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Correlation between seminal lead and cadmium and seminal parameters in idiopathic oligoasthenozoospermic males

Emad A. Taha¹, Sohair K. Sayed², Nagwa M Ghandour³, Ali M. Mahran¹, Medhat A. Saleh⁴, Magdy M. Amin⁵, Rany Shamloul⁶

¹Departments of Dermatology & Andrology; ²Clinical Pathology; ³Forensic Medicine and Clinical Toxicology and ⁴Public Health & Community Medicine, Assiut University, Assiut, Egypt

⁵Department of Obstetrics and Gynecology, Sohag University, Sohag, Egypt

⁶Department of Andrology, Cairo University, Egypt and Department of Urology, University of Ottawa, Canada

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Correspondence

Rany Shamloul
Department of Urology
University of Ottawa
1053, Carling Avenue
Ottawa K1Z8M6, Canada
phone: +1 613 761 45 00
ranyshamloul@gmail.com

Introduction. The exact causes of the decline in semen quality are not yet known, environmental factors have been considered to play an important role. Lead (Pb) and Cadmium (Cd) are two of the well-known reproductive toxicants to which humans are exposed occupationally and environmentally and can lead to negative effects on the testicular functions. The aim of this study was to evaluate lead and cadmium levels in seminal plasma of men with idiopathic oligoasthenozoospermia in comparison to fertile healthy controls and to correlate these levels with conventional semen parameters, sperm hypo-osmotic swelling (HOS) percentage, sperm DNA fragmentation percentage, and semen reactive oxygen species (ROS) levels.

Material and Methods. Thirty infertile male patients with idiopathic oligo and/or asthenozoospermia and thirty healthy fertile men, which was the control group, were included in the study. Lead and cadmium levels in seminal plasma, semen parameters, sperm HOS, sperm DNA fragmentation percentage and semen ROS assay were measured in all subjects.

Results. There was a significant increase in seminal lead and cadmium levels among infertile males in comparison to controls. There were significant negative correlations between seminal lead and cadmium levels on one hand and certain semen parameters especially progressive sperm motility and vitality (HOS). Importantly, significant positive correlations were noted between seminal lead and cadmium levels on one hand and sperm DNA fragmentation percentage and semen ROS level in infertile men and controls on the other hand.

Conclusions. Thus, men with idiopathic male infertility had higher levels of lead and cadmium in their semen which correlated with impairment of sperm motility and vitality percentages and more importantly with higher sperm DNA fragmentation% and semen ROS level.

Key Words: azoospermia ◊ lead ◊ cadmium

INTRODUCTION

Globally, human fecundity appears to be on the decline [1]. The exact causes for the decline in semen quality are not yet known, although environmental factors have been considered to play an important role [2].

Lead (Pb) and Cadmium (Cd) are two of the well-known reproductive toxicants to which humans are exposed occupationally and environmentally [3, 4]. Several studies suggested that the testis and reproductive organs may be exquisitely sensitive to cadmium, which may lead to profound testicular damage and irreversible infertility without affecting any other

organ system [5]. Moreover, cadmium and lead were found to be elevated in the seminal plasma and testes of infertile men with or without varicoceles [6, 7]. Furthermore, exposure to these metals was shown to be associated with oxidative DNA damage in human sperm [3]. Occupational exposure to lead and cadmium has also been associated with impaired semen quality, decreased fertility rates and increased frequencies of spontaneous abortion and male infertility [8]. Most of these studies were based on occupational exposure or in smokers while few studies were concerned with basic environmental exposure, which may have more important and long term impacts on human health,

particularly in a country with non-existent or un-enforced occupational safety and health standards.

Male factors account for nearly half of all infertility cases [9]. The term “idiopathic infertility” designates diagnosis by exclusion, after elimination of all other possible or probable causes of infertility. In idiopathic male infertility, the female partner must be evidently free from any cause of infertility, conventional semen parameters are subnormal, and clinical examination of men reveal no specific etiology, such as varicocele, maldescended testes, male accessory gland infection, or hypogonadism among others [10]. At least half of these cases of idiopathic male infertility may be attributable to various environmental and occupational exposures [10].

