration. The concentration of 9-OH-PHE was found higher in drivers whose job duration was < 4 h/day as compared to those who drive for longer duration i.e., 4-6 h/day and > 6 h/day.

The metabolites of Benzo[a]pyrene i.e., 3-OH-BaP and 9-OH-BaP concentration (3.53 ± 0.62 ng g^{-1} and 2.28 ± 0.68 ng g^{-1}, respectively), was found in all HTV drivers in almost same amount irrespective of their job duration. Although the effect of job duration on urinary concentration of both the metabolites of Benzo[a]pyrene was not significant, the detected values indicate an unprecedented PAH exposure. Benzo[a]pyrene metabolites are usually found in fewer amounts in urine but found in higher concentration in occupational groups who are highly exposed to the PAHs (Maier et al., 2022). Similar trend was noticed for 6-OH-CHR whose variation in the urine samples of all HTV drivers was non-significant and is a clear indication that the concentration of Chrysene did not depend upon the driver's job duration (Fig. 2).

Overall a significant difference in the concentration of PAH metabolites was detected in the urine samples of both exposed and unexposed cohorts. Among exposed group of HTV drivers, 3-OH-PHE (6.51 ± 1.08) was estimated in highest amount followed by 1-OH-PYR (4.50 ± 0.86) (Table 3). All the urinary metabolites were found significantly higher in HTV drivers than unexposed control which is a strong evidence of PAH exposure primarily because benchmark for non-occupational PAH exposure is set using 1-OH-PYR in the range of 0.24 to 0.76 µmol mol^{-1} creatinine (Jongeneelen, 2001). However, in our case, non-occupational exposed controls have values within the benchmark range (0.47 ± 0.03) set by Jongeneelen (2001), whereas the occupationally exposed group i.e., HTV drivers have 1-OH-PYR values 10 times higher than this range (Table 3) which strongly indicate that HTV drivers are occupationally exposed to PAHs.

Health risk and predictive analysis for disease symptoms

The susceptibility of drivers of HTV to three key health indicators revealed that renal diseases are likely to prevail 4.6 times more among exposed group than unexposed control (Table 4). The odds of neurasthenic cases were also predicted very high (OR: 3.98, 95%CI: 1.54–10.28) for drivers (Table 4). This is probably due to inevitable exposure to PAHs among drivers, especially through inhalation. Since almost all the heavy vehicles running on Karakoram Highway burn diesel extensively, drivers of such vehicles are forced to inhale diesel emission containing elevated levels PAHs (Rashid et al., 2018) as their job requires to stay

Table 3 Urinary metabolites (ng $g.^{-1}$ creatinine) in all exposed (HTV drivers) vs control cohorts compared with t test using mean values of PAHs

PAH metabolite	All exposed ($n = 48$)	Control ($n = 49$)	t test (p value)
1-OH-PYR	4.50 ± 0.86	0.47 ± 0.03	< 0.0001
1-OH-PHE	1.82 ± 0.13	0.04 ± 0.01	< 0.001
2-OH-PHE	3.46 ± 0.57	0.34 ± 0.02	< 0.005
3-OH-PHE	6.51 ± 1.08	0.91 ± 0.03	< 0.001
4-OH-PHE	0.47 ± 0.05	0.06 ± 0.01	< 0.005
9-OH-PHE	2.28 ± 0.68	1.01 ± 0.09	< 0.001
3-OH-BaP	3.53 ± 0.62	0.85 ± 0.08	< 0.001
9-OH-BaP	3.69 ± 0.74	0.31 ± 0.03	< 0.001
6-OH-CHR	0.53 ± 0.04	0.36 ± 0.02	< 0.05

Table 4 Logistic regression analysis predicting odds of disease symptoms among exposed population of drivers of heavy vehicles

Symptoms	Control ($n=49$)	Exposed (48)	Odds ratio	β	p value	95% CI	
						Lower	Upper
Renal diseases							
No	39	22					
Yes	10	26	4.61	1.52	0.001	1.87	11.31
Cardiovascular							
No	30	19					
Yes	19	29	2.44	0.89	0.034	1.06	5.44
Neurasthenic[a]							
No	41	27					
Yes	08	21	3.98	1.38	0.004	1.54	10.28

[a]Referred to as nervous exhaustion or nervous weakness (lead to the development of a vast range of symptoms, including tiredness, headaches, palpitations, anxiety, depression, and sexual impotence)

on road for several hours thus causing acute to chronic physical and neurasthenic symptoms due to job-related unprecedented PAH exposure (Rashid et al., 2017). In such cases, where occupational exposure to PAHs and ultrafine particles is coupled with less physical exercising, as in the present case of HTV drivers, the vulnerability of cardiovascular disease rises manifold (Ali et al., 2022; Brito et al., 2010) probably that is why cardiovascular disease symptoms were predicted 2.4 times more among drivers than their unexposed control cohorts (Table 4).

