

Effects of Lead and Cadmium Concentration on Blood and Semen of Male Patients Consulting Fertility Clinic, Abakiliki, South-East Nigeria

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Abstract- The present study aimed to determine the Effects of Lead and Cadmium Concentration on Blood and Semen of Male Patients Consulting Fertility Clinic, Abakiliki, South-East Nigeria. 73 consecutively consenting male partners of women attending fertility clinic (age range, 20-45 years) whose wives were seen by gynecologists at the hospital between July and November 2014 were involved in the study. These men were referred for semen analysis with diagnosis of primary and secondary infertility (13 and 60 respectively). Semen analysis was done in accordance with World Health Organization (WHO) guideline. The semen samples were classified into 3 groups: Azospermia (n = 15), oligospermia (n = 22) and normospermia (n = 36). Atomic absorption spectrophotometer was used to determine the concentrations of lead and cadmium in seminal and blood plasma, respectively. The result obtained showed that the three groups have comparable ($p > 0.05$) age, Body Mass Index (BMI) and sperm volume. The sperm count in the normospermic patients were significant ($p < 0.05$) than the azospermic patients. The same pattern was observed for sperm motility and morphology. Plasma cadmium was higher in azospermic patient in comparison with oligospermic and normospermic patient. However, the plasma cadmium in oligospermic patient was significant than the ones in normospermic patient. It was also observed that the seminal cadmium in oligospermic patients was higher than azospermic patient while the normospermic patients had significant seminal cadmium in comparison with both azospermic and oligospermic patients. For plasma lead, the oligospermic patients had significant ($p < 0.05$) value than the normospermic or the azospermic patients. Furthermore, the normospermic patients were observed to have significant plasma lead in comparison with azospermic patients. The seminal lead was observed to be significant in normospermic patients in comparison with the oligospermic or azospermic patients. The oligospermic and the normospermic patient had comparable values for seminal lead. Plasma cadmium concentration shows a significantly negative correlation with sperm count, sperm motility and sperm morphology. The same pattern was observed with seminal cadmium. Meanwhile, plasma lead was observed to have a significantly negative correlation with sperm count while that of sperm motility and sperm morphology was not significant. In seminal lead, all of the seminal parameters were not significantly related. The result of the present study suggests that Lead and Cadmium have negative effect on sperm parameters. Hence, in avoidance of exposure to these metals, occupational health surveillance should include the assessment of adverse effects on the reproductive system of workers exposed to Lead and Cadmium.

Keywords- Blood and Semen; Lead and Cadmium Concentration; Infertility; Semen quality; Sperm count; Reproductive toxicity.

I. INTRODUCTION

Human infertility appears to be on a rise worldwide. The main cause of this fall in fertility is not yet known, although environmental factors have been found to be one of the causes. A Study in University of Ilorin Teaching Hospital, Ilorin shows that 50% of gynecological consultations are for infertility (Nwabuisi & Onile, 2001).

Infertility by World Health Organization is the failure of a sexually active non-contraception couple to attain pregnancy easily in one year (WHO, 2000). Approximately,

15% of couples attempting their first pregnancy fail and recent data suggest that male-related factors are responsible for about half of all infertility cases, and oligozoospermia and asthenozoospermia were found to be the most common aetiological factors responsible for male infertility (Orisakwe 2014, Ikechebelu, Adinma, Orie, & Ikegwonu, 2003). A study carried out in a fertility clinic Abakiliki, Eastern Nigeria, observed that 70% of the subject in a population of 170 had low sperm count with more abnormal parameters (64%). Asthenozoospermia and teratozoospermia were the most abnormal parameters

discovered. Higher prevalence of oligospermia was discovered in civil servants and the semen (38%) was associated with bacterial infection (Ugwu, Ugwu & Ejikeme, 2008). Furthermore, a study in Mile four hospital, Abakiliki, noted that the prevalence of oligospermia and azoospermia was 15.3% and 2.6% respectively (Ugboma, Obuna & Ugboma, 2012).

Nigerian environments have been found to be extremely polluted with toxic metals, particularly lead and cadmium (Katakura & Sugawara, 1999). Lead and cadmium are some of the known reproductive toxicants that affects fertility that human are occupationally and environmentally exposed to. Studies have shown that cadmium alkalizes the lumen fluid of epididymis and the vas deferens by inhibiting the H-ATPase function which is vital in the maturation and storage of sperm (Herak-kramberger, Sabolic, Blanusa, Smith, Brown & Breton, 2000). To bolster this finding, Burukoglu and Baycu (2008) added that short time exposure to high dose of cadmium causes severe damage of the seminiferous tubule and degenerate and disintegrates sperm cell.

