Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2023; Vol. 21(1)

# The Association between Human Epididymal Protein 4 and Exposure to Poly Aromatic Hydrocarbons in Samples of Al-Daura Refinery Workers-Baghdad

**Ashwak W. Shaker**[1] *PhD*, **Estabraq A. Al-Wasiti**[2] *PhD*

[1]Dept. of Medical Laboratory Technique, Al-Maamoon University College, Baghdad, Iraq, [2]Dept. of Chemistry and Clinical Biochemistry, Al-Nahrain University College, Baghdad, Iraq

## Abstract

**Background** Exposure to polycyclic aromatic hydrocarbons (PAHs) can cause many genetic alterations, which may contribute to cancers because PAHs derivatives can bound to DNA induces mutations and tumorigenesis. Some cancer-related proteins differentially expressed in exposed workers such as Human epididymal protein 4 (HE4) therefore, it considers a good biomarker for PAHs adverse effects that can progressed to tumors.

**Objective** To estimate HE4 level in refinery workers to predict related adverse effect of PAHs that can developed to occupational cancer.

**Methods** This study included 168 participants divided in three groups (control, office workers and in field workers). PAHs and HE4 were measured for each participant by GC/MS, ELISA respectively.

**Results** PAHs were negative in controls blood whereas they were elevated in office and field workers. Concentration of HE4 was significantly higher in field than in office workers and controls, as well as significant correlation between concentration of PAHs and HE4 levels with duration of exposure was found.

**Conclusion** PAHs and HE4 levels were significantly increased in refinery exposed workers (both field and office workers).

**Keywords** Polycyclic aromatic hydrocarbon, air pollution, Human epididymal protein4, mutation, tumor marker

**Citation** Shaker AW, Al-Wasiti EA. The Association between human epididymal protein 4 and exposure to poly aromatic hydrocarbons in samples of Al-Daura Refinery Workers-Baghdad. Iraqi JMS. 2023; 21(1): 23-29. doi: 10.22578/IJMS.21.1.3

**List of abbreviations:** B[A]P = Benzo(a)pyrene, ELISA = Enzyme-linked immunosorbent assay, GC/MS = Gas chromatography coupled with mass spectrometry, HE4 = Human epididymal protein 4, PAHs = Polycyclic aromatic hydrocarbons

## Introduction

One of the most important issues facing humanity and other life forms on our planet today is pollution. Pollutants can be substances that arise naturally or synthetically [(1)]. Toxic exposure alters essential cellular functions and is associated to the etiology of a number of chronic diseases that cause irreversible epigenetic alterations [(2)]. Environmental exposures, such as diet, tobacco, alcohol, stress, genetic factors, infectious agents, and environmental carcinogens like poly aromatic hydrocarbons (PAHs), all influence epigenetic marks such as [acetylation, DNA hypo or hypermethylation and micro-RNA expression]. Some epigenetic alterations alter the expression of tumor suppressor genes and oncogenes, potentially

leading to carcinogenesis [3]. Poly aromatic hydrocarbons like benzo(a) pyrene and its metabolites, known as epoxides, are highly poisonous, mutagenic and carcinogenic to microorganisms and people, thus they've gotten a lot of attention in recent years [4]. They have a poor water solubility, but are extremely lipophilic [5], as a result, they are easily absorbed from animals' gastrointestinal tracts and readily deposited in a range of tissues, including body fat. Because of their high lipophilicity, these chemicals have a high bioavailability after ingestion and inhalation. According to a previous study, detectable levels of PAHs can be found in almost all internal organs, particularly those with a lot of adipose tissue. These organs can act as storage depots for the hydrocarbons, allowing them to be released gradually [6].

Once PAHs enter the body, they must be activated by a multistep metabolic process involving certain enzymes. The mixed-function oxidase system is the enzyme system that is principally responsible for PAH metabolism [7], so to detoxify these chemicals, the initial reaction is an epoxidation, followed by PAHs epoxides coupled with glutathione. Epoxides that are not glutathione conjugated are transformed to phenols and diols as shown in figure (1). PAHs metabolites are not always polar enough to be excreted. As a result, they must be conjugated with glucuronic or sulfuric acids in order to be excreted. The majority of PAHs metabolites are eliminated in the feces and urine [8].