Interactive effect of smoking and occupational exposure on urinary PAH metabolites

In this study, effect of smoking on urinary 1-OH-PYR was also analyzed because smoking is one of the

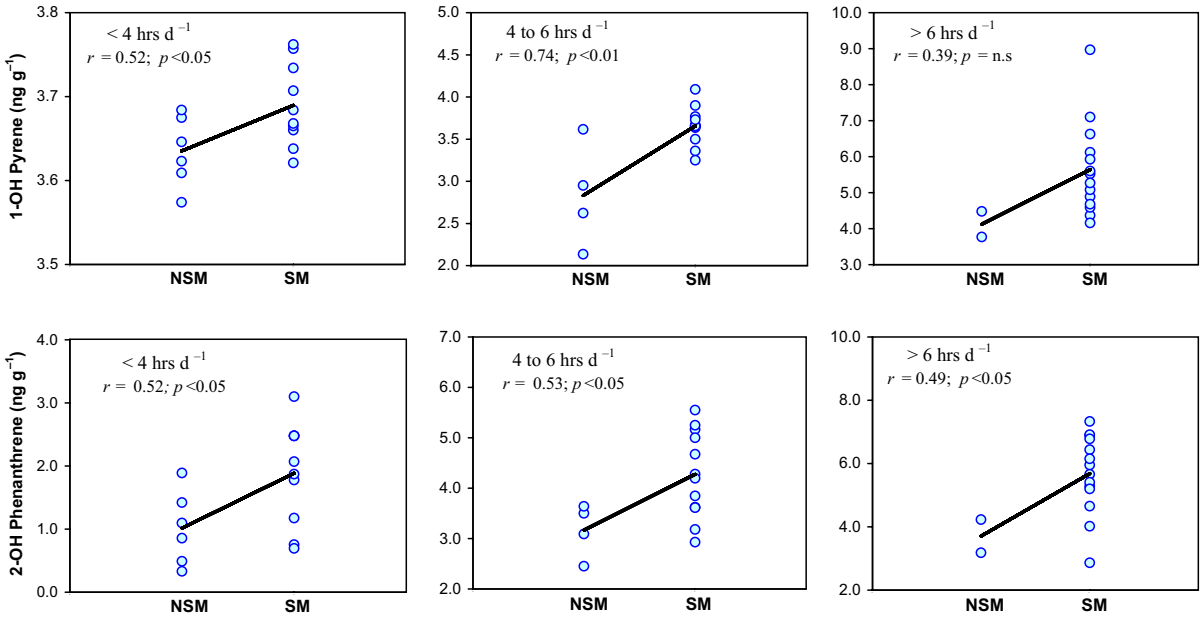

Fig. 3 Smoking effect in relation to urinary concentration of 1-hydroxypyrene and 2-hydroxyphenanthrene detected among HTV drivers comprising of exposed cohorts. NSM = non-smokers, SM = smokers

major sources of PAHs exposure for humans (Alomirah et al., 2010; Kamal et al., 2011). All HTV drivers who smoke had fairly high concentration of 1-OH-PYR than non-smokers (Fig. 3). Nonetheless smoking appears as confounding factor of PAH exposure as non-smokers also had 1-OH-PYR concentration in urine which strengthens the view that the detected amount of 1-OH-PYR in whole exposed population is probably the reflection of exposure to diesel exhaust. The urinary 1-OH-PYR concentration was fairly high during 4–6 h shift in smokers, ($r = 0.7$) followed by those working less > 4 h (Fig. 3). An almost similar trend was observed in the case of urinary 2-OH-PHE (Fig. 3). Interesting, the urinary concentration of both these metabolites was least in those working for 6 or more hours. These findings showed firstly, that the drivers, probably used to smoke more frequently during 4 h to 6 h' drive over a routine ride on this track, so much so, it is highly likely that more exposure to traffic pollution was highly likely (Wang et al., 2017; Kamal et al., 2014), in dense traffic at certain spot away from their destination. Such exposure during 4–6 h period was supposedly more prolonged and supplemented by their smoking episodes. A clear explanation for urinary 1-OH-PYR came from Fig. 3, revealing that almost 70 percent of the variance was accounted for by inhalation of cigarette smoke and 4–6 h of exposure (Cao et al., 2020; Hoseini et al., 2018; Kamal et al., 2011, 2012). However, other sources including traffic pollution itself were also contributing to PAH body burden. Nevertheless, paradoxically, discriminating the individual's exposure to lifetime environmental factors (Fang et al., 2021) and cigarette smoke is not easy to carry out in cross-sectional studies. Interestingly, in past studies, not all smokers with PAH body burdens were active smokers (i.e., 43%) but passive ones, thus passive smoking and environment equally contributed to exposure to greater extent (Huang et al., 2022).

