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SPERM QUALITY AND DNA INTEGRITY OF COKE OVEN WORKERS EXPOSED TO POLYCYCLIC AROMATIC HYDROCARBONS

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Abstract
Objectives: The objective of this study was to assess sperm quality and deoxyribonucleic acid (DNA) integrity of coke oven workers exposed to polycyclic aromatic hydrocarbons (PAHs) as compared to control subjects. **Material and Methods:** The coke oven workers (N = 52) and administrative staff (N = 35) of a steel plant served as the exposed and control groups, respectively. Exposure to PAHs was assessed by measuring 1-hydroxypyren. Analysis of sperm quality (concentration, motility, vitality, and morphology) was performed simultaneously with sperm DNA integrity analysis, including DNA fragmentation, denaturation, bulky DNA adducts, and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dGuo). A questionnaire was conducted to collect demographic and potential confounding data. **Results:** The coke oven workers had lower percentages of sperm motility, vitality and normal morphology than the control group, but the difference was not significant. For DNA integrity, the coke oven workers had significantly higher concentrations of bulky DNA adducts and 8-oxo-dGuo than the control subjects ($p = 0.009$ and $p = 0.048$, respectively). However, DNA fragmentation percentages did not significantly increase as compared to those in the subjects from the control group ($p = 0.232$). There was no correlation between sperm quality parameters and DNA integrity indicators. **Conclusions:** Occupational exposure of the coke oven workers to PAHs was associated with decreased sperm DNA integrity. Int J Occup Med Environ Health 2016;29(6):915–926

Key words:
DNA damage, Semen quality, Sperm, DNA integrity, DNA fragmentation, Bulky DNA adducts

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INTRODUCTION

Occupational exposure to toxic substances that affect the reproductive system still constitutes a significant public health concern. Polycyclic aromatic hydrocarbons (PAHs) are common toxic compounds generated from coal combustion during steel processing. Several PAH compounds have been reported to impair the reproductive capacity of males, including decreased semen quality in terms of concentration, motility and vitality [1–3].

Cellular deoxyribonucleic acid (DNA) has been suggested as a target in the subjects exposed to PAHs. Polycyclic aromatic hydrocarbon active intermediates, the byproducts of PAHs via cytochrome P450 (CYP450) phase I metabolism, have been reported to covalently attach to DNA in tissues and lymphocytes [4]. Also, active intermediates could take the redox cycling pathway and generate excess reactive oxygen species. That, in turn, leads to oxidative stress, which could induce oxidative DNA damage and possible single- and double-DNA strand breaks [5].

Sperm is protected by the blood–testis barrier, which can block environmental toxins from entering testicular tissues. However, animal studies have detected PAH-DNA adducts in testicular tissues simultaneously revealing the possibility that PAHs can compromise the function of the barrier and affect spermatogenesis [6]. Additionally, the studies have shown PAH exposure could form PAH-DNA adducts in human sperm [4]. Prior to this study, limited available data existed about whether environmental and/or occupational exposure to PAHs contributes to the increased levels of bulky DNA adducts in human sperm. Gaspari et al. have reported that occupational exposure to PAHs was associated with higher levels of PAH-DNA adducts in infertile men [7]. Horak et al. have observed an increase in bulky DNA levels in sperm from infertile individuals. However, the bulky DNA levels did not link to environmental and occupational factors [8]. Sperm DNA integrity has been shown to relate to male fertility potential and the risk of increased chromosomal abnormalities, and minor or major birth defects [9–13]. Emerging evidence has suggested that sperm DNA integrity may be a better predictor of male fertility potential than the routine semen parameters [14]. Also, semen parameters may not address integrity of the male genome contained in the sperm head [15]. Despite the importance of sperm DNA integrity in male reproductive health, there is still no reliable, sensitive method for detection of sperm DNA oxidative adducts. The European Standard Committee on Oxidative DNA Damage recommended DNA isolation methods to minimize oxidation during DNA extraction from tissues and cells [16,17]. Such a development is particularly useful for DNA extraction from sperm cells due to the compact nature of sperm chromatin, which could increase the possibility of DNA oxidation during extraction procedures. Deoxyribonucleic acid lesion 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dGuo) is one the most abundant products of oxidatively damaged DNA and has been used as a biomarker to depict oxidative stress in various tissues, e.g., blood, urine, and saliva [18].

