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RESEARCH ARTICLE

RELATIONSHIP BETWEEN BLOOD AND SEMINAL FLUID ZINC LEVELS AND SEMEN PARAMETERS AMONG NIGERIAN MEN

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ABSTRACT

Background: Zinc plays an important role in testicular development, spermatogenesis and sperm motility via its antioxidant properties in scanvenging reactive oxygen species. **Objectives:** This study sets out to determine the correlation of blood and seminal fluid zinc levels and semen parameters in Nigerian men with normal and abnormal semen parameters who were seen for infertility. **Methods:** A prospective case-control study of eighty five men with abnormal semen parameters (cases) and equal number with normozoospermia (controls) enrolled from the Institute of Fertility Medicine and the gynaecological outpatient clinic was carried out. The cases were further sub-grouped as oligozoospermic (n=64), asthenozoospermic (n=13) and azoospermic (n=8). Blood and seminal fluid concentrations of zinc were determined by microwave plasma-atomic emission spectrophotometer. **Results:** The median zinc concentrations in blood and seminal seminal fluid were significantly lower in the cases compared with the controls; 1.7 µmol/L(IQR 0.6,2.7) versus 3.2 µmol/L (IQR 1.6,5.2), p<0.001 and 9.0 µmol/L (IQR 6.1,11.0) versus 19.4 µmol/L (IQR 14.0,25.5), p<0.001 respectively. Blood zinc levels showed a significant positive correlation with sperm count (r=0.311 p=0.012), progressive motility (r=0.252 p= 0.045) and total motility (r=0.285 p=0.022) in the oligozoospermic men. Seminal fluid zinc level however was significantly correlated only with progressive sperm motility (r=0.251 p=0.045) in the oligozoospermic men. **Conclusion:** This study revealed that blood and seminal plasma zinc levels were significantly lower in men with abnormal semen parameters compared with normal controls. In oligozoospermic men, blood zinc levels were significantly positively correlated with semen parameters while the seminal fluid zinc levels showed significant positive correlation only with progressive motility.

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INTRODUCTION

Worldwide, infertility is generally quoted as occurring in 8-12% of couples (Inhorn, 2003). However, the incidence varies from one region of the world to the other, being highest in the so-called infertility belt of Africa that includes Nigeria (Okonofua, 2003). In contrast to an average prevalence rate of 10-15% in the developed countries (Alvarez NC.2006), the prevalence of infertility has been highly variable in sub-Saharan Africa ranging from 20-46% (Idrisa, 2005). The prevalence of infertility in Nigerian is put between 20-25% among married couples and about 40-50% of all consultations in gynaecology clinics are infertility-related (Okonofua, 2003).

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In Nigeria, male factor contribution to the incidence of infertility is put between 20-25% (Nwajiaku *et al*., 2012; Abubakar *et al*., 2014). There are some risk factors leading to defective spermatogenesis, and hence male infertility like; varicocele, cryptorchidism, obstructive lesions, cystic fibrosis, trauma, genitourinary tract infections, environmental agents, and nutritional deficiency of trace elements like, zinc and selenium (Agarwal *et al*., 2007, Wong *et al*., 2000). Zinc which is second only to iron as the most abundant element in the body is found in chicken, nuts, meat, fish, milk, and legumes (Wong *et al*., 2000). Despite this, the World Health Organization estimates that one-third of world population is deficient in zinc (The World Health Report 2002). The zinc content of semen is 87 times that in the blood and has been reported to protect spermatozoa from bacteria and chromosome damage (Mohammad *et al*., 2011). In addition, zinc in the body plays an important role in normal testicular development, spermatogenesis, and sperm motility (Altaher *et al*., 2015, Thomas *et al*., 2011).