This study was undertaken to evaluate the level of lead and cadmium in seminal plasma of men with idiopathic oligo- and/or asthenozoospermia in comparison with fertile controls and to correlate these levels with conventional sperm parameters especially sperm concentration, sperm progressive motility and sperm morphology percentages. Moreover, we tried to clarify the possible relation between these levels and sperm DNA damage, semen ROS levels, and sperm vitality.

MATERIAL AND METHODS

Study design

This is a case control hospital-based study that was conducted during December 2009 to December 2010.

Subject recruitment

Thirty patients with idiopathic male infertility (idiopathic oligo- and/or asthenozoospermia) were recruited randomly from the Andrology Unit of the Dermatology and Andrology Department, Assiut University Hospital. Exclusion criteria included specific genital diseases that may impair reproductive capacity, such as clinical varicocele, genital infection, undescended testis, and testicular atrophy. Patients with other systemic diseases that may impair reproductive capacity, such as hepatic, renal, endocrine, and autoimmune diseases, were also excluded. We meant to exclude all factors that could impair semen quality (e.g. varicocele, genital infection, and undescended testis among others) to highlight the impact of lead and cadmium on semen parameters. Additionally, those with female factor infertility were excluded. Thirty men with proven fertility were considered as controls. Among patients and controls those with special habits (e.g. smoking) or occupational exposure to heavy metals were excluded. The Scientific Research Ethics Committee of Assiut Faculty of Medicine approved the study and all participants signed an informed consent.

History taking and clinical examination

Each participant completed an extensive questionnaire regarding his occupation, residence, social status, and smoking habits. Full detailed medical history was taken from all participants with special emphasis on reproductive history. They were also subject to thorough general medical and genital examination.

Conventional semen analysis

All samples were collected by masturbation in polypropylene containers after three to five days of sexual abstinence. After liquefaction at 37°C, conventional semen analysis was carried out according to WHO's 1999 guidelines [12]. We couldn't use the WHO's 2010 guidelines because most of the patients were included in the study before it was published.

Sperm hypo-osmotic swelling (HOS) test

The hypo-osmotic swelling (HOS) test was performed according to the method described by Jeyendran and associates [13].

Measurement of sperm DNA fragmentation

The sperm DNA fragmentation index was performed on flow-cytometry model PAS DAKO Cytomation by the kit supplied by Coulter (DNA Prep, BECKMANCOULTER Fulterton, CA, USA) based on the fluorescence emission from individual sperm cells after staining with propidium iodide (PI) and excitation with a 488 nm argon laser. The measurement is based upon the ability of PI to bind histochemically to DNA under appropriate staining conditions [14, 15].

Measurement of semen ROS level

ROS levels were measured by detection of the chemiluminescence activity using the luminol (5-amino-2, 3 dihydro-1, 4 phthalazinedione reagent ($C_8H_7N_3O_2$) supplied by MP Biomedicals). Ten milliliters of luminol, used as a probe, was added to the semen pellet. The ROS levels were assessed by measuring chemiluminescence activity with an Autolamat Luminometer (Berthold technologies, Bad-wilbad, Germany) in the integrated mode for 15 minutes. The results were expressed as Relative Light Unit (RLU) per 20 million spermatozoa [16].

Metal analysis

Approximately 1 ml of seminal plasma was digested twice with 5 ml of an acid mixture ($6HNO_3$: $1HClO_4$) in a glass tube. The residue was dissolved in 1 ml of

Table 1. Predicted versus actual 1-year recurrence and progression rates

Semen variable	Infertile patients (n = 30)	Control group (n = 30)	P-value
Semen volume (ml)			
Range	1–4	2–4	
Mean ±SD	2.08 ±0.84	2.52 ±0.53	<0.05
Sperm Concentration (mil/ml)			
Range	2–150	20–220	
Median	20	65	
Mean ±SD	53.4 ±56.02	78.83 ±53.51	<0.01*
Total sperm count (mil/ejaculate)			
Range	4–300	36–600	
Mean ±SD	86.4 ±7.39	185.3 ±137.8	<0.01
Normal sperm morphology (%)			
Range	0–60	50–77	
Mean ±SD	34.83 ±12.51	63.53 ±6.46	<0.001
Progressive sperm motility (%)			
Range	0–40	40–65	
Mean ±SD	23.17 ±11.7	53.33 ±7.23	<0.001
Sperm HOS test (%)			
Range	20–75	60–90	
Mean ±SD	56.77 ±14.64	79.77 ±7.04	<0.001
Sperm DNA fragmentation (%)			
Range	6.8–25.5	3.5–11.4	
Mean ±SD	13.95 ±6.210	6.24 ±1.85	<0.001
Semen ROS level (**RLU)			
Range	1229–7600	180–898	
Mean ±SD	3062 ±1625	359.8 ±217.6	<0.001

