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Some Heavy Metals Correlated Negatively with Total Antioxidant Capacity, Glutathione Peroxidase, Fructose, and Testosterone in Seminal Plasma of Oligospermic and Azoospermic Males

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DO and IE designed the study. Author IE performed the statistical analysis and wrote the protocol while authors EPU and IE wrote the first draft of the manuscript. Authors DO, IE and EPU managed the analyses of the study. Authors EPU and IE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study aimed to investigate the relationship between some heavy metals and total antioxidant capacities, glutathione levels, fructose, and testosterone in seminal plasma of infertile azoospermic and oligospermic males in Akwa Ibom State of Nigeria.
Study Design: A cross-sectional design with a total of 124 males included in the study of which 32 were azoospermic, 38, oligospermic, and 54, normospermic.
Place and Duration of Study: Semen samples were collected from the urology or fertility clinic of UUTH, St. Luke's Hospital, Anua, and Ibom specialist hospital of Akwa Ibom State. However, laboratory assays were performed at the Department of Medical Laboratory Science, Rivers State University, Port Harcourt between May, 2018 and January, 2021.
Methodology: Semen specimens were collected after 3-5 days abstinence according to WHO criteria while seminal plasma were obtained from semen by spinning at 4500 rpm for 10 minutes and stored at -70^{0}C prior to laboratory analysis. Atomic absorption spectrometer (AAS) was used to

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determine the levels of heavy metals while ELISA methods were used to determine testosterone and GSH concentrations. TAC and fructose assays were carried out using spectrophotometric methods.

Results: Results showed that non-essential heavy metals such as lead, mercury, and arsenic correlated negatively with testosterone, fructose, and antioxidant activities of the seminal plasma in azoospermic subjects. In addition, lead and mercury correlated positively in the azoospermic subjects. Meanwhile, selenium, an essential heavy metal, correlated positively with testosterone and antioxidant activities in oligospermic subjects with 11-19 x10^6cells/ml.

Conclusion: The levels of non-essential heavy metals in azoospermic subjects precipitated poor anti-oxidant and testosterone activities inducing oxidative stress while in oligospermic subjects, selenium and antioxidant parameters and testosterone were in good association indicating improved antioxidant activities and testicular function.

Keywords: Correlation; heavy metals; azoospermic and oligospermic males; antioxidants; testosterone; fructose; seminal plasma.

1. INTRODUCTION

Urbanization, agricultural and Industrial revolution have impacted positively on livelihood of societies globally but these revolutions have also introduced several toxic substances directly or indirectly into the environment affecting the ecosystem at a tremendously dangerous pace [1,2]. The influence on the ecosystem by these toxic substances has also been reported to affect human health and reproduction as they bio-accumulate over time [1]. Examples of such toxic substances as a result of environmental pollution are the heavy metals [1,2]. Heavy metals are introduced into the environment through several routes including industrial and domestic waste, municipal sewage, dust, smoke etcetera which pollute air, land and sea. However, industrial wastes and pollution from oil and gas drilling activities have contributed majorly of the pollution particularly in the Niger Delta region of Nigeria [2,3].

Heavy metals (HMs) are defined as trace elements having relatively higher densities compared to the density of water [3,4]. These metals also include metalloids, which are elements that possess metallic and non-metallic characteristics such as arsenic [5,6]. It is important to note that heavy metals are also introduced into the environment through natural phenomena such as earthquakes, volcanoes eruptions, soil erosion, etc. However, the anthropogenic activities involving oil and gas (crude) exploration and spillage have contributed greatly in the pollution of the environment, especially in the Niger Delta that have been linked with acute and chronic heath challenges including infertility [2,7].

Infertility is generally described as a medical problem associated with the inability of couples to reproduce a live offspring after 12 months of unprotected sexual intercourse.

Infertility could be primary or secondary type [7]. Factors associated with infertility are multi-dimensional which include, medical, socioeconomic, environmental, genetics, occupational and so on [7,8]. Male infertility is the inability of a sexually matured male to establish pregnancy when it is clinically proven that the female is fit. It is very vital to know that not all heavy metals are associated with adverse health challenges [8,9]. Therefore, we considered both the non-essential and essential heavy metals. The non-essential heavy metals include lead, mercury, chromium, and arsenic since they are non-degradable, and tend to be associated no homeostasis mechanism, and are not also involved in any beneficial physiological processes. On the other hand, the essential heavy metals include selenium, zinc, iron, etc because of their involvement in human physiological processes [8,9,10].