Deficiency of zinc is associated with hypogonadism and insufficient development of secondary sex characteristics in human beings and can cause atrophy of the seminiferous tubules in the rat, leading to failure in spermatogenesis and impotence (Laura *et al*., 2010). Low seminal zinc levels were correlated with a decrease in fertilizing ability of sperm. There are still controversies regarding zinc levels in both fertile and infertile men, as well as the relationship between seminal fluid zinc and semen parameters. Some authors reported significantly different seminal zinc levels between fertile and infertile groups, indicating low seminal zinc levels in the infertile population (Hasan *et al*., 2007; Doshi *et al*., 2008), while some others have shown that there is no difference between the two groups (Basil OM et 2008). There is therefore the need to find out the relationship between zinc levels in blood and seminal fluid on semen parameters to contribute to the body of knowledge in this emerging field. The hypothesis is that blood and seminal fluid levels of zinc are decreased in men with abnormal semen parameters. This study sets out to compare semen parameters between men with abnormal semen parameters and men with normal semen parameters, determine the concentration of zinc in the blood and seminal fluid of both groups. In addition, to find out the relationship if any between zinc levels in blood and seminal fluid of men with abnormal and normal semen parameters.

MATERIALS AND METHODS

Design and setting: The study was a prospective case-controlled study conducted at the The Institute of Fertility Medicine (IFM) and the gynaecological out patient clinic both of Lagos State University Teaching Hospital (LASUTH), which is one of the four main referral hospitals with obstetrics and gynaecological services in Lagos, Nigeria. The Institute of Fertility Medicine is a public private partnership between The Bridge Clinic and the department of Obstetrics/Gynaecology of LASUTH that offers assisted reproductive technology to low income earners. The two units open between 08.00 and 16,00 hour (Monday to Friday). The average number of 30 clients attend the two clinics each day.

Study population and sample size determination: The study population consisted of two groups: cases and controls. Cases were infertile male subjects, who had adequate unprotected sexual intercourse for at least one year without conception with their partners who were fully investigated and the only identifiable cause was semen abnormality. Controls were age-matched fertile males whose partners had spontaneous conception within one year with normal seminal fluid analysis according to WHO-2010 recommendation (World Health Organization, 2010). Inclusion criteria were absence of factors in the history that adversely affect male fertility as suggested by the World Health Organization (WHO) manual for the standardized investigation, diagnosis and management of the infertile male (Rowe PJ *et al*., 2000), more than one year of failed attempt at conception, normal genital tract examination and exclusion of female factors. Exclusion criteria include refusal of consent, those with history of pelvic surgery or hernia repair, testicular injury, mumps-related orchitis, diabetes mellitus, hypertension, heavy or regular use of alcohol, history of fever in the last three months, occupational exposure to agents that affect spermatogenesis and history of smoking cigarettes or marijuana. The study lasted for twelve months: January 2015-December 2015.

The sample size of 170 (85 in each arm) was calculated based on male infertility prevalence rate of 20-25% in Nigeria as reported by Okonofua (Okonofua, 2003) and with the formula by D.W Taylor (Tailor, 1994). Convenience sampling was used to recruit subjects, following the incl Tailor DW usion and exclusion criteria. One out of every two patients was selected within each group until the desired sample size was reached.

Ethics: Formal approval for the study was obtained from the Research Ethics Committee of the Lagos State University Teaching Hospital.

Collection of samples and seminal fluid analysis: Semen specimens were collected in sterile polystyrene containers through masturbation after 3 days of abstinence. The samples were brought within one hour of production. Period of abstinence, time of ejaculation, spillage during collection (if any) were noted. Semen samples were incubated for 30 minutes at 37 degree centigrade for liquefaction and were subsequently analyzed in the research laboratory within one hour of receipt. The WHO guidelines of 2010 (World Health Organization, 2010) was used to measure volume, sperm concentration, progressive motility, total motility and morphology. After liquefaction, a portion of the semen sample was centrifuged at 2000g for 15- 20 minutes and the supernatant was transferred into fresh tubes and stored at -20 degrees centigrade until analyzed for zinc content. In addition, five mililitres of blood was taken from each subject by venepuncture with metal-free disposable syringe and stored in metal-free bottle. The sample was centrifuged at 2000g for 15-20 minutes and the supernatant transferred into fresh tubes and stored at -20 degrees centigrade until analyzed for zinc content. The samples were labeled with patient's name and identification number. Sample processing and analysis was at the research laboratory of Lagos State University College Of Medicine, Ikeja-Lagos.

