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EVALUATION OF ZINC IN BLOOD, SEMEN AND THEIR RELATIONSHIP TO SPERM QUALITY AMONG MALES ATTENDING INFERTILITY CLINIC IN KENYATTA NATIONAL HOSPITAL
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EVALUATION OF ZINC IN BLOOD, SEMEN AND THEIR RELATIONSHIP TO SPERM QUALITY AMONG MALES ATTENDING INFERTILITY CLINIC IN KENYATTA NATIONAL HOSPITAL

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ABSTRACT

Objectives: **To determine the sperm quality among adult males attending infertility clinic at the Kenyatta National Hospital; To determine zinc concentration in the seminal plasma and the relationship to sperm quality among the study population; To determine zinc concentration in Blood serum and the relationship to sperm quality among the study population.**
Design: **This was a cross-sectional study in which participants were picked as they randomly attended infertility clinic.**
Setting: **Kenyatta National Hospital Infertility clinic**
Participants: **277 Male adults aged 18-65 years.**
Interventions: **Semen and blood was collected from every participant. Semenalysis, Zinc and Testosterone levels were determined by Microscopy, Flame Atomic absorption spectrometry and immunoassay respectively.**
Results: **Zinc levels showed positive correlation with sperm motility, normal morphology, count (concentration), and volume. Blood Zinc levels and sperm concentration (r =0.168, p =0.035). Zinc concentration in semen and normal sperm morphology (r= 0.198, P= 0.013). Positive correlation between semen Zinc-C and sperm motility (r =0.186 P=0.020). Zinc-T and sperm volume (r =0.387, p =0.000), sperm motility (r =0.162, p =0.043) and normal sperm morphology (r =0.152, p = 0.050).**
Conclusions: **Zinc assay should be considered as routine tests in management of male infertility especially where abnormalities are on motility, morphology, sperm concentration and semen volume. Secondly Ministry of Health Kenya, needs to pay special attention to micronutrient malnutrition especially Zinc among the adult males.**

INTRODUCTION

Male infertility is a significant problem in our society today given that it contributes up to 50% of total infertility problems. Sperm quality, which is characterized by seminal volume, viscosity, sperm count, morphology, motility, and viability has a major influence on male infertility. Numerous factors affect sperm quality including essential micronutrients like zinc. Studies have shown that Zinc is vital for proper physiology of spermatozoa, especially the prevention of spermatozoa degradation, stability of sperm membrane, a major contributor of antibacterial activity of the seminal plasma and antioxidants that reduce oxidation of spermatozoa by free radicals. In Kenya there is no official data showing the relationship between sperm quality versus Zinc concentration in blood serum and seminal plasma. This study aimed at evaluating the said relationship and the findings should add vital information in marriage counseling, diagnosis and management of male infertility in Kenya.

MATERIALS AND METHODS

This cross-sectional study was conducted between April 2016 and April 2018 at Kenyatta National Hospital in Nairobi, Kenya in which a total of 277 male adults aged 18-65 years who attended infertility clinic at the hospital were recruited, their individual consent sought, and a standard questioner filled for biodata.

Ethical approval was granted by Kenyatta National Hospital/University of Nairobi Ethical Review Committee.

Out of 277 participants recruited for the study, only 157 samples were fully analysed. About 2.5% (7) were unable to yield semen through masturbation, 5.4 % (15) declined to give blood samples citing fears of being tested for HIV despite having been assured that HIV test was not part of this study, 2.8% (8) produced insufficient semen volume (< 0.2ml), and 32.5 % (90) absconded for unknown reasons.

Semen analysis: The participants were instructed to abstain from sexual intercourse for 4 days after which they collected semen by masturbation into wide mouthed clean polystyrene containers and submitted the semen sample to the lab within 1 hour of collection. Each semen sample was analyzed for visual appearance (colour), liquefaction time (in minutes), volume (ml) motility (%), morphology (both wet preparation and papanicolou stained smears), leukocytes count, and spermatozoa count.

