

ORIGINAL ARTICLE

Correlation between lead and cadmium concentration and semen qualityN. Pant¹, G. Kumar², A. D. Upadhyay³, Y. K. Gupta² & P. K. Chaturvedi¹¹ Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi, India;² Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India;³ Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India**Keywords**

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Summary

There are contrary reports of association of lead and cadmium with the decline in semen quality. This study evaluates whether seminal lead (Pb) and cadmium (Cd) at environmental concentration are associated with altered semen quality. We conducted a study of healthy fertile and infertile men 20–43 years of age attending the Andrology Laboratory of Reproductive Biology Department for semen analysis. The semen analysis was carried out according to the WHO 2010 guidelines. Seminal lead and cadmium were estimated by ICP-AES. The lead and cadmium values were significantly higher in infertile subjects. A negative association between seminal lead or cadmium concentration and sperm concentration, sperm motility and per cent abnormal spermatozoa was found. This study shows that exposure to Pb (5.29–7.25 $\mu\text{g dl}^{-1}$) and cadmium (4.07–5.92 $\mu\text{g dl}^{-1}$) might affect semen profile in men. Age, diet, smoking and tobacco chewing habits may have an influence on the increase in exposure to Pb and Cd in the individual subjects.

Introduction

With the advent of rapid industrialisation, several chemicals are released in the environment and have contaminated air, water and soil. These xenobiotics including lead and cadmium have been suggested playing a vital role in declining semen quality (Mínguez-Alarcón *et al.*, 2012; Martenies & Perry, 2013). Food, water and tobacco and smoking are the primary source of baseline exposure to lead and cadmium (Tong *et al.*, 2000; Bernard, 2008). Although in the developing countries after substantial efforts, the concentrations of these metals have been lowered, but still there is public concern of the toxic effect of the low exposure to these chemicals on the general population. In India after 2000, the lead levels of different environmental strata have decreased due to replacement of leaded petrol by unleaded petrol. This is evident by the data analysis report during the leaded petrol phase (before 1996) and the unleaded petrol phase (2000 onwards). The lead level in air decreased from 1.6 to 0.2 $\mu\text{g m}^{-3}$ while in river water the values decreased from 18.0 to 3.1 $\mu\text{g l}^{-1}$. The mean blood lead levels of children decreased from 18.1 to 12.1 $\mu\text{g dl}^{-1}$ (Singh & Singh, 2006).

The toxicity of these chemicals on human subject is well documented. However, scant and inconsistent infor-

mation exists pertaining to their effect on human semen quality as most of the data available on male reproductive system are from animal models. Studies conducted in Indian, Mexican, Croatian, American and Spanish population showed significant negative effects of low to moderate lead or cadmium exposure levels on semen quality (Telisman *et al.*, 2000; Pant *et al.*, 2003; Hernandez-Ochoa, 2005; Benoff *et al.*, 2009; Mendiola *et al.*, 2011). However, Singaporean or Finnish or German or Nigerian study did not find any significant effect of lead or cadmium exposure on sperm parameters (Xu *et al.*, 1993; Keck *et al.*, 1995; Hovatta *et al.*, 1998; Akinloye *et al.*, 2006).

Overall, those studies which were previously conducted had either small sample size or lacked control population or in some studies only few variables were not taken into consideration. Previous studies reported by us and others about the effect of lead/cadmium exposure on semen quality are based on the World Health Organization (WHO) 1992, 1999 guidelines for semen analysis where the cut-offs of all the sperm parameters were high, that is sperm concentration ≥ 20 millions ml^{-1} ; motility $\geq 50\%$. This study evaluates whether seminal lead (Pb) and cadmium (Cd) at environmental concentration are associated with altered semen quality based on the WHO (2010)

new criteria for semen analysis where the cut-offs of all the sperm parameters are low, that is sperm concentration ≥ 15 millions ml^{-1} ; motility $\geq 40\%$. Besides this, the influence of all the potential confounders viz age, diet, smoking and tobacco chewing habits was considered because they are commonly associated with biomarkers of exposure to lead and cadmium.

Materials and methods

Subject selection and semen analysis

A cross-sectional study was conducted on healthy human males (21–40 years old) attending the Andrology Laboratory of Reproductive Biology Department AIIMS, New Delhi for semen analysis. Men recruited to the study were from New Delhi and its surrounding areas. The proposed study was approved by the Institutional Ethical Committee. Subjects occupationally exposed to metals or with past medical history, mainly testicular dysfunction/history of urogenital abnormality/mumps, tuberculosis, thyroid dysfunction, or surgical operation, using drugs known to affect gonadal function were excluded from the study. Informed consent was obtained from each participant prior to the study. The volunteers were given an option to withdraw from the study at any time. The participation rate of the volunteers was noted. One hundred and ninety men agreed to participate in the study while 71 men failed in the predetermined selection criteria. A total of 119 men selected in the study composed of 46 fertile and 73 infertile men. Infertile males include those whose female partners failed to achieve pregnancy after 1 year of regular unprotected intercourse and had no diagnosed fertility disorder whereas fertile men with proven fertility whose partners had conceived spontaneously within 1 year were considered control groups. Semen of volunteers was collected by masturbation into a sterile wide mouth glass container after at least 3–5 days of sexual abstinence. Abstinence period and spillage, if any, were recorded. Semen analysis of each sample was carried out following the protocols of the WHO (2010). The liquefaction time, pH, odour, viscosity, volume, presence of pus or epithelial cells, sperm agglutination, sperm motility, sperm concentration and sperm morphology were noted. Simultaneously, a detailed questionnaire was filled, which included age, education, social status, occupation, diet, smoking, tobacco chewing habit, medical and surgical history.