The objective of this study was to assess sperm quality and DNA integrity of the coke oven workers exposed to PAHs as compared to the control subjects. Deoxyribonucleic acid fragmentation, denaturation and oxidative adducts were simultaneously assessed to determine nuclear DNA integrity of sperm in the coke oven workers exposed to PAHs. Correlation between DNA integrity and sperm quality was determined. The study employed well established ^{32}P-labeling for DNA adduct detection, while employing the newly established liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) with the on-line solid phase extraction procedure for the 8-oxo-dGuo analysis [19–21].

MATERIAL AND METHODS

Human subjects

The subjects included coke oven workers and administrative staff of a steel plant in southern Taiwan. They

were recruited during their annual health examination at the Kaohsiung Municipal Hsiao-Kang Hospital, a main municipal hospital system providing health care for occupational workers in the southern region of Taiwan. Ninety percent of the subjects who were contacted by mail agreed to participate in this study.

The coke oven workers (N = 52) served as the exposed group that had been chronically exposed to PAHs; while the administrative staff, including administrators and security personnel (N = 35), served as the control group. Our preliminary data showed that PAH concentrations around the coke oven processing area ranged from 15 000 ng/m^3 to 40 000 ng/m^3, while PAH concentrations in the offices were < 50 ng/m^3 [22]. Criteria for the subjects selection included males 25–50 years old, no reproductive dysfunction, and > 1 year of employment in the plant. All the participants were fully informed about the objective of this study and signed a consent form. Information about the subjects selected for the study remains confidential and within the institution. The study was approved by the Institutional Review Boards at both Old Dominion University and Kaohsiung Medical University.

Questionnaire

A questionnaire was used to collect information pertaining to demographics and potential confounding factors. Demographic information included: age, body mass index (BMI), education, marital status, smoking and drinking habits. Questions on employment history emphasized current occupational duties, production processes, respirator usage and job classification. Health history covered the participants and their families' history of cancer, other diseases and treatment history.

Semen and urine sample collection

Semen samples were collected via masturbation. The subjects were instructed to abstain from sex for at least 3 days before the sample collection. Sperm quality analysis was conducted within 1 h after the sample collection according to the World Health Organization (WHO) guidelines. A portion of semen was stored at –20°C for sperm DNA integrity assessment. Two spot urine samples were collected from each individual: first one in the morning before the work shift; the second at the end of the work shift. All the urine samples were stored at 4°C before the analysis.

Human semen analysis

After liquefaction of semen, the standard semen quality analysis was conducted according to the World Health Organization (WHO) recommendations [23]. Quality control for all the measurements was in accordance with the WHO guidelines [23]. Sperm quality parameters included: concentration, motility, morphology and vitality. Sperm concentration, motility and vitality were assessed within 1 h after ejaculate sampling. Manual evaluation of sperm concentration and motility was conducted using a Makler Counting Chamber (Irvine Scientific, USA). The eosin stain method was used to assess sperm vitality. At least 300 sperm/samples were assessed for vitality analysis. For the morphology assessment, 2 slide smears were prepared from each semen sample. Three hundred sperm/slides were evaluated using the air-dried Papanicolaou-stained preparations and classified as either normal or abnormal according to the criteria recommended by the WHO [23]. None of the semen samples had significant leukocytospermia (as specified in the WHO guidelines).

Terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate nick end-labeling (TUNEL)

The TUNEL assay was used to detect sperm DNA fragmentation [16]. A sperm pellet was obtained after 200 μl of semen was centrifuged at 250×g for 5 min. The pellet was re-suspended, washed with 1% human serum albumin in a phosphate buffer solution (PBS), and spread onto slides. Then, the cells were permeabilized with 0.1% Triton X-100

(Sigma-Aldrich, USA) in 0.1% sodium citrate at 4°C for 2 min. A nucleotide labeling mixture, prepared according to the Roche Diagnostic manufacture's instruction, was deployed onto sperm cells. After the cells were incubated for 1 h at 37°C, the cells were washed twice with a 1% human albumin solution in a phosphate buffered solution. Each test included both positive and negative controls. The cells in the positive control were treated with a 50 μl of deoxyribonuclease (DNase) solution, while the cells in the negative control were not treated with the nucleotide labeling mixture. Fluorescence in sperm cells recorded as positive for the TUNEL assay was assessed using an Olympus BX61 fluorescence microscope (Olympus America Inc., USA). At least 300 sperm cells from each sample were taken into account, and the percentage of TUNEL positive cells was calculated as the outcome of interest.