* Mann – Whitney test, **RLU – Relative Light Unit

1% HNO₃ then applied to air–acetylene flame atomic absorption spectrophotometer (Buck model 210 VGP) with hollow cathode lamp (8 mA) current for detection of cadmium and lead. Wavelengths: Lead – 283.2 nm; Cadmium – 228.9 nm [17].

Statistical analysis

Data was processed using SPSS software package version 16. Descriptive statistics were done in the form of mean and SD. The unpaired t–test, Mann–Whitney

U test, and Pearson correlation test were applied to analyze the data. Values were considered significant when *P* values were less than or equal to 0.05.

RESULTS

Sociodemographic data of infertile patients and controls

This study included 30 infertile men with idiopathic oligo– and/or asthenozoospermia and 30 age–matched

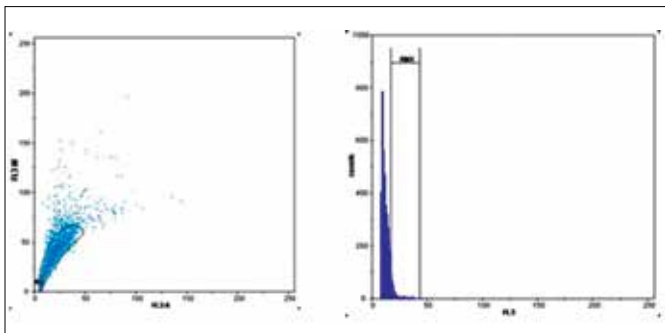


Figure 1. shows flow cytometer scatter plot (left) and histogram (right) in a control subject. Sperm DNA fragmentation % in this control subject was 5.3%.

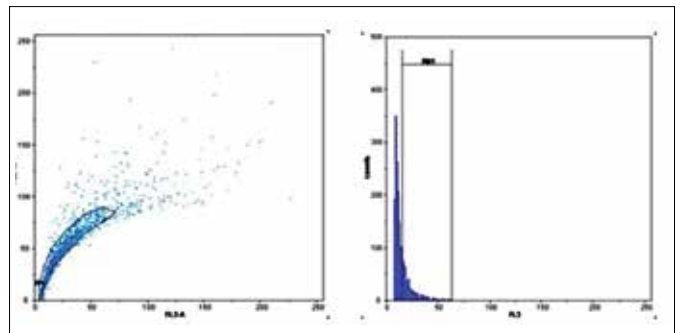


Figure 2. shows flow cytometer scatter plot (left) and histogram (right) in an infertile subject. Sperm DNA fragmentation % in this infertile man was 16%.

Table 2. Semen lead and cadmium levels among infertile patients and controls

Semen heavy metals level	Infertile patients (n = 30)	Control group (n = 30)	P-value
Semen lead ($\mu\text{g/L}$)			
Range	32–60	17–28	
Mean \pm SD	44.4 \pm 8.6	21.7 \pm 2.6	<0.001
Semen cadmium ($\mu\text{g/L}$)			
Range	3.2 – 4.3	2.1–3.4	
Mean \pm SD	3.77 \pm 0.294	2.774 \pm 0.514	<0.001

healthy fertile controls. Patients and controls were comparable as regards to age (34.63 \pm 6.47 years for infertile men *versus* 32.5 \pm 6.1 years for controls) and body mass index (24.27 \pm 3.09 kg/m^2 for infertile men *versus* 25.84 \pm 2.4 kg/m^2 for controls). They all belonged to the same population (Assiut city).

