



To review this article online, scan this QR code with your Smartphone



Urinary and Genetic Biomonitoring of Polycyclic Aromatic Hydrocarbons in Egyptian Coke Oven Workers: Associations between Exposure, Effect, and Carcinogenic Risk Assessment

Aisha Mohamed Samir¹, Dalia Abdel-Hamid Shaker¹,
Mona Mohamed Fathy², Salwa Farouk Hafez³,
Mona Mohsen Abdullatif², Laila Ahmed Rashed⁴,
Hany Ahmed Fouad Alghobary²



Abstract

Background: Coke oven workers are exposed to polycyclic aromatic hydrocarbons (PAHs) with possible genotoxicity and carcinogenicity. Metabolizing enzymes genes and DNA repair genes are suspected to be correlated with the level of DNA damage. They may contribute to variable individual sensitivity to DNA damage induced by PAHs exposure at workplace.

Objective: To investigate the relationship between biomarkers of PAHs: 1-hydroxypyrene (1-OHP), DNA adducts, and 8-hydroxy-2-deoxyguanosine (8-OHdG) in coke oven workers, and to assess the role of cytochrome P2E1 (CYP2E1) gene expression and DNA repairing gene (XRCC1) polymorphism in detecting workers at risk.

Methods: 85 exposed workers and 85 unexposed controls were enrolled into this study. Urinary 1-OHP, 8-OHdG, and BPDE-DNA adduct were measured. CYP2E1 gene expression and genotyping of XRCC1 399 Arg/Gln were evaluated by real-time PCR.

Results: The median urinary 1-OHP levels (6.3 $\mu\text{mol/mol}$ creatinine), urinary 8-OHdG (7.9 ng/mg creatinine), DNA adducts (6.7 ng/ μg DNA) in the exposed group were significantly higher than those in the unexposed group. Carriers of the variant allele (Gln) of XRCC1 had the highest levels of 1-OHP, DNA adducts and 8-OHdG, and the lowest level of CYP2E1 gene expression. In exposed workers, significant positive correlations were found between 1-OHP level and each of the work duration, 8-OHdG, and DNA adducts levels. There was a significant negative correlation between 1-OHP level and CYP2E1 gene expression. Work duration and CYP2E1 gene expression were predictors of DNA adducts level; 1-OHP level and work duration were predictors of urinary 8-OHdG level.

Conclusion: Workers with higher exposure to PAH were more prone to oxidative DNA dam-

Cite this article as: Samir AM, Shaker DAH, Fathy MM, *et al.* Urinary and genetic biomonitoring of polycyclic aromatic hydrocarbons in Egyptian coke oven workers: Associations between exposure, effect, and carcinogenic risk assessment. *Int J Occup Environ Med* 2019;10:124-136. doi: 10.15171/ijoem.2019.1541

¹Department of Occupational and Environmental Medicine, Faculty of Medicine, Cairo University, Egypt

²Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Egypt

³Department of Industrial and Environmental Medicine, National Research Center, Giza, Egypt

⁴Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence to
Dalia Abdel-Hamid Shaker, Assistant Professor in the Occupational and Environmental Medicine Department, Faculty of Medicine, Cairo University, Egypt
E-mail: dalia.shaker@kasralainy.edu.eg
Received: Dec 11, 2018
Accepted: Apr 10, 2019

age and cancer development. DNA adducts level reflects the balance between their production by CYP2E1 and elimination by XRCC1 gene.

Keywords: Bay-Region, polycyclic aromatic hydrocarbon; 1-hydroxypyrene-glucuronide [Supplementary concept]; DNA adducts; 8-oxo-7-hydrodeoxyguanosine [Supplementary concept]; Cytochrome P-450 CYP2E1; X-ray repair cross complementing protein 1; Polymorphism, genetic

Introduction

In the coke production process, thousands of compounds are formed by destructive distillation of coal in the coke ovens. Many of these compounds are polycyclic aromatic hydrocarbons (PAHs) which are complex mixtures of more than 100 compounds that contain fused benzene rings. The main routes of occupational exposure to PAHs are the skin contact and inhalation.¹ The International Agency for Research on Cancer (IARC) classifies coke oven emissions as a group-I human carcinogen.²

Pyrene, a type of PAHs that is formed by four benzene rings, is considered a human carcinogen and is always present in PAHs mixtures. Pyrenes metabolized to 1-hydroxypyrene (1-OHP) and then eliminated into urine and feces.³ Urinary 1-OHP levels are highly correlated to total PAHs levels in ambient air and to the skin contamination level.⁴ Therefore, 1-OHP urinary level is widely used as a metabolite for biological monitoring of occupational exposure to PAHs among coke oven workers.^{5,6} Coal tar contains benzo[a]pyrene (BaP), one of the most studied PAHs. It is a potent human immunosuppressive, pro-inflammatory and carcinogen agent.⁷ Its carcinogenicity starts after phase I biotransformation mediated by the cytochrome P4501 superfamily (CYP1).⁸ By inducible CYP2E1, one of the most abundant isoforms among all cytochrome P450, PAHs form reactive species such as benzo[a]pyrene-7, 8-diol-9, 10-epoxides (BPDE), which attack DNA to form BPDE-DNA adducts. These adducts are subjected to further hydrolytic

reactions catalyzed by phase II conjugations to eliminate PAH metabolites and protect cell genome.⁹