Sperm chromatin structure assay (SCSA)

The SCSA was used to detect DNA denaturation as described by Evenson et al. [24,25]. Briefly, $1–2\times10^6$ sperm cells per ml were treated with a low pH 1.2 detergent solution (0.08 M of hydrogen chloride (HCl), 0.15 M of sodium chloride (NaCl), 0.1% Triton X-100, pH 1.2). After 30 s, the cells were stained by adding 1.2 ml acridine orange stain in a phosphate-citrate buffer, pH 6, for 3 min. Then, the cell suspensions were analyzed using a flow cytometer. Sperm chromatin damage can be quantified by cytometric measurements of the metachromatic shift from green (native double-stranded DNA) to red (denatured, single-stranded DNA) fluorescence using a FACScan flow cytometer (Becton-Dickinson, USA). Flow cytometry measurements of 5000 sperm/samples provided statistically robust data on the ratio of red to green sperm and the extent of the DNA denaturation.

Detection of 8-oxo-dGuo adducts in sperm

Sperm DNA was isolated according to the procedure recommended by the European Standard Committee on Oxidative DNA Damage (ESCODD) [17], with modifications to minimize DNA oxidization during DNA isolation procedures. Sperm samples ($15–30\times10^6$ sperm/ml) were washed with 1% human serum assay in a phosphate buffer solution and centrifuged at 3000×g for 5 min. The resulting pellets were added to 600 μl of ice-cold extraction buffer (10% (weight per volume – w/v) sodium dodecyl sulfate and 1 M dithiothreitol), 30 μl of proteinase K, 30 μl of RNase A (1×10^{-2} mg/l) and 8 μl of RNase T1 (1 U/μl). The mixture was incubated at 37°C for 1 h and then cooled to 4°C for 5 min. Subsequently, 1.2 ml of the sodium iodide (NaI) solution and 2 ml of 2-propanol were added. After centrifugation at 5000×g for 5 min, the DNA pellet was washed with 1 ml of ice-cold 40% (volume per volume – v/v) 2-propanol, collected by centrifugation and dissolved in 200 μl of 0.1 mM deferoxamine overnight.

8-Oxo-dGuo adducts in sperm DNA were determined by a LC-MS/MS with an on-line solid-phase extraction procedure as reported in the recent studies [20]. After automatic sample cleanup, DNA samples were injected into the Agilent 1100 series high performance liquid chromatography (HPLC) system (Agilent Technologies, Germany) interfaced with a PE-SCIEX API 3000 triple quadrupole mass spectrometer (Agilent Technologies, Germany) with an electrospray ion source. Detection was performed in a positive ion multiple reaction monitoring mode for simultaneous quantification of 8-oxo-dGuo and deoxyguanosine (dG). Transitions of the precursors to the product ions were as follows: 8-oxo-dGuo (mass-to-charge ratio (m/z) 284→168), [$^{15}N_5$]-8-oxo-dGuo (m/z 289→173), dG (m/z 268→152), and [$^{15}N_5$]-dG (m/z 273→157). With the use of isotopic internal standards and an on-line SPE, this method exhibited a low detection limit of 1.8 fmol for 8-oxo-dGuo, which corresponds to 0.13 adducts at 10^6/dG^{-1} when using 20 μg of DNA per analysis.

Detection of bulky DNA adducts

Presence of bulky DNA adducts was determined by the ^{32}P-postlabeling method [26,27]. Briefly, DNA

(10 μg) was enzymatically degraded to normal and adducted by deoxyribonucleoside 3'-monophosphates with micrococcal nuclease and spleen phosphodiesterase at pH = 6 and incubated at 37°C for 3.5 h. After the treatment of the mixture with nuclease P1 to convert normal nucleotides to nucleosides, adducted nucleotides were converted to 5'-^{32}P-labeled deoxyribonucleoside 3',5'-bisphosphates by incubation with carrier-free [γ-^{32}P] adenosine triphosphate (ATP) and polynucleotide kinase. Radioactive labeled modified nucleotides were mapped by multidirectional anion-exchange thin-layer chromatography (TLC) on polyethyleneimine-cellulose sheets [28].