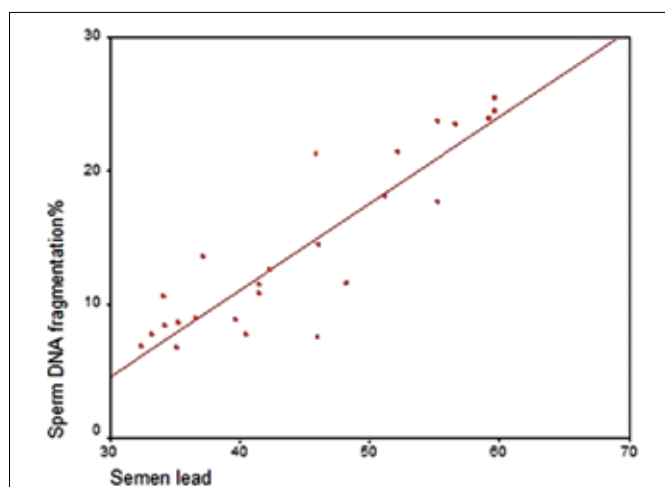
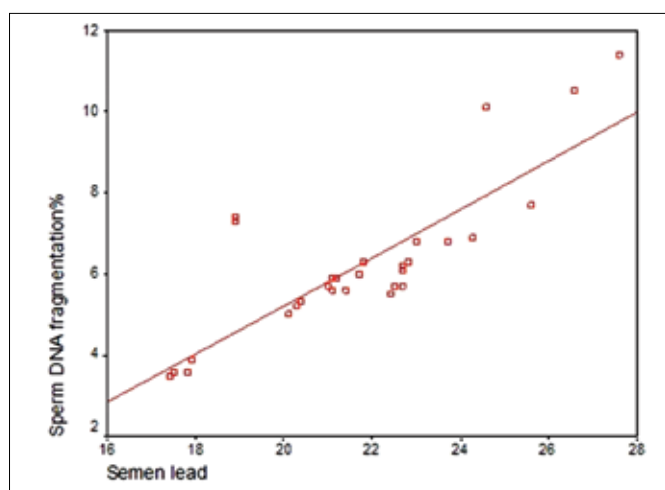
Among the patients, 20 (66%) presented with primary infertility with a mean duration of 7.1 \pm 3.8 years and 10 (33%) presented with secondary infertility with a mean duration of 5.6 \pm 2.3 years.

Comparison of conventional semen parameters, sperm HOS, sperm DNA fragmentation index, and semen ROS in infertile men and controls

Semen volume, progressive sperm motility, normal sperm morphology, and HOS percentages showed a statistically significant decrease in infertile male semen samples when compared to the control group with *p* value (0.05, 0.01, 0.01, and 0.01) (Table 1) respec-

Table 3. Correlations with semen lead level in infertile men and in controls

Variant	Patients		Controls	
	r	P value	r	P value
Age (years)	0.10	N.S	0.52	<0.01
Body mass index (Kg/M2)	0.37	<0.05	0.29	N.S
Semen volume (ml)	0.26	N.S	-0.15	N.S
Sperm concentration (mil/ml)	-0.04	N.S	-0.42	<0.05
Total sperm count (mil/ejaculate)	0.06	N.S	-0.33	N.S
Normal sperm morphology (%)	-0.37	<0.05	-0.34	N.S
Progressive sperm motility (%)	-0.65	<0.001	-0.50	<0.01
Sperm HOS test (%)	-0.62	<0.001	-0.76	<0.001
Sperm DNA fragmentation (%)	0.90	<0.001	0.82	<0.001
Semen ROS level	0.73	<0.001	0.60	<0.001

**Figure 3a.** Correlation between semen lead level and sperm DNA fragmentation in infertile men.**Figure 3b.** Correlation between semen lead level and sperm DNA fragmentation in fertile men (controls).

tively while sperm DNA fragmentation percentage showed a statistically significant increase in infertile male semen samples when compared to the control group with *p* value (0.001) (Figs. 1 & 2) (Table 1).

Comparison between semen lead and cadmium levels in infertile men and controls

Seminal plasma lead and cadmium levels showed a statistically significant increase in infertile men when compared to the control group with *p* value (0.001 for each) (Table 2).

