Effect of smoking on blood lead levels in workers and role of reactive oxygen species in lead-induced sperm chromatin DNA damage

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Objective: To investigate whether cigarette smoking affects the blood lead levels (BLL) and whether exposure to lead introduces sperm chromatin DNA damage in factory workers.

Design: Cross-sectional study.

Setting: A battery plant in Taiwan.

Patient(s): Eighty male workers employed within a battery plant.

Intervention(s): Standard semen analysis was performed according to the World Health Organization guidelines.

Main Outcome Measure(s): Assessment of BLL, sperm chromatin DNA structure, reactive oxygen species generation and other conventional parameters of semen quality.

Result(s): As compared with nonsmoking workers, the BLL were found to be considerably higher among smokers. Statistically significant differences were found in the sperm DNA denaturation (α T) induction and the percentage of sperm with increased DNA denaturation (COMP α T) in workers with moderate BLL ($\geq 25 \mu\text{g}/\text{dL}$). After adjustment for smoking propensity, a positive correlation was discernible between BLL and α T, COMP α T, and morphologic abnormality. Furthermore, α T and COMP α T were also found to have positive correlations with sperm superoxide anion production.

Conclusion(s): Workers with higher BLL were found to be at a higher risk of sperm morphologic abnormality and chromatin DNA integrity. These data are significant because they can facilitate the estimation of lead exposure in reproductive toxicology. (Fertil Steril® 2009;91:1096–103. ©2009 by American Society for Reproductive Medicine.)

Key Words: Lead exposure, smoking, blood lead levels, sperm chromatin DNA

Lead (Pb) is a ubiquitous environmental and industrial pollutant that has been detected in virtually all phases of environmental and biological systems. The occupational exposure of a father just before conception is thought to increase the risk of cancer in his offspring through toxic inheritance (1). One of the causal mechanisms for such an association is thought to be that occupational exposure may bring about a genetic change in the father's sperm, which may ultimately affect the susceptibility of his children to cancer. Although numerous studies have indicated that lead does generally

seem to be genotoxic in mammalian cells (2, 3), epidemiologic information on the genotoxic effects in germ cells has been very limited; indeed, it remains a mystery as to whether lead is carcinogenic to humans.

During the later stages of mammalian spermatogenesis, chromatin structure is found to be completely reorganized through a series of sequential steps that result in the removal of nucleosomal histones and their replacement with small, arginine-rich protamines (4). Lead did not appear to have any association with the sperm oxidative DNA damage when the concentration of lead in seminal plasma was below $1.0 \mu\text{g}/\text{dL}$ (5). However, although much work has been undertaken to determine the clastogenicity of lead compounds through the investigation of the chromosomal aberrations in lymphocytes of workers exposed to lead (6–8), there remains a lack of precise data to facilitate the study of the genotoxic effects on sperm chromatin.

The structure of chromatin in the sperm nucleus is a relevant factor for reproductive toxicology essentially because

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there is the potential for chromatin damage anywhere along the male reproductive tract, thereby compromising male fertility and offspring development. Indeed, several studies have reported that alterations in sperm chromatin conformation may diminish human fertility (9–11). Sperm chromatin structure analysis (SCSA) by means of flow cytometry has become a useful tool for the evaluation of sperm quality in reproductive studies. This is a technique that has been widely used in epidemiologic studies for the identification of potential reproductive hazards (12, 13).

Excessive reactive oxygen species (ROS) generation may prove to be an important mediator of the damage to cell structures, including lipids and membranes, proteins, and nucleic acids (14); indeed, there is growing evidence to suggest that transition metals, particularly iron and copper, can produce ROS, which result in lipid peroxidation, DNA damage, and depletion of the cell antioxidant defense systems. However, the mechanisms that cause lead to induce oxidative stress on sperm DNA damage are not completely understood.

To learn more about the potential relationship between lead exposure and genetic risk in germ cells, we investigated the alterations in sperm chromatin structure and conventional semen quality observed in workers exposed to lead in a battery plant in Taiwan, with blood lead levels (BLL) as the biomarkers of lead exposure. We began by examining the percentage of sperm associated with the excessive production of superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), which was determined as ROS generation. This was then followed by analysis of the correlations among BLL, sperm chromatin DNA integrity, conventional semen quality, and sperm ROS generation.