An alternative PAHs carcinogenic pathway includes metabolizing PAHs to active semiquinones by the cytochrome P450 enzymes. These quinones are free radical intermediates that could go through redox cycling to generate reactive oxygen species (ROS), causing oxidative damage to DNA and lipids.^{10,11} PAHs may therefore exert their mutagenic, genotoxic, and carcinogenic properties by two major mechanisms: One pathway includes formation of specific PAHs-DNA adducts with subsequent replication error and mutation;^{9,10} the second pathway includes induction of oxidative stress.^{11,12} Oxidative stress represents an imbalance in the homeostasis between ROS and the mechanisms of detoxification. Oxidative damage to nucleic acids has been found to be associated with a variety of diseases including aging and cancer. Guanine is the base most prone to oxidation. Critical biomarker of oxidative DNA damage is 8-hydroxy-2-deoxyguanosine (8-OHdG), which is the form of oxidized guanine.¹² Interactions between genetic and environmental factors are important in the development of the majority of human cancers. Consequently, genetic variation in an individual's ability to repair DNA base damage as single-strand breaks (SSB) may confer differential risk for PAH-induced cancer. In humans, the base excision repair (BER) system is responsible for the repair of oxidative DNA damage.¹³ The *XRCC1* (X-ray Repair Cross-Complementing) gene participates in the repair of mammalian DNA and plays an integral role in

BER and SSB repair. *XRCC1* acts as a coordinator in BER, through its interaction with poly ADP-ribose polymerase, DNA polymerase β , and DNA ligase III.¹⁴ A polymorphism at codon 399 of the *XRCC1* gene (exon 10, SNP; c.1316G>A; p.Arg399Gln; rs25487) results in an amino acid substitution from arginine to glutamine, which results in inefficient repair pathway. *XRCC1* gene plays an important role in DNA damage repair. *XRCC1* Arg399Gln polymorphism is the commonest among more than 60 validated SNPs in that gene and has no major ethnic variations.¹⁵ Exposure biomarkers, such as hydroxylated metabolites of toxicants in urine, are used to indicate the internal dose received to estimate the level of exposure to these toxins. Effect biomarkers are measured as the forms that interact with critical targets, such as DNA adducts or cytogenetic alterations. Susceptibility biomarkers include genetic variations of metabolizing enzymes and DNA repairing system such as cytochrome P450s and *XRCC1* genes, respectively.¹⁶ Published data suggest that the metabolizing enzymes genes and DNA repair genes are correlated with the level of DNA damage. They may contribute to variable individual sensitivity to DNA damage induced by PAH exposure at workplace.¹⁷

We conducted the present study to investigate the relationship between

biomarkers of PAH: 1-hydroxypyrene (1-OHP), DNA adducts and 8-hydroxy-2-deoxyguanosine (8-OHdG) in coke oven workers, and to assess the role of cytochrome P2E1 (*CYP2E1*) gene expression and DNA repairing gene (*XRCC1*) polymorphism in detecting workers at risk.

Materials and Methods

The study population was selected from coke oven workers who had been working for at least one year at a coke factory in Helwan district, Cairo, Egypt, between December 2016 and April 2017. The study was conducted on two groups: an exposed group consisting of 85 male workers directly engaged in coke-making operations and occupationally exposed to PAHs; and a control group including 85 male participants from administrative departments of the same company, who had never been occupationally exposed to PAHs or chemicals. Those having malignant, infectious, liver, or kidney diseases during the last six months were excluded from the study. Clinical examination was done with special emphasis on the chest and skin. Chest manifestations included chronic bronchitis, defined as the presence of chronic productive cough for at least three months/year for at least two consecutive years in absence of any other diseases.¹⁸ Occupational asthma was defined as attacks of shortness of breath accompanied with expiratory wheeze that improved after a day off work or on weekends and worsened over the work days.¹⁹ Contact dermatitis was diagnosed if there were erythema, papules, vesicles, exudation and itching. In chronic cases, there was fissuring and lichenification; there may be acute exacerbation of contact with an offending agent.²⁰ A total of 120 people was directly engaged in coke-making operations and exposed to PAHs. They worked either on the top, the side or at the bottom of the oven. Only 99

TAKE-HOME MESSAGE

- Coke oven workers are exposed to polycyclic aromatic hydrocarbons (PAHs) with possible genotoxicity and carcinogenicity.
- The susceptibility to PAHs-associated risk could be attributed to genetic causes that act synergistically leading to alterations in metabolism of carcinogens and/or impairment in DNA repair capacity.
- The most effective measures of controlling coke oven emissions are engineering controls and good work practice.

workers from three shifts accepted to participate in this study. Eighty-five out of 99 were involved in the study according to the inclusion and exclusion criteria set.

Sample Collection

Five mL blood was collected from all subjects and divided into two EDTA-anticoagulated vacutainer tubes. One tube was sent for DNA extraction for the measurement of BPDE-DNA adducts and estimation of *XRCC1* Arg399Gln polymorphism; the second tube was used for RNA extraction for CYT 2E1 gene expression assay. A spot urine sample was collected from the exposed workers and controls for the analysis of 1-OHP and 8-OHdG. All participants were asked to wash their hands prior to urine collection to avoid environmental contamination. Urine samples were centrifuged to remove particulate matter and kept frozen at -80 °C until analysis.

Analysis of Urinary 1-OHP

Urine samples were analyzed for 1-OHP by high-performance liquid chromatography (HPLC) method according to Nguyen, *et al*,⁶ with slight modification. Extraction of 1-OHP from urine samples was done by mixing 1 mL of the urine with 600 µL acetate buffer and 20 µL β-glucuronidase (pH 5). The mixture was incubated for 2 hours at 37 °C with shaking. After incubation, the mixture was applied to a Sep-Pak C18 cartridge that was conditioned with 5 mL methanol, then 10 mL water. The cartridge was washed with 2.5 mL 40% methanol. The 1-OHP was eluted with 5 mL pure methanol. Methanol was evaporated under nitrogen to dryness, then re-dissolved to 1 mL. A 20-µL aliquot of the solution was injected into the HPLC system. We used a Column Hyper-Clone 5 ODS (C18) 120A, DIM: 250×4.60 mm. The wavelengths of excitation and emission were 242 and 388 nm, respectively. The temperature of the column was maintained at 30 °C. The

concentration of 1-OHP in urine extracts was determined from the standard curve, using peak area for quantification. Urine samples were analyzed for creatinine using the kinetic Jaffe's method photometrically (Roche Diagnostics, Mannheim, Germany) to adjust 1-OHP concentrations to creatinine excretion (µmol/mol creatinine).²¹

Analysis of Urinary 8-OHdG

Urinary 8-OHdG was measured with an ELISA kit (MyBioSource Inc, San Diego, USA), using competitive binding enzyme immunoassay technique. The amount of 8-OHdG was calculated by comparison with a standard curve and its concentrations were adjusted for urinary creatinine.