Lead metals are applicable in the production of automobile batteries, domestic and industrial paints, and lead pipes. Since lead is not biodegradable and tends to bio-accumulate overtime, it has been reported to be very toxic to the nervous system [1,9]. The kidneys, liver, heart, brain and other soft tissues like the testes are also targets of lead toxicity [8,9]. However, the greatest percentage of lead is absorbed by the kidney [3,11]. Arsenic as metalloids are applied as colorants in industries in the production of candles, fabric, toys, etc as well as wallpapers. It has also been documented that exposure to inorganic mercury is strongly associated with the development of lung cancers, tremor, anxiety, restlessness, sleep disturbance and depression while prolonged exposure to

elemental mercury is associated with kidney and skin damages [3,10]. However, it should be noted that, the major sources of heavy metal contamination in our present environment (air, water bodies, and soil in particular Akwa Ibom, Niger Delta, South-South of Nigeria) are mainly as a result of many anthropogenic activities involving crude oil exploration, shipping, spills, and leakages from pipelines as well as gas flaring and hydrocarbon soot [12,13]. In addition, the coastal marine environment has also been exposed to high degree of pollution caused by polycyclic aromatic hydrocarbon (PAH) especially from petroleum seeps which had led to their bioaccumulation in the tissue of some fishes and seafoods [14,15]. Since fishing is the major occupation of our people, it is obvious that males are exposed frequently and these contaminated seafoods are made available for consumption [2,11,15].

Selenium and glutathione have been reported to act as antioxidants in biological systems [16,17]. Antioxidants are chemicals that interface with and counteract free radicals, preventing them from cell destruction [16]. An antioxidant slows, averts or moves back oxidative destruction to target molecule by acting on different procedures in the oxidative protocol [16,17]. There are basically the enzymatic and non-enzymatic strategies of antioxidants activities. The enzymatic strategies of antioxidants include superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione reductase while non-enzymatic strategies involve vitamins A, C and E; lipoic acid, mixed carotenoid, glutathione, various bioflavonoids, minerals such as copper, zinc, manganese, and selenium [17]. These antioxidants usually function in combination against diverse free radicals. Therefore, the total activities of the various body's antioxidant functions is referred to the total antioxidant capacity [17]. The optimal activities of these antioxidants have been reported to improve testicular integrity, testosterone production, sperm motility, morphology and structural functionality. Therefore, the aim of this work is to study the relationship of some heavy metals with total antioxidant capacities, selenium concentration, glutathione levels and fructose in seminal plasma of azoospermic and oligospermic male in Akwa Ibom State of Nigeria. In Nigeria, Uadia & Emokae, [18], reported increasing cases of severe oligospermia and azoospermia and that male infertility accounts for 46% of infertility cases in couples in the south-south of Nigeria.

2. MATERIALS AND METHODS

2.1 Materials

The materials employed in this study include binocular microscope (Olympus, Japan), MPW bucket centrifuge Model 351 (MPW Medical Instruments, Poland), Stat Fax 4200 Microplate Reader (Awareness, USA), and Atomic Absorption Spectrophotometer (Buck 158 Model 211, USA). In addition, glutathione peroxidase (GSH) and testosterone ELISA kits purchased from Bioassays Technology Laboratory (Shangai, China) while fructose and total antioxidant capacity (TAC) reagents purchased from Fortress Diagnostics (Belfast Road, Antrim, United Kingdom) were also used.

2.2 Study Area

Semen specimens were collected from males who attended the Urology clinic of Ibom specialist hospital, University of Uyo Teaching Hospital, and St. Luke's Hospital, Anua, Uyo, Akwa Ibom State. However, laboratory assay was performed at the Chemical Pathology laboratory, Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

2.3 Study Design

The study was a cross-sectional study in which a total of 124 males were randomly selected. The subjects were aged between 25 - 45 years. Of the 124 subjects, 70 were infertile males while 54 were used as controls. The subjects were classified after semen analysis as normospermic (n=54), oligospermic (n=38) and azoospermic (n=32) according to the WHO classification [16]. The oligospermic group were further classified into oligospermic group 1 and oligospermic group 2 based on their sperm count of 11-19 x 10^6cells/ml and 1-10 x 10^6cells/ml respectively. The normospermic and azoospermic subjects were grouped based on sperm count of ≥20 x 10^6cells/ml and <1.0 x 10^6cells/ml respectively.