MATERIALS AND METHODS

Patients

Our study sample comprised a population of 167 male workers employed in a battery plant in Taiwan. A total of 80 (48%) men agreed to participate. Our experimental protocol was approved by the institutional review board at the National Cheng Kung University Medical Center Human Subjects Committees. All of the participants were fully aware of the entire experimental procedure, and they were also very clear as to their right to withdraw; all participants signed a written consent form and agreed to provide semen samples for this study. All of the men indicated that they were not taking any medications and that they had not been exposed to any substances that were known to influence the study variables.

Background information on all participants was collected by individual questionnaire interviews, with the questions covering occupational, medical, and reproductive history, alcohol and tobacco use, propensity for hot baths, and marital status. Workers who indicated they consumed beer, wine, liquor, or any other alcoholic beverage at least once a week for the past 12 months were defined as “current drinkers.” None of the participants reported any urologic or andrologic

disorders during the interviews, including history of testicular injury, surgery, or cryptorchidism. Similarly, the reports provided by the annual health examinations on the study participants did not reveal any physician-diagnosed urologic or andrologic disorders.

A Perkin-Elmer model 5100 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT) equipped with an HGA-600 graphite furnace and a deuterium arc background corrector was used to determine blood lead levels, with a 283.3-nm absorption wavelength being adopted. Blood lead standards included bovine whole blood certified materials at concentration between 1 and 100 $\mu\text{g/dL}$. The r^2 of the calibration curve was at least above 0.995. An external quality assurance/quality control (QA/QC) program was used to ensure the accuracy and consistency of BLL; the QA/QC program was accepted by the Institute of Occupational Safety and Health of Taiwan (15, 16).

Analysis of Semen Quality

Semen samples were collected by masturbation in the week after the collection of the blood samples. Participants were instructed to abstain from ejaculation for at least 3 days before their appointment to provide semen specimens. A volume of 10 μL of semen was held in a Makler chamber (Sefi Medical Instruments, Haifa, Israel) for sperm count and motility measurement.

Sperm morphologic characteristics were subsequently evaluated on air-dried smears stained with trypan blue in a 10% (v/v) formaldehyde solution and scored at $\times 1000$ magnification under a light microscope. At least 400 sperm per sample were categorized as normal (or abnormal) according to the presence (or absence) of head, neck, and tail as well as any defects in terms of immaturity. Sperm count, morphology, and motility were evaluated in accordance with World Health Organization guidelines (17). All interviews, examinations, and laboratory tests were performed in a blinded fashion.

Within 1 hour of collection, an aliquot of semen was diluted with at least 1:1 human tubule fluid medium to obtain a concentration suitable for computer-assisted semen analysis (CASA), were loaded into the Makler chamber, and were videotaped with the use of negative phase-contrast microscopy. The videotapes were analyzed later for sperm motion with a Hamilton Thorne Research motility analyzer (version HTM-IVOS Specification, Beverly, MA) at a temperature of 37°C.

The computer-assisted semen analysis was performed for the assessment of sperm motility under the following parameters: [1] the velocity of a cell along the original track (curvilinear velocity, VCL); [2] the velocity along a smoothed path (average path velocity, VAP); [3] the velocity measured along the direct path between two points (straight-line velocity, VSL); [4] the frequency of alternating track direction (beat cross-frequency, BCF); and [5] the average amplitude of the lateral head displacement (ALH).

Sperm Chromatin Structure Assay (SCSA)

The flow cytometry SCSA described by Evenson et al. (18) detects the susceptibility of sperm to in situ acid denaturation of DNA. Briefly, the samples were treated for 30 seconds with 400 μL of a solution of 0.1% Triton X-100, 0.15 M NaCl, and 0.08 N HCl, pH 1.2. After 30 seconds, 1.2 mL of staining buffer was added to the test tube and analyzed by flow cytometry. Excitation with a 488-nm wavelength light causes the acridine orange, intercalated into the double-stranded DNA, to emit a green fluorescence (515–530 nm), whereas single-stranded DNA fluoresces red (≥ 630 nm).