Measurement of zinc concentration in serum and seminal fluid: The serum was first allowed to thaw in room temperature. With the aid of an analytical weighing balance AG 204 METTLER TOLEDO, 0.5g of serum was measured and aspirated with a pipette into a conical flask. Two millilitres each of concentrated nitric acid and percholoric acid were added to the serum for deproteinization, to release the serum zinc from the protein matrix. Subsequently, the suspension of serum in acid in the conical flask was moved to the digestion chamber to continue the digestion process. The sample was heated by hot plate until complete dissolution, this occured when the suspension changed from golden yellow to a transparent solution. A blank sample was also prepared as control, to correct for any background error. The pellet obtained was diluted 100 times with deionized distilled water for measurement of zinc concentration by direct aspiration of the sample into the Microwave Plasma-Absorption Emmision Spectrophotometer instrument model MY14280004. Maker: Agilent technologies,West Germany. The measurement was conducted at 481.053 nm wavelength and the coefficient of variation for the instrument was 0.997. The above procedure was also repeated to determine the level of zinc in seminal fluid.

Data collection and analysis: A proforma was used to collect data on socio-demographic characteristics and the laboratory parameters measured.

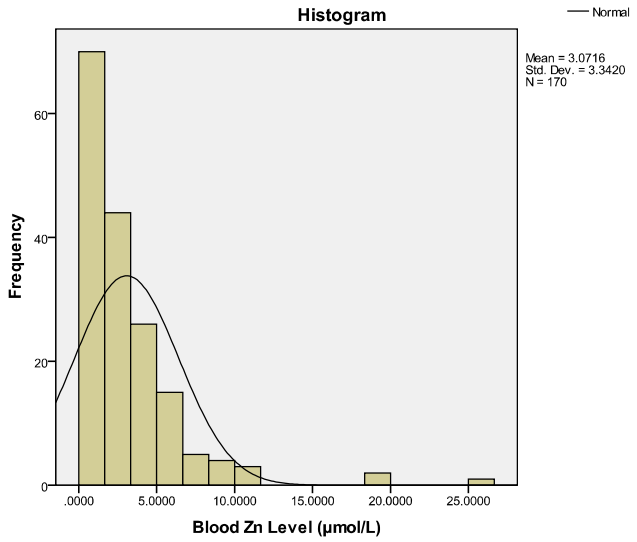

Fig. 1. Histogram showing the distribution of blood zinc levels among respondents

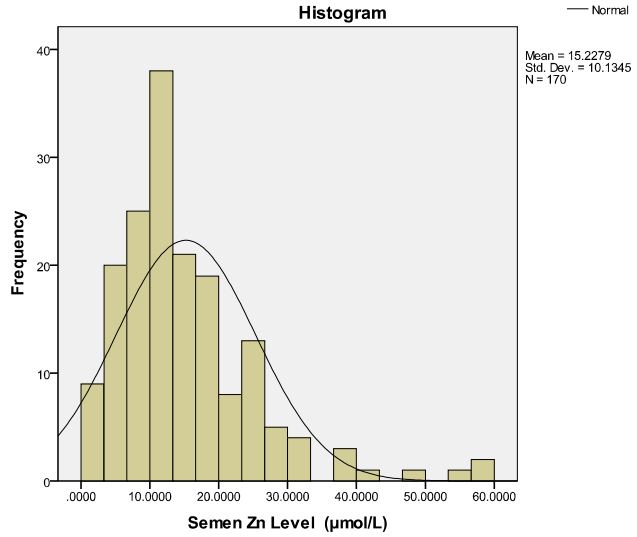

Fig.2.Histogram showing the distribution of seminal plasma zinc levels among respodents