Exposure to lead and cadmium has been found to damage the DNA of human sperm through oxidative reaction (Xu, Shen, Zhu, Chua, Wang, Chia & Ong, 2003). This damage is as a result of the increase in reactive oxygen species (ROS) levels. The damage to the DNA can lead to alteration, histone base modification and obstruction in sperm effectiveness during fertilization (Barratt, Aitkenrj, Bjorndahl, Carrel, De-Boer, 2010). To this end, this study investigates the effects of lead and cadmium concentration on blood and semen of male patients consulting fertility clinic, Abakiliki, SouthEast Nigeria.

A. Purpose of the Study

The aim of the study was to examine the effects of lead and cadmium concentration on blood and semen of male patients consulting fertility clinic, Abakiliki, SouthEast Nigeria. The objectives of the study were enumerated as follows:

- (i) to determine Lead And Cadmium in the blood and semen of Male partners of women consulting at fertility clinic;
- (ii) to determine the semen parameters of Male partners of Women consulting at fertility clinic; and
- (iii) to correlate the levels of cadmium and lead in blood and semen with sperm parameter in Male patient consulting at fertility clinic.

II. MATERIALS AND METHODS

A. Recruitment of Sample

The study was conducted at Our Lady's Hospital Abakiliki, a private specialist hospital. 73 male patients attending fertility clinic (age range, 20-45 years) whose wives were been seen by gynaecologists at the hospital between July and November 2014 were volunteers in the study. Sociodemographic data such as age of couples, occupation, duration of involuntary childlessness, and obstetric history were obtained by structured questionnaire.

B. Inclusion and Exclusion Criteria

Inclusion criteria includes men within reproductive age of 20-45 years, normal testis male partners of couples living together and having regular unprotected sexual intercourse for two or more years. Exclusion criteria includes genital infection, male contraceptive users, testicular varicocele, smoking/chronic alcohol intake, long term medication, surgery, chronic systemic illness that may affect fertility such as hepatic, endocrine diseases etc. and men currently on fertility drugs.

C. Study Consent/Ethical Approval

A written consent of each participant that merited our selection criteria was taken after explaining the aim and objectives of the study and its benefits on individual and society. Also, the Ethics and Research Committees of the Federal Teaching Hospital, Ebonyi State University, Abakiliki approved the study protocol.

D. Material/Reagent

Pipette, Centrifuge (MDCEN-302-SD), Centrifuge Tube, Test Tube, Neubauer Counting Chamber, Microscope (model 163), Absorbent paper, Oil immersion, Perkin-Elmer Model 303 atomic absorption spectrometer, Basophilic thiazine, Glass slide, Coverslip, Eosinophilicxanthenes, Trichloroacetic acid solution, Ammoniumpyrrolidinedithiocarbamate solution, Methylisobutylketone and Petroleum jelly.

E. Sample Analysis

1. Sperm motility

A drop of semen was place on a glass slide and covered with a coverslip that was then ringed with a petroleum jelly to prevent dehydration. It was then examined under 40 × objective. Atleast 200 spermatozoa were counted. The result was expressed as a percent of:

- (a) Rapidly progressive spermatozoa moving fast forward in a straight line.
- (b) Slowly progressive spermatozoa (slow linear or non-linear).
- (c) Non-progressive spermatozoa (movement of tail but with no forward progress).
- (d) Immotile spermatozoa (no movement at all) (WHO criteria).

2. Sperm count

The semen was diluted with sodium bicarbonate formalin diluting fluid at the ratio of 1:20. A coverslip was placed over the improved Neubauer counting chamber and the counting chamber was filled with the well-mixed diluted semen sample using a Pasteur pipette. The chamber was then placed in a humid box for 10-15 min for the spermatozoa to settle. The chamber was then placed on a microscope stage using a 20× objective and an iris diaphragm was lowered sufficiently to give a sufficient contrast. The number of spermatozoa was counted in the 4 large corner squares. Spermatozoa whose heads are touching the left and upper lines of the square are considered to have been part of the square.