Correlations between semen lead level (in $\mu\text{g/L}$) and other variables in infertile men and controls (Table 3) (Figures 3a, 3b, 4a, and 4b)

There were significant positive correlations between semen lead level and sperm DNA fragmentation per-

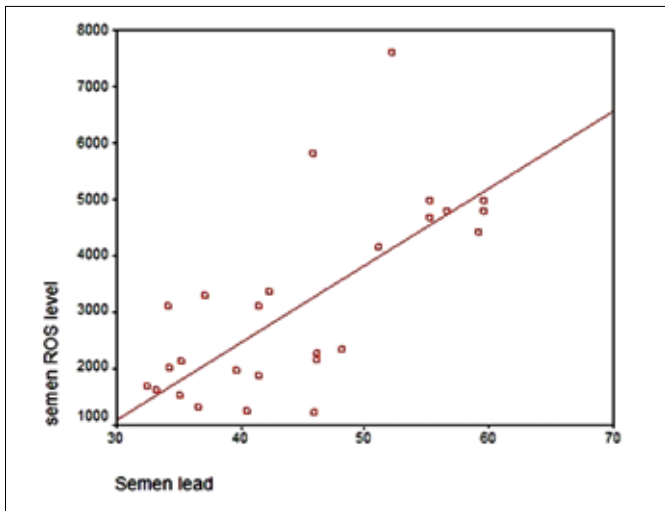


Figure 4a. Correlation between semen lead level and ROS levels in infertile men.

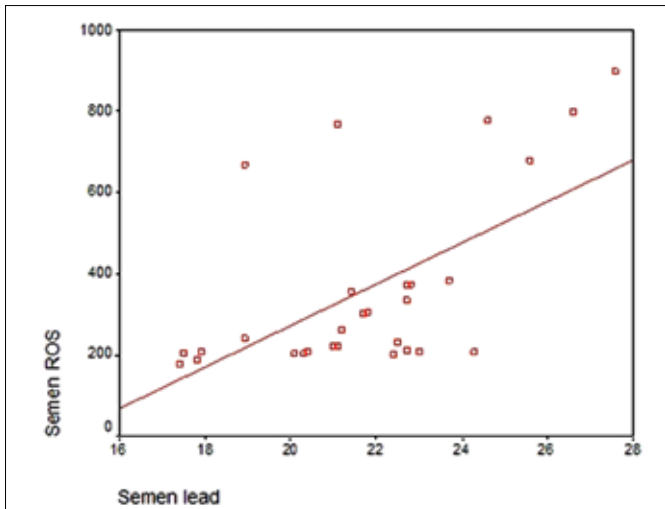


Figure 4b. Correlation between semen lead level and ROS levels in fertile men (controls)

cent in both infertile men ($r = 0.9$, $P < 0.001$) and controls ($r = 0.82$, $P < 0.001$). Significant positive correlations were also noted between semen lead level and ROS level among infertile men ($r = 0.73$, $P < 0.001$) and controls ($r = 0.6$, $P < 0.001$). On the other hand, there were significant negative correlations between semen lead level and each of progressive sperm motility and sperm vitality (HOS) percent in both infertile men ($r = -0.65$ and -0.62 respectively with $P < 0.001$ for each) and controls ($r = -0.5$ and -0.76 with $P < 0.01$ and < 0.001 respectively). Additionally in infertile men, a significant negative correlation was noted between semen lead level and normal sperm morphology ($r = -0.37$, $P < 0.05$) and there was a significant positive correlation between semen lead level and body mass index ($r = 0.37$, $P < 0.05$), while in controls there was a significant positive cor-

Table 4. Correlations with semen cadmium level in infertile men and in controls

Variant	Patients		Controls	
	r	P value	r	P value
Age (years)	-0.06	N.S	0.45	<0.05
Body mass index (Kg/M ²)	0.16	N.S	0.36	N.S
Semen volume (ml)	-0.02	N.S	-0.19	N.S
Sperm concentration (mil/ml)	0.01	N.S	-0.09	N.S
Total sperm count (mil/ejaculate)	-0.07	N.S	-0.11	N.S
Normal sperm morphology (%)	-0.24	N.S	-0.29	N.S
Progressive sperm motility (%)	-0.60	<0.001	-0.08	N.S
Sperm HOS test (%)	-0.53	<0.001	-0.52	<0.01
Sperm DNA fragmentation (%)	0.77	<0.001	0.64	<0.001
Semen ROS level	0.67	<0.001	0.58	<0.01

relation between semen lead level and age in years ($r = 0.52$, $P < 0.01$).