Cytochrome P2E1 (CYP2E1) Gene Expression

RNA extraction and cDNA synthesis by Reverse Transcription (RT)

Ficoll-Hypaque density-gradient centrifugation was used to isolate peripheral blood mononuclear cells (PBMCs). Phosphate-buffered saline was used to wash them. Total RNA was isolated from cell preparations using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The concentration of RNA was determined spectrophotometrically by monitoring UV absorbance at 260 nm. Purity was assessed by the absorbance ratio measured at 260 nm/280 nm. One to two µg of total RNA was used for RT to synthesize cDNA using MMLV RT (Promega). Briefly, 0.5 µg of oligo dT18 was added to each tube and annealed to RNA by incubating at 70 °C for 5 min to melt the secondary structure within the template, and then quick chilled on ice. RT of RNA was performed in a final volume of 25 µL containing 5 µL of MMLV 5× reaction buffer, 1.25 µL of 10 mM dNTP mixture, 25 U of ribonuclease inhibitor (Sigma), 200 U of MMLV (Promega), and RNase-free water. The cDNA was synthe-

99% collection efficiency using high volume samplers (General Metal Works Inc, USA). The average flow rate of sampling was 40–50 cft/m, (1.12 to 1.4 m³/min) for eight hours. Before sampling, the cleaned glass fiber filters were stored in desiccators. The clean filters were weighed. After sampling, the filters were dried in darkened desiccators for 24 hours, reweighed until constant weight to obtain the weight of TSP. PAHs were extracted from the TSP samples by Soxhlet apparatus and extracted with DCM/n-hexane mixture (50/50 v/v) for 24 hours according to the US National Institute for Occupational Safety and Health, method 5506.²² The organic extracts were then concentrated using a rotary evaporator, cleaned by clean silica gel/alumina columns consisting of 5 g anhydrous sodium sulfate, 20 g silica, 10 g alumina (deactivated 1% with distilled water), 5 g sand, and glass wool. The extracts were concentrated, exchanged to 2 mL hexane, placed on the columns, and eluted with dichloromethane (200 mL). The eluted extracts were then concentrated on a rotary evaporator and exchanged to 1 mL hexane and stored in a freezer until analysis. For PAHs analysis, 1 µL of the extract was withdrawn from the samples, including the blank samples, and injected into a Hewlett-Packard gas chromatography (GC; model HP6890), fitted with a flame ionization detector (FID). A HP-5 (30 m × 320 µm × 0.25 µm) capillary column was used with hydrogen as the carrier gas. The concentrations of the target PAH compounds were quantified by an external standard solution of 15 PAH compounds (PAH mixture, Supelco, Inc). The concentrations of the following PAH compounds in the particulate phases were determined: naphthalene (NA), acenaphthylene (ACY), acenaphthene (ACE), fluorine (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA),

chrysene (CRY), benzo[b]fluoranthene (BbF), benzo[a]pyrene (BaP), dibenzo[a, h]anthracene (DBA), benzo[ghi]perylene (BGP), and indeno[1, 2, 3,-cd]pyrene (IND). The geometric means of the measurement of total PAHs were 2.2 (SD 0.02; range 2.1 to 0.9) mg/m³. The limit threshold for workplace PAHs level²² set by the National Institute for Occupational Safety and Health is 0.1 mg/m³.

Ethics

Written informed consent was obtained from all the included participants. The study was approved by the Ethical Committee of Occupational and Environmental Medicine Department, Cairo University, Egypt. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Statistical Analysis

SPSS® for Windows® ver 15 was used for data analysis. Continuous variables with normal distribution are presented as mean (SD); those not normally distributed are presented as median (IQR). Categorical variables are presented as frequencies and percentages. Comparison of numerical variables was made using *Student's t* test for parametric variables and Mann-Whitney U test for nonparametric variables. χ^2 test was used to compare categorical variables. A p value <0.05 was considered statistically significant. Spearman's ρ was used to assess the correlation between two non-normally distributed variables. One-way analysis of variance (ANOVA) was used to compare means of three groups or more; Kruskal Wallis test was used for non-parametric variables. Multivariate regression analysis was used to identify independent predictors of oxidative DNA damage in coke oven workers.

Table 1: Demographic, clinical, biochemical and *XRCC1* (Arg399Gln) genotyping characteristics of the studied groups. Values are either mean (SD), median [IQR], or n (%).