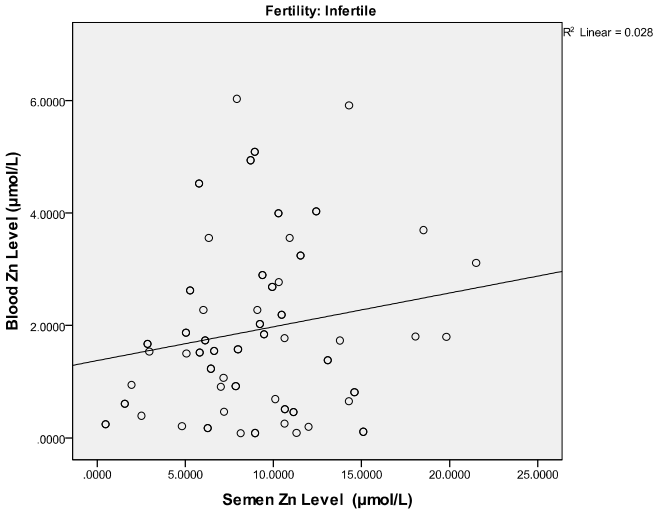

Fig 3.Satter plots showing correlation between blood zinc and semen zinc levels among cases.Spearman rho's correlation coefficient = 0.115, p = 0.297

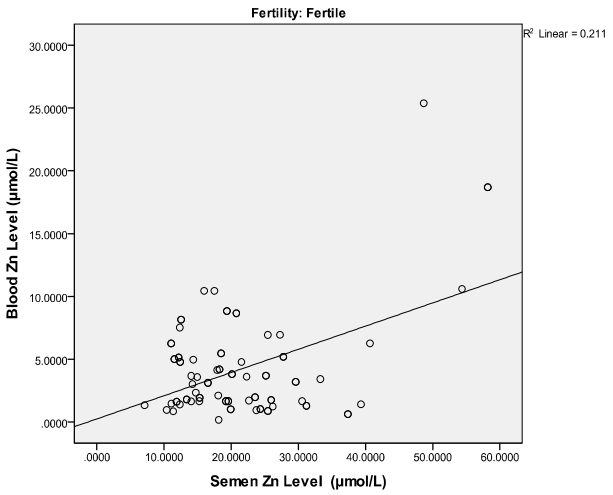

Fig 4. Scatter plots showing correlation between blood zinc and semen zinc levels among Controls. Spearman rho's correlation coefficient = 0.008,p = 0.940

The data obtained were entered into the computer and analyzed using the Statistical Package for Social Sciences (SPSS) IBM version 19. Histogram of outcome variables were plotted to determine if they were normally distributed and hence the appropriate statistical test to use. Percentages, mean, median, standard deviation and inter quartile range of numerical variables were determined. Chi square test was used to compare categorical variables and t-test was used to compare means. Mann Whitney U test or Kruskal wallis test were used to compare median of two or more than two numerical variables respectively. Spearman rho correlation was used to determine association between parameters.

RESULTS

Socio-demographic characteristics of the study population revealed that the mean age was 38.5+ 5.9 years in the cases and 38.6 + 6.3 years in the control. Majority had tertiary education in both groups and variables like occupation, tribe, religion and duration of infertility were not statistically significant. Males with abnormal semen parameters (cases) were divided into the following three sub-groups according to their sperm count, motility and morphology. Group I: Azoospermic (sperm count= zero), Group II: Oligozoospermic (sperm count $<15x10^6$/ml), Group III: Asthenozoospermic sperm count$\geq$ $15x10^6$/ml, progressive motility <32%, irrespecctive of morphology). Blood and seminal fluid zinc levels were not normally distributed as depicted by the histograms in Figs 1 and 2, hence the use of median and inter quartile range as a measure of average. Table 1 showed the median blood and seminal fluid zinc levels and the median semen parameters among cases and controls. The median blood zinc level was 1.7 µmol/L (IQR 0.6, 2.7) in the cases and 3.2 µmol/L (IQR 1.6, 5.2) in the controls with a p value of <0.001 which was statistically significant. The median seminal fluid zinc levels among the cases and controls is as shown in Table 1. The median sperm count, progressive motility and morphology were all statistically significant between cases and controls as shown in Table 1. Table 2 showed blood and seminal fluid zinc levels in cases (abnormal semen group) and the controls (normozoospermic group). The median blood zinc level was 1.7µmol/L (IQR 0.6, 2.7) in the oligozoospermic,