Correlations between semen cadmium level (in $\mu\text{g/L}$) and other variables in infertile men and controls (Table 4) (Figures 5a, 5b, 6a, and 6b)

There were significant positive correlations between semen cadmium level and sperm DNA fragmentation percent level in both infertile men ($r = 0.77$, $P < 0.001$) and controls ($r = 0.64$ & $P < 0.001$). Significant positive correlations were also noted between semen cadmium and ROS levels among infertile men ($r = 0.67$, $P < 0.001$) and controls ($r = 0.58$, $P < 0.01$). On the other hand, there was a significant negative correlation between semen cadmium level and each of progressive sperm motility and sperm HOS percent in infertile men ($r = -0.6$ and -0.53 respectively with $P < 0.001$ for each), while in controls we noted only a significant negative correlation with sperm HOS percent ($r = -0.52$, $P < 0.01$). Additionally in controls only, there was a significant positive correlation between semen cadmium level and age in years ($r = 0.45$, $P < 0.05$).

Correlations between semen cadmium and lead levels in infertile men and controls

There were significant positive correlations between the levels of the two metals in semen in both infertile men ($r = 0.67$, $p < 0.001$) and controls ($r = 0.56$, $p < 0.01$).

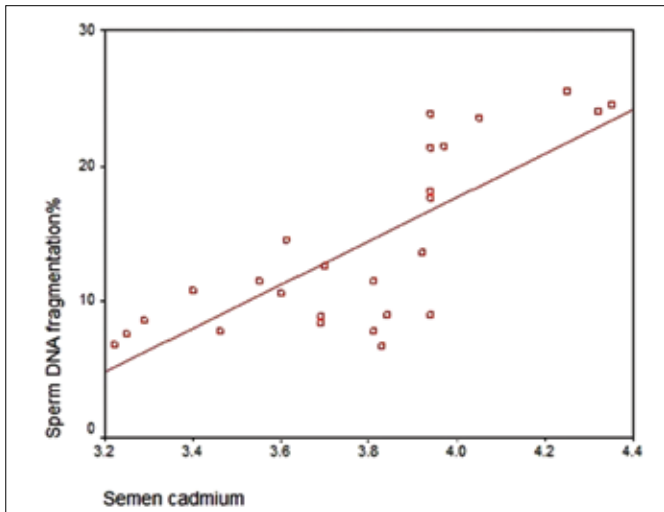


Figure 5a. Correlation between semen cadmium level and sperm DNA fragmentation in infertile men.

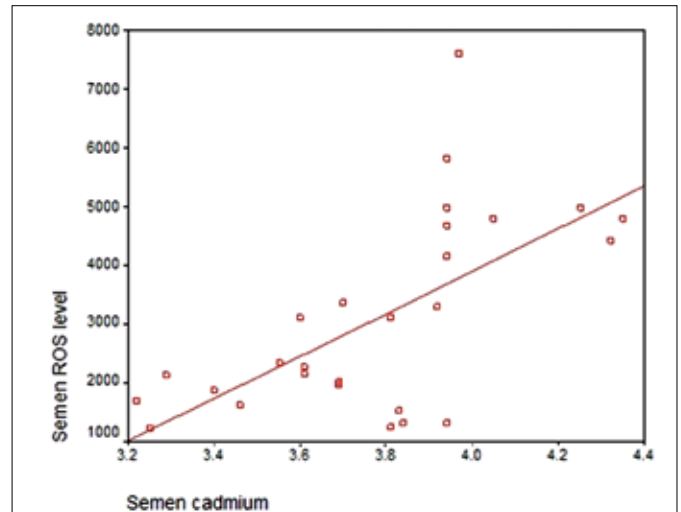


Figure 6a. Correlation between semen cadmium level and ROS levels in infertile men.

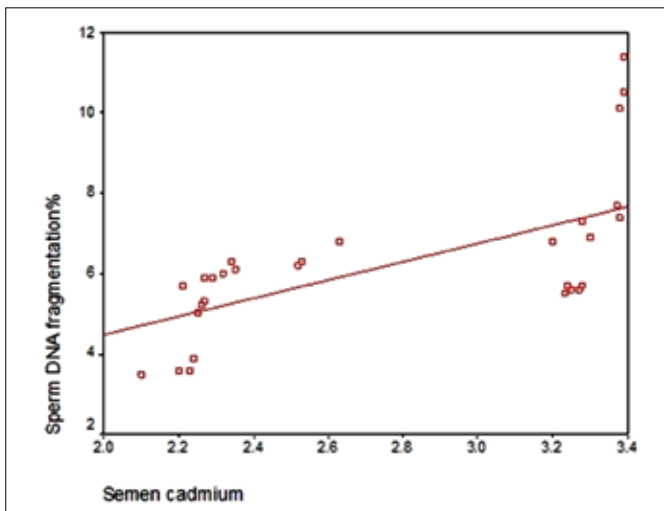


Figure 5b. Correlation between semen cadmium level and sperm DNA fragmentation% in fertile men (controls).