Parameter	Exposed group (n=85)	Control group (n=85)	p value
Age, yrs	45.6 (10.1)	44.9 (10.5)	0.669
Duration of exposure, yrs	20.8 (8.8)	—	—
Using personal protective equipment	14 (16%)	—	—
Smoking habit	37 (44%)	41 (48%)	0.538
Chest manifestations	53 (62%)	17 (20%)	<0.001
Dermatitis	30 (35%)	6 (7%)	<0.001
Urinary 1-OHP, $\mu\text{mol/mol}$ creatinine	6.3 [2.9 to 9.8]	0.6 [0.04 to 1.8]	<0.001
CYP2E1 expression level	1.1 [0.6 to 1.8]	3.4 [2.4 to 5.3]	<0.001
BPDE-DNA adducts, ng/ μg DNA	6.7 [2.6 to 7.9]	0.4 [0.13 to 0.89]	<0.001
Urinary 8-OHdG, ng/mg creatinine	7.9 [2.9 to 12.4]	2.1 [1.6 to 2.5]	<0.001
<i>XRCC1</i> (Arg399Gln) genotype			
Arg/Arg (Wild)	50 (59%)	67 (79%)	0.005
Arg/Gln (Heterozygous)	20 (24%)	13 (15%)	0.175
Gln/Gln (Mutant)	15 (18%)	5 (6%)	0.017
Allele			
Arg	120 (70.6%)	147 (86.5%)	<0.001
Gln	50 (29.4%)	23 (13.5%)	

Results

The geometric mean of the workplace total PAHs level was 2.2 (SD 0.02, range 2.1 to 0.9) mg/m³, significantly higher than the exposure limit threshold set by the National Institute of Occupational Safety and Health Administration (NIOSH), 0.1 mg/m³.

All 170 participants were age-matched males (Table 1). The age of the exposed group ranged from 26 to 60 years; that of control's ranged from 22 to 60. There were no significant differences between the exposed and controls in terms of smoking habit (43% vs 48%). The minority (16.5%) of the exposed workers were us-

ing protective equipment. Compared with the control group, the exposed group had a significantly higher prevalence of chest manifestations (20% vs 62%) and dermatitis (3% vs 11%). The urinary concentrations of 1-OHP, DNA adducts, and urinary 8-OHdG in the exposed group were significantly ($p < 0.001$) higher than the controls. CYP2E1 expression level in the exposed group was significantly ($p < 0.001$) lower than the controls. There was a significant difference in wild and mutant genotypes in *XRCC1* Arg399Gln gene polymorphism (Table 1). Carriers of variant allele Gln (Arg/Gln + Gln/Gln genotypes) had significantly higher values of urinary 1-OHP, DNA adducts, and urinary 8-OHdG lev-

Table 2: Median (IQR) levels of measured biochemicals measured in the studied participants based on their XRCC1 (Arg399Gln) genotypes.

Biochemicals	Wild allele (Arg/Arg), (n=117)	Variant allele (Arg/Gln + Gln/Gln), (n=53)	p value
1-OHP, (µmol/mol creatinine)	1.7 (0.05 to 3.6)	4.9 (2.2 to 9.5)	<0.001
CYP2E1 expression level	2.4 (1.6 to 4.2)	1.7 (0.6 to 2.3)	0.001
BPDE-DNA adducts, ng/µg DNA	1.2 (0.4 to 4.5)	6.3 (0.7 to 7.8)	<0.001
8-OHdG, ng/mg creatinine	2.5 (2.1 to 4.6)	4.9 (2.5 to 12.2)	0.001

els; they had significantly lower levels of CYP450 expression compared with those homozygous for the wild type allele (Arg/Arg, Table 2). There were significant positive correlations between 1-OHP level and each of age, duration of exposure, BPDE-DNA adducts, and 8-OHdG levels in the exposed workers. CYP2E1 expression level had a significant negative correlation with age, duration of exposure, 1-OHP, BPDE-DNA adducts, and 8-OHdG levels (Table 3). A significant increase in 1-OHP level was observed in smoker controls compared with non-smokers controls (1.8 vs 0.1 ng/mg creatinine) (Table 4). After adjusting for confounders (regression analysis), only duration of work among exposed group (β 0.124, 95% CI 0.01 to 0.23) not smoking was found an independent predictor for 1-OHP. Independent predictors for BPDE-DNA adducts in the exposed group, were duration of exposure and CYP2E1 expression level. Independent predictors of

8-OHdG level were duration of exposure and urinary 1-OHP concentration (Table 5).

Discussion

We studied the urinary 1-OHP level as a biomarker for exposure level to PAHs. Urinary 1-OHP levels were significantly higher in the exposed group compared to the control (Table 1). Comparing the urinary 1-OHP levels of the exposed workers with those exposed in other countries, the exposure levels in Egyptians appeared to be higher than those in Japan (2.2 µmol/mol creatinine with 5-fold increase between workers and controls)⁶ and Turkish oven workers (3.2 µmol/mol creatinine with 5-fold increase).²³ In Chinese coke oven workers, Hanaoka, *et al*,²⁴ reported higher levels of urinary 1-OHP compared with what we found in the current study; a mean of 20.9 µmol/mol creatinine and

Table 3: Spearman's correlation matrix for the studied variables in the exposed group

Variable	Age	Duration of exposure	1-OHP	CYP2E1 expression	BPDE-DNA adducts
Duration of exposure	0.83*	1			
1-OHP	0.47*	0.61*	1		
CYP2E1 expression	-0.52*	-0.71*	-0.60*	1	
BPDE-DNA adducts	0.62*	0.81*	0.63*	-0.88*	1
8-OHdG	0.42*	0.51*	0.71*	-0.59*	0.68*

*p<0.001

Table 4: Comparison between smokers and non-smokers among the studied groups. Values are either mean (SD) or median [IQR].