Table 1. Semen parameters of Cases and Controls

Variables	Cases n = 85 (%)	Controls n = 85 (%)	x2	p
Semen volume				
Low(<1.5mls)	14 (16.5)	18 (21.2)	0.616	0.433
Normal (≥1.5 mls)	71 (83.5)	67 (78.8)		
Sperm count				
Normal (>15x10^6/ml)	13 (15.3)	85 (100)	124.898	<0.001
Low (<15x10^6/ml)	72 (84.7)	0 (0)		
Progressive motility				
Normal (≥32%)	18 (21.2)	85 (100)	110.583	<0.001
Low (<32%)	67 (78.8)	0 (0)		
Total motility(%)				
Normal	37 (43.5)	85 (100)	59.953	<0.001
Low	48 (56.5)	0 (0.0)		
Morphology				
Normal (≥4%)	6 (7.1)	85 (100)	147.582	<0.001
Low (<4%)	79 (92.9)	0 (0.0)		

Table 2. Median blood and seminal plasma zinc levels and median semen parameters in Cases and Controls

Variables	Cases Median (IQR)	Controls Median (IQR)	U	p
Blood zinc(µmol/L)	1.7 (0.6, 2.7)	3.2(1.6,5.2)	2095.0	<0.001
Seminal plasma zinc (µmol/L)	9.0 (6.1, 11.0)	19.4 (14.0,25.5)	488.0	<0.001
Volume(mls)	2.5 (1.8, 3.2)	2.0 (1.5,3.1)	3210.0	0.207
Sperm count(x 10^6)	6.0 (2.5, 11.0)	28 (23.0,40.0)	636.0	<0.001
Progressive motility(%)	20.0 (10.0, 30.0)	40.0 (35.0,45.0)	767.0	<0.001
Total motility(%)	35.0 (26.0, 45.5)	56 (50.0,64.0)	778.5	<0.001
Morphology(%)	1.0 (0.0, 2.0)	5 (5.0,5.0)	198.0	<0.001

NB: U = Mann Whitney U test
IQR = Interquartile range

Table 3. Correlation of blood and seminal plasma zinc level with semen parameters among Cases and Controls

Variable	Cases spearman's rho correlation	p	Controls spearman's rho correlation	p
	Blood Zinc level			
Volume(mls)	0.057	0.604	-0.186	0.088
Sperm count (x10^6)	0.008	0.941	-0.008	0.940
Progressive motility(%)	0.016	0.883	0.189	0.083
Total motility(%)	0.065	0.553	0.080	0.469
Morphology(%)	-0.029	0.794	-0.014	0.896
	Seminal plasma zinc level			
Volume(mls)	0.031	0.775	0.065	0.557
Sperm count(%)	0.014	0.898	-0.054	0.626
Progressive motility(%)	0.136	0.215	-0.193	0.077
Total motility(%)	0.118	0.282	-0.056	0.613
Morphology(%)	0.155	0.157	0.088	0.423

Table 4. Correlation of blood and seminal plasma zinc level with semen parameters among different groups of the study population

Variable	Azoospermic spearman's rho	p	Oligozoospermic spearman's rho	p	Asthenozoospermic spearman's rho	p	Normozoospermic spearman's rho	P
Blood Zinc level	**correlation (p)**		**correlation (p)**		**correlation (p)**		**correlation (p)**	
Volume	0.282	0.498	-0.045	0.722	0.298	0.322	-0.186	0.088
Sperm count	-		0.311	0.012	0.014	0.964	-0.008	0.968
Progressive motility		-	0.252	0.045	0.063	0.838	0.189	0.083
Total motility			- 0.285	0.022	0.367	0.217	0.079	0.472
Morphology		-	-0.109	0.389	-0.322	0.283	-0.014	0.896
Seminal plasma zinc level								
Volume	-0.233	0.578	0.064	0.613	-0.307	0.308	0.065	0.557
Sperm count		-	0.086	0.497	0.438	0.134	-0.054	0.626
Progressive motility		-	0.251	0.045	0.172	0.574	-0.193	0.077
Total motility	-		0.218	0.083	0.391	0.187	-0/056	0.613
Morphology		-	0.165	0.192	0.096	0.756	0.088	0.423