A minimum of 7000 cells in each sample were analyzed by flow cytometry (Becton Dickinson, San Jose, CA) with a data handler (CellQuest software program, Becton Dickinson). The extent of DNA denaturation per cell was quantified as alpha-T (αT), expressed as a ratio, or percentage, of the quantity of (red/[red + green]) fluorescence reported on an expanded scale from 0 to 1000. The percentage of sperm cells falling outside the main population (COMP αT) reflects the percentage of cells with increased sensitivity to denaturation as compared with the cells characterizing that sample.

Sperm ROS Generation

The sperm superoxide anion (O₂^{•-}) level was measured using a modification of a method previously described elsewhere (19). Hydroethidine (HE) is rapidly oxidized by O₂^{•-} to yield ethidium, which intercalates into DNA. The HE stock, which dissolves in dimethyl sulfoxide DMSO at 0.33 mM, can be directly oxidized into ethidium bromide by the O₂^{•-} produced by sperm. The spermatozoa (1 × 10⁶) were incubated in 1 mL of buffer containing HE dye (2 μM) for 5 minutes. The sperm hydrogen peroxide (H₂O₂) level, on the other hand, can be measured in most cells through the use of a fluorescent probe, 2',7'-dichlorofluorescein diacetate (DCFH-DA). The DCFH-DA probe is a stable dye that passively diffuses into cells and is hydrolyzed by intracellular esterase to yield DCFH, which is trapped inside cells. The H₂O₂ produced by cells oxidizes DCFH to the highly fluorescent compound 2',7'-dichlorofluorescein (DCF) with a green color.

In this study, the H₂O₂ level in the sperm was evaluated by modifying the method proposed by Royall and Ischiropoulos (20). The spermatozoa (1 × 10⁶) were incubated in 1 mL of buffer containing DCFH-DA dye (12.5 μM) for 5 minutes. The mixture was set aside and maintained at 34°C for 30 minutes. Intracellular O₂^{•-} and H₂O₂ levels were measured using flow cytometry. The excitation wavelength for DCF and ethidium fluorescence was 488 nm, DCF emission was 530 nm, and ethidium emission was 630 nm. The percentage of red or green fluorescent spermatozoa revealed the respective percentages of sperm with excessive O₂^{•-} or H₂O₂ production.

Statistical Analysis

The values were expressed as mean ± standard deviation (SD). The men's BLL were compared in relation to the vari-

ables of "cigarette smoking" and "alcohol drinking" using Student's *t*-test or one-way analysis of variance (ANOVA). The comparisons between the semen quality of the low, moderate, and high BLL groups and the semen quality in relation to the number of cigarettes smoked per day were undertaken by the ANOVA method, followed by the Tukey test. Multiple linear regression models were then used to examine the relationship between BLL and semen parameters, after adjusting for smoking propensity.

To determine the relationship between sperm ROS generation and sperm chromatin DNA integrity, we applied a linear regression analysis on the sperm O₂^{•-} and H₂O₂ production levels and on the levels of αT and COMP αT. Analysis of the data was undertaken using the JMP statistical package (SAS Institute, NC). All *P* values were two-sided.

RESULTS

Demographic Data and BLL

The descriptive statistics on the study population in terms of propensity for cigarette smoking and alcohol drinking are provided in Table 1. The background characteristics of the participants and nonparticipants were similar. The men were routinely exposed to lead in their workplace; the average length of exposure to lead was 1.7 years, and the average BLL was 40.2 μg/dL. Approximately 52% of the study participants reported that they were current smokers, and 83% were current drinkers. As shown in Table 2, when the workers were divided into smoking and nonsmoking groups, the BLL of the 42 smokers was 43.0 ± 13.1 μg/dL, a level that was statistically significantly higher than the 37.1 ± 11.9 μg/dL BLL for the 38 men in the nonsmoking group (*P* = .042).

