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Seminal Plasma Chromium, Cadmium and Lead Levels in Infertile Men

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Heavy metals, have continued to pose health problems and hazards to humans. In recent years, there has been an increasing interest in the contribution of occupational and environmental exposures to toxic pollutants such as chromium, cadmium and lead to declining sperm concentration and human male infertility. A total of one hundred and forty two subjects were used for this study. Eighty of the subjects were infertile men attending the Gynaecological Clinic of the University of the Calabar Teaching Hospital. The infertile men were categorized as oligospermic azoospermic and asthenoligospermic while sixty two fertile men who had fathered at least one child 62 were used as control subjects. The mean seminal plasma chromium levels of oligospermic and asthenoligospermic infertile men was significantly higher ($p < 0.05$) than that of the controls. The mean seminal plasma lead level for the subjects was asthenoligospermic $26.81 \pm 7.5 \mu\text{g dL}^{-1}$, oligospermic $15.4 \pm 1.5 \mu\text{g dL}^{-1}$ and azoospermic $12.1 \pm 0.8 \mu\text{g dL}^{-1}$ while that of the control was $17.12 \pm 0.7 \mu\text{g dL}^{-1}$. There was no significant difference between the infertile men and control ($p < 0.05$). The mean seminal plasma cadmium levels for the subjects was asthenoligospermic $3.65 \pm 0.2 \mu\text{g dL}^{-1}$, oligospermic $3.32 \pm 0.3 \mu\text{g dL}^{-1}$ and azoospermic $3.49 \pm 0.5 \mu\text{g dL}^{-1}$ while that of control was $3.66 \pm 0.2 \mu\text{g dL}^{-1}$. There was no significant difference between the infertile men and control. High chromium concentration in seminal plasma has adverse effect on sperm production, motility and sperm count in oligospermic and azoospermic infertile men.

Key words: Male infertility, semen, chromium, cadmium, lead

INTRODUCTION

Infertility can be defined as the failure to conceive (regardless of cause) within one year of unprotected intercourse (De Melo-Martin, 2002). Thus, an estimated 15-20% of couples attempting their first pregnancy have difficulty achieving it (Morley and Anthony, 2008). For many couples there may be contributing factors from both the man and women, with a cumulative impact on conception. Male factors account for 30-40% of infertility, being the sole cause in about 20% of cases (Jarow *et al.*, 2002). Deficiencies in the semen is the most common cause of male infertility and semen quality is therefore used to measure male reproductive ability (Cooper *et al.*, 2010). Certain cases of male infertility are due to congenital causes such as varicocele, absent vasa differentia and undescended testes, or acquired causes such as endocrine disorders, chronic or acute illness, trauma and surgery. Other acquired causes of male infertility include erectile dysfunction and lifestyle (Balen and Jacob, 2003). Recent studies show that some cases of infertility are caused by deficiencies and/or excess of trace elements. Trace elements implicated in male infertility include chromium, zinc and selenium. The human body requires chromium as a cofactor for a variety of important enzymes, deficiencies of this trace element have been associated with reduced sperm quality in rodents and humans (Benoff *et al.*, 2000). Humans are exposed both occupationally and environmentally to metal aerosols which accumulate in male reproductive organs and may cause infertility. Epidemiological studies have been equivocal about the effects of Pb^{2+} and Cd^{2+} on hormone concentrations, male fertility and sperm parameters (Benoff *et al.*, 2000; Naha and Chowdhury, 2006; Ten *et al.*, 2008). Globally, research information concerning the role of trace elements in male infertility especially in Africans is scarce (Oguntibeju *et al.*, 2009). Thus, there is great need for more research works on this subject matter, hence the need for this study.

This study was conducted to determine the levels of lead, chromium and cadmium in seminal plasma of infertile men and to assess the desirability or otherwise of estimation of trace element as a routine investigation in management of male infertility.

MATERIALS AND METHODS

Subject selection: Eighty Nigerian males attending fertility clinic, University of Calabar Teaching Hospital (UCTH) with confirmed infertility were used as subjects. They were classified as oligospermic, oligoasthenozoospermic and azoospermic. Sixty-two apparently fertile men with history of fathering at least a

child were used as controls. The method of selection was simple random sampling informed consent was obtained from all the participants.

Collection of semen samples: The subjects and controls were advised to abstain from sex for 3 days. The semen samples were collected by masturbation into universal containers and properly labeled. The time of sample collection was also noted by the patient and the sample delivered in the laboratory within one h. The samples were analyzed for motility and total sperm count after which they were centrifuged at 1000 rpm for 10 min. The seminal plasma was separated and stored frozen pending analysis of cadmium, lead and chromium. The deposit was discarded.

Materials: Chromium, Cadmium and Lead standards were obtained from Buck Scientific Inc., East Norwalk, USA. Ultra-pure water were obtained from Sterilin, Britain.

Macroscopic and microscopic analysis of semen: Ten millilitres graduated falcon glass tube (0.1 mL accuracy) was used to measure the semen volume. The pH was measured using a narrow range pH paper. A drop of liquefied sample (15 μ L) was placed on a clean, grease-free slide and cover slip was applied. The preparation was examined microscopically using 40x objectives. The motility of the spermatozoa were graded as:

- Rapidly progressive motility
- Slow or sluggish progressive motility
- Non-progressive motility
- Non-motile

At least 200 scores were performed. Also, 0.02 mL of the semen was diluted with 0.38 mL of sodium bicarbonate-formalin diluent. A new improved Neubaur counting chamber was charged with the diluted fluid using a Pasteur pipette and the preparation allowed settling for 3 min. The spermatozoa in 2 large squares were counted using 10x objectives and the number of cells counted multiplied by 10,000 to give the number of sperm cells per milliliters of sample.