Zinc analysis in Semen: All plastic and glassware for Zinc analysis were made chemically clean by first soaking in 10% nitric acid for 12hours then thoroughly rinsed in distilled water.

The semen was digested using 3.7 M Trichloroacetic acid for 2 hours followed by centrifugation at 1000 rpm for 10min. The supernatant was diluted 150 times using distilled water. Zinc Concentration in micrograms per milliliter (Zn-C μg/ml) was analyzed using Flame Atomic Absorption Spectrometry (FABS) in the range of 0.5 – 1.5 ppm using commercial standards. Levels of Zinc per ejaculate or total zinc (Zn-T μg/ejaculate) was obtained by multiplying Zn-C with the respective semen volume.

Zinc analysis in Blood: The whole blood was coagulated at room temperature then centrifuged at 1000rpm for 5min to yield serum. The serum was digested using 3.7M Trichloroacetic acid for 2 hours, centrifuged to yield a supernatant which was diluted 150 times using distilled water and zinc analysis done using FABS in the range of 0.5 to 1.5 ppm using commercial standards.

Testosterone Analysis was done using Cobas Integra® 400 plus (Roche Diagnostics, Mannheim, Germany) using fresh plasma obtained from the whole blood.

Statistics: Data collected was analyzed in SPSS Version 23. Descriptive statistics for mean,

Standard error of mean, Standard deviation, kurtosis and Skewness. Test of normality was used to determine the type of inferential statistics to be performed. A p-value for the difference between Seminal Zinc and Blood Zinc sperm qualities was estimated using using Kruskal Wallis test where $p < 0.05$ was considered significantly different, while Mann Whitney U test and Bonferroni correction was used as post hoc tests for paired comparison of seminal Zinc and Blood Zinc respectively. Spearman's rank correlation coefficient was used to establish any correlation between the different sperm quality parameters and significant correlations reported at $p < 0.05$.

RESULTS

Sperm quality among the study participants.

The sperm quality parameters which were determined included semen volume, sperm concentration, sperm total count, motility and morphology.

Table 1

Descriptive statistics of Sperm quality parameters among study population

	Sperm quality parameters								
Descriptive statistics	Semen. Vol. (ml)	Sperm Conc. (10^6/ml)	Sperm count (10^6/ml)	Motility > 50% (%)	Rapid Movt. (%)	Slow Movt. (%)	Immotile (%)	Normal Morph (%)	Abnormal Morph. (%)
N	157	157	157	157	157	157	157	157	157
Mean	2.91	20.49	72.72	43.66	13.03	26.91	50.32	15.83	79.07
Std. Error of Mean	0.123	1.563	10.37	1.851	1.111	1.319	1.922	1.57	2.12
Median	2.00	15.00	40.00	50.00	10.00	30.00	50.00	11.00	88.00
Mode	2	20	0.00[a]	50	10	40	50	12	88
Std. Deviation	1.54	19.59	129.93	23.20	13.918	16.53	24.09	19.61	26.51
Variance	2.36	383.57	16881.53	538.10	193.72	273.17	580.18	384.55	702.71

a. Show presence of multiple mode. The smallest value is shown.

From the semen analysis results, 3 % (n=11) were Azoospermia, 52% (n=144) were teratozoospermia, 30% (n=82) were Oligozoospermia, 7 % (n=19) were Asthenozoospermia and8 %(n=21) were normozoospermia (Control group).

This categorization was done as per nomenclature of semen by World Health Organization criteria (WHO, 2010) (Table 1).

Distribution of Zinc levels among the sperm quality categories

The distribution of Zinc levels was determined among the sperm quality categories and their statistics summarized on table ii below.