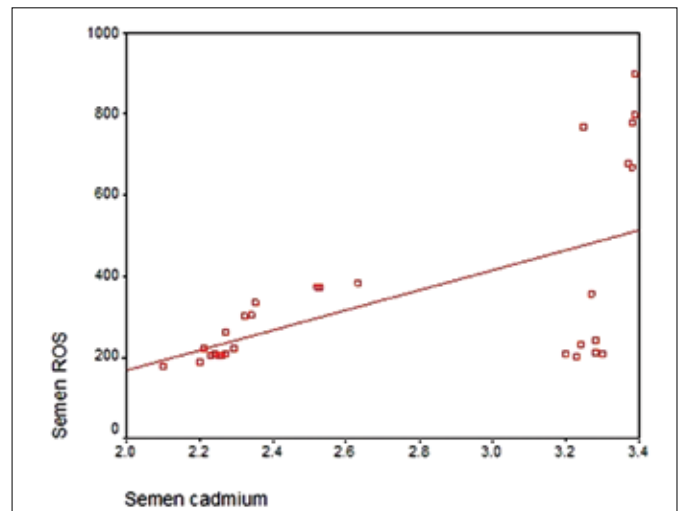


Figure 6b. Correlation between semen cadmium level and ROS levels in fertile men (controls).

DISCUSSION

Previous studies concerned with the effect of lead and cadmium on reproductive function in males focused on passive changes induced by exposure to heavy metals on testis (histology and function in animals) [18–20] or semen parameters (in humans and animals) [17, 21–25] with fewer studies on the effect of these metals on pregnancy rate [26].

Conflicting evidence in the literature exists regarding the association of lead and cadmium with semen quality [3, 11, 17, 22–26]. The results of our study revealed a significant increase in lead and cadmium levels in the semen of patients with idiopathic oligo- and/or asthenozoospermia in comparison to fertile controls. This is consistent with five of the above studies [11, 17, 23, 24, 26].

In spite of lack of significant correlations between semen volume and either of semen lead and cadmium levels in different groups in our study, semen volume was significantly lower in patients compared to controls. This might be due to an adverse effect of high level of heavy metals in seminal plasma on the prostate and seminal vesicles, which secrete a significant amount of seminal plasma. This has been shown in previous studies that reported impairment of the secretory function of accessory sexual glands by lead and cadmium exposure in experimental animals [27] and in humans [22].

As in case of semen volume, significant decreases in sperm concentration and total sperm count were observed in patients compared to controls in this study, with a significant negative correlation found only between semen lead and sperm concentration in control group. Plechaty and associates [21] and Saarenen

and associates [25] reported no association between seminal lead or cadmium concentration and sperm concentration (in healthy men of the general population and in smokers respectively). Similarly, Xu and coauthors [22] failed to establish a relationship between heavy metal seminal concentration and semen quality. On the other hand, Kuo and coauthors [23] and Alexander and coauthors [24] observed an inverse relationship between lead concentration and sperm concentration in lead battery workers. Also, Pant and coauthors [17] reported a significant negative correlation between seminal lead and cadmium concentration and sperm concentration in oligoasthenozoospermic men in the general population. Moreover, Xu and coauthors [3], Akinloye and coauthors [11] and Wu and coauthors [26] reported a significant inverse correlation between Cadmium and semen quality. The negative impact of these metals on sperm concentration and count might be due to their deleterious effect on testicular structure and function as shown in previous studies [18, 19, 20].

In the present study we observed negative correlations between semen lead and cadmium levels on one hand and normal sperm morphology percentage on the other hand. However, these correlations were significant only with semen lead in patients. In their study, Acharya and coauthors [28] also found a decrease in the percentage of sperm cells with normal morphology in mice injected with cadmium. Similar results were reported in another two studies in animals exposed to Cd [29, 30].