Parameter	Exposed group			Control group		
	Smoker	Non-smoker	p value	Smoker	Non-smoker	p value
Age, yrs	45.1 (9.4)	46.1 (10.7)	0.58	45.7 (9.7)	44.2 (11.3)	0.60
Duration of exposure	22 [15 to 26]	23.5 [11 to 27]	0.67	—	—	—
Urinary 1-OHP, $\mu\text{mol/mol}$ creatinine	6 [3.2 to 8]	7.5 [2.9 to 10.5]	0.28	1.8 [1.6 to 2.1]	0.1 [0 to 0.1]	<0.001
CYP2E1 expression level	1.1 [0.6 to 1.8]	1.1 [0.7 to 1.9]	0.56	3.4 [2.4 to 5.3]	3.3 [2.5 to 5.2]	0.88
BPDE-DNA adducts, $\text{ng}/\mu\text{g}$ DNA	6.8 [4.5 to 7.9]	6.6 [2.2 to 7.9]	0.32	0.4 [0.1 to 0.7]	0.5 [0.2 to 1.1]	0.16
Urinary 8-OHdG, ng/mg creatinine	8.5 [4.3 to 12.1]	6.4 [2.9 to 13]	0.74	2.1 [1.7 to 2.5]	2.1 [1.5 to 2.5]	0.89

21.8-fold increase between workers and controls. A level of 1.0 $\mu\text{mol/mol}$ creatinine (equivalent to 1.93 $\mu\text{g}/\text{creatinine}$ or 1.93 $\mu\text{g}/\text{L}$ urine) for urinary 1-OHP is recommended for occupational exposure by Jongeneelen.⁵ In the current study, the level exceeded this proposed limit in 59% (50/85) of the exposed workers. The total PAHs measured in the workplace air of the coke oven areas exceeded the exposure limit threshold set by the National Institute of Occupational Safety and Health.²² This could explain the higher levels of urinary 1-OHP observed in the exposed workers; it could be attributed to lack of sufficient ventilation at the workplace. Moreover, only a minority (16%) of exposed workers were using protective equipment.

One of the most important explanations for inconsistent results in human biomonitoring studies is the inter-individual differences in xenobiotics metabolizing enzymes and DNA repair capacities that may mask or augment the effects of the exposures. Identifying inter-individual differences help to provide information for making decisions aiming to limit risks for sensitive populations. Therefore, this study measured the expression level of *CYP2E1* gene involved in xenobiotic biotransfor-

mation. The results showed significantly lower *CYP2E1* mRNA expression levels in the exposed group compared with the controls. Carriers of the variant allele (Gln) of *XRCC1* gene (Arg/Gln + Gln/Gln) had significantly lower *CYP2E1* expression levels than those homozygous for the wild allele (Arg/Arg) (Table 1). Interestingly, *CYP2E1* mRNA expression levels had a significant negative correlation with individual's age, duration of exposure, urinary 1-OHP level, BPDE-DNA adduct, and 8-OHG concentration (Table 3).

Liu, *et al*,²⁵ showed that expression of *CYP2E1* is declined along with the initiation, promotion and progression of hepatocellular carcinoma (HCC). Studying the clinical specimens taken from 85 HCC patients, Ho, *et al*,²⁶ found that 70% of tumor tissues have low expression of *CYP2E1*. This low expression of *CYP2E1* is associated with poor prognosis of HCC. A plausible explanation for this observation would be that the lack of *CYP2E1* may decrease xenobiotics metabolism, including PAH and other pro-carcinogens, leading to prolonged injurious effects, which enhances cytotoxicity and genotoxicity, making the person more susceptible to cancer. However, the exact mechanism of downregulation

Table 5: Multivariate regression analysis to identify predictors for 8-OHdG and BPDE-DNA adducts among (coke oven workers) the exposed group.

Variable	β (95% CI)	
	Predictors for 8-OHdG (r^2 0.644)	Predictors for BPDE-DNA (r^2 0.760)
Age, yrs	0.07 (-0.04 to 0.19)	-0.01 (-0.06 to 0.04)
Smoking habit	0.65 (-0.68 to 2.00)	0.45 (-0.18 to 1.09)
Duration of exposure	-0.17 (-0.3 to -0.01)	0.15 (0.07 to 0.22)
XRCC1 (Arg399Gln)	-0.63 (-2.01 to 0.74)	0.01 (-0.65 to 0.67)
Urinary 1-OHP	0.79 (0.51 to 1.07)	0.06 (-0.09 to 0.22)
CYP2E1 expression level	0.01 (-0.65 to 0.67)	-0.49 (-0.78 to -0.19)

lation has not been clearly elucidated yet. Therefore, CYP2E1 expression levels allow us to identify individual's risk susceptibility to minimize PAHs exposure.

Benzo[a]pyrene (BaP) is notable for being a famous PAH present in coal tar and is the first chemical carcinogen discovered.⁸ Its mechanism of carcinogenicity depends on an enzymatic metabolism in steps to a final mutagen; benzo[a]pyrene-7, 8-diol-9, 10-epoxide (BPDE). The very reactive BPDE binds covalently to lipids, proteins, and DNA to produce BPDE-DNA adducts. If not repaired, DNA adducts may lead to persistent mutations resulting in cell transformation and ultimately tumor development.⁷

The results of this study support the above findings; significantly higher values of BPDE-DNA adducts (\approx 17 folds) were presented by the exposed workers relative to the controls (Table 2). This indicates an increased hazard of genotoxic effects related to PAH exposure. A strong positive correlation was found between BPDE-DNA adducts and 1-OHP level in the exposed group; BPDE-DNA adducts was also correlated with age, duration of exposure (indicating that DNA adducts can be a sensitive marker of exposure) and 8-OHdG (genotoxic effect). This is confirmed by

multivariate regression analysis that revealed that the duration of exposure was a predictor of DNA adducts in coke oven workers (Table 5). In concordance, Talaska, *et al*,²⁷ reported that coke oven workers who were heavily exposed to PAHs had higher BPDE-DNA adducts compared with the control subjects.