The sperm count per ml was calculated as follows:

$$\begin{aligned}\text{Sperm count} &= \frac{\text{sperm counted} \times \text{correction factor} \times 100}{\text{Number of squares counted} \times \text{volume of 1 square}} \\ &= \frac{\text{sperm counted} \times 40 \times 1000}{4 \times 0.1} \\ &= \text{sperm counted} \times 50000\end{aligned}$$

3. Sperm morphology

A smear was prepared by spreading a drop of seminal fluid on a glass slide. It was stained with staining solution 1 (eosinophilic xanthene) for 10 seconds, then with rapid solution 2 (basophilic thiazine) for 5 seconds. It was later passed through a running tap water for 10 to 15 times to

remove the stain. The excess solution was drained at each step by placing it vertically on blotting paper. The slide was allowed to dry. Oil immersion was placed on the stained smear and the percentage of normal and abnormal forms of spermatozoa were counted using the 100× objective under a microscope. At least 200 spermatozoa were counted (WHO, 2000).

Table I: WHO Criteria for Semen Analysis

Semen volume	2ml or more
Sperm concentration	> 20×10 ⁶ cell/ml
Sperm motility	> 50% forward movement
Ph	7.2-7.8
Sperm morphology	> 30% normal form
WBC	< 1×10 ⁶

Table II: Abnormal Sperm Parameter

Aspermia	no ejaculate
Azoospermia	no sperm cell
Oligospermia	< 20×10 ⁶ sperm/ml
Severe oligospermia	< 5×10 ⁶ sperm/ml
Asthenozoospermia	abnormal motility
Teratozoospermia	alteration in morphology

F. Sample Collection

Semen samples were collected by masturbation into a sterile metal-free glass container after 3-4 days of abstinence. The use of antibiotic prior to sample collection was avoided. Fasting venous blood (5 ml) was also collected from each participant in to a lithium heparin sample bottle free from metal for the determination of lead and cadmium. The samples were centrifuged at 2000rpm for five minutes after which plasma was isolated and stored frozen (-20°C) until they were analyzed.

G. Reagent Preparation

- **Trichloroacetic acid solution, 5gm/dl:** 50gm of metal-free trichloroacetic acid was dissolved in 1liter of water.
- **Methylisobutylketone saturated with trichloroacetic acid:** 400ml of methylisobutylketone was added to 100ml of trichloroacetic acid in a separation funnel. The mixture was shaken and allowed to stand in a refrigerator for 1 hour. The methylisobutylketone phase was removed and centrifuges to eliminate trace of aqueous phase.
- **Ammonium pyrrolidinedithiocarbamate solution, 2gm/dl:** 1gm of ammonium pyrrolidinedithiocarbamate dissolved into 50 ml of water and the solution was extracted twice using 5 ml of methylisobutylketone. The extracts were discarded.
- **Methylisobutylketone – ammonium pyrrolidinedithiocarbamate solution:** 10ml of trichloroacetic acid solution was transferred in to a centrifuge tube and its PH was adjusted to 2.5 by adding ammonium hydroxide solution. 5 ml of ammonium pyrrolidinedithiocarbamate solution (2 gm/dl) and 30 ml of methylisobutylketone were added. It was mixed and the centrifuge at 900 rpm for 15 mins. the methylisobutylketone phase was removed.

H. Sample Preparation for Metal Analysis

8ml of trichloroacetic acid solution was dissolved in to 2ml of heparinized blood and seminal plasma and mixed respectively. It was allowed to stand for 1hour at room temperature. It was then centrifuge at 900rpm for 10min. the protein-free supernatant was decanted in to a tube. The precipitated blood and seminal plasma were washed with 4ml of trichloroacetic acid solution and allowed to stand for 5mins respectively. It was then centrifuge at 900rpm for

10mins. Blank and standard samples were prepared by dissolving 0ml, 1m and 2ml of lead stock standard solution to 12ml of trichloroacetic acid solution in three test tubes respectively. These samples correspond to 0.25 and 50 $\mu\text{g}/\text{Pb}/\text{dl}$ of blood. Concentrated ammonium hydroxide solution was added to adjust the PH to 2.5. Ammoniumpyrrolidinedithiocarbamate solution was added to each tube, shaken and allowed to stand on a testtube racks for 5mins. 2ml of methylisobutylketone was then added and mixed with a vortex mixer, and then it was centrifuge at 900rpm for 15min. methylisobutylketone was aspirated into the burner of the atomic absorption spectrometer and the flame was adjusted to a dark blue color with 5mm bright blue segment. The reagent methylisobutylketone was aspirated for 20min in order to stabilize flame. Methylisobutylketone added with trichloroacetic acid is then aspirated for 10min with the recorder reading ON in order to verify stability. Finally methylisobutylketone saturated with ammonium pyrrolidinedithiocarbamate solution was aspirated for 1min to remove residue of lead from burner. Methylisobutylketone extracts was aspirated into the burner and the absorbance recorder. The height of the peaks was measured and the concentration of lead in the samples was calculated by proportionality with the standard samples. (Thompson & Banniga, 2008)

