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THE IMPACT OF SELECTED HEAVY METALS TO DYSPERMIA IN NIGERIA

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Declining male fertility is of global concern and has been linked to the effects of some heavy metals which are recognised as testicular toxins. Selenium and zinc play specific roles in heavy metal detoxification, testosterone metabolism, sperm formation and motility. This study was aimed at identifying the possible contribution of cadmium (Cd), lead (Pb), selenium (Se) and zinc (Zn) to sperm defects in Nigerian men. 120 males (20-54 years) were recruited after informed consent. These were age-matched 77 dyspermics and 43 normospermics. Semen samples were collected from subjects by masturbation after 3-5 days of abstinence from sexual intercourse. Spermiogram and sperm morphological characteristics were done using WHO guidelines and Tygerberg Strict criteria respectively. 10 ml of blood was obtained from each participant. Serum and seminal plasma were obtained by centrifugation of clotted blood and semen respectively. Cd, Pb, Se and Zn were assayed in serum and seminal plasma by atomic absorption spectrophotometry. Data were analysed using t-test, ANOVA and multiple regressions at p=0.05. Increased serum Zn/Cd (p=0.04) and Se/Cd (p=0.03) significantly predicted increased semen volume in dypermics. Increased seminal plasma Se/Pb (p=0.05) significantly predicted increased normal sperm morphology. Increased serum Cd significantly predicted increased tail defects (p=0.008) whereas, increased serum Se/Cd significantly predicted decreased tail defects (p=0.01) in normospermics only. Increased serum Zn/Cd significantly predicted (p=0.048) decreased Teratozoospermia index (TZI). Increased seminal plasma Zn (p=0.04) and Zn/Pb (p=0.04) significantly predicted decreased sperm deformity index (SDI) respectively. Reduced levels of selenium and zinc in dyspermic males may account for the loss of their protective effect against cadmium and lead toxicity to the testes.

Keywords: Cadmium, Lead, Selenium, Semen quality, Sperm quality, Trace elements, Toxic metals, Zinc.

INTRODUCTION

The negative impact of the environment on the human reproductive ability has been of great concern globally (Carlsen et al., 1992; Itoh et al., 2001). Heavy metals such as cadmium (Cd) and lead (Pb) are toxic to the testis and have been shown to cause testicular damage and irreversible infertility. Epidemiological studies have been equivocal about the effects of Cd^{2+} and Pb^{2+} on male fertility and sperm parameters (Benoff et al., 2000). Changes in human and animal sperm morphology and motility have been associated with toxic occupational and environmental exposures which may relate to damage of differentiating cells or over time to stem cells (Carreau et al., 2007). Although it is known that various toxic and essential metals are interactive, very little information is available on their

possible combined effect on human male reproductive function.

OBJECTIVE

Dyspermia is common in African males with mechanisms that are not well defined (Ilesanmi et al., 1996). It is postulated that Cd and Pb deplete Zn and Se thus adversely affecting semen quality in man. This study was therefore, designed to identify the possible interactions of Cd and Pb with essential trace elements (Zn and Se) and their effects on semen quality with a view to improving our understanding of the aetiology of poor semen quality as well as provide novel and rational approaches to preventing and treating infertility in men.

MATERIALS AND METHODS

STUDY DESIGN AND SELECTION OF SUBJECTS

This prospective cross-sectional survey was conducted using 120 male subjects after informed consent. The subjects were recruited from the Urology clinic of the University College Hospital, Ibadan and Urology department of the University of Port Harcourt Teaching Hospital, Port Harcourt and their environs. The study protocol was approved by the University College Hospital/ University of Ibadan ethical review committee prior to commencement of the study in accordance with the Helsinki Declaration (1983).

The controls comprised apparently healthy males without any history of fertility problems, the partners of whom had a spontaneous pregnancy within one year of regular unprotected intercourse and were pregnant at the time of the male's inclusion into the study. Cases (subjects) comprised apparently healthy males with/without any history of fertility problems, the partners of whom have not had a spontaneous pregnancy within one year of regular unprotected intercourse and were not pregnant at the time of the male's inclusion into the study. A baseline semen analysis was carried out for all subjects and this was repeated within two weeks following the World Health Organisation (WHO, 1999) guidelines. Semen was examined macroscopically for appearance, liquefaction, consistency and volume; and microscopically for concentration, motility and morphology. Based on the outcome of the semen analysis, the subjects were then classified as normospermics {fertile and infertile} and dyspermics {azoospermics, oligospermics, asthenoteratozoospermics and oligoasthenoteratozoospermics}.

