REPRODUCTIVE BIOLOGY

ORIGINAL RESEARCH

Cadmium toxicity: a possible cause of male infertility in Nigeria

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Received: 2 November 2005; accepted: 1 March 2006

SUMMARY

Serum and seminal plasma cadmium (Cd) concentrations were estimated by atomic absorption spectrophotometry in 60 infertile adult male Nigerians (40 oligozoospermics and 20 azoospermics). The results were compared with Cd level in 40 normozoospermic subjects (matched age, with proven evidence of fertility). The relationship between Cd levels and spermatograms or the hypothalamic-pituitary-gonadal (HPG) -axis was investigated by correlating serum and seminal plasma Cd levels with semen characteristics and hormone levels. The seminal plasma Cd level was significantly higher than those of serum in all studied groups ($p<0.001$). The serum and seminal plasma Cd levels were increased

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($p<0.001$) in azoospermics in comparison to oligozoospermic and control subjects. A significant negative correlation was observed between serum Cd level and all examined biophysical semen characteristics except sperm volume. A positive correlation was also observed between seminal plasma Cd and FSH. Results of the study for the first time implicate cadmium as a cause of infertility in male Nigerians as well as extend and support previous findings concerning cadmium toxicity and male infertility. The strong deleterious effect of cadmium on spermatogenesis may be due to the systemic and cellular toxicity. A possible relationship between this element and the HPG axis is also suggested. *Reproductive Biology 2006 6 (1): 17–30.*

Key words: cadmium toxicity, male infertility, reproductive hormones, spermatogram, Nigeria

INTRODUCTION

In the field of toxicology, the adverse effects of greatest concern are those of chronic toxicity, cancer and reproductive dysfunction[1]. It has long been suggested that at least half of the cases of human male infertility of unknown etiology may be attributable to various environmental and occupational exposures [11, 33, 34]. The possibility that exposures to multiple environmental agents are associated with reproductive and developmental disorders in human populations has recently generated much public interest[2] [8, 24, 26, 30, 36]. Epidemiological studies provided equivocal results concerning the effects of lead (Pb) and cadmium on hormone concentration, male infertility and sperm parameters [3]. Geographic differences in the amount of naturally occurring cadmium have been correlated with incidence rates of prostate cancer [2, 12].

[1] Rees TJ **1993** Toxicology of Male Reproduction. Literature Review in Applied Toxicology. M.Sc. Review Text, Portsmouth University, www.brighton73.freeserve.co.uk/tomsplace/scientific/msc-review/msr-top.htm

[2] Andersson AM, Grigor KM, Rajpert De Meytz E, Leffers H, Skakkebaek NE **2001** Hormones and endocrine disrupters in food and water. Possible impact on human health. Munksgaard, Copenhagen. A collection of scientific meeting in Copenhangen, May 27-30, 2000.

Cadmium is a non-essential toxic element. It has a toxic effect on many enzymes dependent on iron as a co-factor, one of these being cytochrome P450 [18]. Leydig cells contain ten times more of P450 than Sertoli cells, hence are more sensitive to increased Cd level[1]. Since cytochrome P450 is required for the functioning of 17-α-hydroxylase and 17-20 lyase, its disruption may well interfere with testicular steroidogenesis.

Major changes in the levels of toxic elements in seminal fluid have been related to abnormal spermatozoa function and fertilizing capacity [22]. Aboushakra et al [1], working on wide range of toxic elements including cadmium concluded that the role of these elements in infertility may be more directly related to sperm and whole serum than seminal plasma level. The role of cadmium in male infertility is uncertain. Omu et al [22] detected a significantly high level of Cd in serum of men who were smokers and implicated this metal as one of the causes of asthenoteratozoospermia. Nigerian environments have been reported to be highly polluted by toxic metals, especially lead and cadmium[1] [15]. This study was therefore designed to determine the relationship between serum/seminal plasma Cd levels and spermatogram as well as the pituitary-gonadal-axis (HPG) in infertile Nigeria men.

SUBJECTS AND METHODS

Sixty male volunteers of infertile couples attending the infertility clinics of the Department of Obstetrics and Gynaecology and Urology Clinic of Surgical Outpatient, Department of Surgery, University College Hospital, Ibadan, Nigeria, who conformed to the selection criteria (tab. 1) were recruited into the study. The study design included three groups based on their sperm counts. Group 1 consisted of male partners of infertile couples with sperm counts less than 20 million/cm^3 (oligozoospermia;

[1]Anetor JI **2002** Endocrine Disruption. The probable situation in the highly polluted Nigerian environment. SCOPE/IUPAC (Scientific committee on problems of the environment/International union of pure and applied chemistry). International symposium of endocrine active substances and supplementary workshop. Program and collective abstract 2002, Nov 17-21, Yokohama, Japan, pp 67-68.

