Serum and Seminal Fluid Zinc in Males Presenting with Infertility in a Nigerian Population

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Abstract

Background: *The usefulness of serum and seminal fluid zinc measurements, in investigating male infertility in the Nigerian population has been evaluated, by determining the relationship between zinc levels in these body fluids and % spermatozoa headpiece abnormality, % spermatozoa motility and sperm count. We have also explored the possibility of substituting serum zinc for seminal fluid zinc measurement, in investigating this condition, by determining the relationship between serum and seminal fluid zinc levels.*

Materials and Methods: *Sixty (60) infertile males, aged 32 – 53 years, were recruited for the study by random selection. The subjects were classified on the basis of sperm cunt into three groups: azospermic (3.3%); oligospermic (75%) and normospermic (21.7%). Pairs of samples (blood sample and semen) were obtained from the subjects for laboratory analysis. Atomic absorption spectroscopy was used to measure zinc levels. Semen analysis was done by employing the conventional method (World Health Organisation-1987). Statistical analysis of all data was done using SPSS 14.*

Results: *Mean serum zinc concentration in the three groups was not significantly different, likewise mean seminal zinc level. There was a positive correlation between serum and seminal fluid zinc levels in the normospermic and oligospermic groups ($r=0.4$; $p < 0.05$ and $r=0.6$; $p<0.01$)_respectively. Furthermore, a strong positive correlation existed between seminal zinc concentration and percentage spermatozoa headpiece abnormality ($r=0.84$, $p < 0.01$), and also between serum zinc levels and percentage headpiece abnormality ($r=0.60$, $p < 0.05$). However, a significant negative correlation exists between seminal zinc level and percentage motile spermatozoa ($r=0.57$, $p < 0.05$).*

Conclusion: *Zinc is one of the major components that determine semen quality. Routine seminal zinc analysis is useful as a complementary test in investigating male infertility, especially in the normospermic group, while serum zinc could serve as substitute for seminal fluid zinc analysis.*

Keywords: Serum; semen; zinc; infertility

Introduction

Zinc is an essential trace element and acts as a co-factor for several enzymes in the body[1]. It is required for the maintenance of proper DNA conformation, growth, wound healing, immune functions and fertility[2-5]. Deficiency states from nutritional and non-nutritional effect result in diverse diseases including

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acrodermatitis enteropathica, growth retardation, vulnerability to infection, impaired wound healing, sensory alteration, coronary artery disease, insulin resistance, mental lethargy and poor appetite[6-8]. Zinc plays critical role in male and female reproduction and fetal development, thus its deficiency is associated with infertility and gonadal abnormality. In males, deficiency is associated with testicular atrophy, hypogonadism and infertility[9-11]. Effect of zinc on fertility stems from its influence on sperm quality, especially sperm concentration and motility[12-16]. It also to a lesser extent affects semen volume and sperm morphology. Based on fertility, adult males are classified into fertile or infertile group or into azospermic, oligospermic, and normospermic groups based on sperm count. Effect of zinc concentration on semen quality results from its influence on spermatogenesis and steroidogenesis and had been found to vary among the three groups[17,18]. Conflicting reports exist on the role of zinc in spermatozoa function. Important role of zinc in spermatozoa function and fertility has been stated by some workers[13,14]. This role has also been refuted[19,20]. Male factor infertility is linked with oxidative stress[21]. Zinc has been stated to have antioxidant effect on spermatozoa, as it acts as a co-factor for antioxidants[22-25]. Apoptotic effects on prostate cells and antimicrobial properties of zinc have also been reported[26]. It has also been opined to be more relevant in assessing prostatic function[3,14,18,26].

The relevance of zinc measurement in serum and semen in comparison with other parameters of semen quality in infertile males and the possibility of substituting serum (blood sample) for semen in case of any correlation is the main purpose of this study.