2.4 Subject Selection Criteria

2.4.1 Inclusion criteria

A structured questionnaire was provided to all participants to get their demographic details, medical history and lifestyle. Those included in the study as infertile subjects were those attending urology clinic due to established male

factor infertility without presence of other diseases like obesity, diabetes mellitus, thyroid disorder, cardiovascular diseases or any other chronic disorder. Also, they had not achieved established pregnancy within the last 2 years with sperm count of <1.0 x 10^6 cells/ml. In addition, these subjects were non-smokers, non-alcoholics and without any history of substance abuse. On the other hand, the control subjects were males that have had at least established pregnancy within the study period with sperm count of ≥20 x 10^6 cells/ml. They were also non-smokers, non-alcoholics as well as without history of any diseases as stated above.

2.4.2 Exclusion criteria

Subjects excluded from the study include those that did not give their consent, males that did not abstain for 3-5days or did spill in the course of semen collection. Also, males that were obese (BMI of > 30.0kg/m^2) as described by Elekima & Ugwu [19]; Elekima & Inokon, [20] were excluded. More so, subjects excluded from the study include those with history of or have diabetes mellitus (FBS>7mmol/L), prostate hyperplasia and prostate disorders, thyroid disorders, cardiovascular diseases, on anti-hypertensive or lipid lowering drugs.

2.5 Specimen Collection and Preparation

Participants were educated before their semen specimens were collected by means of masturbation after abstaining from sex for 5 days into a sterile universal container according to WHO [21]. The time of production, reception, and examination were also noted. All semen specimen collected were allowed to liquefy in the incubator at 37^0C and examined within 45 minutes. The examination covered the determination of sperm volume, viscosity, concentration, motility, morphology and viability according to the WHO manual for semen analysis [21]. Seminal plasma specimens were obtained by centrifugation of the semen at 4500rpm for 10 minutes and were carefully pipetted into another clean, dry plain container and stored at -70^0C prior to analysis.

2.6 Laboratory Assays of Semen and Seminal Plasma

2.6.1 Establishment of Total Sperm Count

Semen samples were examined macroscopically and microscopically according to the WHO [21] criteria. The total sperm count was estimated manually using Neubauer haemocytometer. The semen samples were properly mixed and diluted 1 in 20 using semen diluting fluid consisting of sodium bicarbonate, formalin and distilled water. The counting chamber was prepared and charged with the diluted seminal fluid and allowed to stand for 15 minutes. The counts were established in four secondary squares and five tertiary squares (four at corner and one central) of the central secondary square as described by WHO [21]. The total number of spermatozoa per ml was calculated by multiplying the number of complete, morphologically mature sperm cells counted x dilution factor (20) x multiplication factor (50,000). The total count was then established as n x 10^6 sperm cells/ ml. According to WHO classification, participants with <1.0 x 10^6 sperm cells/ ml, were grouped as azoospermic, between 1-19 x 10^6 sperm cells/ ml as oligospermic and ≥20 x 10^6 sperm cells/ml as normospermic.

2.6.2 Evaluation of Biochemical Parameters in Seminal Plasma

Lead, Arsenic, Mercury, and Selenium were estimated using atomic absorption spectrometer (AAS) with samples digestion done according to the method described by Kayne et al. [22]. More so, the concentration of testosterone and glutathione peroxidase enzyme in the seminal plasma was determined using ELISA technique as described in the work done by Adele et al. [23]. The estimation of fructose was performed by the resorcinol spectrophotometric method as documented by Yaphe & Arsenault [24], while that of total antioxidant capacity (TAC) was determined using Mahfouz et al. [25] method.

2.7 Statistical Analysis

Correlation analyses were done using Graphpad Prism 8.02 (California, USA). One-way ANOVA and Pearson's correlation were employed. Statistical significance was set at p=.05.

3. RESULTS

The one-way ANOVA indicated that values of Pb and As in oligospermic, and azoospermic subjects were significantly higher when compared against normospermic subjects at p=.05. However, significant reductions were observed in Se, TAC, GSH and Testosterone concentration in the oligospermic and azoospermic subjects when compared against normospermic subjects (Table 1).