I. Atomic absorption spectrophotometry

The sample to be analyzed is atomized in either a flame or a graphite furnace. Upon the absorption of ultraviolet or visible light, the free atoms undergo electronic transitions from ground state to excited electronic states. To obtain the best results in atomic absorption, the instrumental and chemical parameter of the system must be geared toward the production of neutral ground state atoms of the element of interest. A common method is to introduce a liquid sample into the flame. Upon introduction, the sample solution is dispersed into a fine spray, the spray is then dissolved into salt particle in the flame and the particles are subsequently vaporized into neutral atoms, ionic species and molecular species. The light produce by the hollow cathode lamp is emitted from excited atoms of the same element which was determined. (Haswell, 1991, Schrenk, 1975)

J. Method of Data Analysis

The data obtained was statistically evaluated using Statistical Package for Social Sciences (SPSS^(R)) version 20.0. Values are represented as mean $\pm$ standard deviation for all variables. One-way Analysis of Variance (ANOVA) was used to compare the means while relationship between parameters was established using Pearson correlation

analysis. Values were considered statistically significant at p value < 0.05 .

III. RESULTS

Table III shows the general characteristics of the sample engaged in the study. It represents their age, body mass index and sperm.

Table III: Age, Body Mass Index and Sperm Characteristics of the Sample

Parameter	Azoospermia (n = 13)	Oligospermia (n = 26)	Normospermia (n=36)
Age	39.54±7.3	37.32±6.9	36.08±6.96
BMI	28.4±3.6	27.99±4.63	26.62±5.36
Volume	2.2±0.99	2.91±1.38	2.92±1.32
Count	0.00±0.00 ^a	26.77±15.75 ^b	117.97±48.55 ^c
Motility	0.00±0.00 ^a	44.32±16.78 ^b	55.83±16.63 ^b
Morphology	0.00±0.00 ^a	46.82±9.46 ^b	58.33±13.63 ^b

Values with different superscript along row are significant; $p < 0.05$.

From Table III, the three groups have comparable ($p > 0.05$) age, body mass index (BMI) and sperm volume. The semen count in the normospermic patients were significant

($p < 0.05$) than the oligospermic and azoospermic patients. The same pattern was observed for sperm motility and morphology.

Table IV: Plasma, Seminal Cadmium and Lead Levels µg/dl

Parameter	Azoospermia (n = 13)	Oligospermia (n = 26)	Normospermia (n = 36)
Plasma cadmium	0.98±0.49 ^a	0.77±0.36 ^a	0.43±0.29 ^b
Seminal cadmium	0.89±0.41 ^a	1.14±0.43 ^a	0.49±0.31 ^b
Plasma lead	1.71±0.98 ^a	2.05±0.97 ^a	1.49±0.59 ^b
Seminal lead	0.44±0.49 ^a	1.22±0.81 ^b	0.85±0.86 ^b

Plasma cadmium was higher in azoospermic patient in comparison with oligospermic patient and normospermic patients. However, the plasma cadmium in oligospermic patients was significant than the normospermic patient (Table IV). It was also observed that the seminal cadmium in oligospermic patient were significant than azoospermic patient while the normospermic patient is has significant ($p < 0.05$) seminal cadmium in comparison with both azoospermic patient and oligospermic patients.

For plasma lead, the oligospermic patients had significantly ($p < 0.05$) higher value than normospermic or the azoospermic patients. However, the normospermic patients were observed to have a significant ($p < 0.05$) plasma lead in comparison with azoospermic patients. The seminal lead was observed to be significant ($p < 0.05$) in normospermic patients in comparison to the normospermic or the azoospermic patient. Meanwhile, the azoospermic and the normospermic patients have comparable values for seminal lead (Table IV).

Table V: Pearson Correlation Analysis of Semen Parameters with Plasma, Seminal Cadmium and Lead

Semen parameters	Plasma cadmium	Seminal cadmium	Plasma lead	Seminal lead
Count	$r = -0.455, p = 0.001$	$r = -0.476, p = 0.001$	$r = -0.280, p = 0.015$	
Motility	$r = -0.385, p = 0.001$	$r = -0.249, p = 0.032$	-	
Morphology	$r = -0.413, p = 0.001$	$r = -0.304, p = 0.010$	-	

r = correlation coefficient

p = p - value

In Table V, using Pearson correlation analysis, plasma cadmium showed a significant ($p = 0.001$) negative correlation with sperm motility, sperm count, sperm morphology among all the three groups (azoospermic, oligospermic, and normospermic). The same pattern was observed with seminal cadmium. Meanwhile, plasma lead was observed to have a significantly negative correlation with sperm count while that of sperm motility and sperm morphology was not significant. However, in seminal lead all of the seminal parameters had no significant correlation with sperm parameters.