Table 1. Criteria for inclusion and exclusion of subjects

Inclusion Criteria	Exclusion Criteria
• men within the reproductive age of 20–45 years; • male partners of couples in good marital harmony, living together and having regular unprotected coitus for two or more years; • normal descended testes.	• male contraceptive users; • testicular varicoele; • genital infections; • long term medications; • previous groin/scrotal surgery; • known HIV positive men; • smoking/chronic alcohol intake (other than occasional alcohol intake); • chronic and serious systemic illness; • men currently on fertility drugs or steroid preparations.

n=40); group 2 consisted of male partners of infertile couples with no spermatozoa in their semen (azoospermia; n=20) and group 3 consisted of healthy fertile control males with sperm count greater than 20 million/cm^3 (normozoospermia; n=40). Male partners of infertile couples with sperm count greater than 20 million/cm^3 (normozoospermia) were regarded as subfertile and hence excluded from this study.

The control subjects were recruited from semen donors for intra-uterine fertilization and male partners of pregnant women and nursing mothers attending the Antenatal Clinics of the Department of Obstetrics and Gynaecology, University College Hospital, Ibadan. Control subjects were recruited from a similar population as those of oligozoospermia and azoospermia groups, except that they did not have to exhibit two years of unprotected intercourse. Having at least two living children were used as additional criteria for selecting control subjects in addition to the exclusion criteria used for cases. All subjects gave their informal consent. The study received the approval of the ethical committee of the College of Medicine, University of Ibadan and University College Hospital, Ibadan. On the first visit to the clinic, a complete medical history was obtained and a physical examination was performed for each subject including measurement of body weight, height and blood pressures.

Biophysical analysis of semen samples

Semen analysis was performed according to the World Health Organization (WHO) guidelines [40] with slight modifications. The mean progressive motility (MPM) graded by WHO (A-D) was modified (1-4) for statistical convenience. Evaluation of percentage motility was done by systematic manual assessment of microscopic field and estimating the approximate percentage of moving spermatozoa compared with non-motile cells for at least five fields. In addition to motility, the experiment also reported on volume of ejaculate, sperm counts per ml, percentage of spermatozoa with normal morphology, and living spermatozoa (viability).

Hormonal assays and determination of cadmium

The hormone assays were carried out using enzyme immunoassay techniques developed for the Special Program Research in Human Reproduction by the WHO [41]. Cadmium levels in serum and seminal plasma samples were determined by Atomic Absorption Spectrophotometer (Buck Scientific, East Norwalk; Buck Model 210 – VGI Spectrophotometer with wavelength of 226 for Cd, high standard and ABS energy, 1 ppm/0.75 detection limit, 0.01, linear range 2.0 and typical stability±0.005).

Statistical analysis

The mean of duplicate readings of all measurements with coefficient of variation (CV)<15% were analyzed. Analysis was performed with Statistical Package for Social Sciences software (SPSS for Windows, version 10.0). Since sperm biophysical characteristics and hormone levels do not exhibit normal distributions (tested by Shapiro-Wilk test) in large groups of adult males [4, 5, 6], the data were log transformed. The results were expressed as means (95% confidence interval; CI). Significant differences among the three groups were analyzed first by one-way ANOVA and, if significant difference were found, student `t´ test was used to compare the groups. Relationships between data were measured with Pearson's correlation. Statistically significant level was set at $p<0.05$.

RESULTS

The admission characteristics of each group of subjects were similar. The sperm biophysical parameters of infertile subjects were significantly lower than those of controls, except for semen volume which was similar in all groups (tab. 2). Apart from testosterone there were no significant differences in the seminal plasma hormone levels in the three groups. The androgen was lower in normozoospermic subjects compared to oligo- and azoospermics. Significant increases in the serum hormone levels were observed in infertile subjects compared to those of controls (tab. 3). The serum testosterone level was the highest in azoospermics.