Materials and Methods

Sixty (60) infertile Nigerian males, aged 32 – 53 years, were recruited into the study by random selection, at the Omni Medical Centre/ Advanced Fertility Clinic, Lagos, Nigeria. The main inclusion criterion is diagnosis of infertility (inability to achieve conception after one year of regular unprotected sexual intercourse). Exclusion criteria include: hypertension, diabetes mellitus, recent systemic disease and positive report for chlamydia, toxoplasmosis, syphilis and HIV. Informed consent (signed consent form) was obtained from each subject, after the study was explained in detail. Sixty pairs of samples (blood sample and semen from each subject) were collected, after each subject had observed an overnight fast and abstained from sexual intercourse for 2 – 3 days.

All samples were collected within the hospital premises, to avoid undue delay in processing. Early morning venous blood samples, were collected from the subjects into plain specimen tubes, without stasis or haemolyis. The blood was then spun at 3000 rpm for 5 minutes using gallenkamp bench-centrifuge, after clot retraction. The supernatant (serum) was extracted using Pasteur pipettes into storage tubes and stored frozen at -20^{0}C prior to analysis. Semen sample collection was done by masturbation with instructions. Each sample was divided into two: one for immediate semen analysis and the other was centrifuged at 3000rpm to obtain the supernatant which was then stored frozen at -20^{0}C prior to zinc assay. Semen analysis was done by the conventional method prescribed by the World Health Organisation[27]. Semen samples were processed and analyzed promptly after collection. Macroscopic evaluation was done for appearance, consistency, volume and $_P$H.

Microscopic examination was then done as follows: wet preparation of a drop of semen on a slide, covered with a slip was examined under the microscope for the percentage motile spermatozoa and percentage abnormal forms, i.e. percentage headpiece and percentage midpiece abnormalities. Sperm count was determined by charging a 1 in 20 dilution of semen into new improved neubauer counting chamber after which it was examined under Nikon microscope to count the number of spermatozoa. Samples with significant number of leucocytes (>1 × 10^6) were excluded. Serum and seminal fluid zinc concentrations were determined by atomic absorption

spectrophotometry. Statistical analysis of all data was done using SPSS14.

Results

The mean of all seminal fluid parameters studied (with the exception of volume) was lower than the respective lower reference limits (serum zinc: 7.6 – 22.7µmol/l; seminal zinc: > 2.4µmol/ejaculate; sperm count: > 20 × 10^6/ml; volume: > 2.0mls; percentage abnormality: < 70%) confirming infertility in the studied subjects. Table 1 shows the descriptive characteristics of all the variables. The frequency distribution of serum and seminal fluid zinc levels and sperm count were skewed (non-gaussian), necessitating log-transformation. There was no significant difference in mean serum zinc concentration in the study groups, with the subjects classified on the basis of sperm count, into azospermic (3.3%); oligospermic (75%); and normospermic (21.7%). Mean seminal fluid zinc concentration was also not significantly different in the three study groups. Table 2.

Pearson moment correlation shows significant positive correlation between seminal zinc and serum zinc concentrations in the normospermic and oligospermic groups

Table 1. Descriptive statistics of all variables

Variable	N	Minimum	Maximum	Mean	SEM
AGE (years)	55	32	53	39.15	0.80
SEMINAL Zn (µmol/L)	60	0	28.3	4.67	0.89
SERUM Zn (µmol/L)	60	0	25.2	4.37	0.68
VOLUME (mls)	60	0	7.0	2.78	0.18
SPERM COUNT (10^6/ml)	60	0	114.0	12.33	2.14
% MOTILITY	60	0	85.0	46.58	2.67
% HEADPIECE	60	0	60.0	23.08	1.91
% MIDPIECE	60	0	60.0	15.42	1.20
LOG AGE	55	1.5	1.72	1.59	0.01
LOG SEMINAL ZINC	60	1.00	1.45	0.33	0.07
LOG SERUM ZINC	60	0.70	1.40	0.34	0.07
LOG SPERM COUNT	60	0	2.06	0.84	0.06