BIOLOGICAL SAMPLE COLLECTION AND EVALUATION

SEMEN

Semen was collected in a clean, dry, sterilized, wide mouth, well stoppered glass vial by masturbation after 3 - 5 days of abstinence. The sample was labelled with participant's identification number, date and time of collection and delivery, completeness of the collection. Physical characteristics of semen, sperm count, motility, viability, morphology and corresponding morphometry was measured at 400x and 1000x magnification after liquefaction of the sample following the WHO guidelines (World Health Organisation, 1999).

Seminal plasma was prepared from the whole liquefied semen after centrifugation at 500g for 15 minutes in IEC centrifuge (International Equipment Company, Boston, USA). Aliquot of the seminal plasma was separated into plastic sample containers and stored at -20ºC in lead free storage vial for heavy metal content analysis.

Blood

Blood sample (10 ml) was drawn from a large cubital vein in the sitting position from the subject between 8.00 am and 11.00 am as the semen sample was being submitted for analysis. The blood was collected directly into the vacuum tube and allowed to clot and retract completely before centrifugation at 500g in IEC centrifuge for 15 minutes. The serum sample was separated into plastic sample containers and stored at -20ºC until further analysis. Lead, cadmium, zinc and selenium content of serum and seminal plasma was measured by atomic absorption spectrophotometer (AAS), Perkin-Elmer AAS model 703 (Perkin-Elmer

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Table 1. Distribution of Subjects Based on their Semen Quality.

Semen Quality	Nature of Dyspermia	N (%)
Total Male Subjects		120 (100%)
Normospermics		43 (35.8%)
	Fertile (Controls)	27 (62.85%)
	Infertile (Subjects)	16 (37.2%)
Dyspermics		77 (64.2%)
	Oligospermics	17 (22.1%)
	Azoospermics	12 (15.6%)
	Asthenoteratozoospermics	17 (22.1%)
	Oligoasthenoteratozoospermics	31 (40.2%)

n = number of subjects; % = percentage of subjects.

Oak Brown, Illinois, USA) equipped with AS 60 atomic sampler and hollow cathode lamp. When the atoms in the vapour are excited, they return to the ground state by emitting light of the same wavelength. The amount of light absorbed by the metal is proportional to its concentration in the solution and is determined at a specific wavelength in the AAS. Lead was determined by the modified methods of Pleban and Mei (1983) using AAS. Cadmium was determined by the modified methods of Ediger and Coleman (1973); Alfaro and Heaton (1973), a modification of the method of Piper and Higgins (1967) using AAS. Selenium in serum and seminal plasma was determined with AAS by the method of Pleban et al. (1982). Zinc in serum and seminal plasma was determined by the method of Smith et al., (1979) using AAS.

STATISTICAL ANALYSES

All the data obtained from the study participants were collated and analysed using the computer based software SPSS version 19 (SPSS Inc., USA). Most of the data were expressed in mean ± standard error of mean (sem). Analyses of variance (ANOVA) and Post Hoc were used for comparison of multiple variables. Student *t*-test was used for comparison of paired variables. Multiple regression analysis was used to calculate the interrelationships of toxic metals (Cd, Pb), essential elements (Zn, Se) considered as possible explanatory variables (simultaneously introduced in the model) with respect to each of the measured semen parameter. The measured differences were considered to be statistically significant when $p<0.05$.

RESULTS

PATTERN OF DYSPERMIA IN THE STUDY POPULATION

Table 1 showed that 43 (35.8%) of the subjects were normospermic while 77 (64.2%) were dyspermic. 27 (62.8%) of the normospermic men were fertile while the remaining 16 (37.2%) were infertile. Among the dyspermic subjects, 17 (22.1%), 12 (15.6%), 17 (22.1%), 31 (40.2%) were oligospermic, azoospermic, asthenoteratozoospermics and oligo-astheno-teratozoospermics respectively.