There were significant differences among the mean (95% CI) serum Cd levels in the three examined groups ($F=16.16$, $p<0.001$). The mean level in azoospermics increased significantly compared with those of control and oligozoospermic subjects ($p<0.001$). The mean (95% CI) seminal plasma Cd levels also differed significantly ($F=36.57$, $p<0.001$) among groups. In comparison to control subjects, seminal plasma Cd was significantly reduced in oligozoospermics and increased ($p<0.001$) in azoospermics. A highly significant decrease in the mean seminal plasma Cd level was noted in oligozoospermics compared with that of azoospermics ($p<0.001$). Generally, the mean seminal plasma Cd levels

Table 2. Semen biophysical characteristics in the three groups of subjects

Characteristic	Normozoospermics Mean (CI)	Oligozoospermics Mean (CI)	Azoospermic Mean (CI)
n	40	40	20
Volume of ejaculate (ml)	**2.43** (1.97-2.88)	**2.15** (2.06-2.95)	**2.89** (1.94-3.04)
Sperm count ($\times 10^6$/ml)	**72.70** (64.38-81.02)	**5.16** (3.88-7.04)	NA
Viability (%)	**80.50** (78.59-82.40)	**46.80** (41.25-54.84)	NA
Morphology (%)	**84.50** (82.89-86.11)	**55.00** (53.32-66.67)	NA
Motility (%)	**79.25** (76.68-81.82)	**35.75** (28.74-42.75)	NA
MPM	**4.40** (4.29-4.61)	**1.80** (1.42-2.17)	NA

Values expressed as means (95% confidence interval; CI); NA=not applicable; MPM=mean progressive motility

Table 3. Serum and seminal plasma hormonal levels in the three groups of subjects

Hormone	Normozoospermics Mean (CI)	Oligozoospermics Mean (CI)	Azoospermic Mean (CI)	F
SERUM				
LH (mmol/L)	**8.60** (7.87-9.89)	**13.04*** (10.97-15.40)	**13.90*** (9.37-15.82)	3.71*
FSH (mmol/L)	**8.44** (7.71-9.98)	**25.60**** (19.99-32.72)	**30.94**** (18.61-32.77)	7.72**
PRL (mmol/L)	**246.40** (206.63-296.71)	**426.50*** (346.03 – 572.36)	**465.27*** (354.04-585.75)	2.81*
Testosterone (iu/L)	**5.92** (4.91 – 6.61)	**6.70** (5.27 – 7.01)	**20.27*** (14.39 – 32.13)	2.87**
SEMINAL PLASMA				
LH (mmol/L)	**0.22** (0.16-0.34)	**0.50** (0.10-0.60)	**0.42** (0.03-0.95)	0.41
FSH (mmol/L)	**0.26** (0.22-0.31)	**0.81** (0.37-0.89)	**0.42** (0.37-0.49)	2.92
PRL (mmol/L)	**97.85** (87.27-107.62)	**112.40** (93.27-144.12)	**88.73** (70.59-111.95)	0.79
Testosterone (iu/L)	**4.02** (3.99 – 4.51)	**14.62**** (12.06 – 18.71)	**14.63**** (13.59 – 15.48)	10.34**

Values expressed as means (95% confidence interval; CI); statically significant at *p<0.05, **p<0.001;
Normozoospermia (controls) vs oligozoospemia or azoospermia (student ´t` test); all groups compared by ANOVA (F-values)
iu/L= international unit/L

Table 4. Serum and seminal plasma cadmium levels in normozoospermic, oligozoospermic and azoospermic subjects

Parameters	Normozoospermics Mean (CI)	Oligozoospermics Mean (CI)	Azoospermics Mean (CI)	F
Serum cadmium (mg/L)	**0.21** (0.18-0.23)	**0.23**$^{+}$ (0.18-0.29)	**0.46***$^{+}$ (0.44-0.49)	16.16
Seminal plasma cadmium (mg/L)	**1.10** (1.01-1.18)	**0.65**$^{+}$ (0.59-0.71)	**1.57***$^{+}$ (1.44-1.83)	36.57
Ratio	**1 : 5**	**1 : 3***	**1 : 3***	28.13

Values expressed as means (95% confidence interval; CI); $^{+}$p<0.001, oligozoospermia vs. azoospermia; *p<0.001, normozoospermia (controls) vs oligozoospermia or azoospermia