1.7µmol/L (IQR 0.9, 3.8) in the asthenozoospermic and 0.2 µmol/L (IQR 0.1, 1.6) in the azoospermic group while it was 3.2 µmol/L (IQR 1.6, 5.2) in the normozoospermic with a p value of < 0.0001 which was not statistically significant. The median seminal fluid zinc level in the oligozoospermic, asthenozoospermic, azoospermic group and the normozoospermic was statistically significant as shown in Table 2. Table 3 showed the correlations of blood and seminal fluid zinc levels with semen parameters among the cases and controls. Blood zinc levels among the cases showed a weak positive correlation with semen volume (r= 0.057 p= 0.604), sperm count (r= 0.008 p= 0.941), progressive motility (r = 0.016 p = 0.883), total motility (r= 0.065 p= 0.553) and a weak negative correlation with morphology (r = - 0.029 p = 0.794). These correlations were not statistically significant. Blood zinc levels among the controls showed a weak negative correlation with semen volume (r = - 0.186 p = 0.088), sperm count (r = - 0.008 p = 0.940) and morphology (r = -0.014 p = 0.896); and a weak positive correlation with progressive motility (r = 0.189 p = 0.083) and total motility (r = 0.080 p = 0.469) with p values that were not statistically significant. Seminal fluid zinc levels among the cases showed a weak positive correlation with all the semen parameters studied but were not statistically significant. Seminal plasma zinc levels among the controls showed a weak negative correlation with sperm count, progressive motility, total motility and a weak positive correlation with semen volume and morphology but were not statistically signifcant. Figure 3 and figure 4 are scatter plots of correlation between blood zinc level and seminal fluid zinc level among the cases and the controls respectively. Blood zinc levels showed a weak positive correlation with seminal fluid zinc level in both cases (r = 0.115 p = 0.297) and controls (r = 0.008 p = 0.940). These correlations were not statistically significant. Table 4 showed the correlation coefficient of blood and seminal fluid zinc levels with semen parameters in azoospermic, oligozoospermic, asthenozoospermic and normozoospermic men.

There were no significant correlations between blood zinc levels and semen parameters in the azoospermic and normozoospermic groups. However, blood zinc showed a significant positive correlation with sperm count (r = 0.311 p = 0.012), progressive motility (r = 0.252 p = 0.045) and total motility (r = 0.285 p = 0.022) and a non- significant negative correlation with semen volume (r = -0.045 p = 0.722) and morphology (r = -0.109 p = 0.389) in the oligozoospermic men. The remaining is as shown on the table. Among the oligozoospermic group, seminal fluid zinc level was significantly positively correlated with progressive motility (r = 0.251 p = 0.045). It was also positively correlated with semen volume (r = 0.064 p = 0.613), sperm count(r = 0.086 p = 0.497), total motility(r = 0.218 p = 0.083) and morphology(r = 0.165 p = 0.192), but not statistically significant. The remaining is as shown on Table 4.