Table 2 showed that all the semen parameters except volume were significantly different between normospermic and dyspermic groups ($p = 0.001$). Normal morphology was significantly higher in fertile normospermics than infertile normospermics ($p = 0.001$), while head defects, mid-piece defects, tail defects, teratozoospermia index and sperm deformity index were significantly higher in infertile normospermics ($p = 0.001$) as shown in Table 3.

LEVELS OF TOXIC METALS AND ESSENTIAL ELEMENTS IN SERUM AND SEMINAL PLASMA

Serum and seminal plasma essential elements (Zn, Se) and toxic metals (Cd, Pb) as well as their ratios were compared between normospermic and dyspermic subjects using the student's *t*-test as

Table 2. Semen Biophysical Characteristics of Normospermic and Dyspermic Men.

Semen Biophysical Characteristics	Normospermics n=43	Dyspermics n=77		
	Mean (sem)	Mean (sem)	t	p
Semen volume (ml)	3.50 (0.15)	3.20 (0.13)	1.57	0.12
Sperm viability (%)	64.28 (1.85)	40.82 (3.09)	5.74	0.001*
Sperm count (x 10^6/ml)	88.03 (4.26)	29.44 (3.78)	10.12	0.001*
Total sperm count (x 10^6)	308.26 (19.91)	91.78 (12.58)	9.67	0.001*
Sperm motility (%)	62.77 (1.36)	33.63 (2.50)	8.93	0.001*
Normal Morphology (%)	19.51 (0.53)	10.68 (0.54)	11.19	0.001*
Head defects (%)	80.49 (0.53)	89.35 (0.54)	11.22	0.001*
Mid-piece defects (%)	11.09 (0.59)	17.22 (0.71)	6.15	0.001*
Tail defects (%)	3.72 (0.27)	9.31 (0.49)	8.65	0.001*
Cytoplasmic droplets (%)	1.33 (0.14)	4.66 (0.49)	5.48	0.001*
Teratozoospermia index	1.19 (0.01)	1.30 (0.01)	6.39	0.001*
Sperm deformity index	0.83 (0.01)	0.92 (0.01)	12.73	0.001*

t = Student's *t*-test, p = Probability value, *= significant at p<0.05, sem = standard error of mean.

Table 3. Semen Biophysical Characteristics of Fertile and Infertile Normospermic Men.

	Fertile Normospermics n = 27	Sub-fertile Normospermics n=16		
Semen Parameters	**Mean (sem)**	**Mean (sem)**	**t**	**P**
Semen volume (ml)	3.47 (0.18)	3.62 (0.27)	0.45	0.65
Sperm viability (%)	67.04 (1.87)	59.63 (3.63)	2.01	0.05
Sperm count (x 10^6/ml)	93.01 (4.57)	79.64 (8.25)	1.54	0.13
Total sperm count (x 10^6)	318.07 (22.08)	291.70 (30.07)	0.64	0.53
Sperm motility (%)	62.96 (1.40)	62.44 (2.83)	0.19	0.85
Normal Morphology (%)	20.89 (0.61)	17.19 (0.68)	3.91	0.001*
Head defects (%)	79.11 (0.61)	82.81 (0.68)	3.91	0.001*
Mid-piece defects (%)	9.19 (0.44)	14.31 (1.00)	5.38	0.001*
Tail defects (%)	2.70 (0.17)	5.44 (0.38)	7.58	0.001*
Cytoplasmic droplets (%)	1.22 (0.16)	1.50 (0.26)	0.96	0.34
Teratozoospermia index	1.15 (0.01)	1.26 (0.02)	7.567	0.001*
Sperm deformity index	0.82 (0.01)	0.86 (0.01)	4.39	0.001*

t = Student's *t*-test, p = Probability value, *= significant at p<0.05 Sem = standard error of mean.

shown in Table 4. The serum and seminal plasma toxic metals were all significantly lower while the essential elements were all significantly higher in normospermics than dyspermics (p<0.002). All the essential and toxic metal ratios in serum and seminal plasma were significantly higher in normospermics than dyspermics (p = 0.0001). There was no significant difference in the levels of essential elements and toxic metals in serum as well as their ratios between fertile and infertile

Table 4. Comparison of mean (sem) Toxic Metals and Essential Elements in Serum and Seminal Plasma between Normospermics and Dyspermics.