The results of Pearson's correlation in the seminal plasma of the azoospermic subjects indicated significant negative relationship between lead (Pb) and testosterone (r= -0.76, p=0.038). Mercury and arsenic also indicated significant negative correlation with total antioxidant capacity (TAC) correlation indices of r= -0.56, p=0.04 and r= -0.66, p=0.01 respectively. Other parameters correlated, did not indicate any significant relationship in the azoospermic subjects.

In oligospermic subjects with sperm count between 1-10 x 10^6 sperm cells/ ml, significant negative correlation (r= -0.41, p=0.03) was observed between lead and TAC. Meanwhile, selenium, TAC and GSH had significant positive correlation with testosterone with correlation indices of r=0.43, p=0.03, r=0.65, p=0.02 and r=0.75, p=0.04 respectively at p=.05. However, on the other hand, the oligospermic subjects with sperm count of 11-19 x 10^6 sperm cells/ ml, indicated significant negative correlation between mercury and fructose (r= -0.44, p=0.04) and also between mercury an GSH (r= -0.47, p=0.02) while significant positive correlation was observed between selenium and testosterone (r=0.51, p=0.009) as well as between selenium and GSH (r=0.54, p=0.002). In addition, TAC and GSH had significant positive correlations with testosterone with correlation indices of r=0.56, p=0.002 and r=0.16, p=0.03 respectively.

Finally, in normospermic subjects, no significant correlations were observed in the seminal parameters considered except TAC that significantly correlated positively withtestosterone with r=0.86, p=0.04 at p=0.05. In all of the groups whether azoospermic, oligospermic or normospermic subjects, no correlations were observed between the heavy metals except between lead and mercury (r=0.09, p=0.005) in azoospermic subjects.

3.1 Post Hoc Analysis (Turkey's Test)

Pb, Se, GSH &Testo: Values in the same row with the different superscripts (a, b) differ significantly when normospermic (≤20x10^9 cells/ml) values were compared with oligospermic (11-19 x 10^6cells/ml), oligospermic (1-10 x 10^6cells/ml) and azoopermic (<1.0 x 10^6cells/ml). However, values in the same row with the same superscripts (b) do not differ significantly. **AS &TAC:** Values in the same row with the different superscripts (a, b) differ significantly when normospermic (≤20x10^9 cells/ml) values were compared with oligospermic (11-19 x 10^9cells/ml), oligospermic (1-10 x 10^6cells/ml) and azoopermic (<1.0 x 10^6cells/ml). Aslo, values in the same row with different superscripts (c, d) differ significantly when oligospermic (11-19 x 10^9cells/ml) values were compared with oligospermic (1-10 x 10^9cells/ml) and azoopermic ((<0.1 x 10^6cells/ml). However, values in the same row with the same superscripts (e) do not differ significantly when oligospermic (1-10 x 10^6cells/ml) values were compared with azoopermic (<1.0 x 10^6cells/ml) while that of (e, f) differ significantly in Arsenic. Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone, S=Significant, NS=Not Significant at *p*=.05.

Table 1. ANOVA of heavy metals, anti-oxidative enzymes, fructose, and testosterone in normospermic, oligospermic and azoopermic subjects

Parameters	Normospermic (≥20x10^6 cells/ml)	Oligospermic (11-19 x 10^6cells/ml)	Oligospermic (1.0-10 x 10^6cells/ml)	Azoospermic (<1.0 x 10^6cells/ml)	P value
Pb (mg/L)	0.017±0.002^{a}	0.133±0.009^{b}	0.163±0.002^{b}	0.107±0.001^{b}	0.0412
Hg (mg/L)	0.002±0.001	0.004±0.003	0.003±0.001	0.003±0.002	0.7310
As (mg/L)	0.003±0.003^{a}	0.020±0.010^{bc}	0.006±0.004^{bde}	0.010±0.001^{bdf}	0.0012
Se (mg/L)	0.57±0.06^{a}	0.26±0.41^{b}	0.15±0.03^{b}	0.02±0.01^{b}	0.0009
Fructose (mg/ml)	5.07±1.69	5.09±1.57	5.22±1.69	5.08±1.97	0.7320
TAC (mmol/L)	5.85±3.41^{a}	5.53±3.59^{ac}	2.47±2.32^{bde}	1.89±1.24^{bde}	<0.0001
GSH (mg/L)	8.04±1.47^{a}	6.70±1.79^{b}	6.00±1.80^{b}	5.98±1.92^{b}	0.0001
TESTO (ng/ml)	2.15±2.16^{a}	0.70±0.58^{b}	0.40±0.32^{b}	0.29±0.23^{b}	<0.0001