Table 2. Descriptive statistics of study groups based on sperm count

	AZOSPERMIA		OLIGOSPERMIA		NORMOSPERMIA	
Sperm Count	0 × 10^6		1-20 × 10^6		> 20 × 10^6	
% of Subjects	3.3%		75%		21.7%	
Statistics	**Log-Mean**	**SD**	**Log-Mean**	**SD**	**Log-Mean**	**SD**
Log-Serum Zinc	0.4	0.52	0.39	0.56	0.52	0.56
Log-Seminal Zinc	0.17	1.65	0.39	0.55	0.25	0.46
Log-Sperm Count	0	-	0.70	0.35	1.48	0.20
Volume(ml)	2	0	2.91	1.44	2.61	1.26
% Motility	0	-	44.00	18.73	62.69	12.35
% Headpiece Abnormality	0	-	25.22	15.11	19.23	10.96
% Midpiece Abnormality	0	-	15.44	10.49	15.38	6.28

Table 3. Relationship between seminal and serum zinc concentrations and other parameters of semen quality

Variable 1	Variable 2	r-value	p-value
Log-Seminal Zinc	Log-Age	0.02	0.92
,,	Log-Serum Zinc	0.40	<0.001
,,	Log-Sperm Count	0.02	0.87
,,	Volume	0.09	0.48
,,	% Motility	0.07	0.61
,,	% Headpiece Abnormality	0.16	0.22
,,	% Midpiece Abnormality	-0.08	0.53
Log-Serum Zinc	Log-Age	-0.13	0.36
,,	Log-Sperm Count	0.22	0.09
,,	Volume	0.00	0.97
,,	% Motility	0.13	0.34
,,	% Headpiece Abnormality	-0.01	0.95
,,	% Midpiece Abnormality	-0.17	0.19

Significance set at p < 0.05

Table 4. Relationship between seminal and serum zinc concentrations and other parameters of semen quality among the normospermic group

Variable 1	Variable 2	r- value	p-value
Log-Serum Zinc	Log-Seminal Zinc	0.59	<0.05
,,	Log-Sperm Count	0.09	0.77
,,	% Motility	-0.46	0.12
,,	Volume	-0.47	0.10
,,	% Headpiece Abnormality	0.60	<0.05
,,	% Midpiece Abnormality	0.15	0.64
Log-Seminal Zinc	Log-Sperm Count	0.15	0.62
,,	% Motility	-0.57	<0.05
,,	Volume	-0.47	0.10
,,	% Headpiece Abnormality	0.84	<0.001
,,	% Midpiece Abnormality	0.37	0.22

Significance set at p < 0.05.

Table 5. Relationship between seminal and serum zinc concentrations and other parameters of semen quality among the oligospermic group

Variable 1	Variable 2	r-value	p-value
Log-Serum Zinc	Log-Seminal Zinc	0.38	<0.01
,,	Log-Sperm Count	0.09	0.57
,,	% Motility	0.07	0.64
,,	Volume	-0.03	0.85
,,	% Headpiece Abnormality	-0.17	0.27
,,	% Midpiece Abnormality	-0.26	0.08
Log-Seminal Zinc	Log-Sperm Count	-0.08	0.61
,,	% Motility	-0.01	0.95
,,	Volume	-0.14	0.37
,,	% Headpiece Abnormality	-0.04	0.81
,,	% Midpiece Abnormality	-0.14	0.35

Significance set at p<0.05

(r=0.40, p < 0.05 and r=0.6; p<0.01) respectively. Table 3. All other semen quality parameters showed no significant correlation with either serum or seminal fluid zinc concentrations. Correlation studies (tables 4 & 5) between either the serum or seminal fluid zinc concentration and other parameters of semen quality for each of the subject groups,, showed that in the normospermic group, both serum and seminal fluid zinc concentrations had significant positive correlation with the percentage headpiece abnormality (r = 0.60; p < 0.05 and r = 0.84; p < 0.01) respectively. However, a significant negative correlation exists between seminal zinc level and percentage motile spermatozoa (r= 0.57, p < 0.05).