In the exposed group, we found a negative correlation between DNA adducts level and its metabolizing enzyme, CYP2E1, expression level (Table 3); the enzyme expression level was also a strong predictor of DNA adducts (Table 5). Carriers of the variant allele (Gln) of XRCC1 (DNA repairing gene) showed significantly higher DNA-adducts levels (Table 2). The possible explanation for this observation is that inefficient repairing mechanism (variant XRCC1 gene) leads to increased BPDE-DNA adducts, which markedly downregulates CYP2E1 expression level in a way to reduce more adducts production. Therefore, BPDE-DNA adducts level represents the balance between exposure, production and elimination, which will determine individual's cancer susceptibility. Urinary 8-OHdG is considered a biochemical marker for oxidative DNA damage caused by ROS.²⁸ Marczyński, *et al*,²⁹ reported the relationship between PAHs exposure

sized at 42 °C for 60 min, heated to 95 °C for 5 min, and stored at -20 °C.

Quantitative Real-Time PCR

The gene expression of CYP2E1 was determined by a quantitative PCR analysis, using SYBR Green method on Step One plus (Applied Biosystems, Foster City, CA, USA). Primers of PCR were designed with Gene Runner Hasting Software (Hasting, NY, USA) from RNA sequences from GenBank as follows: CYP2E1 Forward: 5'-ACCTGCCCCATGAAGCAACC-3'; Reverse: 5'-GAAACAACCTCCATGCGAGCC-3' Gene bank Acc. Number NM 00073. β -actin Forward: 5'-CCTGGCACCCAGCA-CAAT-3'. Reverse: 5'-GCCGATCCACACG-GAGTACT-3'. Genebank Acc. Number NM 00111.

All primer sets had a calculated annealing temperature of 60 °C. Quantitative Real-Time PCR was performed in a 25 μ L reaction volume consisting of 12.5 μ L of SYBR green PCR Master mix, 1 μ L of each primer, and 5 μ L of cDNA. Amplification conditions were 2 min at 50 °C and 40 cycles of 15 sec at 95 °C, 1 min at 60 °C, and 1 min at 72 °C for the amplification step. The threshold cycle (Ct) was calculated using the Applied Biosystems SDS software ver 2.1. Relative expression of CYP2E1 was calculated using the comparative cycle threshold method ($\Delta\Delta$ Ct).

Measurement of Benzo[a]pyrene-7, 8-diol-9, 10-epoxide-DNA Adducts (BPDE-DNA Adducts)

The extracted DNA samples were subsequently diluted to a concentration of 4 μ g/mL in 1X TE Buffer. The level of BPDE-DNA adducts was determined using sandwich-based immunoassay OxiSelect BPDE-DNA Adduct ELISA kit, Cat No MBS169130 (MyBioSource, Inc, USA). To minimize background signals from unadducted DNA non-specific binding, the Reduced DNA Standard was used as an

absorbance blank. For quantification, an eight-point BPDE-DNA standard curve (measuring range 0 to 100 ng/mL) was included in each run. The results were expressed as ng of BPDE-DNA adducts per μ g of DNA with detection limit of 1.56 ng BPDE-DNA/ μ g of DNA.

Genotyping of XRCC1 Arg399Gln Polymorphism by Real-Time PCR

Extraction of genomic DNA from sterile EDTA-anticoagulated blood samples was done using a QIA amp DNA blood mini-kit (Qiagen, Hilden, Germany) by silica-gel spin columns.

Analysis of XRCC1 Arg399Gln polymorphism (exon10 SNP; G 28152 A; p.Arg399Gln; rs25487) was done by Real-Time PCR fluorogenic 5' nuclease allelic discrimination technique using TaqMan Single-Nucleotide Polymorphism (SNP) Genotyping Assay (Applied Biosystems) and performed on Step One TM Real-Time PCR system. The final volume of each reaction was 25 μ L consisting of 12.5 μ L of TaqMan Universal Master Mix (2X), which contained AmpliTaq-Gold DNA polymerase; 1.25 μ L of assay mix (20X), which contained primers and probes; 5 μ L genomic DNA; and 6.25 μ L nuclease-free water. Negative control (no DNA template) was run to ensure that there was no amplification of contaminating DNA. The amplification reactions were carried out with an initial hold step at 95 °C for 10 min to activate AmpliTaq-Gold DNA polymerase followed by 40 cycles of three steps: denaturation at 92 °C for 15 sec, annealing at 60 °C for 30 sec, and extension at 60 °C for 30 sec.

Workplace Monitoring

Air samples for total PAHs level were assessed by the industrial hygienist of El-Tebbin Institute for Metallurgical Studies (TIMS) in Egypt. Glass fiber filters (20 \times 25 cm) were used to collect samples with

and 8-OHdG levels in coke-oven workers. They found levels 1.38 times higher than those in the controls. The exposed workers in this study had levels 3.7 times higher than those in the controls (7.9 vs 2.1 ng/mg creatinine, Table 1). This agrees with previous studies,^{6,11,30} where the effect of PAHs on the formation of 8-OHdG has been investigated. Similarly, the current study found a positive correlation between urinary 1-OHP and 8-OHdG (Table 3); this urinary marker of PAHs exposure was a strong predictor for oxidative DNA damage (Table 5). On the other hand, Zhang, *et al.*,³¹ found no relationship between urinary 1-OHP and 8-OHdG. This could be explained by the different exposure levels of PAHs, which depend on the amount of PAHs in the workplace air. Another possibility is that several other carcinogens are present in coke-oven emissions; examples are benzene, nitrosamine and arsenic compounds. These substances can also influence the amount of oxidative DNA damage.⁶

Individual's genetic build up may overcome oxidants through creation of a balanced antioxidant gene defense system. Our study found a positive correlation between 8-OHdG urinary excretion and age (Table 3), which may be due to the higher duration of exposure in older workers than younger ones. This was strengthened by the positive correlation found between urinary 8-OHdG and duration of exposure in coke oven workers (Table 3). Multivariate regression analysis confirmed that duration of exposure was an independent predictor for 8-OHdG.