TABLE 1		
Characteristics of study participants and nonparticipants.		
Parameters	Participants (n = 80)	Nonparticipants (n = 87)
Age (years)	29.2 ± 3.9	33.2 ± 5.2
Body height (cm)	166.3 ± 4.5	168.5 ± 6.6
Body weight (kg)	60.7 ± 6.5	63.2 ± 7.1
Body mass index (kg/m ²)	21.9 ± 2.1	22.3 ± 2.8
Working duration (years)	1.7 ± 0.7	2.1 ± 1.3
Blood lead levels (μg/dL)	40.2 ± 12.8	(no data)
Cigarette smoking (% , Y/N)	52.5% (42/38)	48.3% (42/45)
Alcohol drinking (% , Y/N)	82.5% (66/14)	78.2% (68/19)

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TABLE 2

Blood lead levels ($\mu\text{g/dL}$) in relation to the variables of cigarette smoking and alcohol consumption.

Parameters	Mean $\pm$ SD	P value
Cigarette smoking		
Yes (n = 42)	43.0 $\pm$ 13.1 ^c	.042 ^a
No (n = 38)	37.1 $\pm$ 11.9	
Number of cigarettes smoked per day		
1–5 cigarettes per day (n = 21)	38.8 $\pm$ 12.5	.006 ^b
6–10 cigarettes per day (n = 14)	42.6 $\pm$ 9.3	
>10 cigarettes per day (n = 7)	56.4 $\pm$ 13.9 ^{d,e}	
Current drinkers ^f		
Yes (n = 66)	40.7 $\pm$ 12.6	.387 ^a
No (n = 14)	37.5 $\pm$ 13.2	

^a By Student's *t*-test.

^b By one-way analysis of variance (ANOVA).

^c $P < .05$ as compared with nonsmoking group.

^d $P < .05$ as compared with 1 to 5 cigarettes per day group.

^e $P < 0.05$ as compared with 6 to 10 cigarettes per day group.

^f Consumed beer, wine, liquor, or any other alcoholic beverage at least once a week for the past 12 months.

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A positive association was also observed in this study between the BLL and the total number of cigarettes smoked per day. The mean BLL for the seven men who consumed the highest quantities of cigarettes per day (>10 cigarettes per day) was $56.4 \pm 13.9 \mu\text{g/dL}$. Compared with the men consuming 6 to 10 cigarettes per day ($42.6 \pm 9.3 \mu\text{g/dL}$) and 1 to 5 cigarettes per day ($38.8 \pm 12.5 \mu\text{g/dL}$), the difference was statistically significant ($P = .006$). Conversely, there were no statistically significant differences between the BLL for the 66 alcohol drinkers and the 14 nondrinkers.

Semen Quality Between the Three BLL

To better visualize the relationship between BLL and semen quality outcomes, we categorized the workers into three groups in accordance with the average level ($25 \mu\text{g/dL}$) from the lowest level suggested by CDC for the toxic effects of lead ($10 \mu\text{g/dL}$) and the BLL biological exposure index used in Taiwan ($40 \mu\text{g/dL}$ for males). These values are presented in Table 3, which shows the three groups corresponding to low-level exposure ($<25 \mu\text{g/dL}$), moderate-level exposure ($25\text{--}45 \mu\text{g/dL}$), and high-level exposure ($>45 \mu\text{g/dL}$). The respective numbers of workers in each group were 14, 38, and 28.

As the table shows, as compared with those with low-level and moderate-level exposure, there was a statistically significant increase in the percentage abnormality of sperm morphology and head abnormality among those with high-level exposure ($P < .05$). The data on the sperm chromatin DNA structure assay indicated a statistically significant increase in the levels of αT and COMP αT among those with moderate-level and high-level exposure as compared with those with low-level exposure ($P < .05$). However, no statistically significant differences were found in semen volume, sperm count, motility, velocity, or percentage of sperm with excessive $\text{O}_2^{\cdot-}$ or H_2O_2 production among the three BLL groups.

Sperm Chromatin DNA Damage and Occupational Lead Exposure

To clarify the effect of lead exposure on sperm quality, the correlation between BLL and semen quality parameters was calculated. The results, after adjusting for smoking propensity, are reported in Table 4. Both before and after adjustment for the propensity for smoking, the percentages of sperm with morphologic abnormality, head abnormality, αT , and COMP αT were found to have positive correlations with BLL. After adjusting for smoking propensity, a 10-fold increase in BLL was found to be associated with [1] a 14.7-fold increase in αT ; [2] a 2.7-fold increase in the percentage of sperm with morphologic abnormality; [3] a 2.4-fold increase in head abnormality; and [4] a 2.3-fold increase in COMP αT . However, no statistically significant correlation was found between BLL and semen volume, sperm count, motility, velocity, or the percentage of sperm with excessive $\text{O}_2^{\cdot-}$ or H_2O_2 production either before or after adjusting for the subjects' propensity for smoking.