Table 2. Correlation matrix (Pearson's) of heavy metals, anti-oxidative enzymes, fructose, and testosterone in azoopermic subjects

Parameters	Pb (mg/L)	Hg (mg/L)	As (mg/L)	Se (mg/L)	FRUCTOSE (mg/ml)	TAC (mmol/L)	GSH (mg/L)	TESTO (ng/ml)
Pb (mg/L)	r= 1.00 p= 0.00							
Hg (mg/L)	r= 0.09 p=0.005	r= 1.00 p= 0.00						
As (mg/L)	r= 0.27 p= 0.38	r= -0.08 p= 0.80	r=1.00 p=0.00					
Se (mg/L)	r= -0.05 p= 0.85	r=0.17 p=0.58	r=0.05 p=0.85	r= 1.00 p= 0.00				
Fructose (mg/ml)	r= -0.56 p= 0.04	r= 0.45 p= 0.11	r=0.45 p=0.12	r= -0.31 p= 0.30	r=1.00 p=0.00			
TAC (mmol/L)	r= -0.10 p= 0.73	r= -0.56 p= 0.04	r=-0.66 p=0.01	r= -0.12 p= 0.71	r=0.16 p=0.60	r=1.00 p=0.00		
GSH (mg/L)	r= -0.31 p= 0.29	r= 0.28 p= 0.36	r=-0.14 p=0.63	r= 0.11 p= 0.71	r=-0.27 p=0.37	r=0.06 p=0.84	r=1.00 p=0.00	
TESTO(ng/ml)	r= -0.76 p=0.038	r= -0.54 p= 0.52	r=0.07 p=0.63	r=-0.56 p=0.13	r=0.76 p=0.12	r=0.43 p=0.14	r=0.47 p=0.32	r=1.00 p=0.00

Pb correlated significantly positive with Hg, significantly negative with Fructose and Testosterone. Hg correlated significantly negative with TAC. More so, As also related significantly negatively with TAC at p<0.05. Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone

Table 3. Correlation matrix (pearson's) of heavy metals, anti-oxidative enzymes, fructose, and testosterone in oligospermic (1-10 x 10^6cells/ml) subjects

Parameters	Pb (mg/L)	Hg (mg/L)	As (mg/L)	Se (mg/L)	Fructose (mg/ml)	TAC (mmol/L)	GSH (mg/L)	TESTO (ng/ml)
Pb (mg/L)	r= 1.00 p= 0.00							
Hg (mg/L)	r= -0.08 p=0.70	r= 1.00 p=0.00						
As (mg/L)	r= -0.17 p=0.40	r=- 0.17 p= 0.38	r =1.00 p=0.00					
Se (mg/L)	r= -0.21 p=0.29	r= 0.13 p= 0.52	r= -0.20 p=0.32	r=1.00 p=0.00				
Fructose (mg/ml)	r= -0.09 p=0.65	r= 0.13 p= 0.53	r= 0.29 p=0.14	r= 0.05 p=0.80	r= 1.00 p=0.00			
TAC (mmol/L)	r= -0.41 p=0.03	r= 0.03 p= 0.89	r= 0.20 p=0.33	r= 0.13 p=0.53	r= 0.21 p=0.28	r=1.00 p=0.00		
GSH (mg/L)	r= -0.05 p=0.82	r= -0.08 p= 0.68	r= -0.10 p=0.68	r= 0.06 p=0.77	r= -0.05 p=0.82	r= -0.09 p=0.65	r=1.00 p=0.00	
TESTO(ng/ml)	r= -0.54 p= 0.81	r= -0.18 p= 0.08	r=-0.04 p=0.06	r= 0.43 p=0.03	r=0.65 p=0.14	r=0.65 p=0.02	r=0.75 p=0.04	r=1.00 p=0.00

Pb correlated significantly negative with TAC. Se correlated significantly positive with Testosterone. More so, TAC also related significantly positive with Testo at p<0.05.
Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone

Table 4. Correlation matrix of heavy metals, anti-oxidative enzymes, fructose, and testosterone in oligospermic (11-19 x 10^6cells/ml) subjects