In the current study, we observed a significant negative correlation between lead and cadmium levels on one hand and progressive sperm motility on the other hand especially in patients. Similar results were obtained in another study [17]. Additionally, Leoni and coauthors [31] suggested that sperm motility is a sensitive parameter to cadmium toxicity. Cadmium was shown to disturb microtubules sliding and assembly [32]. It also affects sperm mitochondrial function and structure [29]. Furthermore, it has been established that cadmium competes with calcium for calmodulin binding, which results in a decrease in sperm motility [33].

Cadmium and lead also induce oxidative stress due to ROS accumulation, mostly superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$) which affect sperm motility [29, 34]. Moreover, cadmium may also lead to premature acrosome reaction in sperms [31]. Cadmium competition for calcium binding sites may explain the premature acrosome exocytosis in sperms, which affects sperm motility [29].

In this study, there was a significant negative correlation between sperm vitality as measured by HOS on one hand and each of lead and cadmium on the

other hand in all groups. This is consistent with prior findings regarding the effect of cadmium on sperm viability in vitro [31], which showed that the vitality of ram sperm was significantly affected by exposure to cadmium. The oxidative stress induced by lead and/or cadmium may explain the reduction in sperm vitality [34].

One of the main findings of our study was that there was a significant positive correlation between ROS level on one hand and the seminal plasma level of each, lead and cadmium, on the other hand. Results from previous studies support our findings [34]. Lead and cadmium are known to induce the production of nitric oxide (NO) and ROS such as hydroxyl radicals ($\cdot OH$), superoxide anions radicals (O_2^-) and hydrogen peroxide (H_2O_2) [35]. Furthermore, they are associated with a decrease in components of the anti-oxidant defenses in the sperm of infertile males [34]. The decrease in the activity and/or intracellular levels of antioxidants caused by lead and cadmium, together with the generation of ROS may cause lipid peroxidation and DNA damage in sperms [34].

In our study, there was a significant positive correlation between sperm DNA fragmentation percentage and seminal plasma level of each of lead and cadmium. Moreover, sperm DNA fragmentation percentage was significantly higher in semen samples of infertile patients compared to fertile control. Few reports have discussed the correlation between seminal plasma lead and cadmium levels and sperm DNA integrity in humans. A previous study by Xu and coauthors [3] suggested that oxidative DNA damage in human sperm is related to cadmium in seminal plasma, which is consistent with our study. However, their data did not conclusively indicate that lead in semen induces oxidative DNA damage in human sperm. This might be due to the lower mean concentrations of lead and cadmium in seminal plasma of their patients compared to that among our patients and controls.

Other studies have reported that lead induces alterations in sperm chromatin structure in occupationally and environmentally exposed men [36] and smokers [37]. The exact mechanism was not clear yet. Hernandez-Ochoa and coauthors [36] found that lead reaches the sperm nucleus in the epididymis by binding to nuclear sulfhydryl groups from the DNA-protamine complex, and this binding delays nuclear decondensation in vitro, which might be the cause for fertilization failures observed after lead exposures. Finally, it has been stated that lead's compromise on sperm chromatin structure depends on the timing of its incorporation into sperm nuclei during spermatogenesis, epididymal maturation, or even at ejaculation [35].

As in case of lead, studies also reported that cadmium exposure induced sperm DNA fragmentation in animals [29] and in smokers [39]. However, the study performed by Sergerie and coauthors [38] showed that the presence of cadmium of semen fluid and blood of smokers was not related to increasing sperm DNA fragmentation. This is contradictory to our results and to what was described by other authors [3, 39]. The mechanism by which cadmium increases DNA fragmentation was suggested through inhibiting sperm chromatin condensation reflecting the defective chromatin packaging during spermiogenesis [40] and generation of ROS with oxidative DNA [29, 34].

CONCLUSIONS

In conclusion, men with idiopathic male infertility had higher levels of lead and cadmium in their semen, which correlated with impairment of sperm motility and vitality percentages and more importantly with higher sperm DNA fragmentation and semen ROS level. Therefore, we should always consider the impact of cadmium and lead on semen parameters especially in cases of idiopathic oligo and/or asthenozoospermia. Further long-term studies in larger-sized random populations are needed to evaluate the effect of heavy metals and other environmental toxins on male reproductive capacity.

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