Correlation Between Sperm ROS Generation and DNA Damage

The percentage of sperm with excessive $\text{O}_2^{\cdot-}$ production was found to be highly correlated with αT ($r = 0.47$; $P < .0001$), as illustrated in Figure 1A, and COMP αT ($r = 0.35$; $P = .0013$), as illustrated in Figure 1B; however, no statistically significant correlation was found between the percentage of sperm with excessive H_2O_2 production and the semen quality parameters (data not shown).

DISCUSSION

In this study, workers with a propensity for smoking were found to have statistically significantly higher BLL than their nonsmoking counterparts. The greater the quantity of cigarettes smoked per day, the higher the BLL found among the smoking workers. However, the main finding of this study is that a statistically significant association exists between BLL and the number of spermatozoa showing greater susceptibility to DNA denaturation and morphologic abnormality in workers exposed to lead. Furthermore, a positive correlation is identified between sperm chromatin DNA denaturation and the percentage of sperm with excessive $\text{O}_2^{\cdot-}$ production.

TABLE 3

Comparisons of semen quality from three blood lead levels (BLL) in 80 battery plant workers.

Parameters	Blood lead levels ($\mu\text{g/dL}$)			P value
	< 25 (n = 14)	25–45 (n = 38)	> 45 (n = 28)	
BLL mean (min/max)	21.3 (10.5/24.9)	37.3 (26.9/45.0)	53.3 (45.7/70.9)	
Semen volume (mL)	2.3 $\pm$ 1.2	2.2 $\pm$ 1.2	2.4 $\pm$ 1.4	.771
Sperm count ($10^6/\text{mL}$)	50.5 $\pm$ 41.4	78.5 $\pm$ 63.0	58.5 $\pm$ 48.9	.184
Motility (%)	43.6 $\pm$ 22.9	48.7 $\pm$ 18.8	44.5 $\pm$ 19.1	.598
Morphologic abnormality (%)	30.4 $\pm$ 6.2	34.4 $\pm$ 7.7	39.4 $\pm$ 6.9 ^{a,b}	< .001
Head abnormality (%)	9.7 $\pm$ 5.8	12.3 $\pm$ 7.2	18.1 $\pm$ 6.7 ^{a,b}	< .001
Neck abnormality (%)	3.8 $\pm$ 2.8	3.7 $\pm$ 2.2	4.1 $\pm$ 2.4	.812
Tail abnormality (%)	9.4 $\pm$ 6.3	12.6 $\pm$ 9.2	11.9 $\pm$ 8.3	.480
Immaturity (%)	7.5 $\pm$ 1.6	5.8 $\pm$ 3.9	5.3 $\pm$ 3.5	.143
Computer-assisted semen analysis (CASA)				
VCL ($\mu\text{m/s}$)	97.7 $\pm$ 29.9	90.5 $\pm$ 26.7	100.1 $\pm$ 29.2	.384
VAP ($\mu\text{m/s}$)	49.7 $\pm$ 16.6	46.5 $\pm$ 16.9	54.3 $\pm$ 20.8	.248
VSL ($\mu\text{m/s}$)	36.1 $\pm$ 15.0	34.0 $\pm$ 15.0	41.9 $\pm$ 21.6	.209
ALH (μm)	6.1 $\pm$ 3.0	5.4 $\pm$ 4.0	4.9 $\pm$ 3.0	.604
BCF (Hz)	37.1 $\pm$ 15.3	40.1 $\pm$ 14.4	38.4 $\pm$ 12.5	.782
Sperm chromatin structure assay (SCSA)				
αT	429.4 $\pm$ 58.4	480.6 $\pm$ 62.8 ^a	488.0 $\pm$ 60.4 ^a	.012
COMP αT (%)	65.2 $\pm$ 8.9	77.0 $\pm$ 11.8 ^a	75.6 $\pm$ 9.2 ^a	.002
Percentage of sperm with reactive oxygen species (ROS) production				
$\text{O}_2^{\cdot-}$ (%)	70.3 $\pm$ 10.4	75.3 $\pm$ 15.7	78.2 $\pm$ 9.9	.188
H_2O_2 (%)	7.8 $\pm$ 13.4	8.6 $\pm$ 17.3	8.9 $\pm$ 21.7	.983