The ^{32}P-labeled I-compounds were visualized by screen-enhanced autoradiography at –80°C using Kodak Bio-Max XAR film (Sigma-Aldrich, USA) or with the aid of an InstantImager (Packard Instruments, USA). Radioactivity of TLC fractions from individual sperm samples was determined with the aid of an InstantImager electronic autoradiography system as previously described [28]. The extent of covalent DNA adducts was estimated by calculating relative adduct labeling values from the sample count rates, the amount of DNA assayed (expressed as pmol DNA monomer units or DNA-P), and the specific activity of [γ-^{32}P]ATP according to Zhou et al. [29]. Each set of the analyzed samples included positive controls on liver DNA from mice treated with PAHs to ensure the labeling procedures worked appropriately.

Urinary 1-hydroxypyrene (1-OHP) measurement

Urinary 1-OHP served as a biomarker assessing PAH exposure of the coke oven workers. Urinary 1-OHP was analyzed using the HPLC with a fluorescent detector described in several studies [22,23]. The procedures included purification and enrichment using the C18 reverse-phase liquid chromatograph and quantified concentrations using the HPLC system with an auto-injector and a fluorescence detector. The detection and qualification limits were 0.1 ng/l and 1 μg/l, respectively. Normalized concentrations of urinary 1-OHP were expressed as μg/g creatinine.

Statistical analysis

Data analysis was performed using the SAS version 9.1 (SAS Institute Inc., Cary, USA) program with the results expressed as the mean ± standard deviation (M±SD). Distributions of semen quality parameters were examined for normality to determine if transformation would be needed. Certain measures were log-transformed if their skewness prevented their normality. To determine any significance in terms of semen quality parameters and sperm DNA integrity measurements, the multivariate regression analysis was conducted while controlling for confounding factors, i.e., age, alcohol consumption and smoking. Also, the multivariate regression analysis was used to assess the relationship between semen quality parameters and DNA integrity parameters, while controlling for the confounding factors. All hypothesis testing was two-sided with a probability value of 0.05 considered as significant.

RESULTS

Table 1 shows that the mean age and mean working years of the subjects in the exposed group were similar to those in the control group. There was a significant difference in the smoking status between the 2 groups ($p = 0.03$). The exposed group had more subjects who drank regularly than the control group ($p = 0.007$). Mean level of urinary 1-OHP in the exposed group was significantly lower than that in the control group ($p = 0.007$). The exposed group had lower percentages of sperm motility, vitality, and normal morphology than the control, but there was no significant difference between the 2 groups on the sperm quality parameters.

Table 1. Demographic characteristic and routine semen quality of the coke-oven workers exposed to polycyclic aromatic hydrocarbon (PAH) and the control group

Variable	Group		p
	exposed (N = 52)	control (N = 35)	
Age [years] (M±SD)	48.0±8.0	34.0±10.0	0.057
Body mass index (BMI) (M±SD)	24.7±3.5	24.0±3.3	0.353
Smoking status [%]			
non-smokers	43.8	71.4	0.012*
smokers	56.2	28.6	0.030*
Alcohol consumption [%]			
yes	55.0	4.6	0.007*
no	43.3	95.4	
Working history[a] [years] (M)	6.5	10.5	
Education [%]			
elementary school	3.2	0.0	
junior high school	22.6	0.0	
high school	34.7	29.2	
college	39.5	43.2	
post-college	0.0	27.6	
Semen parameters (M±SD)			
total sperm count [$\times 10^{-6}$/ejaculation]	216.6±96.7	309.10±101.30	0.231
concentration [$\times 10^{-6}$/ml]	113.7±99.6	121.90±102.70	0.790
motility [% motile]	55.9±20.6	67.50±17.80	0.056
vitality [% viable]	72.4±19.4	82.10±13.30	0.068
morphology [% normal]	15.3±6.8	30.90±5.60	0.032
Urinary 1-hydroxypyrene (1-OHP) [µg/g of creatinine] (M±SD)	8.6±11.2	0.24±0.67	0.007*

M – mean; SD – standard deviation.
[a] Length of time of employment at the steel plant.
* $p < 0.05$.