Estimation of lead, cadmium and chromium: This was done using atomic absorption spectrophotometric method.

Quality control: The atomic absorption spectrophotometer also has internal lithium controls as well as quality control standards used to calibrate the equipment.

Table 1: Comparison of age, semen parameters, seminal plasma chromium, lead and cadmium levels of fertile and infertile men

| Parameter | Infertile men | | | | Cal-f | Crit-f | p-value | Remarks |
|-----------------------------------|-------------------------|---------------------|--------------------------|--------------------------|---------|--------|---------|---------|
| | Normo-spermic men | Asthen-oligospermic | Oligo-Spermic | Azoo spermic | | | | |
| Age (Years) | 36.77±1.03 | 36.35±1.13 | 36.89±1.66 | 34.30±1.65 | 0.575 | 2.671 | >0.05 | NS |
| Sperm count (m cm ⁻³) | 46.60±3.39 [#] | 11.00±0.83 | 15.78±0.61 | 0.00 | 46.191 | 3.074 | <0.05 | S |
| Motility (%) | 68.36±2.06 [*] | 13.45±2.11 | 64.40±3.09 | 0.00 | 176.195 | 3.073 | <0.05 | S |
| Cr (µg dL ⁻¹) | 7.63±0.26 | 7.38±0.24 | 12.34±1.83 ^{y*} | 10.59±1.54 ^{y*} | 10.788 | 2.671 | <0.05 | S |
| Cd (µg dL ⁻¹) | 3.66±0.22 | 3.65±0.23 | 3.32±0.40 | 3.49±0.53 | 0.212 | 2.671 | >0.05 | NS |
| Pb (µg dL ⁻¹) | 17.13±0.69 | 26.81±7.55 | 15.41±1.48 | 12.20±0.82 | 1.888 | 2.671 | >0.05 | NS |
| n | 62 | 40 | 18 | 20.00 | | | | |

Mean±SD, ^{*}Higher than that in asthenoligosperms, [#]Higher than that in Oligosperms, ^y: Higher than that in normosperms

Statistical analysis: Statistical analysis was carried out using SPSS 11 analysis pack. Correlation analysis was carried out using Pearson’s correlation.

RESULTS

The mean seminal plasma level of chromium for the control and infertile groups is shown in Table 1. The seminal plasma chromium level of the oligospermic, azoospermic and asthenoligospermic infertile men were 12.34±1.83, 10.59±1.54 and 7.38±0.24 µg dL⁻¹, respectively while that of controls was 7.63±0.26 µg dL⁻¹. The seminal plasma chromium levels of the oligospermic and azoospermic infertile men were significantly higher than those of the controls (p<0.05). However, mean seminal plasma chromium levels of the asthenoligosperms were significantly lower (p>0.05) than those of the azoosperms and oligosperms.

The mean percentage motility for the infertile group was: asthenoligospermic, 13.45±2.10%; oligospermic, 64.40±3.09%; azoospermic, 0%; while that of the control was 68.36±2.06%. The mean percentage motility of the control was significantly higher (p<0.05) than those of other groups as expected.

The mean seminal plasma lead level for the controls and subjects was as shown in Table 1. There was no significant variation among the groups (p>0.05). Seminal plasma cadmium levels for the controls and subjects (Table 1) also showed no significant variation (p>0.05) among the groups. Correlation analyses showed no significant correlations.

DISCUSSION

This study determined the seminal plasma levels of lead, cadmium and chromium in infertile men and also tried to establish the relationship or otherwise among these parameters and male infertility. The results showed that the sperm count and percentage motility was significantly higher (p<0.05) in the normospermic controls compared to the infertile men. This was expected because sperm count determines the density and the motility determines the probability of fertilization occurring.

Seminal plasma chromium concentration was significantly higher in the oligospermic and azoospermic subjects compared to the controls (p<0.05). However levels in the oligospermic subjects were also significantly higher than those of the azoospermic group. The oligospermic subjects had the highest mean seminal chromium level of all the groups. Other studies have made similar findings about chromium and sperm characteristics (Umeyama *et al.*, 1985, 1986; Skandhan *et al.*, 2005). This raises a pertinent question about the ‘hype’ around chromium-containing supplements. Are they really safe?

No significant association was found between seminal plasma lead, cadmium and sperm motility/count. This also agrees with previous studies (Umeyama *et al.*, 1985; Skandhan *et al.* 2005). Similar findings were reported by Alexander *et al.* (1996) who reported no significant relationship between seminal plasma cadmium between fertile and infertile men. However, this finding does not agree with those of Akinloye *et al.* (2006) who reported that Cadmium levels were higher in infertile men compared to normozoospermic men. This is probably because Cadmium and Lead exposure around this part of the country may not be as high as those found elsewhere.

CONCLUSION

High chromium concentration in seminal plasma has adverse effect on sperm production, motility and sperm count in oligospermic and azoospermic infertile men.

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