**Figure 1. Formation of toxic and mutagenic epoxide from Benzo(a)pyrene (B[A]P)**

A number of suspected cancer-related proteins have been shown to be differently expressed in PAHs-exposed workers [9]. According to pathway and gene ontology studies, cell movement, cell migration and cell adhesion were the most common biological processes linked with differentially expressed cancer-related proteins [such as human epididymal protein 4(HE4)] between exposed employees and non-exposed persons [10]. This secretory protein, HE4, is abundant in the human epididymis [11], it is a protease inhibitor that is found in the epididymis epithelium and plays a role in sperm maturation and it is an antimicrobial peptide that plays an important role in the immune system [12,13]. PAHs have recently been found to cause apoptosis in a variety of cell types. Apoptosis is a natural and active method of cell death marked by chromatin condensation in the nucleus and DNA fragmentation [14]. Intracellular cysteine proteases, such as HE4, are thought to be critical components of the intracellular apoptotic signaling pathways generated by PAHs, which result in the accumulation of the tumor suppressor protein p53, which may be important for apoptosis induction [14,15].

The objectives of this study was to estimate HE4 level in refinery workers to predict related adverse effect of PAHs.

## Methods

One hundred and twelve (112) Iraqi male workers at Midland Refineries Company-Daura Refinery-Baghdad with age range (25-65 years) and fifty-six 56 healthy individuals who have been lived in distant areas that away from Al-Dura Refinery, with matched age range were enrolled in this study, during the period from 16 July to 2 September 2020.

Subjects were divided into three groups as the following:

Group1: 56 workers in the oil refining field.

Group2: 56 workers in the office (around field).

Group3: 56 apparent healthy individuals as controls, these controls have been lived in distant areas away from Al-Dura Refinery.

Serum concentration of HE4 was determined by enzyme-linked immunosorbent assay (ELISA) kit (Catalog Number: MBS771364), which is designed for the quantitative measurement of HE4 concentrations in serum, plasma, saliva, urine, tissue homogenate, cell culture supernates and other biological fluids, while PAHs concentration in serum was determined by gas chromatography mass spectrometry (GC-MS) after the extraction of these compounds from the serum specimens by liquid - liquid extraction technique [16].

The statistical analysis was performed with SPSS Statistics software (version 24.0, Chicago, USA). Chi squared test was performed to study the correlation between variables and student T test to compare between two groups while analysis of variance (ANOVA) test was used to compare between more than two groups. The level of Statistical significance was set when the p-value was less than 0.05.

## Results

There is no statistically difference in age among the groups (controls, office workers and field workers) as shown in table (1).

**Table 1. Comparison of the mean age among the groups**

| Group | N. | Age (Mean±SD) | P value |
|---|---|---|---|
| Control | 56 | 33.96±3.33 | |
| Office | 56 | 38.81±4.00 | 0.976 |
| Field | 56 | 40.86±3.12 | |

P value by ANOVA

Mean concentration of PAHs and HE4 for the three groups (controls, office workers and field workers) shown in table (2), it was found that PAHs concentration for field workers was significantly higher than office workers (p=0.0001) as well as HE4 concentration(p=0.012). However, PAHs was not detected in controls

**Table 2. The mean concentration of PAHs and HE4 among the groups**

| Parameter | Group | Mean±SE | P value |
|---|---|---|---|
| PAHs (ppm) | Control | - | 0.0001* |
| | Office | 0.35±0.05 | |
| | Field | 2.14±0.09 | |
| HE4 (pmol/l) | Control | 17.45±0.81 | 0.012** |
| | Office | 18.21±0.75 | |
| | Field | 20.67±0.82 | |

* p value by unpaired ttest, **p value by ANOVA, PAHs: Polycyclic aromatic hydrocarbons, HE4: Human epididymal protein 4

PAH and HE4 levels was compared according to the duration of exposure for (≤10 years) and (>10 years) for field workers as shown in table (3), it was found that PAHs and HE4 levels were significantly higher in (>10 years group) than (≤10 years group) (p= 0.003 and p= 0.006 respectively). While in office workers, it was found that HE4 level was significantly higher in (>10 years group) than (≤10 years group) (p= 0.001), whereas there was no significant difference in PAHs in the two different duration groups (p=0.09).