Table 2 summarizes the status of sperm DNA integrity. For DNA fragmentation, the exposed group had 36.32% of sperm positive for DNA fragmentation, which was not significantly higher than 30.4% of sperm positive for DNA fragmentation in the control group ($p = 0.232$). Bulky DNA adducts were detected both in the exposed workers and in the control subjects. The mean level of bulky DNA adducts was 74.72 in 10^9 nucleotides in the exposed group, which was significantly higher than that in the control group (26.05 in 10^9 nucleotides) ($p = 0.009$), while controlling for smoking, alcohol consumption and age. The mean value of 8-oxo-dGuo for the exposed group was 24.29/10^6 dG as compared to the control with 12.14/10^6 dG. A significantly increased 8-oxo-dGuo

Table 2. Sperm deoxyribonucleic acid (DNA) integrity of the coke-oven workers exposed to PAH and the control group

Sperm DNA integrity	Group (M±SD)		p[a]
	exposed (N = 52)	control (N = 35)	
DNA fragmentation [%]	36.32±22.36	30.40±8.59	0.232
DNA denaturation [%]	11.28±10.34	9.39±8.90	0.329
8-oxo-dGuo/10^6 dG [n]	24.29±19.89	12.14±8.05	0.050*
Bulky DNA adducts/10^9 nucleotides [n]	74.72±31.29	26.05±12.93	0.009*

8-oxo-dGuo – 8-oxo-7,8-dihydro-2'-deoxyguanosine; dG – deoxyguanosine.
Other abbreviations as in Table 1.
[a] The p values indicated the comparison between the 2 groups.
* $p \leq 0.05$.

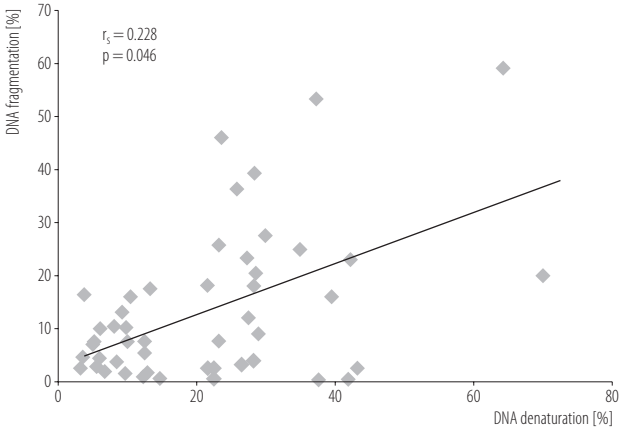
Table 3. Pearson's correlation between routine semen parameters and sperm DNA integrity of the coke-oven workers exposed to PAH and the control group[a]

Sperm DNA integrity	Conventional semen quality (Pearson's coefficient (p))*			
	concentration	motility	vitality	morphology
DNA fragmentation	0.03 (0.79)	–0.19 (0.09)	–0.18 (0.09)	–0.07 (0.54)
DNA denaturation	0.05 (0.87)	–0.23 (0.12)	–0.26 (0.14)	–0.14 (0.64)
8-oxo-dGuo	–0.05 (0.68)	–0.18 (0.12)	–0.14 (0.25)	–0.01 (0.92)
Bulky DNA adducts	–0.10 (0.59)	–0.17 (0.34)	–0.24 (0.30)	–0.09 (0.20)

Abbreviations as in Table 1 and 2.
[a] Smoking, age, and drinking status were controlled in the correlation analysis.
* $p < 0.05$ as a significant level.

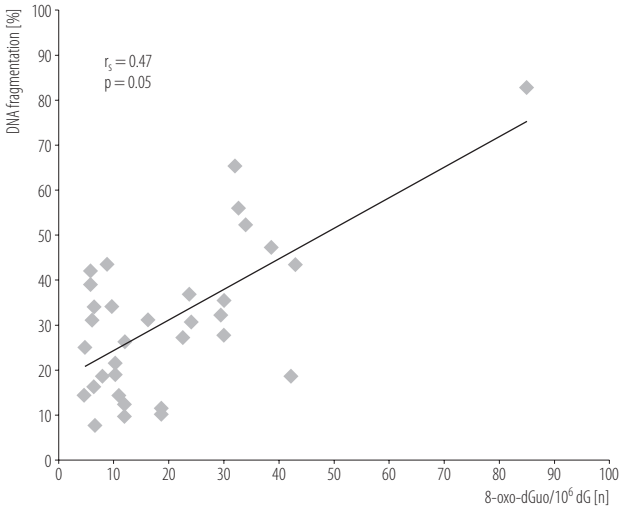
level was detected in the exposed as compared to the control group (p = 0.05).