The association between urinary 8-OHdG, CYP2E1 expression and XRCC1 399 Arg/Gln polymorphism was highlighted. A strong negative correlation was found between urinary 8-OHdG and CYP2E1 gene expression level (Table 3). CYP2E1 is a highly inducible enzyme the expression of which changes under vari-

ous circumstances. As CYP2E1 is a potent generator of ROS, the finding that CYP2E1 gene expression actually decreased in the exposed group is rather surprising. A possible explanation is that during oxidative stress and high 8-OHdG production, an antioxidant defense mechanism may be induced to suppress CYP2E1 gene in an attempt to reduce ROS. Carriers of variant allele (Gln) of *XRCC1* gene had significantly higher levels of urinary 8-OHdG compared with those homozygous for the wild allele (4.9 vs 2.5 ng/mg creatinine respectively, Table 2). This underlined the increased hazard of oxidative stress related to inefficient DNA repairing. Our findings were inconsistent with results of Jongeneelen, *et al.*,⁵ who reported that NOGEL (no observed genotoxic effect) at 1 µmol/mol creatinine and LOGEL (lowest observed genotoxic effects level) at 1.9 µmol/mol creatinine. This limit was recommended in workplaces where the pyrene/Bap ratio is 2.5 such as that observed in coke oven plants, where the studies were conducted. Unfortunately, we did not make this measurement. It is one of the limitations of our study.

Tobacco smoke contains the most widespread human carcinogens and is supposed to cause oxidative DNA damage. Smoking at work may increase urinary 1-OHP among smokers as one cigarette contains approximately 50–200 ng of pyrene. Our study found significantly higher levels of urinary 1-OHP among smoker controls compared to nonsmoker controls (Table 4). However, after adjusting for confounders (regression analysis), only duration of work among the exposed group not smoking was found an independent predictor for 1-OHP.

The current study showed a significantly higher frequency of variant Gln/Gln genotype among the exposed group compared with that in controls (18% vs 6%) with no significant difference in the

heterozygous genotype. The variant allele (Gln) presented higher frequency among the exposed group compared with the controls (29% *vs* 13%, $p < 0.001$). Carriers of the variant allele (Gln) had higher levels of 1-OHP, BPDE-DNA adducts and 8-OHdG, and a lower CYP2E1 gene expression level compared with those homozygous for the wild allele (Arg/Arg). This means that a higher frequency of the variant genotypes (Arg/Gln and Gln/Gln) of *XRCC1* gene in the exposed workers decreased repairing system for DNA damage, making them more vulnerable to malignant disorders as it is a predisposing factor for oxidative DNA damage and DNA adducts. These results were consistent with previous studies reporting that Gln/Gln genotype is a risk for various environmentally caused cancers (*eg*, lung cancer).^{15,32} The current study provided an additional evidence for gene-environment interactions between *XRCC1* gene polymorphisms and PAHs exposure among coke oven workers.

We showed that the susceptibility to PAHs associated risk could be attributed to genetic causes which act synergistically leading to alterations in the carcinogen metabolism and/or impairment in the capacity of DNA repair. Urinary 1-OHP level of 59% of our workers exceeded the occupational exposure limit and only 16% were using protective equipment. Therefore, countermeasures should be implemented at workplace to reduce exposure; those include improvement of the manufacturing process, regular use of personal protective equipment, and reduction in time of exposure to prevent long-term adverse effects. The most effective measures of controlling coke oven emissions are engineering controls and good work practices. Furthermore, annual monitoring for exposure to PAHs should be conducted.

Acknowledgments

The authors would like to thank all partici-

pants for their cooperation. They are also grateful to Prof. Dr. Nermin Zawilla in Occupational and Environmental Medicine Department in Cairo University, Egypt, for providing facilities during this study.

Conflicts of Interest: None declared.

Financial Support: None.

References

1. Hansen AM, Mathiesen L, Pedersen M, Knudsen LE. Urinary 1-hydroxypyrene (1-HP) in environmental and occupational studies-a review. *Int J Hyg Environ Health* 2008;**211**:471-503.
2. International Agency for Research on Cancer (IARC). Chemical agents and related occupations: a review of human carcinogens. In WHO monographs on the evaluation of carcinogenic risks to humans. Lyon, France: 2012; IARC. Available from www.monographs.iarc.fr/ENG/Monographs/vol100F/mono100F.pdf (Accessed December 1, 2018).
3. Solaraj G, Mohanraj R. Urinary PAH metabolites as potential biomarkers for PAH exposure- A pilot study in three cities of Southern India. *Austin Environ Sci* 2017;**2**:1023.
4. Lafontaine M, Gendre C, Morele Y, Laffitte-Rigaud G. Excretion of urinary 1-hydroxypyrene in relation to the penetration routes of polycyclic aromatic hydrocarbons. *Polycyclic Aromatic Compounds* 2002;**22**:579-88.
5. Jongeneelen FJ. A guidance value of 1-hydroxypyrene in urine in view of acceptable occupational exposure to polycyclic aromatic hydrocarbons. *Toxicol Lett* 2014;**231**:239-48.
6. Nguyen T, Kawakami S, Kawai K, *et al*. Urinary 1-hydroxypyrene and 8-hydroxydeoxyguanosine levels among coke-oven workers for 2 consecutive days. *J Occup Health* 2014;**56**:178-85.
7. Elovaara E, Mikkola J, Stockmann-Juvala H, *et al*. Polycyclic aromatic hydrocarbon (PAH) metabolizing enzyme activities in human lung, and their inducibility by exposure to naphthalene, phenanthrene, pyrene, chrysene, and benzo(a)pyrene as shown in the rat lung and liver. *Arch Toxicol* 2014;**81**:169-82.
8. Beranek M, Fiala Z, Kremlacek J, *et al*. Genetic polymorphisms in biotransformation enzymes for benzo[a] pyrene and related levels of benzo[a] pyrene-7,8-diol-9,10-epoxide-DNA adducts in Goeckerman therapy. *Toxicol Lett* 2016;**255**:47-51.