**Table 3. Levels of PAHs and HE4 correlated with duration of exposure for field workers**

| Group | Parameter | Duration of exposure | N | Mean±SE | p value |
|---|---|---|---|---|---|
| Field workers | PAHs (ppm) | ≤10 years | 28 | 1.54±0.06 | 0.003 |
| | | >10 years | 28 | 2.75±0.08 | |
| | HE4 (pmol/l) | ≤10 years | 28 | 18.45±1.24 | 0.006 |
| | | >10 years | 28 | 22.89±0.91 | |
| Office workers | PAHs (ppm) | ≤10 years | 28 | 0.03±0 | 0.09 |
| | | >10 years | 28 | 0.67±0.06 | |
| | HE4 (pmol/l) | ≤10 years | 28 | 15.7±0.81 | 0.001 |
| | | >10 years | 28 | 20.72±1.09 | |

P value by unpaired ttest, PAHs: Polycyclic aromatic hydrocarbons, HE4: Human epididymal protein 4

Correlation between PAHs and HE4 for field and office workers together was positive but this correlation was not significant (r= 0.159 and p value=0.093) as shown in figure (2).

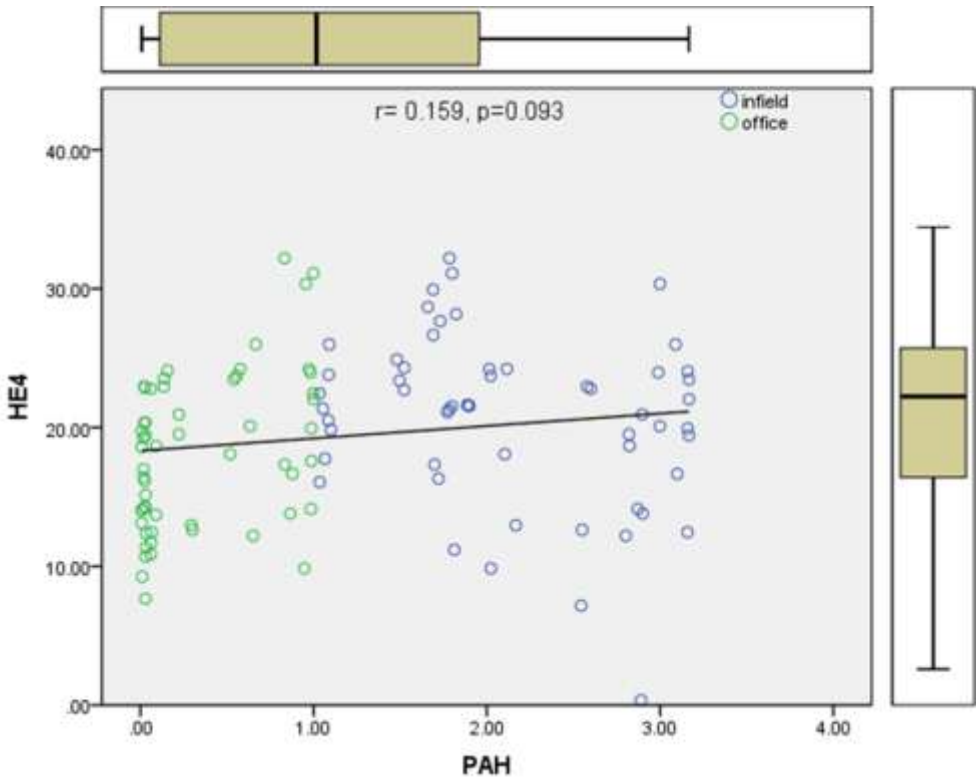


**Figure 2. Correlation between PAHs and HE4 in field and office groups**

## Discussion

Workers' occupational exposure to PAHs, such as during coke manufacture, roofing with bituminous materials, oil refining, and coal gasification, has frequently resulted in human exposure to toxicants and associated carcinogenicity [17]. PAHs have been linked to malignancies of the skin, lungs, bladder, liver, and stomach [18], but at first, they must be converted by enzymes to electrophilic metabolites that can bind to nucleophilic DNA sites to exert their mutagenic or carcinogenic effects [19]. A range of unstable and reactive intermediates, such as diol epoxides and o-quinones, are generated during PAHs metabolism by xenobiotic metabolizing enzymes, which can assault DNA and cause cell toxicity and transformation [20,21] as well as modulate the gene expression through the induction of mutations [22]. This study results showed that PAHs concentration was negative in controls blood while field and office refinery workers have elevated blood concentration of these pollutants and their derivatives, these results are in line with Al-Ani and Al-Wasiti who found the same results [23]. Also, current study found that HE4, which has been used as a biomarker in this study for the adverse effects of PAHs is higher in field and office refinery workers than in controls, this is agree with Talhout et al. who found that there is a number of putative cancer-related proteins differentially expressed in workers exposed to PAHs like HE4 [9] because it regulates cell movement, cell migration and cell adhesion, according to gene ontology analyses [10]. Duration of exposure has a significant correlation with HE4 levels as shown by current results, which