Discussion

Frequency distribution of all the measured variables was non-guassian i.e. skewed, necessitating a log-transformation. This agrees

Table 6. Relationship between seminal and serum zinc concentrations and other parameters of semen quality among the oligospermic subgroup (SG-1)

Variable 1	Variable 2	r-value	p-value
Log-Serum Zinc	Log-Seminal Zinc SG-1	0.39	<0.02
,,	Log-Sperm Count SG-1	0.15	0.37
,,	% Motility SG-1	0.09	0.61
,,	Volume SG-1	0.08	0.65
,,	% Headpiece Abnormality SG-1	-0.17	0.30
,,	% Midpiece Abnormality SG-1	-0.29	0.07
Log-Seminal Zinc	Log-Sperm Count SUBGRP-1	-0.08	0.62
,,	% Motility SG-1	-0.05	0.78
,,	Volume SG-1	-023	0.16
,,	% Headpiece Abnormality SG-1	0.02	0.89
,,	% Midpiece Abnormality SG-1	-0.14	0.35

Significance set at p < 0.05; abnorm -abnormality

with findings of Solberg that distributions of most biological data are often skewed[28]. Mean seminal zinc level was not significantly different in the three study groups i.e. nomospermic, oligospermic, and azospermic subjects. This finding is contrary to the earlier reported decreasing order of seminal zinc level among these groups by Stanwell-Smith *et al*[13], who stated that infertile normospermic subjects had higher mean concentration of seminal zinc than infertile oligospermic subjects. The finding is also contrary to that of Ali *et al*, who reported that the level of seminal zinc is greater in infertile oligospermic subjects than in azospermic infertile subjects[29]. Significant positive correlation between serum and seminal zinc concentration in this study is similar to the findings of Mohan *et al*[4], but contrary to that of Ali *et al*[29]. This positive linear relationship can be explained by zinc distribution in the body[28,29]. Prostate cells have affinity for zinc while the main source of seminal zinc is blood. Any loss or decline in seminal zinc during ejaculation would be replaced by prostatic secretion. Thus any decrease in blood or plasma zinc will result in decline in seminal zinc[28,29]. These findings about body zinc distribution have been reported by other workers[12,22,30-32].

The finding of a significant negative correlation between seminal zinc concentration and percentage motility among the infertile normospermic subjects in this study is similar to that of Carreras and Mendoza[33], Sorensen *et al*[34]. It is however in disagreement with Senada and Yoshida[14] who reported that normospermic subjects with low seminal zinc have reduced sperm motility but no significant correlation between these two parameters among their oligospermic group. Available reports on the relationship between seminal/serum zinc levels and spermatozoa headpiece abnormality are contradictory. In this study, a significant correlation was observed between both serum and seminal zinc levels and percentage headpiece abnormality among the normospermic group. This is in line with finding of Wong *et al*[18] but contrary to that of Fuse *et al*[35], and Ali *et al*. It has also been explained by Sorensen *et. al.*[34] that the sperm head accumulates high concentration of zinc which is needed for chromatin stability. No significant correlation was observed between serum or seminal zinc and other parameters of semen quality in the oligospermic patients in this study, an observation similar to that of Eggert-Kruise[36]. Also in this study, no significant correlation was found between seminal zinc level and sperm count at either the different group levels or for total subjects, which is similar to the findings of Stanwell-Smith *et al*[13] who reported that a significant positive correlation can be found between seminal zinc and sperm count only in fertile but not in infertile subjects.

Finally, from this study, it is obvious that semen analysis remains an indispensable tool in investigating male infertility and zinc is one of the major components that determine semen quality especially in the normospermic infertile subjects. A high positive correlation exists between serum and seminal zinc

concentrations. We thus advise that routine zinc analysis be incorporated as complementary test in investigating male infertility especially in the normospermic group as this will enhance prognosis and treatment in those with zinc deficiency and serum zinc could serve as substitute to seminal zinc assay.