Toxic and Essential Elements	Normospermics n = 43	Dyspermics n = 77		
Serum	**Mean (sem)**	**Mean (sem)**	**t**	**P**
Cd (mg/L)	0.13 (0.01)	0.32 (0.02)	7.43	0.001*
Pb (µg/L)	28.24 (0.74)	34.81 (0.55)	7.13	0.001*
Zn (mg/L)	7.89 (0.16)	7.21 (0.12)	3.25	0.002*
Se (mg/L)	0.86 (0.01)	0.80 (0.01)	3.81	0.001*
Zn/Cd ratio	90.75 (10.71)	29.37 (1.96)	7.31	0.001*
Zn/Pb ratio	0.29 (0.01)	0.21 (0.01)	7.49	0.001*
Se/Cd ratio	9.91 (1.14)	3.30 (0.21)	7.35	0.001*
Se/Pb ratio	0.03 (0.00)	0.02 (0.00)	8.18	0.001*
Seminal plasma				
Cd (mg/L)	1.16 (0.07)	2.00 (0.07)	7.43	0.001*
Pb (µg/L)	31.94 (0.87)	39.21 (0.61)	6.99	0.001*
Zn (mg/L)	161.92 (5.16)	140.91 (2.77)	3.93	0.001*
Se (mg/L)	0.27 (0.01)	0.22 (0.01)	3.89	0.001*
Zn/Cd ratio	170.23 (13.84)	81.27 (4.07)	7.63	0.001*
Zn/Pb ratio	5.24 (0.22)	3.66 (0.10)	7.51	0.001*
Se/Cd ratio	0.30 (0.03)	0.12 (0.01)	7.17	0.001*
Se/Pb ratio	0.01 (0.00)	0.006 (0.00)	6.49	0.001*

Cd = cadmium, Pb = Lead, Zn = Zinc, Se = Selenium, Zn/Cd = Zinc/Cadmium ratio, Zn/Pb = Zinc/Lead ratio, Se/Cd = Selenium/Cadmium ratio, Se/Pb = Selenium/Lead ratio, t = student's t-test, p = significance level, * = significant at $p<0.05$.

normospermics. However, significant differences in seminal plasma Pb, Se and Se/Pb ratio were observed. Seminal plasma Pb was significantly lower while Se and Se/Pb ratio were significantly higher in fertile normospermics than infertile normospermics ($p<0.008$), (Table 5). Comparatively, all essential elements and toxic metals as well as their ratios in serum and seminal plasma were significantly different ($p<0.0001$) except for serum Zn level which was similar between fertile normospermics and oligospermics; and seminal plasma Zn between fertile normospermics, oligospermics and oligoasthenoteratozoospermics as shown in Table 6. Tables 7 and 8 showed the relationships between semen parameters and the heavy metals in serum and seminal plasma which might predict the semen quality of normospermic and dyspermic adult Nigerian men. There was no significant relationship between the heavy metals and semen volume in normospermics, but among dyspermics, semen volume was positively associated with serum Zn/Cd and Se/Cd ratios. Serum Se/Cd ratio had the largest beta coefficient of 1.51 and Zn/Cd ratio had the least beta coefficient of 1.45. Thus, 1.0 SD increase in serum Zn/Cd and Se/Cd ratio was significantly associated with 1.45 SD ($p = 0.04$) and 1.51 SD ($p = 0.03$) in predicted increase in semen volume respectively with other variables held constant.

Sperm vitality was positively associated with serum Se/Cd ratio and seminal plasma Zn/Cd ratio in normospermics only. Seminal plasma Zn/Pb ratio had the largest beta coefficient of 1.94 and serum Se/Cd ratio had the least beta coefficient of 1.49. Thus, 1.0 standard deviation (SD) increase in serum Se/Cd ratio was associated with 1.49 standard deviation increase in predicted sperm vitality ($p = 0.04$) and 1.0 SD increase in seminal plasma Zn/Cd ratio was associated with 1.94 SD increase in predicted sperm vitality with other variables held constant. Sperm density was negatively associated with serum Pb level in normospermics only.

Table 5. Comparison of mean (sem) Toxic Metals and Essential Elements in Serum and Seminal Plasma between Fertile and Infertile Normospermics.