Metal analysis

Multi-element standard solution IV purchased from Merck Chemicals, Darmstadt, Germany (product no. 1.11355.0100, Batch no. HC061563 dated 30.07.2010) was

used as reference standard. Suprapure nitric acid and hydrogen peroxide were purchased from Merck Chemicals, Darmstadt, Germany.

Approximately 1 ml of semen was digested with nitric acid (65%) and hydrogen peroxide (30%) ($\text{HNO}_3:\text{H}_2\text{O}_2$, 5 : 1) in a Aurora Transform 800 Microwave Digestion System (MW 800 closed vessels). The digested samples were cooled, diluted up to 5 ml with deionised water and analysed by inductively coupled plasma-atomic emission spectrometry (ICP-AES JY 2000). The precision for lead and cadmium was calculated to be 1.41 and 0.32 while the limit of detection was 1.9 and 0.28 ppb respectively. A sample blank was prepared with each set of samples to control for possible metal contamination from external sources.

Statistical analysis

The data were analysed by STATA 11.1 versus (Stata corps, Lakeway Drive College Station, TX, USA). The data are expressed as mean (SD) and frequency (percentage). Fisher exact/Chi-square test was utilised for categorical variables. In case of continuous variables, the average between two groups was compared by 't'-test and analysis of covariance was performed to see the difference between the two groups after adjusting the confounders. Correlation between two variables was performed by Pearson correlation. *P* value < 0.05 was considered statistically significant.

Results

No significant differences between fertile and infertile groups were evident in socio-demographic characteristics. The majority of men completed high school education. The subjects from both groups were in the private sector and belonged to the middle-class family. No statistically significant difference between the groups was found for age, smoking and consumption of tobacco. However, most men (66%) were nonvegetarian in the infertile group (Table 1).

No significant difference in pH or odour, viscosity, pH volume and liquefaction time was observed for both groups. The liquefaction time varied between 15–20 min, mean volume ranged from 3.04 to 3.01 ml in fertile and infertile men respectively. The sperm concentration was 39.37 ± 15.03 , 11 ± 8.9 millions ml^{-1} , motility was 54.35 ± 11.08 , $20.11 \pm 12.13\%$, normal morphology was 32.24 ± 7.21 , $28.09 \pm 5.28\%$ in fertile and infertile men respectively.

The mean concentration of lead and cadmium was significantly high in infertile men as compared to fertile men. The trend remained statistically significant after adjustment for the potential confounders, that is age, diet, smoking and tobacco chewing habits. The fertile and

Table 1 Demographic characteristic of participants in the study

	Fertile (N = 46)	Infertile (N = 73)	P value	
Age years (Mean ± SD)	31.82 ± 6.56	32.90 ± 5.94	0.52	NS
Diet				
Vegetarian	26 (56)	25 (34)*	0.014	P < 0.05*
Nonvegetarian	20 (44)	48 (66)*		
Smoking				
Yes	4 (9)	11 (15)	0.234	NS
No	42 (91)	62 (85)		
Tobacco chewing				
Yes	10 (22)	15 (20)	0.526	NS
No	36 (78)	58 (80)		

N, number of subjects; NS, Nonsignificant.

Mean ± SD. Value in parentheses is expressed in percentage.

*P < 0.05 considered to be statistically significant.

infertile men in the study ($n = 119$) were then divided into two groups based on parameters of sperm concentration and sperm motility to examine the effect of environmental exposure of lead or cadmium concentration in semen amongst different categories. Amongst men having sperm count ≤ 15 millions ml^{-1} or sperm motility $\leq 40\%$, a significant negative association between these sperm parameters and lead and cadmium concentration in semen was observed. This association remained statistically significant for lead and cadmium after adjustment for the confounding variables (Table 2).

There was no correlation between the lead and cadmium concentration in semen and age. However, significant inverse correlation between the lead and cadmium level and sperm concentration (-0.37 , -0.33) and motility (-0.40 , -0.33) was observed.