References

1. Prasad AS. Discovery of human zinc deficiency and studies in an experimental human model. Amer J Clin Nutr 1991; 53 (2): 403-412
2. Jacob AR. Trace elements. *In:* Burtis CA, Ashwood ER. (Eds) Teitz Textbook of Clinical Chemistry. 4th edn, Philadelphia, W.B Saunders, 2006; 975-981
3. Fervier AE. Role of zinc in reproduction; hormonal mechanism. Biol Trace Elem Res 1992; 3: 363-382
4. Mohan H, Verma J, Singh I, Mohan P, Marwah S, Singh P. Inter-relationship of zinc level in serum and semen in oligospermic infertile patient and fertile males, Indian J Pathol & Microbiol.1997; 40 (4): 451-45
5. Mankad M, Sathawara NG, Dosh H, Saived HN, Kumar S. Seminal plasma zinc concentration and alpha-glycosidase activity with respect to semen quality. Biol Trace Elem Res 2006; 110 (2): 97-106
6. Martin WD Water and minerals: *In*: Martin WD, Mayes P, Rodwell VW. (Eds) Harper's Review of Biochemistry.19th edn, USA, Lange Med. Publication. 1983; 583-586
7. Singh RB, Niaz MA, Rastogi SS, Bajaj S, Gaoli Z, Shoumin Z. Current zinc intake and risk of diabetes and coronary artery disease. J Amer Coll Nutr 1998; 17: 6564-6570
8. Alcock NW. Trace elements *In*: Kaplan LA, Pesce AJ, Kazmierczack SC. (eds) Clinical Chemistry: Theory, Analysis, Correlation. 4th edn, Mosby USA. 2003; 707-715
9. Hunt CD, Johnson PE, Herbel J and Mullen LK. Effects of dietary zinc depletion on seminal volume and zinc loss, serum testosterone concentration and sperm morphology in young men, Amer J Clin Nutr 1992; 26: 148-157
10. Chia SE, ONQ CN, Chua LH, Ho LM and Tay SK. Comparison of zinc concentration in blood and seminal plasma and various sperm parameters between fertile and infertile men, J Androl. 2000; 21 (1): 53-57
11. Ali H, Baig M, Rana MF, Alim OR, Khem AK. Relationship of serum and seminal plasma zinc level, and serum testosterone in oligospermic and azospermic infertile men. J Coll Phys & Surg Pakistan. 2005; 15:671-673
12. Stegmayr B, Berggren PO, Ronquist G and Hellman B. Calcium, magnesium, and zinc contents in organelles of prostatic origin in human seminal plasma. Scand J Urol and Nephrol. 1982; 16(3): 199-203
13. Stanwell-Smith R, Thompson SG, Haines AP, Ward RJ, Cashmore G, Stedronska J *et al.* A comparative study of zinc, copper, cadmium and lead level in infertile men, Fertil and Steril 1983; 40(5): 670-677
14. Sanada S and Yoshida O. Zinc concentration and total amount of zinc in seminal plasma of infertile men with reference to prostatic secretory function. Hinyokika Kiyo. 1985; 31: 1971-1987
15. Xu B, Chia SE, Tsakok M and Ong CN. Trace elements in blood and seminal plasma and their relationship to sperm quality. Reprod Toxicol. 1993; 6: 613-618
16. Venkatesh S, Singh G, Gupta NP, Kumar R, Deecara-man M and Dada R. Correlation of sperm morphology and oxidative stress in infertile men. Iranian J Reprod Med 2009; 7(1): 29-34.
17. Zhao RP and Xiong CL. Zinc content analysis in serum, seminal plasma, and spermatozoa of azospermic, normospermic and oligospermic patients. Zhonghua Nan Ke Xue 2005; 9: 680-682