Table 5. Correlation of cadmium levels and semen biophysical parameters

Biophysical parameters	Serum		Seminal plasma	
	r	p	r	p
Volume of ejaculate (ml)	+0.049	>0.05	+0.134	>0.05
Sperm counts ($\times 10^6$/ml)	-0.320*	<0.05	-0.061	>0.05
Motility (%)	-0.605*	<0.001	-0.189	>0.05
MPM	-0.585*	<0.001	+0.172	>0.05
Viability (%)	-0.575*	<0.001	+0.004	>0.05
Morphology (%)	-0.596*	<0.001	-0.214	>0.05

+ positive correlation; - negative correlation; *significant correlation; MPM: mean progressive motility

Table 6. Correlation between cadmium and hormones' levels in serum and seminal plasma

Hormone	Cadmium levels in	
	Serum	Seminal plasma
Serum	r	
LH	+0.108	-0.020
FSH	+0.234	+0.041
PRL	+0.168	-0.066
Testosterone	+0.054	-0.098
Seminal plasma	r	
LH	-0.040	+0.106
FSH	-0.164	+0.355*
PRL	-0.247	-0.182
Testosterone	+0.168	-0.282

+ positive correlation,; – negative correlation;
*significant correlation, $p<0.05$

were significantly higher than the mean serum levels in all examined groups ($p<0.001$; tab. 4).

Serum cadmium showed a significant negative correlation with all semen biophysical parameters investigated in the study except for semen volume (tab. 5). However, seminal plasma Cd level had no significant correlation with any of the parameters. Seminal plasma Cd levels demonstrated positive significant correlations (tab. 6) only with seminal plasma FSH ($p<0.005$).

DISCUSSION

Toxicology data on cadmium are scanty in several important aspects including its effect on reproduction [39]. The few available studies present conflicting findings [3]. Some authors described lack of significant differences between seminal plasma Cd concentration in fertile and infertile men [16]. In contrast, others reported an increase in Cd concentration in infertile men compared to fertile men [14, 28, 37]. Our results are in agreement with the data obtained with the later authors and indicate that Cd exhibits a deleterious effect on the reproductive system of Nigerian males. Omu et al [22] reported no significant differences in the Cd level in normozoospermic, oligozoospermic and azoospermic semen; contrary to those findings, our results show a significant difference in Cd level in both serum and seminal plasma levels in the three groups of subjects. We found a significant increase in serum Cd level of azoospermics compared to oligozoospermics and controls. This is consistent with the findings of Xu et al [44]. These authors also reported a significant negative correlation between blood Cd level and sperm density. A more recent study by Pant et al [23] demonstrated an increase in lead and cadmium levels in the seminal plasma of infertile men. They also reported a significant negative correlation of these toxicants with sperm motility and concentration in oligoasthenozoospermic men. An increase in blood plasma Cd concentration has also been associated with teratozospermia [7]. We have found significant negative correlation between serum Cd and a number of biophysical parameters i.e. sperm density and motility, mean progressive motility, sperm viability and morphology. This suggests that cadmium has a strong toxic effect on spermatogenesis.

It has long been established that agents such as cadmium, which are known reproductive toxicants, implicated in occupational hazards are found to accumulate in human semen [11]. However, the subjects in this study were recruited from occupationally unexposed population. The role of increased seminal plasma trace metals concentration is poorly understood in the regulation of reproductive function in the occupationally unexposed males [3]. The geographic differences in the amount of naturally occurring cadmium

have been correlated with incidence rate of prostate cancer [2, 12]. This may also be applicable to male infertility. Environmental discharge of cadmium due to the use of petroleum products, combination of fossil fuels (petroleum and coal) and municipal refuge contribute to airborne cadmium pollution [13, 19, 25] and possibly introduce high concentrations of this potential reproductive toxicant into the environment. This may be particularly true for Nigeria. In addition, humans may be unwittingly exposed to cadmium via contaminated food or paper [43], cosmetics and herbal folk remedies [17, 42]. All these factors put Nigerian population at high risk of cadmium toxicity[1] [20] and also may be responsible for the observations reported in this study.

Saksena et al [29] reported that epididymis and seminal vesicles contained the highest concentration of cadmium in the body. This explains the higher Cd level in seminal plasma compared to serum in all the three groups of examined subjects and possibly supports the importance of this element in reproduction. Thus, cadmium appears to have a high affinity for the male reproductive system. Such a notion probably explains why men are more sensitive than women to environmental and occupational exposures to this toxicant [31, 32, 90]. It is then possible that in highly polluted environments like Nigeria[1] [20], cadmium may accumulate markedly in the testicular tissue of the male population.