Abbreviations: αT : the extent of DNA denaturation per cell; ALH: amplitude of lateral displacement for all sperm after adjustment; BCF: beat frequency for all sperm after adjustment; COMP αT : the percentage of sperm with increased sensitivity to DNA denaturation; VAP: average path velocity for all sperm after adjustment; VCL: curvilinear velocity for all sperm after adjustment; VSL: straight-line velocity for all sperm after adjustment.

^a $P < .05$ as compared with low exposure group (<25 $\mu\text{g/dL}$).

^b $P < .05$ as compared with moderate exposure group (25–45 $\mu\text{g/dL}$).

Hsu. Lead exposure and sperm chromatin DNA damage. *Fertil Steril* 2009.

The present study clearly demonstrates the statistically significant differences in BLL between smokers and non-smokers, providing strong support for the results reported in previous studies (21–23). Tobacco plants, like many other plants, will invariably contain certain amounts of lead absorbed from the soil; however, lead may also be deposited on the surface of the leaves. The widespread use of lead arsenate as a pesticide on tobacco crops has, in the past, resulted in lead contamination of tobacco products. The 80 workers at the factory fell into various job categories, including battery assembly, analysis, packaging, and lead cutting; and, in addition to occupational exposure, smoking may be another major source of lead exposure. Chuang et al. (24) reported that, as health promotion programs generally reduced worker exposure to lead, the greatest reduction in lead exposure was achieved as a result of banning smoking within the workplace.

Many other studies have also demonstrated that the poor personal hygiene habits among workers, such as smoking

and food consumption in the workplace, as well as body and clothing-mediated contamination, may represent a substantial contribution to the overall uptake of lead through inadvertent contact and ingestion (25–27). Of all of the methods of uptake, lead contamination of the mouth and hands has been recognized as the most immediate source for exposed workers (25). In this study, we have found a positive association between the BLL of workers and the number of cigarettes smoked per day, which suggests a strong association between lead absorption and smoking, either from smoking lead-contaminated cigarettes or as a result of touching the lips with contaminated hands. Hence, from a practical standpoint, lead-handling workers should be advised to improve their hygiene by refraining from smoking in the workplace, regularly washing their hands and face, and bathing immediately after their day's work.

Several epidemiologic studies have demonstrated that lead may induce DNA strand breaks in the lymphocytes of

TABLE 4

Correlation of blood lead levels with sperm morphology and chromatin DNA integrity characteristics among battery plant workers before and after adjusting for smoking status.

Parameters	Crude analyses		Adjusted analyses	
	β (SE β)	P values	β (SE β)	P values
Morphologic abnormality (%)	0.294 (0.061)	<.0001	0.271 (0.062)	<.0001
Head abnormality (%)	0.255 (0.060)	<.0001	0.237 (0.066)	.0002
α T	1.366 (0.544)	.014	1.469 (0.560)	.011
COMP α T (%)	0.232 (0.096)	.017	0.233 (0.099)	.021

Abbreviations: α T: the extent of DNA denaturation per cell; ALH: amplitude of lateral displacement for all sperm after adjustment; COMP α T: the percentage of sperm with increased sensitivity to DNA denaturation.