IV. DISCUSSION

The present findings show that plasma and seminal cadmium and lead have significant effect on sperm parameters. The results are in agreement with the findings of Oluyemi, Ayodele, Olayiwola and John (2006), which reported significant mean seminal and plasma Cadmium level in oligozoospermics compared with that of azoospermics. The significant seminal cadmium in oligospermic patients in comparison to azoospermic patients but significant seminal cadmium in normospermic patient in comparison with both azoospermic patient and oligospermic patients observed in the present study are in disagreement with the findings of Omu, Dashtu, Mohammed and Mattappalli (1995), where it was reported that no significant differences in seminal cadmium level among the normozoospermic, oligozoospermic and azoospermic patients. A study by Pant, Upadhyaya, Pandey, Mathur, Saxena, and Srivastava (2003) showed an increase in cadmium concentration in the seminal plasma of infertile men. However, the results of this study are in accordance with the data recovered by Umeyama, Ishikawa, Takashima, Yoshii and Koiso (1986), Sanaranen, Suistomaa, Kantola, Saarikoski and Vanha-Perttula (1987) and Jockenhovel, Bals-Pratsch, Bertram and Nieschlag (1990). Therefore this indicates that cadmium exact a deleterious effect on the reproductive system of Nigerian men and other population worldwide. It has been stated by Herak-kramberger et al. (2000) that cadmium, reproductive toxicants are found to be in increasing number in human semen which alkalizes the lumen fluid of epididymis and the vas deferens by inhibiting the H-ATPase function.

The reason for the significant value of plasma lead in oligospermic patient in comparison with the normospermic or azoospermic patients and the significant plasma lead in normospermic patients in comparison with the azoospermic patients in the present study is not quite obvious. However, the accompanying altered sperm qualities support the

detrimental effects of heavy metals on semen quality (Apostoli, Kiss, Porru, Bonde & Vanhoorne 1998). In a Danish study (Bonde & Kolstad, 1997), exposure to lead was associated with reduced fertility among older men but not among younger ones (cut off point at 30 years of age) in a subset of battery workers with at least one PbB > 20 µg/dl. There were a number of mechanisms by which lead may affect male reproductive health. Direct toxic effects on sperm and gonads have been observed in animal tests. Furthermore, lead exposure has been linked with chromosomal aberrations in exposed populations. Both animal and human studies suggest that the sperm chromatin structure is altered at low exposure. A biological rationale for this finding is that lead and other cations (mercury, copper etc.) may cause a partial replacement of zinc which is essential for sperm head chromatin stabilization. Failure of or delay in sperm chromatin decondensation may lead to decreased fertility or different kinds of DNA damage in the fertilization process (Markku, 2001).

Additionally, the negative correlations observed between seminal and plasma lead and cadmium and sperm parameters in the present study reaffirm the detrimental effect of these metals on reproductive health. This observation is consistent with the findings of Xu et al. (1993). These authors also reported a significant negative correlation between blood cadmium concentration and sperm density as well as a significant negative correlation of these toxicants with sperm motility and concentration in oligoasthenozoospermic men. An increase in blood/ plasma cadmium level has been connected with teratozoospermia reported that epididymis and seminal vesicles contains high level of cadmium in the body. Still in agreement to this study, Oluyemi et al. (2006) observed significant negative correlation between serum cadmium and a number of biophysical parameters i.e. sperm density and motility, mean progressive motility, sperm viability and morphology. This shows that cadmium is a strong reproductive toxicant. Katakura and Sugawara (1999) reported the toxic effect of Cd (0.012 mmol/kg/day for 2 days) on wistar rat. Testicular dysfunction was the main observation by histological finding that disclose prevalent of severe necrosis three days after the final infusion 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.

V. CONCLUSION

The results of this study support the body of evidences that heavy metals (cadmium and lead) have negative effects

on sperm parameters and suggest a role for these metals in the reproductive function of Nigerian males especially Abakiliki, South-East Nigeria. Occupational health surveillance must be involved in the assessment of the harmful effects of lead and cadmium on the reproductive system of workers, particularly those with significant environmental exposures. Sources of cadmium and lead such as tobacco, cigarette, and chemicals that contain lead should be avoided by young adolescents and concentrations of cadmium and lead should be evaluated when diagnosing subjects with infertility of unknown etiology in males.

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