Table 2

Statistics of Zinc levels in semen and blood among the sperm quality categories

		Sperm quality categories				
	Statistics	Normo	Astheno	Oligo	Terato	Azoo
Semen Zinc-C	N	21	19	82	144	11
	Mean	124.83	67.87	81.48	79.61	74.08
	SEM	8.53	16.50	6.82	5.04	23.37
	SD	39.09	71.90	61.77	60.53	18.91
	Skewness	-0.589	2,163	1.293	1.357	2.153
	Kortosis	0.882	4.328	1.904	1.877	5.073
	Mean rank	208.50	106.68	136.00	133.72	153.64
	Chi-square	20.008	20.008	20.008	20.008	20.008
	Df	4	4	4	4	4
	p =0.05	<0.01	<0.01	<0.01	<0.01	<0.01
Semen Zinc-T	N	21	19	82	144	11
	Mean	286.68	169.10	248.68	243.68	161.06
	SEM	37.90	37.62	26.80	19.70	55.15
	SD	173.68	164.00	242.68	236.31	182.91
	Skewness	1.663	0.49	1.419	1.705	1.293
	Kurtosis	2.675	1.014	1.322	3.381	0.145
	Mean rank	176.95	112.82	139.40	139.59	101.00
	Chi-square	9.229	9.229	9.229	9.229	9.229
	Df	4	4	4	4	4
	p =0.05	0.56	0.56	0.56	0.56	0.56
Blood Zinc	N	21	22	98	143	11
	Mean	1.48	0.91	1.01	0.99	0.91
	SEM	0.13	0.15	0.08	0.07	0.64
	SD	0.60	0.68	0.83	0.77	2.12
	Skewness	-0.66	0.11	2.18	1.86	-2.76
	Kurtosis	-0.39	-0.65	12.13	11.05	8.50
	Mean rank	190.68	142.36	142.97	135.77	200.31
	Chi-square	14.89	14.89	14.89	14.89	14.89
	Df	4	4	4	4	4
	p =0.05	0.005	0.005	0.005	0.005	0.005

Zinc-C (µg/ml) in seminal plasma among the sperm quality categories

The results showed a decreasing trend of the mean of Zinc-C among the Normozoospermia (control group) at 124.83 ± 8.53 µg/ml, followed by Oligozoospermia at 81.48 ± 6.82 µg/ml, Terato- zoospermia at 79.61 ± 5.04 µg/ml, Azoospermia at 74.80 ± 23.38 µg/ml and Asthenozoospermia at 67.87 ± 16.50 µg/ml. Determination of differences among the Zinc C levels tested using Kruskal-Wallis test revealed significant variation among the sperm quality categories (x^2 = 20.008; df= 4; p =0.000) (Table 2).

A Further paired post hoc analysis by Mann Whitney U test (*p = 0.05)* with benferroni adjustment at *p* = 0.005 showed significant statistical difference between the mean of Semen Zinc-C of control (normozoospermia) and Teratozoospermia (*p* = <0.001), normazoospermia and Oligozoospermia (*p* = <0.001), Normozoospermia and Asthenozoospermia (*p* = <0.001). However there was no significant (p > 0.05) statistical difference among the mean of the other 7 pairs, which were Normo vs Azoo (*p* = 0.238), Terato vs Oligo (*p* = 0.83), Terato vs Astheno (*p* = 0.154), Terato vs Azoo (*p* = 0.49), Oligo vs

Astheno (p = 0.148), Oligo vs Azoo(p = 0.483), Astheno vs Azoo (p = 0.149) respectively (Table 3).