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exposed workers (6–8). Although such data suggest potential genotoxicity to lymphocytes, it is not clear whether occupational exposure to lead affects sperm chromatin DNA structure, and if so, by what mechanisms. Foster et al. (28) reported that chronic lead exposure, at a mean BLL of 56 μ g/dL, resulted in the alteration of sperm chromatin structure in cynomolgus monkeys. Our study used SCSA to track the changes in sperm chromatin as a result of lead exposure, essentially because all of its parameters are assumed to reflect abnormalities in the mature sperm chromatin structure. Our results indicate that, after adjusting for smoking propensity, mean α T and COMP α T values were associated with BLL, with a 10-fold increase being linked to a 14.7-fold increase in α T and a 2.3-fold increase in COMP α T, thereby indicating that chromatin condensation was altered and suggesting that protamines may be molecular targets for lead. In addition, lead might reach the sperm nucleus in the epididymis by binding to nuclear sulfhydryl groups from the DNA-protamine complex, increasing sperm chromatin condensation and interfering with the sperm maturation process (29). It therefore seems likely that such altered chromatin structure would be reflected in an increased number of cells with greater susceptibility to DNA denaturation.

The increased susceptibility of sperm to lead-induced denaturation has been found to have correlations with both semen quality and infertility in mammals (11). The increased sensitivity of sperm DNA to in situ denaturation has also been strongly correlated with DNA strand breaks (30, 31) and lower nuclear maturity (32). This suggests that sperm DNA fragmentation has relevance to sperm quality, fertility potential, and proper genome transfer.

Our study found that the percentage of sperm with excessive $O_2^{\cdot -}$ production was highly correlated with α T and COMP α T. The primary product of the immature spermatozoa system of generating free radicals appears to be $O_2^{\cdot -}$, which has secondary dismutation to H_2O_2 through the catalytic action of superoxide dismutase (33). The ability to

generate $O_2^{\cdot -}$ has been linked to the presence of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-like activity (34, 35); Said et al. (36) reported a model of ROS induction in which it was clear that the exogenous addition of a high amount of NADPH caused early DNA strand breaks in immature sperm.

The presence of higher DNA damage in immature sperm fractions that exhibit a higher rate of cytoplasmic residues may be attributable to high endogenous NADPH oxidase activity; these events are possibly attributable to the presence of the increased $O_2^{\cdot -}$ production, presumably caused by sperm chromatin DNA damage. Although animal studies have suggested that sperm ROS generation was significantly higher among lead-exposed rats (37–39), our study found no statistically significant association between BLL and the percentage of sperm with excessive $O_2^{\cdot -}$ or H_2O_2 production.

The role of heavy metals (Co^{2+} , Mn^{2+} , V^{2+} , Cu^{2+} , Fe^{2+} , Fe^{3+}) in oxidative damage was suggestive of a potential mechanism indicating that lead was in some way involved in the oxidative deterioration of biological macromolecules; however, it is known that lead cannot readily undergo valance changes. Therefore, the role of lead exposure in ROS-mediated sperm chromatin DNA damage, along with the question of whether antioxidant consumption results in reduced sperm ROS generation in lead-exposed workers, still requires further clarification.

Guzick et al. (40) used classification-and-regression-tree analysis to estimate threshold values for subfertility and fertility with respect to the sperm concentration, motility, and morphology in 765 infertile couples and 696 fertile couples. They found the fertile ranges were a concentration of more than 48.0×10^6 sperm/mL, greater than 63% motility, and greater than 12% normal morphologic features. In comparing the sperm parameters to those in the Guzick study, we found only 16.3% of workers' values (sperm count, motility, and morphology) were all well above the "fertile male" values, suggesting that these workers have potential risk of infertility.

FIGURE 1

Relationship between percentage of sperm with excessive superoxide anion production and (A) chromatin DNA denaturation per cell and (B) percentage of sperm with DNA denaturation ($r = 0.47$ and 0.35 ; $P < .0001$ and $P = .0013$, respectively). Observations from three groups of blood lead levels (BLL) are shown by open circles, solid triangles, and solid circles, respectively.

Hsu. Lead exposure and sperm chromatin DNA damage. *Fertil Steril* 2009.

The findings of our study suggest that workers who are routinely exposed to lead within the workplace are at a higher risk of damage to sperm morphology and chromatin DNA integrity. Our results also suggest that cigarette smoking has a clear association with elevated BLL. We therefore propose that BLL can be used as a biomarker for predicting sperm abnormalities because, after adjusting for smoking propensity, we find that BLL is directly related to morphologic abnormality as well as αT and COMP αT levels.

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