Toxic and Essential Elements	Fertile Normospermics n = 27	Infertile Normospermics n = 16		
Serum	**Mean (sem)**	**Mean (sem)**	**t**	**P**
Cd (mg/L)	0.12 (0.01)	0.14 (0.02)	0.97	0.34
Pb (µg/L)	28.23 (1.00)	28.26 (1.12)	0.02	0.99
Zn (mg/L)	8.08 (0.22)	7.53 (0.23)	1.66	0.11
Se (mg/L)	0.87 (0.01)	0.83 (0.02)	1.55	0.13
Zn/Cd ratio	94.77 (14.26)	83.98 (16.25)	0.48	0.63
Zn/Pb ratio	0.29 (0.01)	0.27 (0.01)	1.28	0.21
Se/Cd ratio	9.90 (1.37)	9.92 (2.09)	0.01	0.99
Se/Pb ratio	0.03 (0.00)	0.03 (0.00)	0.91	0.37
Seminal plasma				
Cd (mg/L)	1.11 (0.08)	1.25 (0.14)	0.97	0.34
Pb (µg/L)	30.20 (1.11)	34.87 (1.08)	2.81	**0.008***
Zn (mg/L)	159.66 (6.21)	165.73 (9.28)	0.56	0.58
Se (mg/L)	0.29 (0.01)	0.22 (0.01)	3.87	**0.001***
Zn/Cd ratio	171.24 (16.59)	168.54 (25.23)	0.09	0.93
Zn/Pb ratio	5.46 (0.29)	4.85 (0.34)	1.35	0.19
Se/Cd ratio	0.33 (0.04)	0.25 (0.05)	1.29	0.20
Se/Pb ratio	0.01 (0.00)	0.006 (0.00)	4.61	**0.001***

Cd = cadmium, Pb = Lead, Zn = Zinc, Se = Selenium, Zn/Cd = Zinc/Cadmium ratio, Zn/Pb = Zinc/Lead ratio, Se/Cd = Selenium/Cadmium ratio, Se/Pb = Selenium/Lead ratio, t = student's t-test, p = significance level, * = significant at $p<0.05$.

Thus, 1.0 SD increase in serum Pb level was associated with 3.23 decrease in predicted sperm density (p = 0.007) with other variables held constant.

In normospermics, normal sperm morphology was positively associated with seminal plasma Zn, Se, Zn/Cd and Zn/Pb ratio. Seminal plasma Zn/Pb ratio had the largest beta coefficient of 3.98 and seminal plasma Se had the least beta coefficient of 2.40. Thus, 1.0 SD increase in seminal plasma Zn, Se, Zn/Cd and Zn/P were significantly associated with 3.94 SD (p =0.01); 2.40 SD (p = 0.02); 2.67 SD (p = 0.03) and 3.98 SD (p = 0.01) respectively in predicted increase in normal sperm morphology with other variables held constant. On the other hand, normal sperm morphology was positively associated with seminal plasma Se/Pb ratio in dyspermics. Thus, 1.0 SD increase in seminal plasma Se/Pb ratio was significantly associated with 1.49 SD (p = 0.05) increase in predicted normal sperm morphology with other variables held constant. Sperm head defect was negatively associated with seminal plasma Zn, Se and Zn/Pb ratio in normospermics. Seminal plasma Zn/Pb ratio had the largest beta coefficient of 3.98 and Se had the least beta coefficient of -2.40. Thus, 1.0 SD increase in seminal plasma Zn Se and Zn/Pb ratio was significantly associated with 3.94 SD(p = 0.01),

Table 6. Comparison of Cd, Pb, Zn and Se levels in normospermic and dyspermic subjects using the one-way anova.