9. Pauk N, Klimesova S, Kara J, *et al.* The relevance of monitoring of antibodies against the polycyclic aromatic hydrocarbon (PAH) and PAH-DNA adducts in serum in relation to lung cancer and chronic obstructive pulmonary disease (COPD). *Neoplasia* 2013;**60**:182-7.
10. Marczynski B, Raulf-Heimsoth M, Spickenheuer A, *et al.* DNA adducts and strand breaks in workers exposed to vapors and aerosols of bitumen: associations between exposure and effect. *Arch Toxicol* 2011;**85**:53-64.
11. Kuang D, Zhang W, Deng Q. Dose-response relationships of polycyclic aromatic hydrocarbons exposure and oxidative damage to DNA and lipid in coke oven workers. *Environ Sci Technol* 2013;**47**:7446-56.
12. Roszkowski K. Oxidative DNA damage-the possible use of biomarkers as additional prognostic factors in oncology. *Front Biosci (Landmark Ed)* 2014;**19**:808-17.
13. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature* 2001;**411**:366-74.
14. Taylor RM, Thistlethwaite A, Caldecott KW. Central role for the XRCC1 BRCT I domain in mammalian DNA single-strand break repair. *Mol Cell Biol* 2002;**22**:2556-63.
15. Uppal V, Mehndiratta M, Mohapatra D, *et al.* XRCC-1 Gene polymorphism (Arg399Gln) and susceptibility to development of lung cancer in cohort of North Indian population: A pilot study. *J Clin Diag Res* 2014;**8**:17-20.
16. Lee HS, Yang M. Applications of CYP-450 expression for biomonitoring in environmental health. *Environ Health Prev Med* 2008;**13**:84-93.
17. Arif E, Vibhutin A, Alam P, *et al.* Association of CYP2E1 and NAT2 gene polymorphisms with chronic obstructive pulmonary disease. *Clin Chim Acta* 2007;**382**:37-42.
18. Nicholas A. Chronic obstructive pulmonary disease. Chapter 85. In: Lee G, Dennis A ed. *Cecil Textbook of Medicine*. 22nd ed. Saunders, Philadelphia, **2004**:509.
19. Tarlo SM, Lemiere C. Occupational asthma. *N Eng J Med* 2014;**370**:640-9.
20. English J, Williams J. Occupational diseases of the skin. In: Baxter PJ, Aw TC, Cockcroft A, *et al* eds. *Hunter's Diseases of Occupations*. 10th ed. CRC Press, London, **2010**:1059-79.
21. Syal K, Banerjee D, Srinivasan A. Creatinine estimation and interference. *Ind J Clin Biochem* 2013;**28**:210-1.
22. National Institute for Occupational Safety and Health (NIOSH). 2003. Polycyclic Aromatic Hydrocarbons by HPLC; 2003: Method 5506. Available from www.cdc.gov/niosh/docs/2003-154/pdfs/5506.pdf (Accessed December 1, 2018).
23. Ada AO, Yilmazer M, Suzen S, *et al.* Cytochrome P450 (CYP) and glutathione S-transferases (GST) polymorphisms (CYP1A1, CYP1B1, GSTM1, GSTP1 and GSTT1) and urinary levels of 1-hydroxypyrene in Turkish coke oven workers. *Genet Mol Biol* 2007;**30**:511-9.
24. Hanaoka T, Yamano Y, Pan G, *et al.* Cytochrome P450 1B1 mRNA levels in peripheral blood cells and exposure to polycyclic aromatic hydrocarbons in Chinese coke oven workers. *Sci Total Environ* 2002;**296**:27-33.
25. Liu H, Lou G, Li C, *et al.* HBx Inhibits CYP2E1 Gene Expression via Downregulating HNF4a in Human Hepatoma Cells. *PLoS ONE* 2014;**9**:e107913.
26. Ho JC, Cheung ST, Leung KL, *et al.* Decreased expression of cytochrome P450 2E1 is associated with poor prognosis of hepatocellular carcinoma. *Int J Cancer* 2004;**111**:494-500.
27. Talaska G, Thoroman J, Schuman B, Käfferlein HU. Biomarkers of polycyclic aromatic hydrocarbon exposure in European coke oven workers. *Toxicol Lett* 2014;**231**:213-6.
28. Wu MT, Pan CH, Huang YL, *et al.* Urinary Excretion of 8-Hydroxy-2-deoxyguanosine and 1-Hydroxypyrene in Coke-Oven Workers. *Environ Mol Mutagen* 2003;**42**:98-105.
29. Marczynski B, Rihs HP, Rossbach B, *et al.* Analysis of 8-oxy-7,8-dihydro-2'-deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: comparison with ambient monitoring, urinary metabolites and enzyme polymorphisms. *Carcinogenesis* 2002;**23**:273-81.
30. Chao MR, Wang CJ, Wu MT, *et al.* Repeated measurements of urinary methylated/oxidative DNA lesions, acute toxicity, and mutagenicity in coke oven workers. *Cancer Epidemiol. Biomarkers Prev* 2008;**17**:3381-9.
31. Zhang J, Ichiba M, Hanaoka T, *et al.* Leukocyte 8-hydroxydeoxyguanosine and aromatic DNA adduct in coke-oven workers with polycyclic aromatic hydrocarbon exposure. *Int Arch Occup Environ Health* 2003;**76**:499-504.
32. Divine KK, Gilliland ED, Crowell RE, *et al.* The XRCC1 399 glutamine allele is a risk factor for adenocarcinoma of the lung. *Mutat Res* 2001;**461**:273-8.