Table 3

Mann Whitney U test and Bonferroni correction for Semen Zinc-C

	Groups	**N**	**Mean Rank**	**Sum Rank**	**Mann whitney**	**Z**	**Sig. *P*=0.05**	**Bonferroni *p*=0.005**
1	Normo vs Terato	21 144	122.86 77.19	2580.0 11115.0	675.0	-4.00	<0.001	<0.001
2	Normo vs Oligo	21 82	73.60 46.47	1545.0 3810.0	407.5	-3.72	<0.001	<0.001
3	Normo vs Astheno	21 19	27.10 13.21	569.00 251.00	61.00	-3.75	<0.001	<0.001
4	Normo vs Azoo	21 11	17.95 13.73	377.00 151.00	85.00	-1.21	.238	0.238
5	Terato vs Oligo	144 82	112.81 114.72	16244 9407	5804	-0.21	.832	0.832
6	Terato vs Astheno	144 19	83.91 67.50	12083.50 1282.50	1092.50	-1.43	0.154	0.154
7	Tearato vs Azoo	144 11	77.31 87.00	957.00 11133.00	693.00	-0.69	0.49	0.490
8	Oligo vs Astheno	82 19	53.03 42.24	4348.50 802.50	612.50	-1.45	0.148	0.148
9	Oligo vs Azoo	82 11	46.26 52.36	3795.00 576.00	392.00	-0.70	0.483	0.483
10	Astheno vs Azoo	19 11	13.74 18.55	261 204	71	-1.44	0.149	0.149

Zinc-T (µg/ejaculate) in seminal plasma among the sperm quality categories

Zinc-T was obtained by multiplying Zinc-C levels and the semen volume of each sample. Normozoospermia (control group) had highest Zinc-T mean at 286.68 ± 39.70 µg/ejaculate, followed by Oligozoospermia at 248.68 ± 26.80 µg/ejaculate, Teratozoospermia at 243.68 ± 19.70 µg/ejaculate, Asthenozoospermia at 169.10 ± 37.62 µg/ejaculate and Azoospermia at 161.06 ± 55.15 µg/ejaculate (Table 2).

Kruskal-Wallis test was used to compare the mean of Semen Zinc-T and it showed no significant statistical differences among the means of different sperm qualities (x^2 = 9,229; df= 4; p =0.56) (Table 2).

Blood zinc distribution among sperm quality categories

The sperm quality categories had blood zinc levels within the WHO reference range of 0.66 to 1.10 µg/ml. However the Control group (Normo.) had the highest blood zinc at 1.48+0.13 µg/ml followed by Oligo (1.01+0.08 µg/ml), Terato (0.99+0.07 µg/ml), Astheno 0.91+0.50 and Azoo had 0.91+0.064 µg/ml.(Table 2).

The mean of the blood Zinc levels among the sperm categories were compared using

Kruskal-Wallis test and showed that there was a significant difference among them ($x^2 = 20.008$; $df = 4$; $p = 0.005$) (Table II).

Post hoc test on the data was done using Mann Whitney U test and showed that there was significant statistical difference between the mean of Control (Normo.) versus Terato ($p = 0.001$), Oligo ($p = 0.002$) and Astheno ($p = 0.008$). There was also significant difference between Terato and Azoo ($p = 0.044$). However there was no significant difference between the mean of blood Zinc for the remaining 6 combinations of Normo versus Azoo,Terato versus Oligo,Terato versus Astheno, Oligo versus Astheno, Oligo versus Azoo and Astheno versus Azoo (Table 4).

Upon performing Bonferroni correction (p =0.05 ÷10= corrected $p = 0.005$) there was only significant difference between the mean of Control (Normo.) versus Terato ($p = 0.001$), Oligo ($p = 0.002$) and Astheno ($p = 0.008$). (Table 4).