Interestingly, the ratio in cadmium concentrations in serum and seminal plasma was similar in infertile subjects i.e. oligozoospermic and azoospermic (1:3) and differed in control men (1:5). Such a ratio indicates a higher cadmium burden in the oligozoospermia and azoospermia and implies the possibility of systemic toxicity in infertile subjects. Cadmium ultimately reaches the principal target site - the testes. This phenomenon is consistent with the suggestion of Saksena et al [29] that cadmium toxicity is an important factor in male infertility. Nutritional deficiency in essential elements (micronutrients e.g. zinc) may further aggravate the effect of cadmium burden. Interaction between cadmium and other trace elements, especially zinc, had long been established.

[1] Anetor JI **2002** Endocrine Disruption. The probable situation in the highly polluted Nigerian environment. SCOPE/IUPAC (Scientific committee on problems of the environment/International union of pure and applied chemistry). International symposium of endocrine active substances and supplementary workshop. Program and collective abstract 2002, Nov 17-21. Yokohama, Japan pp 67-68.

Zinc, a critical element in male reproductive function, is normally secreted in enormous amounts by the prostate gland. Since cadmium can interact with the cellular metabolism of zinc, a low level of the latter may cause cadmium to shift from blood to seminal plasma compartment or vice versa.

A high level of cadmium had been reported as the possible cause of asthenozoospermia in smokers [22]. Cigarette smoking is an important variable when considering the effect of both lead and cadmium exposure on human health [35]. Cigarette smoke is a major source of airborne environmental lead and cadmium exposure. A single cigarette has been reported to contain 1.5 μg of cadmium [7, 27]. Moreover, one tenth of the metal content of a cigarette is inhaled [10]. Although smoking was excluded in the subjects investigated in our study, the incidence of unwilling exposure to second-hand cigarette smoke is very high in Nigeria. Unlike in the most developed countries, there are no smoking restrictions in public places in Nigeria except hazardous areas such as petrochemical filling stations.

The serum Cd levels in our infertile groups were higher compared to controls. There was also a corresponding significant increase in seminal plasma Cd of azoospermics, indicating that there is systemic and cellular Cd toxicity in azoospermics. Since the testes are among the principal target sites for cadmium, it is likely that cadmium elicits its toxic effect, probably expressed as infertility. Increased seminal plasma cadmium concentration may be one of the causes of the disruption of spermatogenesis, expressed as infertility in Nigerians. Recently, Katakura and Sugawara [15] reported the toxic effect of Cd (0.012 mmol/kg/day for 2 days) on mice. Testicular dysfunction was the major finding noticed by histological observation which revealed widespread and severe necrosis three days after the final injection of cadmium. Two months later, the changes were further aggravated. According to authors the toxic effect of cadmium was due to oxidative damage and lipid peroxidation.

The relative high testosterone and gonadotropin concentrations seen in oligozoospermia and azoospermia suggest a derangement in the mechanism for testosterone uptake at the cellular level either in the pituitary or testes. There was no evidence in this study to suggest that cadmium toxicity may play a role in this phenomenon since no significant correlation was observed between cadmium and testosterone levels in blood and seminal plasma.

This observation further strengthens the suggestion that the spermatogenic process may be damaged without much disturbance to Leydig cell function. However, a positive correlation observed in this study between seminal plasma Cd and FSH levels might be an indication of a possible effect of cadmium on the hypothalamic – pituitary – gonadal (HPG) axis, outside the Leydig cell. The possible mechanism and clinical implications of cadmium-caused seminal plasma FSH increase are not clear.

Results of this study, for the first time demonstrate the role of cadmium in infertility in male Nigerians as well as extend and support previous findings implicating cadmium-related male infertility. Infertility is currently a serious global phenomenon which is modulated by nutritional status. In Nigeria this problem may be more deleterious because of the well recognized deficiency of protective micronutrients in this sub-region [21, 38].

AKNOWLEDGEMENTS

We wish to acknowledge the technical assistance of Mr A.Ö Adeleye of WHO Collaboration Center for Reproductive Medicine, Sagamu, Nigeria, who helped in hormonal assay procedures. The assistance of the laboratory staff and the Resident Doctors of the Department of Surgery (Urology Clinic) and Fertility Research Unit, Obstetrics and Gynaecology Department, U.C.H, Ibadan Nigeria in sperm analysis and subjects recruitment respectively, are also appreciated. The miscellaneous assistance of Mr. Jude Onuegbu is also greatly acknowledged.

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