18. Wong WY, Flik G, Groener PM, Swinkles DW, Thomas CM, Copius-Pererboon JH *et al*. The impact of calcium, magnesium, zinc, and copper in men. Reprod Toxicol 2001; 15 (2): 131-136
19. Omu AE, Dashti H, Mohammed AT and Mattapallil AB. Significance of trace elements in seminal plasma in infertile men. Nutr 1995; 11(5): 502-505
20. Lin YC, Chang TC, Tseng YJ, Huang FJ, Kung FT and Chang SY. Seminal plasma zinc level and sperm motion characteristics in infertile samples. Chang Gung Me J 2000; 5: 260-266
21. Joffe M. What has happened to human fertility? Hum Reproduction, 2010; 25 : 295-307.
22. Ebisch IM, Thomas CMG, Peters WHM and Braat DDM, Steegers-Theninissen RPM. The importance of folate, zinc, and antioxidants in the pathogenesis and prevention of subfertility. European Soc Hum Reprod and Embryol, 2006; Online ISSN1460-2369 Print ISSN 1355-4786
23. Kumar R, Venkatesh S, Kumar M, Tanwar M, Shasmsi MB, Gupta NP, Sharma RK, Talwar P and Rima D. Oxidative stress and sperm mitochondrial DNA mutation in idiopathic oligoasthenozoospermic men, Indian J Biochem and Biophys 2009; 46: 172-177
24. Nabil H, Lekaa A, Moemen-Manal H and Abu E. Studying the levels of malondialdehyde and antioxidant parameters in normal and abnormal human seminal plasma. Australian J Basic and Appl Sci 2008; 2(3): 773-778.
25. Colagar AH, Marzony ET and Chaichi MJ. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. Nutr Res 2009; 29: (2), 82-88
26. Feng P, Liang JY, LI TL, Guan ZX, Zon J, Franklin R and Costello LC. Zinc induces mitochondria apoptogenesis in prostate cells. Mol Urol Spring. 2000; 4 (1): 31-36
27. World Health Organisation. Towards more objectivity in diagnosis and management of male infertility. Int J Androl 1987; 7: 1-35.
28. Solberg HE. Establishment and use of reference values *In:* Burtis CA, Ashwood ER. (Eds) Tietz Fundamentals of Clinical Chemistry. 3rd edn, Philadelphia, W.B Saunders, 1989; 202-212
29. Ali H, Ahmed M, Baig M and Ali M. Relationship of zinc concentration in blood and seminal plasma with various semen parameters in infertile subjects. Pakistan J Med Sci 2007; 23 (1):111-114
30. Robert AJ. Trace elements *In*: Burtis CA, Ashwood ER (Eds) Teitz Textbook of Clinical Chemistry. 3rd edn, Philadelphia, W.B Saunders, 1986; 975-981
31. King JC, Shames DM, Woodhouse LR. Zinc homeostasis in human. J Nutr 2000; 130 (5): 1360S -6S PMID 10801944 (pubmed-index for medline)
32. Toffaletti GJ. Trace elements In: Bishop ML, Fordy EP, Schoeff L.(eds) Clinical Chemistry: Principle, Procedure, Correlations. 5th edn,, USA. Lippincott William Wilkins. 2005; 365-439
33. Carreras A, Mendoza C. Zinc levels in seminal plasma of fertile and infertile men. Andrologia. 1990; 22(3): 279-283
34. Sorensen MB, Bergdahl IA, Hjollund NHI, Bonde JPE, Stoltenberg M, Ernst E. Zinc, magnesium, and calcium in human seminal fluid: relations to other semen parameters and fertility. Mol Hum Reprod. 1999; 5(4): 331-337
35. Fuse H, Kazama T, Outa S, Fujiuchi Y. Relationship between zinc concentrations in seminal plasma and various sperm parameter. Int J Urol and Nephrol. 1999; 31: 401-408
36. Eggert-Kruse W, Zwiick EM, Bat Schulat K. Rohr G, Ambruster FP, Petzoldt D *et al*. Are zinc levels in seminal plasma associated with seminal leucocytes and other determinants of semen quality? Fertil and Steril. 2002; 77(2): 260-269