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Association of seminal plasma cadmium levels with semen quality in non-occupationally exposed infertile Nigerian males

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ABSTRACT

Aim: Male infertility of unknown etiology may be attributed to various environmental exposures to toxic substances such as metals. Very few human studies have evaluated the relationship between male reproductive function and toxic metal concentrations in seminal plasma in Nigeria. The objective of this study was to evaluate the levels of cadmium in seminal plasma of non-occupationally exposed infertile Nigerian males and correlate their levels with semen quality. **Materials and Methods:** A total of 80 subjects were enrolled, which includes 60 infertile males on a routine visit to the University of Benin Teaching Hospital, Benin City and 20 fertile males (controls). The concentration of cadmium in seminal plasma was measured using atomic absorption spectrophotometer. Semen analyses were performed according to World Health Organization standard. **Results:** Mean seminal plasma cadmium level was significantly higher ($P < 0.001$) in infertile males compared with controls. The toxic metal correlated negatively ($P < 0.001$) with sperm count, progressive motility, total motility and morphology but not with semen volume. **Conclusion:** It appears that cadmium may contribute to infertility in occupationally unexposed infertile Nigerian males and levels of toxic metals may be assayed when evaluating subjects with idiopathic infertility.

KEY WORDS: Cadmium, male infertility, seminal plasma, seminal quality

INTRODUCTION

Cadmium is a toxic metal known to cause serious environmental and health effects such as hepatic, respiratory, renal and reproductive damage with the testes being particularly sensitive to its effects which may be due to disruption of the blood-testis barrier via specific signal transduction pathways and signaling molecules [1]. The general population is exposed to metals at low concentrations either voluntarily through supplementation or involuntarily through intake of contaminated food (from galvanized pipes or water coolers) or contact with contaminated soil, dust or the air. Cigarette smoke is a major source of airborne environmental lead and cadmium exposure. A single cigarette contains 0.6-2.0 μg of lead [2] and 1-4.5 μg of cadmium [3] and at least one tenth of the metal content of a cigarette is inhaled. Alcoholic beverages, including wine can be contaminated with metals in concentrations exceeding the allowable limits and causing toxic effects, particularly in heavy drinkers. With rapid industrialization and motorized vehicular traffic, a lot of toxic metals may be released into the environment. Because widespread human exposure and increased levels, there is growing concern for adverse health effects associated with low-level exposures encountered in the environment. Järup and Alfvén [4] showed that Cadmium has an extremely long biological half-life of more than 15 years in humans with only a very small fraction excreted, and the total body content increases with age. It was also observed that cadmium may have adverse impacts on human and animal male reproductive health even at low-levels exposure [5] and males appear to be more susceptible than females to the effects of occupational or environmental exposures to reproductive toxicants [6]. Poor semen quality contributes up to 25% of the cases of infertility in male [7]. The causes of poor semen quality are multifactorial, and studies have suggested that environmental, industrial, and dietary agents may affect male fertility [8,9]. This poor semen quality may be due to direct an effect of testicular dysfunction or hormonal changes as a result of exposure to toxic metals [10]. Studies have shown that toxic metals levels in the blood may be inadequate to reveal their accumulation in the male reproductive tract [11,12]. Therefore, cadmium levels in seminal plasma may provide a better index of exposure and effect in the reproduction.

Data of the adverse effect of cadmium level on fertility in our setting are very few, since seminal fluid parameters clearly differ by geographical location, which may still be influenced by environmental factors [13], and Nigerian environments have been reported to be highly polluted by toxic metals, especially lead and cadmium [14]. Hence, this study was designed to determine the level of cadmium in infertile males in Nigeria and assess the relationship between this non-essential toxic metal and semen quality among infertile men at environmental exposure level.

MATERIALS AND METHODS

The study was conducted at the Department of Medical Laboratory Science, School of Basic Medical Science, College of Medical Sciences, University of Benin, Nigeria. The study was approved by the Research Ethics committee of the College of Medical Sciences, and informed consent was given by the individual subjects. Demographic and clinical examination findings were obtained using structured questionnaires. Those with other specific genital and systemic disease such as genital infection, undescended testis, hepatic, renal, endocrine, autoimmune disorders that may impair the reproductive capacity were excluded. Subjects with a history of toxic metals exposure or who resided in areas known to have toxic metals contamination and cigarette smokers were also excluded from this study. Semen was obtained by masturbation after three to five days of abstinence into universal container ensuring that the sperm rich first part was not lost. The sample was left on the bench at 37°C for 30 min for liquefaction after which semen analysis was done according to World Health Organization (WHO) [15]. Then, the sample was spun at 12000 g for 20 min to obtain seminal plasma. The seminal plasma was stored at −20°C prior to toxic metal analysis.