Parameters	Pb (mg/L)	Hg (mg/L)	As (mg/L)	Se (mg/L)	Fructose (mg/ml)	TAC (mmol/L)	GSH (mg/L)	TESTO (ng/ml)
Pb (mg/L)	r=1.00 p=0.00							
Hg (mg/L)	r=0.04 p=0.84	r= 1.00 p=0.00						
As (mg/L)	r= -0.07 p=0.72	r= -0.20 p=0.33	r= 1.00 p=0.00					
Se (mg/L)	r= -0.21 p= 0.34	r= 0.08 p=0.71	r= -0.23 p=0.27	r= 1.00 p=0.00				
Fructose (mg/ml)	r= -0.17 p= 0.44	r= -0.44 p= 0.04	r= 0.32 p=0.14	r= -0.28 p=0.21	r= 1.00 p=0.00			
TAC mmol/L)	r= -0.26 p =0.19	r= 0.08 p=0.72	r= -0.07 p=0.75	r= -0.08 p=0.71	r= -0.20 p=0.36	r= 1.00 p=0.00		
GSH (mg/L)	r= -0.24 p=0.24	r= -0.47 p= 0.02	r= -0.127 p=0.55	r= 0.51 p=0.009	r= -0.34 p=0.10	r= 0.65 p=0.09	r= 1.00 p=0.00	
TESTO(ng/ml)	r= -0.56 p= 0.12	r= -0.54 p= 0.03	r= -0.34 p=0.14	r=0.54 p=0.002	r=0.63 p=0.34	r=0.56 p=0.002	r=0.16 p=0.03	r=1.00 p=0.00

Hg correlated significantly negative with Fructose and GSH. Se correlated significantly positive with GSH and Testosterone. More so, TAC also related significantly positive with Testo at p<0.05. GSH also related significantly positive with Testo. Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone

Table 5. Correlation matrix of heavy metals, anti-oxidative enzymes, fructose, and testosterone in normospermic (≥20x10^6 cells/ml) subjects

Parameters	Pb (mg/L)	Hg (mg/L)	As (mg/L)	Se (mg/L)	Fructose (mg/ml)	TAC (mmol/L)	GSH (mg/L)	TESTO (ng/ml)
Pb (mg/L)	r= 1.00 p=0.00							
Hg (mg/L)	r= 0.22 p=0.24	r= 1.00 p=0.00						
As (mg/L)	r= -0.04 p=0.83	r= 0.20 p=0.29	r= 1.00 p=0.00					
Se (mg/L)	r= -0.08 p=0.67	r= -0.17 p=0.38	r= -0.05 p=0.81	r= 1,00 p=0.00				
Fructose (mg/ml)	r= 0.24 p=0.20	r= -0.24 p=0.23	r= -0.19 p=0.32	r= 0.02 p=0.90	r= 1.00 p=0.00			
TAC (mmol/L)	r= -0.09 p=0.64	r= -0.12 p=0.53	r= -0.12 p=0.52	r= 0.01 p=0.96	r=0.25 p=0.20	r= 1.00 p=0.00		
GSH (mg/L)	r= -0.01 p=0.96	r= -0.11 p=0.56	r= 0.14 p=0.48	r= 0.24 p=0.22	r= -0.28 p=0.14	r= -0.19 p=0.35	r= 1.00 p=0.00	
TESTO (ng/ml)	r= -0.26 p=0.20	r= -0.20 p=0.29	r= -0.17 p=0.18	r= 0.24 p=0.20	r= 0.29 p=0.20	r=0.86 p=0.04	r=0.19 p=0.34	r=1.00 p=0.00

No significant correlations at p<0.05. Pb=Lead, Hg=Mercury, As= Arsenic, Se=Selenium, TAC=Total Antioxidant Activity, GSH= Glutathione peroxidase, Testo=Testosterone