Variables	Fertile normospermics n = 27	Infertile normospermics n = 16	Oligospermics n=17	Azoospermics n=12	Asthenoteratozoospermics n = 17	Oligoasthenoteratozoospermics n = 31	F	p- value
Serum								
Cd (µg /L)	0.12 (0.01)	0.14 (0.02)	0.31 (0.04)	0.42 (0.05)	0.32 (0.04)	0.28 (0.02)	13.80	0.0001*
Pb (µg/dL)	28.23 (1.00)	28.26 (1.12)	33.49 (1.46)	37.50 (0.51)	35.49 (1.05)	34.11 (0.87)	11.57	0.0001*
Zn (mg/L)	8.08 (0.22)	7.53 (0.23)	7.58 (0.27)	7.14 (0.26)	7.05 (0.25)	7.12 (0.21)	3.20	0.01*
Se (mg/L)	0.87 (0.01)	0.83 (0.02)	0.77 (0.02)	0.82 (0.02)	0.82 (0.01)	0.81 (0.01)	4.87	0.0001*
Zn/Cd ratio	94.77 (14.26)	83.98 (16.25)	32.84 (5.05)	20.27 (2.45)	26.13 (2.82)	32.77 (3.40)	10.75	0.0001*
Zn/Pb ratio	0.29 (0.01)	0.27 (0.01)	0.23 (0.01)	0.19 (0.01)	0.20 (0.01)	0.21 (0.01)	13.08	0.0001*
Se/Cd ratio	9.90 (1.37)	9.93 (2.09)	3.31 (0.14)	2.41 (0.34)	3.08 (0.33)	3.77 (0.38)	10.66	0.0001*
Se/Pb ratio	0.03 (0.00)	0.03 (0.001)	0.02(0.00)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)	13.93	0.0001*
Seminal plasma								
Cd (µg /L)	1.11 (0.08)	1.25 (0.14)	1.99 (0.20)	2.11 (0.14)	2.11 (0.16)	1.90 (0.11)	15.85	0.0001*
Pb (µg/dl)	30.20 (1.11)	34.87 (1.08)	37.90 (1.60)	40.21 (0.95)	39.32 (1.13)	39.47 (1.01)	11.39	0.0001*
Zn (mg/L)	159.66 (6.21)	165.73 (9.28)	146.49 (5.17)	126.93 (8.34)	134.15 (4.82)	146.98 (4.32)	12.04	0.0001*
Se (mg/L)	0.29 (0.01)	0.22 (0.01)	0.20 (0.01)	0.22 (0.02)	0.23 (0.02)	0.23 (0.01)	4.49	0.001*
Zn/Cd ratio	171.24 (16.59)	168.54 (25.23)	85.47 (8.40)	79.72 (15.52)	70.02 (6.11)	85.73 (5.94)	6.20	0.0001*
Zn/Pb ratio	5.46 (0.29)	4.85 (0.34)	3.95 (0.18)	3.14 (0.14)	3.43 (0.11)	3.84 (0.19)	11.48	0.0001*
Se/Cd ratio	0.33 (0.04)	0.25 (0.05)	0.12 (0.01)	0.11 (0.01)	0.12 (0.01)	0.13 (0.01)	13.51	0.0001*
Se/Pb ratio	0.01 (0.00)	0.01 (0.00)	0.006 (0.00)	0.006 (0.00)	0.006 (0.00)	0.006 (0.00)	11.19	0.0001*

Cd = cadmium, Pb = Lead, Zn = Zinc, Se = Selenium, Zn/Cd = Zinc/Cadmium ratio, Zn/Pb = Zinc/Lead ratio, Se/Cd = Selenium/Cadmium ratio, Se/Pb = Selenium/Lead ratio, F = one-way anova, p = significance level, * = significant at p<0.05.

2.40 SD (p = 0.02) and 3.98 SD (p = 0.01) respectively in predicted decrease in sperm head defects with other variables held constant. In dyspermics, sperm head defect was negatively associated with seminal plasma Se/Pb ratio with a beta coefficient of

Table 7. The relationships between essential and toxic metals in serum and seminal plasma with the spermatogram in normospermics adult Nigerian men using a Linear Multiple Regression model.

	Sperm Vitality	Sperm Density	Normal Morphology	Head Defects	Tail Defects	Teratozoospermia index	Sperm Deformity Index
Serum Cd					β= 0.95 p= 0.008		
Serum Pb		β= -3.23 p= 0.007					
Serum Zn/Cd						β= -1.11 p= 0.048	
Serum Se/Cd	β= 1.49 p= 0.04				β= -1.71 p= 0.01		
Seminal plasma Zn			β= 3.94 p= 0.01	β= -3.94 p= 0.01			β= -3.09 p= 0.04
Seminal plasma Se			β= 2.40 p= 0.02	β= -2.40 p= 0.02			
Seminal plasma Zn/Cd	β= 1.94 p= 0.07		β= 2.67 p= 0.03				
Seminal plasma Zn/Pb			β= 3.98 p= 0.01	β= -3.98 p= 0.01			β= -3.13 p= 0.04

1.49. Thus, 1.0 SD increase in seminal plasma Se/Pb ratio was significantly associated with -1.49 SD decrease in predicted sperm head defects with other variables held constant.