Table 2 Mean lead and cadmium levels in fertile and infertile subjects

	Lead ($\mu\text{g dl}^{-1}$)		Cadmium ($\mu\text{g dl}^{-1}$)	
	Unadjusted	Adjusted	Unadjusted	Adjusted
Fertility				
Fertile (N = 46)	5.29 ± 0.34	5.30 ± 0.30	4.07 ± 0.30	4.03 ± 0.32
Infertile (N = 73)	7.25 ± 0.24*	7.24 ± 0.27*	5.92 ± 0.25*	5.91 ± 0.25*
Sperm concentration				
>15 millions ml^{-1} (N = 60)	5.70 ± 0.29	5.69 ± 0.30	4.52 ± 0.29	4.50 ± 0.28
≤ 15 millions ml^{-1} (N = 59)	7.23 ± 0.30*	7.30 ± 0.30*	5.90 ± 0.28*	5.91 ± 0.29*
Motility				
>40% (N = 48)	5.40 ± 0.33	5.40 ± 0.21	4.13 ± 0.29	4.11 ± 0.31
$\leq 40\%$ (N = 71)	7.23 ± 0.27*	7.22 ± 0.28*	5.93 ± 0.26*	5.94 ± 0.25*

N, number of subjects.

Mean ± SE. The values were adjusted for age, diet, smoking and tobacco chewing habits.

*P < 0.05 considered to be statistically significant.

Discussion

The overall results of this study report that even moderate levels of seminal lead (5.29 – $7.25 \mu\text{g dl}^{-1}$) and cadmium exposure (4.07 – $5.92 \mu\text{g dl}^{-1}$) might reduce the human semen quality. After adjustment for potential confounders (age, diet, smoking and tobacco chewing habits), significant association was found between seminal lead and cadmium and sperm concentration, sperm motility and abnormal sperm ($P < 0.05$). The analysis confirmed that lead or cadmium exposure might play a significant risk factor for infertility. The lead and cadmium related effects on semen quality have also been reported by other investigators. Mendiola *et al.* (2011) found a significant association between the % immotile spermatozoa and seminal lead ($2.9 \mu\text{g dl}^{-1}$) after controlling for appropriate confounders (age, body mass index and smoking habits). Similarly, Hernandez-Ochoa *et al.* (2005) reported that even low lead concentrations in seminal fluid ($0.2 \mu\text{g dl}^{-1}$) were associated with low semen volume and increase in sperm chromatin condensation. Telisman *et al.* (2000) reported that seminal lead correlated with sperm concentration and per cent motile spermatozoa. Benoff *et al.* (2009) and Mendiola *et al.* (2011) reported that cadmium concentration at the level of 0.028 or $0.08 \mu\text{g dl}^{-1}$ in seminal plasma respectively might affect one or more seminal parameters. On the contrary, Hovatta *et al.* (1998) reported that seminal lead and cadmium concentration did not affect sperm concentration, sperm motility and morphology. Other investigators (Keck *et al.*, 1995) also indicated that no correlation was found between semen cadmium level of $0.04 \mu\text{g dl}^{-1}$ and sperm parameters. Xu *et al.* (1993) found no relationship between semen lead concentration and sperm variables. The discrepancy in the value might be due to variations

in exposures, heterogeneity of the population, and different analytical methods of detection. As previously discussed, all the earlier studies were undertaken according to the previously published WHO guidelines where the cut-offs of all the sperm parameters (sperm concentration, motility and per cent abnormal spermatozoa) were high whereas the current study is based on the WHO (2010) new criteria for semen analysis where comparatively the cut-offs of all the sperm parameters are low.

The exact mechanism of male reproductive toxicity due to heavy metals has not been specified. Animal studies suggest that these xenobiotics might induce mitochondrial dysfunction, increase free radical production or decrease antioxidant levels, enhance the lipid peroxidation of the cell membrane and contribute to the oxidative damage of DNA or inhibit androgen biosynthesis in Leydig cells. These parameters likely represent the interrelated aspects of the overall status of spermatozoa, that is increased morphology defects, loss of motility and fertilising potential (Stohs & Bagchi, 1995; Marchetti *et al.*, 2002; Sanocka & Kurpisz, 2004; Aitken & Roman, 2008; Kasperczyk *et al.*, 2008; Hsu *et al.*, 2009; Patra *et al.*, 2011; Vigh *et al.*, 2011).

Although substantial measures have been undertaken to control lead, the metal still exists within the environment and will continue to affect humans for many years. Due to the widespread exposure of humans and the known toxicity of lead, concern is growing about the effect on male reproductive outcomes after using unleaded petrol. In India, the general population is being exposed to lead or cadmium, mainly through water, food and air. Tobacco smoke is an important source of cadmium exposure.

With regard to the new WHO-proposed guidelines of semen analysis, the results for this study indicate that exposure to Pb and cadmium might affect semen profile in men. Age, diet, smoking and tobacco chewing habits may have an influence in the increase in exposure to Pb and Cd in the individual subjects. In spite of these intriguing findings, the results should be discussed with caution as human beings are exposed to a volley of chemicals which can interact additively, synergistically or antagonistically and it is difficult to assign the role of a particular metal. Susceptibility to toxicity is also influenced by genetic factors which are being considered in our future studies.

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