DISCUSSION

This study set out to determine the level of zinc in blood and seminal fluid, correlating these with semen parameters among men with abnormal and normal semen parameters in an infertility clinic. Using the World Health Organization (2010) criteria, men with abnormal semen parameters had values well below normal except for the volume. This could explain the reason for the inability of the couples with abnormal semen parameters to achieve pregnancy in one year of having regular unprotected sex. The result of this study showed that there were significantly lower level of seminal fluid zinc in men with abnormal semen parameters compared with men with normal semen parameters. This finding was in agreement with the work done by Hassan *et al* (Hassan *et al*., 2007) and Chia *et al* (Chia *et al*., 2000) who observed significant decrease in seminal fluid zinc in infertile males. Though seminal fluid zinc was decreased in infertile patients, it is not clear whether the decrease in concentration was as a result of male factor infertility or the decrease in zinc concentration led to male

infertility. This, however is at variance with what was reported by Akinloye *et al* (Akinloye *et al*., 2011), who observed higher levels of blood and seminal fluid zinc in infertile males compared to fertile male controls. This difference could be due to the smaller sample size of their study. Seminal plasma zinc levels in the oligozoospermic and azoospermic men were significantly lower than in normozoospermic men. These findings were similar to results obtained by Hasan *et al* (Hassan *et al*., 2007), though with a wide variation in values of the control group in the two studies. The control group for the study by Hassan *et al* (Hassan *et al*., 2007) were men with normal sperm count selected from the general population after semen analysis, while the controls for our study were men with normal sperm count and proven fertility.

This could account for the wide variation in the seminal plasma zinc concentrations in the control group of both studies. In this study, seminal fluid zinc levels were positively correlated with sperm count in oligozoospermic and asthenozoospermic men, though the relationships were not statistically significant contrary to the findings in other studies (Eggert-Kruse *et al*., 2002; Lin *et al*., 2000). Sperm counts are largely variable and are influenced by various factors such as past illnesses, smoking status, use of medication, and time of abstinence. In this study, time of abstinence was kept constant and special care was taken to ensure identical conditions during sampling, storage, and analysis. This study revealed a significant positive correlation between seminal fluid zinc levels and progressive sperm motility in the oligozoospermic group. This is in accordance with some studies (Zhao *et al* 2016; Lin *et al*., 2000). In addition to the role of zinc in the maintenance of germ cells and spermatogenesis, it also plays an important role in regulation of sperm motility (Yamagushi *et al*., 2009). This study revealed a weak positive correlation between seminal fluid zinc levels and sperm morphology in the normozoospermic, oligozoospermic and the asthenozoospermic subjects. This was in agreement with studies done in Singapore by Chia *et al* (Chia *et al*., 2000) and in India by Doshi *et al* (Doshi *et al*., 2008).

This study revealed a significant positive correlation between blood zinc and sperm count and between blood zinc and sperm motility (progressive and total) in oligozoospermic subjects. These findings were not in agreement with what was reported by Hassan *et al* (Hassan *et al*., 2007) who reported no significant correlation between blood zinc and semen parameters. This difference may be due to the small sample size for the fertile control group in their study. A weak positive correlation was observed between blood zinc levels and seminal fluid zinc levels among men with abnormal and normal semen parameters in this study. However, this was not statistically significant. Zinc is present in high concentration in seminal fluid and there is evidence that it may act in vivo as a scavenger of excessive oxygen production by defective spermatozoa and/or leukocytes in semen after ejaculation and may also play a multifaceted role in sperm functional properties (Mohammad *et al*., 2009). A clinical study demonstrated that adult males experimentally deprived of zinc showed a disturbance of testosterone synthesis in the Leydig cells. The authors concluded that adequate seminal concentration of zinc is required for normal sperm function (Akinloye *et al*., 2011). This study however had a number of limitations. They include; measurement of zinc should have been done three times with the mean value calculated to limit intra assay coefficient of variation and unknown dietary intake of participants could affect the level of zinc.

Conclusion

This study has demonstrated that blood and seminal fluid zinc levels were significantly lower in men with abnormal semen parameters compared with those with normal semen parameters. In oligozoospermic men, blood zinc levels were significantly positively correlated with semen parameters while the seminal fluid zinc levels showed significant positive correlation with progressive motility. Further studies are needed to determine whether zinc supplementation will improve semen quality in males with sub normal semen parameters. Such studies will be useful in deciding the necessity for zinc supplementation in cases of male infertility.

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Conflict of interest

The authors declare that they have no competing interest.

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