Semen Analysis

Routine semen analysis was performed to assess sperm quality parameters, including semen quantity, sperm count, sperm motility, and sperm morphology according to WHO [15] method, after liquefaction at 37°C for 30 min and within 1hr of semen collection. Semen parameters evaluated included: Sperm concentration, progressive motility, total motility, and the percentage of normal forms. The criteria for normozoospermia were a concentration of $\geq 20 \times 10^6$/ml, with sperms of progressive motility more than 32% of spermatozoa, total motility more than 40% and normal morphology with oval-shaped head and no irregularities of tail in at least 30% of the spermatozoa.

Determination of Cadmium

The concentration of cadmium in seminal plasma was determined with electrothermal atomic absorption spectrophotometer (Perkin Elmer analyst 800, Norwalk, U.S.A). Approximately, 100 µl of seminal plasma was digested with 500 µl of super-grade 0.8 M HNO_3 in a glass tube. The residue was dissolved in 1 ml of 1% HNO_3 and applied to a graphite tube for detection of cadmium. The recovery of cadmium in spiked semen samples was 97%. The instrument was calibrated using 0 μg/L, 3 μg/L, 5 μg/L and 20 μg/L cadmium standards respectively. A sample blank was prepared with each set of samples to control for possible metal contamination from external sources. The instrument was allowed to process the sample and display the concentration in μg/L.

Quality Control

Standard sample for the element was diluted to obtain serial dilutions of each sample and was used to calibrate and standardize the electrothermal atomic absorption spectrophotometer before running the analysis, and a graph was generated. Before being used all volumetric polyethylene (including the auto-sampler cups) and glass material were cleaned by soaking in 20% (v/v) HNO_3 for 24 h. They were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container. Certified reference materials from (Le Centre de toxicologie du, Quebec) were analyzed. 3.05 ng/mL was obtained as cadmium measured level from whole blood while 3.38 ng/mL is the certified value. In this study, we did not control for lead exposure that is a likely co-founder to the results.

Statistical Analysis

The data obtained were statistically evaluated using statistical package for Social Science Program (SPSS) version 16.0. Values obtained in this study are represented as mean±standard error mean for both test and controls. Student's *t*-test was used to compare the means while correlation was done with Pearson correlation. The $P < 0.05$ was considered as statistically significant.

RESULTS

Sixty infertile males on routine visit to the University of Benin Teaching Hospital, Benin City, aged 38.9 ± 0.98 form the subject group while 20 fertile males aged 38.2 ± 0.68 with evidence of parity were enrolled as controls.

Table 1 shows the mean concentration of cadmium in seminal plasma and semen parameters in infertile males and control subjects. Cadmium levels were significantly higher in infertile males ($P < 0.001$) compared with controls. Sperm counts, Progressive motility and total motility were lower ($P < 0.001$) in infertile males than controls. There was however no significant different in the semen volume between infertile and fertile males.

Table 2 shows a significant inverse correlation between cadmium and sperm counts, progressive motility, total motility and morphology with ($P < 0.001$) while the correlation between cadmium and semen volume was however not significant ($P = 0.401$).

Table 3 shows no significant correlation between cadmium and sperm counts, progressive motility, total motility, semen volume and morphology of fertile males.

DISCUSSION

This study evaluated the levels of cadmium in non-occupationally exposed infertile males and correlated the

Table 1: Seminal plasma cadmium levels and seminal parameters in infertile males and control subjects (Mean±SEM)

Measured parameters	Infertile males Subjects n=60	Fertile Controls n=20	P value
Age (years)	38.9±0.98	38.2±0.68	=0.720
Cadmium (ug/L)	0.103±0.004	0.050±0.003	<0.001
Sperm count (cells/mL)	$16.8\times10^6\pm2.51\times10^6$	$74\times10^6\pm13.31\times10^6$	<0.001
Progressive motility (%)	9.150±1.789	57.100±3.422	<0.001
Total motility (%)	21.500±2.369	67.050±2.399	<0.001
Morphology (%)	15.217±2.272	54.250±2.572	<0.001
Semen volume (mL)	2.983±0.186	3.040±0.318	=0.879

SEM: Standard error of mean

Table 2: Correlation of cadmium levels with semen parameters in infertile males

Parameters	R value	P value
Sperm count (cells/mL)	−0.436	<0.001
Progressive motility (%)	−0.516	<0.001
Total motility (%)	−0.517	<0.001
Morphology (%)	−0.493	<0.001
Semen volume	0.095	=0.401