Table 3 shows that DNA fragmentation, denaturation, 8-oxo-dGuo and DNA adduct did not correlate with semen parameters, including concentration, motility, morphology and vitality, when adjusting for smoking status, age and drinking. There was a positive correlation between the percentages of DNA fragmentation as determined using the TUNEL assay and the percentage of DNA denaturation as determined using the SCSA assay (p = 0.046) (Figure 1). In addition, the percentages of DNA fragmentation and 8-oxo-dGuo correlated significantly (p = 0.05 (Figure 2). However, no correlation existed between DNA fragmentation and bulky DNA adducts (p = 0.08) (Figure 3).



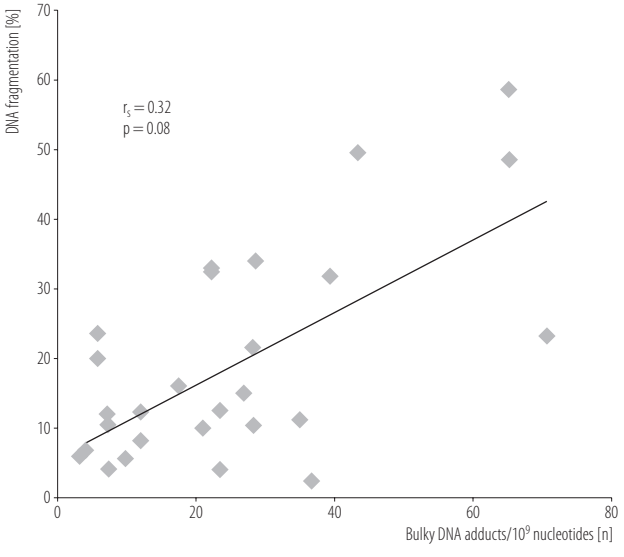
r_s – Spearman's r correlation coefficient.
Other abbreviations as in Table 1 and 2.

Fig. 1. Correlation between the DNA fragmentation and DNA denaturation of sperm from the coke-oven workers exposed to PAH and the control group



Abbreviations as in Table 1 and 2 and Figure 1.

Fig. 2. Correlation between DNA fragmentation and 8-oxo-dGuo/10^6 dG of sperm from the coke-oven workers exposed to PAH and the control group



Abbreviations as in Table 1 and 2 and Figure 1.

Fig. 3. Correlation between the DNA fragmentation and bulky DNA adducts in sperm from the coke-oven workers exposed to PAH and the control group

DISCUSSION

To our knowledge, this is the first occupational exposure study simultaneously employing DNA fragmentation, denaturation, bulky DNA adducts and 8-oxo-dGuo for the purpose of examination of sperm nuclear integrity in relation to exposure to PAHs. Although the coke oven workers self-reported using personal protection equipment (PPE) during working hours, our study showed the existing PPE practice of the workers did not reduce PAH exposure to the degree that protects the workers from an impact on sperm DNA integrity. The study highlights the need to examine whether:

- coke oven workers properly use PPE,
- the PPE is sufficient to reduce PAH exposure,
- job rotation is needed to minimize workers' exposure to PAHs.

The coke oven workers had only 15% normal morphology as compared to the control group with their 35%. Despite the significant reduction, majority of the coke oven workers met the WHO strict criteria for an acceptable level of sperm morphology, which is > 14% normal morphology. When comparing the sperm quality with DNA integrity, we observed no correlations between sperm quality parameters (concentration, motility, vitality, and morphology) and DNA integrity (DNA fragmentation, bulky DNA adducts, and 8-oxo-dGuo). Other studies have observed similar findings and suggested that the routine sperm quality analysis may be insufficient to assess integrity of sperm genome [14,15].

The study indicated that the sperm genome was the target of the effect of PAHs on the male reproductive system. We found that the workers exposed to PAHs experienced an increased mean percentage of DNA fragmentation measured by the TUNEL assay as compared to the control subjects, but the increased level did not reach the significance level when controlling for smoking, alcohol consumption and age. Deoxyribonucleic acid denaturation *in situ* measured by SCSA followed a similar pattern. Our results contrasted with the observations of Hsu et al.'s study, which has concluded that smoking augmented the effect of PAH exposure on DNA fragmentation [1].