Tail defect was positively associated with serum Cd and negatively associated with serum Se/Cd ratio in normospermics only. Serum Se/Cd ratio had the largest beta coefficient of 1.71 and serum Cd had the least beta coefficient of 0.95. Thus, 1.0 SD increase in serum Cd was significantly associated with 0.95 SD (p = 0.008) increase in predicted tail defects whereas, 1.0 SD increase in serum Se/Cd ratio was significantly associated with -1.71 SD (p = 0.01) decrease in predicted tail defects with other variables held constant.

Teratozoospermia index (TZI) was negatively associated with serum Zn/Cd ratio with a beta coefficient of -1.11 in normospermics only. Thus, 1.0 SD increase in serum Zn/Cd ratio was significantly associated with -1.11 SD (p = 0.048) predicted decrease in TZI with other variables held constant.

In normospermics, sperm deformity index (SDI) was negatively associated with seminal plasma Zn and Zn/Pb ratio. Seminal plasma Zn/Pb ratio had the largest beta coefficient of 3.13 and Zn had the least beta coefficient of 3.09. Thus, 1.0 SD increase in seminal plasma Zn and Zn/Pb ratio was significantly associated with -3.09 SD (p = 0.04) and -3.13 SD (p = 0.04) in predicted decrease in SDI respectively with other variables held constant.Whereas in dyspermics, SDI was negatively associated with seminal plasma Se/Pb ratio with a beta coefficient of -1.63. Thus, 1.0 SD increase in seminal plasma Se/Pb ratio was significantly associated with -1.63 SD decrease in predicted SDI (p = 0.03) with other variables held constant.

DISCUSSION

The findings reported here have demonstrated that toxic metals namely Cd and Pb which were

Table 8. The relationships between essential and toxic metals in serum and seminal plasma with the spermatogram in dyspermic adult Nigerian men using a Linear Multiple Regression model.

	Semen volume	Normal morphology	Sperm Head Defects	Sperm Deformity Index
Serum Zn/Cd	β=1.45 p=0.04			
Serum Se/Cd	β=1.51 p=0.03			
seminal plasma Se/Pb		β=1.49 p=0.05	β=-1.49 p=0.05	β=-1.63 p=0.03

significantly higher in serum and seminal plasma ofdyspermic than normospermic adult Nigerians may significantly affect the semen quality. This is in consonance with the reports of other researchers who have expressed great concerns about the negative impact of the environment on the human reproductive ability (Carlsen et al., 1992; Itoh et al., 2001). Endocrine disrupting chemicals such as Cd and Pb are toxic to the testis and have adverse effects on wildlife reproduction (Migliarini et al., 2005), disrupt steroidogenesis and spermiogenesis in laboratory animals (Thompson and Bannigan, 2008). Changes in human and animal sperm morphology and motility have been associated with toxic exposures and may relate to damage to differentiating cells or over time to stem cells (Carreau et al., 2007). Cadmium in seminal plasma has been associated with low semen volume and sperm motility (Xu et al., 1993). Increasing seminal plasma cadmium was significantly predicted to cause abnormal sperm morphology (Moorman et al., 1998). This may explain the significant increase in abnormal morphology in infertile normospermics compared to the fertile normospermics in this sudy.

In recent years, several investigators have examined the concentration of metals and other chemicals in the seminal fluid both of occupationally and non-occupationally exposed individuals and have attempted to correlate the concentrations of the elements present in human seminal fluid with conventional semen parameters, reproductive hormones and/or fertility levels. In this study, increased serum Pb level significantly predicted a decrease in sperm density (p = 0.007) while increased serum Cd significantly (p = 0.008) predicted an increase in tail defects in normospermics with other variables held constant. this agrees with the results of several studies which suggest that relatively high occupational exposure to Pb, as indicated by blood Pb levels can reduce human semen quality i.e. decreased number, motility and altered morphology of sperm (Viskum et al. 1999; Telisman et al., 2000).