Table 3: Correlation of cadmium levels with semen parameters in fertile males

Parameters	R value	P value
Sperm count (cells/mL)	−0.064	=0.782
Progressive motility (%)	0.352	=0.117
Total motility (%)	0.241	=0.295
Morphology (%)	0.051	=0.826
Semen volume	0.144	=0.534

levels of this toxic metal with semen parameters. There was a significant increase (P < 0.001) in the seminal plasma cadmium concentration in infertile males compared with controls. Statistically significant inverse correlation (P < 0.001) was observed between cadmium and sperm count, progressive motility, total motility and morphology. These increased level of cadmium may have contributed to low sperm counts, progressive motility, total motility and morphology in these subjects. This observation is consistent with other authors [16-18].The observation did not agree with that of Omu *et al.*[19] who observed no significant differences between seminal cadmium levels in infertile males and fertile males. However, there was no significant difference in the semen volume in infertile males and controls. The findings from this study suggest that cadmium may be a reproductive toxicant. Although it has long been suggested that cadmium affects the male reproductive function of occupationally exposed individuals [20], the role of increased seminal plasma cadmium levels is poorly understood in occupationally unexposed males [8]. It was reported that testicular dysfunction was the major finding when cadmium was injected into mice. The authors hypothesized that the toxic effect of cadmium was due to oxidative damage and lipid peroxidation [21]. Cadmium produces oxidative stress by depleting glutathione and protein-bound sulfhydryl groups, which results in the enhanced production of reactive oxygen species such as superoxide ions, hydroxyl radicals, and hydrogen peroxide. These reactive oxygen species result in increased lipid peroxidation, excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA damage, membrane damage, altered gene expression, and apoptosis which may result to abnormal semen quality [22]. Exposure to Cadmium can induce germ cell apoptosis, which may account for the decline in sperm counts observed among occupationally unexposed infertile males in this study [18,23]. In addition, Cadmium can directly inhibit primary Leydig cell testosterone levels, but the mechanism of this effect is not clear which may be a secondary reason for the reduction of sperm number in seminiferous tubules [24]. Cadmium has been recognized as an endocrine disruptor by binding to androgen and oestrogen receptors thereby inhibiting steriodogenesis and spermatogenesis, which may affect semen quality [13,25]. A positive correlation was earlier observed between seminal plasma cadmium and follicle stimulating hormone levels [18] which was adduced possibly to effect of cadmium on hypothalamic - pituitary - gonadal axis outside the leydig cells. Cadmium is known to enter chromatin of developing spermatozoa and also affect microtubules and mitochondrial function of sperms which may explain the decreased sperm motility observed in this study [26,27]. Cadmium has been shown to have a toxic effect on many enzymes dependent on iron as a co-factor, one of these being cytochrome P450 [28]. Leydig cells contain ten times more of P450 than Sertoli cells, hence are more sensitive to increased cadmium level [29]. Since cytochrome P450 is required for the adequate function of 17-α-hydroxylase, a key enzyme in the steroidogenic pathway that produces androgens; its disruption may well interfere with testicular steroidogenesis, consequently reduced fertility [28]. However, we observed no significant change in the semen volume in the subjects, which infer that toxic metals may have no effect on the seminal vesicle.

In Nigeria, male factor is responsible for 40-50% of all infertility and the prevalence varies from place to place with higher prevalence in the urban than centers than rural communities [30,31]. In a study conducted in mid-western Nigeria, a prevalence of 50% was observed in 780 couples evaluated [32]. Male factor was reported to be responsible for 42.4% in the south-west while 40.8%, 46% and 55-93% were reported in Kano [33], Ife [34] and Enugu [35] respectively among couples attending infertility clinics. Environmental discharge of cadmium-containing products such as petroleum products, the use of combination of fossil fuels (petroleum and coal) as well as municipal wastes may be contributing factors to airborne cadmium pollution [36].

Treatment options for cadmium toxicity in infertile males are evolving. There is currently no agreement regarding the treatment of cadmium toxicity since not much human studies have been done. However, clinical protocols are available for the use of EDTA, DMPS and DMSA chelators, which had proved to be therapeutically beneficial [37].

CONCLUSION

It was observed that cadmium levels were significantly higher in infertile males than control subjects. This toxic metal inversely correlated with sperm counts, progressive motility, total motility and morphology which infers that cadmium may represent reproductive toxicants in non-occupationally exposed males. The increased levels of toxic metals observed in this study may be due to passive inhalation of cigarette, environmental exposure from petroleum products and vehicular exhaust smoke and wastes. The levels of the toxic metal may be assayed when evaluating subjects with idiopathic infertility.

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