revealed that field and office workers who spent more than ten years in the refinery have higher levels of HE4 than those who spent less than ten years and these results are in line with Bingle et al. who suggested that HE4 elevated levels in exposed workers might be involved in the innate immunity defenses of the respiratory tract, nasal and oral cavities to prevent the development of lung adenocarcinoma [24], also, current results agree with LeBleu et al, Moore et al and Grondin et al. who reported that HE4 was increased upon chronic exposure to chemical pollutants such as benzo(a)anthracen, benzo(a) pyren and their metabolites as well as smoke and because its encoding gene was the most upregulated gene in fibrosis-associated myofibroblasts therefore, over expression of this gene and high levels of HE4 according to Solhaug et al. and Chhikara et al. considered to be essential components of the intracellular apoptotic signaling pathways that induced by PAHs and resulting in accumulation of the tumor suppressor protein p53 that may be important for the induction of apoptosis [14,15] and prevent tumorigenesis through interacts with insulin-like growth factor receptor (IGF1R) to arrest the invasion, metastasis and angiogenesis of cancer, so because IGF1R signal promotes non-cancerous cells to malignantly transform and possesses anti-apoptotic and mitogenic activity, therefor, the elevation of HE4 and it's interacting with IGF1R have a beneficial effect in tumorigenesis inhibition [25-27].

In conclusions, PAHs as one of the air pollutants was higher in refinery workers whether they were office or field workers. HE4 levels were significantly increased in refinery exposed workers (both field and office workers). It is recommended to do more investigations to find out the cause of HE4 elevation whether due to tumorigenesis or inflammation.

## Acknowledgement

Special thanks to all the participants in this study for their cooperation as well as to all the staff of the Environmental Research Center at Iraqi Ministry of Science and Technology for their unlimited help during our work.

## Author contribution

Shaker and Dr. Al-Wasiti: designed the study and contributed to the acquisition of data. Shaker: contributed to sample preparation and was the main person in writing the manuscript. Both authors provided critical feedback and helped shape the research, interpreted the data and read and approved the final manuscript.

## Conflict of interest

No potential conflict of interest was reported by the authors.

## Funding

Authors of this study did not receive financial reward from any institute or organization.