Synergetic effect from smoking on PAHs could depend on the number of consumed cigarettes. Potts et al.'s study has reported that smoking ≥ 10 cigarettes/day could induce DNA damage [30]. In our study, although the exposed group had a significant number of workers who smoked, the average number of cigarettes consumed (N = 4.5 cigarette/day) was much lower than the level suggested in the Potts et al.'s study. Further studies may examine the potential role of cigarette smoking as related to the impact of PAHs on the genetic integrity of human sperm by considering the number of cigarettes smoked, duration and urinary nicotine.

The mean levels of bulky DNA adducts and 8-oxo-dGuo in sperm were significantly higher in the individuals exposed to PAHs than in those who weren't exposed. Evidence from this study suggests that exposure to PAHs increases the level of bulky DNA adducts in sperm. At the same time, bulky DNA adducts were detected in the control group. That indicates that other genotoxic chemicals from other sources, e.g., second-hand smoking and air pollution, may also contribute to DNA adduct formation [31]. For example, steroid estrogens and their metabolites have been reported to bind covalently to DNA and form bulky DNA adducts that can be detected by ^{32}P-postlabeling [32].

The 8-oxo-dGuo adducts have been successfully used to assess oxidative damage in tissues and cells. In the present study, we employed ESCODD recommended DNA isolation procedures and our newly established LC-MS/MS with an on-line solid-phase extraction method for detecting 8-oxo-dGuo adducts in sperm. Such a combination of methods minimized oxidation of sperm DNA during extraction and analytical detection procedures. Also, the method was very specific, sensitive and required a relatively small amount of sperm DNA at 10 μg. We observed that the coke oven workers had significantly higher 8-oxo-dGuo levels than the control subjects ($p = 0.048$). The results suggested that exposure to PAHs is associated with oxidative DNA damage. Also, the levels of 8-oxo-dGuo significantly and positively correlated with the levels of DNA fragmentation. To date, the sources contributing to DNA strand breakage have not been fully explored. However, this study provided evidence that 8-oxo-dGuo may be associated with DNA fragmentation in sperm. This result was similar to that reported in other studies suggesting an association between the 2 types of DNA damage and supporting the notion that reactive oxygen species (ROS) can reach DNA nucleotides leading to DNA fragmentation [5,19,33]. The proposed mechanism for ROS induced DNA fragmentation is such that formation of 8-oxo-dGuo leads to the creation of basic sites and destabilization of the DNA backbone [30]. DNA fragmentation was found to discrete into smaller fragments in the 20–25 kb range rather than much larger 50-kb toroid fragments detected by other studies [3,34]. Increased 8-oxo-dGuo and bulky DNA adducts in sperm suggested that PAHs probably used metabolic pathways, including phase I metabolism, to form active meditates, and redox cycling to form excessive reactive oxygen species. A limitation of this study is the lack of identification of specific bulky DNA adducts and reactive oxygen species in relation to the possible metabolic pathways. Another limitation is that the study included one semen sample that may not fully depict the impact of PAH exposure during the entire period of spermatogenesis. Nonetheless, the study demonstrated that exposure to PAHs could induce oxidative damage to sperm DNA and covalently form DNA adducts. Sperm cells are resistant to exogenous genotoxic factors due to the blood–testis barrier and the compact nature of sperm chromatin, which can effectively restrict DNA reacting compounds and minimize DNA adduct formation. The observed increase in bulky DNA levels in this study raises 2 possibilities for the findings: PAHs could pass through the blood–testis barrier and/or ineffective repair of DNA damage occurred during early stages of spermatogenesis.

CONCLUSIONS

The current study thoroughly examined the status of DNA integrity by measuring DNA fragmentation, DNA oxidative damage and DNA adducts. In doing so, the study adds to the existing literature by showing sperm genomes were the target of PAH effect. Our study underscored the importance of monitoring sperm DNA integrity as part of an assessment of the impact of occupational and environmental exposures on sperm. Detecting such DNA damage in sperm could provide new elements, besides sperm quality analysis, in assessing environmental toxins' effect on male reproductive health. Sperm DNA integrity is crucial for the accurate transmission of paternal genetic information to the offspring. However, our study results suggest that additional research is needed to better understand the impact of sperm DNA integrity in relation to overall male reproductive capacity and the health of offspring. We hope that the current study will encourage further research in this area.

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