It is noteworthy that benzo[a]pyrene exposure is mostly used for exposure to external PAHs. However, internally its traces could be studied for potential carcinogenesis health hazards (Forster et al., 2008). Our results also unveiled that 60 percent of smoker's urinary 3-OH-BaP levels were related to the smoking factor, especially among those who worked for more than 4 h per day. The data also showed that such concentration of the above-mentioned metabolite gradually decreased among those working for prolonged hours (i.e., 50 during 4–6 working hrs). However, a highest urinary level of 9-OH-BaP was observed only among those smokers working for more than 6 h

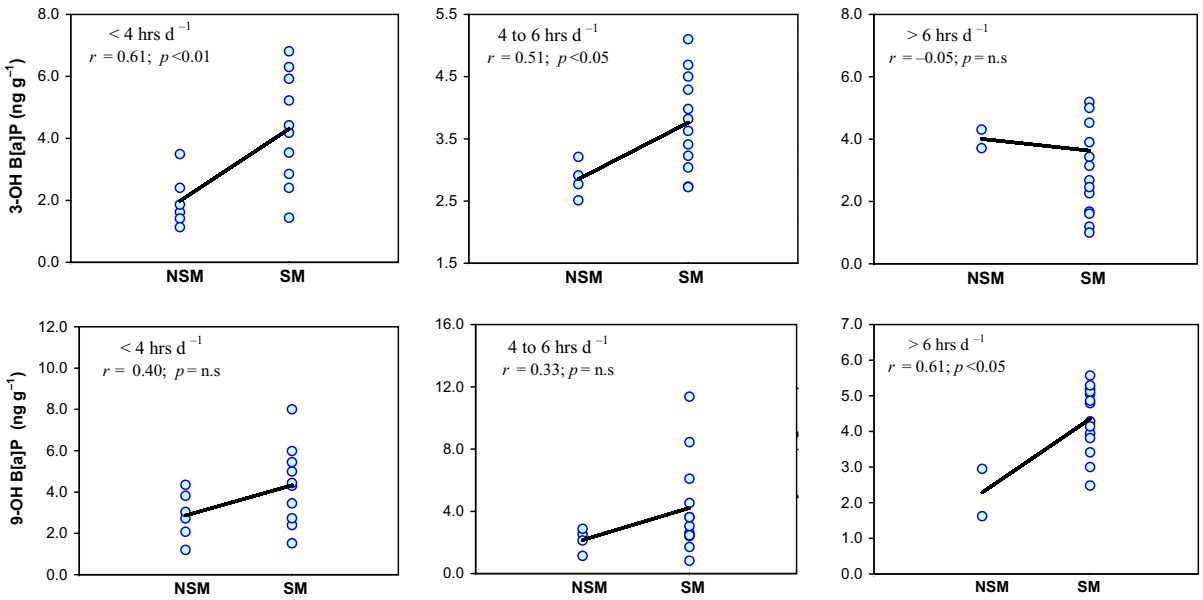

Fig. 4 Smoking effect in relation to urinary concentration of 3-hydroxy benzo[a]pyrene and 9-hydroxy benzo[a]pyrene detected among HTV drivers comprising of exposed cohorts. NSM = non-smokers, SM = smokers

(Fig. 4). These findings on one hand showed possible metabolic lapse of different PAH's congeners, and also personal demographic differences and work routine (Poursafa et al., 2018; Ramesh et al., 2004). For instance some medium weight PAHs metabolites may require longer exposure duration before accumulation and subsequent elimination from the body, and therefore inter-metabolite difference in elimination is possible when we consider multiple-contaminant exposure over diverse duration of being exposed, provided that personal characteristic remains constant.

Conclusion

The current study focuses on PAH exposure along the Karakoram Highway, a vital route in Pakistan. The drivers of heavy traffic vehicles (HTVs) on the Karakoram Highway spend lengthy hours in dense traffic and, as a result, inhale a large amount of PAH carcinogens. The urinary metabolites of PAHs were compared in such drivers (satisfying selection criterion $n=48$) and a control group. Ninety-six percent of HTV drivers had increased urine biomarkers, indicating that they had been exposed to PAHs. Urinary benzo[a]pyrene metabolites were found to be higher in HTV drivers' samples (3-OH-BaP = 3.53 ± 0.62 ng g^{-1} and 9-OH-BaP = 3.69 ± 0.74 ng g^{-1}) than in controls (3-OH-BaP = 0.85 ± 0.08 and 9-OH-BaP = 0.31 ± 0.03 ng g^{-1}), peers. Urinary benzo[a] pyrene metabolites were also greater ($p < 0.001$) among HTV drivers who worked more than 6 h per day (1.79 0.08 ng g^{-1}) than those who worked less than 4 h per day (0.58 0.04 ng g^{-1}). Low molecular weight volatile PAHs, which are often found in vehicle exhaust, were detected in these metabolites in a consistent pattern. The increased PAH body burden was directly connected to the nature of their work and the Karakoram Highway's long-distance pollution. Furthermore, HTV drivers' health susceptibility was worsened by their poor economic condition and smoking, and their health capability was severely reduced. There was conclusive proof that HTV drivers were exposed to PAHs while riding back and forth on the Karakoram highway, an element not previously documented.

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Declarations

Conflict of interest The authors confirm that there are no known conflicts of interest associated with this manuscript and there has been no significant financial support for this work that could have influenced its outcome.

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