Since Zn and Se levels in serum and seminal plasma were significantly higher in normospermics than dyspermics (p = 0.001); the mean serum Zn value was significantly higher in fertile normospermics than azoospermics (p = 0.01), asthenoteratozoospermics (p = 0.001) and oligoasthenoteratozoospermics (p = 0.002) and mean seminal plasma Zn value was significantly higher in fertile normospermics than azoospermics (p = 0.001) and asthenoteratozoospermics (p=0.001). It is reasonable to postulate that the depletion of these trace elements and poor semen quality may be as a result of the increased levels of Cd and Pb. Saaranen et al., (1987) found Zn concentrations to increase with increasing sperm density.

However, Low levels of Zn have been reported in oligospermic and azoospermic patients but no significant difference was found in mean Zn levels in fertile and infertile patients, and between normospermic and dyspermic infertile men (Charles-

Davies, 1999; Akinloye et al., 2011), nor between idiopathic infertile and normal men (Chia et al., 1994). And no significant correlation was found between semen and blood Zn concentration and the fertility potential between normospermic, oligospermic and azoospermic infertile men (Adejuwon et al., 1996). Although seminal Zn may be associated with seminal and prostatic function, its role in infertility is considered controversial (Burnazian et al., 1992) in the light of these conflicting findings.

Furthermore, seminal plasma Se was significantly higher in fertile normospermics than infertile normospermics ($p = 0.008$) and increase in seminal plasma Se significantly predicted decrease in % cytoplasmic droplets ($\beta = -2.27$) and increase in sperm deformity index ($\beta=0.24$) in dyspermics only. In this study, increased seminal plasma Zn ($p = 0.01$) and Se ($p = 0.02$) significantly predicted increased normal sperm morphology and a decrease in sperm head defects as well as a decrease in SDI ($p = 0.04$) with other variables held constant in normospermics only. These findings may explain the essence of Selenium-vitamin E supplementation in infertile oligo-asthenoteratozoospermic men which caused statistically significant increases in sperm motility, percent live, and percent normal spermatozoa (Vezina et al., 1996).

Various toxic and essential metals are interactive, leading to metal detoxification and depletion of Zn and Se and sperm formation and motility (Telisman, 1995; Behne et al., 1996). In this study, the interactions of trace elements and toxic metals in serum and seminal plasma with the spermiogram demonstrate very complex mechanisms which may determine the sperm quality of the participants. Similarly, in their study, Omu and Fernandes, (2001) found that a high zinc/cadmium (Zn/Cd) ratio of more than 200 was associated with a normal sperm count and motility suggesting an inverse relationship between the Zn/Cd ratio and impairment of spermatozoa motility. Therefore, it may be reasonable to suggest that Zn/Cd ratio may be a better index of assessing sperm quality than seminal zinc and cadmium independently.

CONCLUSION

Exposure to environmental pollutants such as Cd and Pb which may occur occupationally or indirectly through the food chain, affects human health including male fertility. Reduced levels of selenium and zinc in dyspermic males as demonstrated in this study may account for the loss of their protective effect against Cd and Pb toxicity to the testes. Dyspermic Nigerian men have significantly higher levels of Cd and Pb in serum and seminal plasma. These toxicants were associated with poor semen quality (sperm count, motility and morphology). These findings suggest that the depletion of the Zn and Se levels maybe one of the mechanisms explaining the poor semen quality in dyspermic males.There is need therefore, to understand other conditions which may predispose some people to accumulate high amounts of toxicants from exposures no higher than what most of us encounter. These conditions which may be dietary resulting in altered bioavailability of these toxicants or genetic, involving isoforms of proteins involved in transport, membrane passage or storage of metals. All these considered, it may be better to evolve better strategies to prevent the continuous contamination of our environment by these toxicants.

LIMITATIONS AND FUTURE PROSPECTS

A major portion of male infertility is thought to have an underlying genetic basis. Exposure to occupational and environmental metal aerosols including Pb^{2+} and Cd^{2+} can influence gene expression directly by binding various metal response elements in the target gene promoters. Recent research suggests that metals can also influence gene expression through epigenetic mechanisms; this adds a new twist to the complexity of metal-mediated gene expression. A consequence of the low essential element/toxic metal ratio as seen in dyspermics in this study may be a metabolic disorder or the lack of efficient DNA repair systems or epigenetic events leading to poor semen quality.

Further studies may identify and characterise the relevant genes, determine their functions in normal human reproduction as well as identify functional pathways and the nature of the interactions and consequences of mutations or dysregulation for sperm production and function.

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