4. DISCUSSION

The correlation study indicated significant correlation of heavy metals with biochemical parameters in seminal parameters in azoospermic and oligospermic subjects. The observed significant negative relation seen between heavy metals (lead, mercury, and arsenic) and total antioxidant capacity, GSH and testosterone in azoospermic and oligospermic subjects is in line with the finding of Tark & Kullisaar, [26]. They reported in their study that significant increases in heavy metals in male infertile males induced a significant fall in total antioxidant capacity and GSH. This submission is also in line with our comparative analysis indicating significantly lower values of Se, GSH and TAC in azoospermic and oligospermic subjects while on the other hand, heavy metals like Pb and As were seen to be significantly higher in the aforementioned subjects. The significant negative correlation seen between heavy metals and antioxidants suggest that the presence of these heavy metals in seminal plasma probably enhance the activities of free radicals and oxidative stress leading to the depletion of the antioxidant enzymes such as GSH and total antioxidant capacity of the seminal plasma. Oxidative stress occurs when the antioxidant capacity of the system is overwhelmed with free radicals in course of metabolic processes or toxic substances such as heavy metals in the biological system. Fatima et al. [27], reported in their study that >40µg/L of lead in blood induces significant reduction in sperm count, motility, and morphological derangements. In 2012, Minguez-Alarcon [28], also reported negative correlation between different heavy metals and human sperm parameters. In addition, the negative correlation between heavy metals with testosterone is also an indication of their effect or direct oxidative damages on the testicular germ cells, steroidogenic enzymes as well as spermatogenesis. The germ cells (leydig and sertoli cells) of the testes are responsible for the production and regulation of testosterone along the hypothalamus-pituitary-adrenal/gonadal axis. Therefore, it possible that the toxicities of these metals (such as lead and mercury) due to oxidative stress have negatively affected the production of testosterone in both azoospermic and oligospermic subjects by altering the plasma concentration of gonadotrophins such as FSH and LH and other androgens. More so, the negative correlation between lead and fructose also indicate depletion of nutrients in the seminal plasma as the lead tends to increase in the plasma. In addition, the significant correlation of lead and mercury also explains the synergistic effects of these heavy metals on GSH and TAC in azoospermic subjects.

Meanwhile, the significant positive correlation between selenium and testosterone in oligospermic subjects with 1-10x10^6cells/ml as well as that between selenium and GSH, TAC, and testosterone in oligospermic subjects with 11-19x10^6cells/ml is an indication of improved antioxidative activities in the seminal plasma. Unlike in the azoospermic subjects, in the oligospermic subject with 11-19x10^6cells/ml, had improved antioxidative capacities which in turn correlated positively with selenium and testosterone concentration. Our findings concur with the reports of Akinloye et al. [29], Qazi et al. [30]. They also reported in their separate work significant positive correlation of selenium with testosterone and sperm motility, viability and morphology. Selenium is an antioxidant that is capable of eradicating free radicals from the biological system. Selenium has been shown to be vital for normal mammalian spermatogenesis and protection against oxidative DNA damage and this vital role is mainly mediated by selenoproteins. Our results further suggest that an increased selenium levels in the seminal plasma, will induce a corresponding increase in the antioxidant capacities of the seminal plasma. And an improved antioxidant capacity in the seminal plasma is necessary for spermatogenesis and testicular integrity. Kumar, [31]; Alahmar, [32], reported heavy metal-associated decline in sperm functions and spermatogenesis due to oxidative stress induction were mitigated by improved antioxidant activities and selenium supplementation. Therefore, these results indicate that the infertile men may benefit from selenium supplementation which in turn will enahnce antioxidant capacities of the seminal fluid fighting against lipid peroxidation and sperm cells membrane distortions easily observed in lead induced toxicity and oxidatve stress.

5. CONCLUSION

Non-essential heavy metals such as lead, mercury, and arsenic correlated negatively with testosterone, fructose, and antioxidant activities of the seminal plasma in azoospermic subjects. In addition, synergetic effect of lead and mercury were also observed as also observed. Meanwhile, selenium, an essential heavy metal,

correlated positively with testosterone and antioxidant activities in oligospermic subjects with 11-19 x10^6cells/ml.

FUTURE WORK

Our future research will be aimed at improving the sperm parameters of infertile men who have azospermia, oligospermia towars improving their sperm parameters and achievement of pregnancy. Therefore, we will investigate semen parameters and testicular biopsies of azoospermic and oligospermic subjects histopathologically after been administered with selenium, vitamin C, and vitamin E orally singularly and in combination over a period of 2 years to arcertain if the treatment will improve on semen quality and testicular integrity. In addition, as supportive therapy, oral administration of chelating agents such alpha lipoic acid (as supplement) in azoospermic and oligospermic subjects will also be investigated within the stated period.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

The authors hereby declare that all experiments have been examined and approved by the ethical review Boards of University of Uyo Teaching Hospital (UUTH) and Akwa Ibom State Ministry of Health with approval file no of UUTH/AD/S/96/VOL.I/401 and MH/PRS/99/VOL.V/923 respectively. The study, therefore were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. In addition, we declare that written informed consent was obtained from all the participants before enrollment into the study and for the publication of the results.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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