## References

1. Tan C, Lu S, Wang Y, et al. Long-term exposure to high air pollution induces cumulative DNA damages in traffic policemen. Sci Total Environ. 2017; 593-594: 330-6. doi: 10.1016/j.scitotenv.2017.03.179.
2. Shukla A, Bunkar N, Kumar R, et al. Air pollution associated epigenetic modifications: Transgenerational inheritance and underlying molecular mechanisms. Sci Total Environ. 2019; 656: 760-77. doi: 10.1016/j.scitotenv.2018.11.381.
3. Mathers JC, Strathdee G, Relton CL. Induction of epigenetic alterations by dietary and other environmental factors. Adv Genet. 2010; 71: 3-39. doi: 10.1016/B978-0-12-380864-6.00001-8.
4. Rengarajan T, Rajendran P, Nandakumar N, et al. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pacific J Tropical Biomed. 2015; 5(3): 182-9. doi: https://doi.org/10.1016/S2221-1691(15)30003-4.
5. Srogi K. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ Chem Lett. 2007; 5(4): 169-95. doi: 10.1007/s10311-007-0095-0.
6. Abdel-Shafy HI, Mansour MSM. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egyptian J Petroleum. 2016; 25(1): 107-23. doi: https://doi.org/10.1016/j.ejpe.2015.03.011.
7. Höner A. Polycyclic aromatic hydrocarbon (PAH) metabolite. In: Kleiböhmer W. (ed). Handbook of analytical separations. 2001; 3: 99-121.
8. Campo L, Rossella F, Pavanello S, et al. Urinary profiles to assess polycyclic aromatic hydrocarbons exposure in coke-oven workers. Toxicol Lett. 2010; 192(1): 72-8. doi: 10.1016/j.toxlet.2008.12.018.
9. Talhout R, Schulz T, Florek E, et al. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health. 2011; 8(2): 613-28. doi: 10.3390/ijerph8020613.
10. Alhamdow A, Tinnerberg H, Lindh C, et al. Cancer-related proteins in serum are altered in workers occupationally exposed to polycyclic aromatic hydrocarbons: a cross-sectional study. Carcinogenesis. 2019; 40(6): 771-81. doi: 10.1093/carcin/bgz022.
11. Shu Y, Wang W. serum human epididymal protein 4 is associated with depressive symptoms in patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2020; 15: 3417-22. doi: 10.2147/COPD.S282214.
12. Kirchhoff C, Habben I, Ivell R, et al. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. Biol Reprod. 1991; 45(2): 350-7. doi: 10.1095/biolreprod45.2.350.
13. Hua L, Liu Y, Zhen S, et al. Expression and biochemical characterization of recombinant human epididymis protein 4. Protein Expr Purif. 2014; 102: 52-62. doi: 10.1016/j.pep.2014.08.004.
14. Solhaug A, Refsnes M, Låg M, et al. Polycyclic aromatic hydrocarbons induce both apoptotic and anti-apoptotic signals in Hepa1c1c7 cells. Carcinogenesis. 2004; 25(5): 809-19. doi: 10.1093/carcin/bgh069.
15. Chhikara N, Saraswat M, Tomar AK, et al. Human epididymis protein-4 (HE-4): a novel cross-class protease inhibitor. PLoS One. 2012; 7(11): e47672. doi: 10.1371/journal.pone.0047672.
16. Oluremi OI, Idowu AO, Adedoyin KM, et al. Determination of polycyclic aromatic hydrocarbons in blood plasma of neurology patients. J Toxicol EnvironmentHealth Sci. 2019; 11(1): 1-8.
17. Zhang Y, Ding J, Shen G, et al. Dietary and inhalation exposure to polycyclic aromatic hydrocarbons and urinary excretion of monohydroxy metabolites--a controlled case study in Beijing, China. Environ Pollut. 2014; 184: 515-22. doi: 10.1016/j.envpol.2013.10.005.
18. Boström CE, Gerde P, Hanberg A, et al. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ Health Perspect. 2002; 110 Suppl 3(Suppl 3): 451-88. doi: 10.1289/ehp.110-1241197.
19. Melendez-Colon VJ, Luch A, Seidel A, et al. Cancer initiation by polycyclic aromatic hydrocarbons results from formation of stable DNA adducts rather than apurinic sites. Carcinogenesis. 1999; 20(10): 1885-91. doi: 10.1093/carcin/20.10.1885.
20. Arenas-Huertero F, Apátiga-Vega E, Miguel-Pérez G, et al. Molecular markers associated with the biological response to aromatic hydrocarbons from urban air in humans. Air Pollution: New Developments. 2011: 87-112. doi: 10.5772/17999.
21. Shimada T, Takenaka S, Murayama N, et al. Oxidation of pyrene, 1-hydroxypyrene, 1-nitropyrene and 1-acetylpyrene by human cytochrome P450 2A13. Xenobiotica. 2016; 46(3): 211-24. doi: 10.3109/00498254.2015.1069419.
22. Valavanidis A, Vlachogianni T, Fiotakis K, et al. Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. Int J Environ Res Public Health. 2013; 10(9): 3886-907. doi: 10.3390/ijerph10093886.
23. Al-Ani RH, Al-Wasiti EA. 7, 8-dihydro-8-oxoguanine (8-oxoG) as biomarker of oxidative DNA damage caused by air pollution with PAH in Midland Refineries Company-Daura Refinery workers.

Materials Today: Proceedings. 2021. doi: https://doi.org/10.1016/j.matpr.2021.05.446.

**24.** Bingle L, Cross SS, High AS, et al. WFDC2 (HE4): a potential role in the innate immunity of the oral cavity and respiratory tract and the development of adenocarcinomas of the lung. Respir Res. 2006; 7(1): 61. doi: 10.1186/1465-9921-7-61.

**25.** LeBleu VS, Teng Y, O'Connell JT, et al. Identification of human epididymis protein-4 as a fibroblast-derived mediator of fibrosis. Nat Med. 2013; 19(2): 227-31. doi: 10.1038/nm.2989. Epub 2013 Jan 27. PMID: 23353556; PMCID: PMC4457508.

**26.** Moore RG, Hill EK, Horan T, et al. HE4 (WFDC2) gene overexpression promotes ovarian tumor growth. Sci Rep. 2014; 4: 3574. doi: 10.1038/srep03574.

**27.** Grondin CJ, Davis AP, Wiegers TC, et al. Accessing an Expanded Exposure Science Module at the comparative toxicogenomics database. Environ Health Perspect. 2018; 126(1): 014501. doi: 10.1289/EHP2873.

**Correspondence to Ashwak W. Shaker**
**E-mail: waheebashwak@gmail.com**
**Received Jul. 27th 2021**
**Accepted Oct. 24th 2021**