Table 4

Mann Whitney U test and Bonferroni correction for level of Zinc in blood

	Groups	**N**	**Mean Rank**	**Sum Rank**	**Mann whitney U**	**Z**	***p*=0.05**	**Bonfferoni *p*=0.005**
1	Normo vs Terato	21 144	110.81 78.34	2327 11203	907.00	-3.255	0.001	0.001
2	Normo vs Oligo	21 82	79.02 55.92	1659 5480	629.5	-3.075	0.002	0.002
3	Normo vs Astheno	21 19	26.76 17.45	562 384	131.00	-2.655	0.008	0.008
4	Normo vs Azoo	21 11	16.71 16.09	351 177	111.00	-0.198	0.876	0.876
5	Terato vs Oligo	144 82	120.87 121.27	17276.50 11884.50	6980.50	-0.056	0.955	0.955
6	Terato vs Astheno	144 19	83.50 79.73	11941.00 1754.00	1501.00	-0.387	0.699	0.699
7	Terato vs Azoo	144 11	75.70 100.86	10825.00 1109.50	529.50	-2.015	0.044	0.044
8	Oligo vs Astheno	82 19	61.04 58.09	5982.00 1278.00	1025.00	-0.401	0.689	0.689
9	Oligo vs Azoo	82 11	53.23 70.73	5217.00 778.00	366.00	-1.930	0.054	0.054
10	Astheno vs Azoo	19 11	15.00 21.00	330.00 231.00	77.00	-1.833	0.097	0.097

Blood testosterone levels and the relationship with the sperm quality

The levels of testosterone was determined among the sperm quality categories and summarized in table v below.

Table 5

Statistics for blood testosterone among sperm quality categories

Sperm Quality Categories					
Statistics	Azoo	Terato	Oligo	Astheno	Normo (Control)
N	11	143	82	19	21
Mean(μg/ml)	3.22	4.64	4.39	5.41	5.508
Std. Error of Mean	0.095	0.138	0.187	0.409	0.175
Median	3.10	4.40	4.10	5.90	5.50
Std. Deviation	0.316	1.66	1.70	1.79	0.801
Variance	0.100	2.75	2.88	3.19	0.62
Skewness	1.314	0.796	1.169	-0.255	0.154
Kurtosis	0.995	0.581	1.69	-1.44	-0.861
Range	1.00	9.55	9.54	5.51	2.80.
Mean Rank	54.91	140.17	125.09	173.61	198.02
Chi-Square	29.608	29.608	29.608	29.608	29.608
Df	4	4	4	4	4
p=0.05	<0.01	<0.01	<0.01	<0.01	<0.01

The pathozoospermia (AStheno, Oligo, Terato, and Azoospermia) had lower blood testosterone levels than the normozoospermia (Control group) even though all the Sperm quality categories have testosterone levels within WHO normal reference value
(3 -10.6ng/ml) (Table 5).

From this data, skewness and kurtosis shows that all the data were nonparametric hence Kruskal Wallis test was used to test the statistical significance of the mean. The differences among the mean were found to be statistically significant using Kruskal Wallis test where p-values of $p < 0.05$ were considered statistically significant ($x^2 = 20.08$; df=4; $p = 0.00$) (Table 5).

The mean of Testosterone among the sperm quality categories was subjected to Mann Whitney U test and showed that there were significant statistical difference among all the combinations except between Terato versus Oligo ($p = 0.160$) and Normo versus Astheno ($p = 0.789$) (Table 7).

Table 6

Mann Whitney U test and Bonferroni correction for blood Testosterone

	Groups	N	Mean Rank	Sum rank	Mann whitnney	Z	Significance *p*=0.05	Bonferroni *p*=0.005
1	Normo vs Terato	21 144	113.95 78.49	2393.00 11302.00	862.00	-3.180	0.001	0.001
2	Normo vs Oligo	21 82	75.07 46.09	1576.50 3779.50	376.50	-3.969	<0.001	<0.001
3	Normo vs Astheno	21 19	20.00 21.05	420.00 400.00	189.00	-0.285	0.789	0.789

4	Normo vs Azoo	21 11	22.00 6.00	462.00 66.00	0.00	-4.590	<0.001	<0.001
5	Terato vs Oligo	144 82	118.11 105.40	17008.00 8643.00	5240.00	-1.406	0.160	0.160
6	Terato vs Astheno	144 19	79.68 99.55	11474.50 1891.50	1034.50	-1.726	0.008	0.008
7	Terato vs Azoo	144 11	81.39 35.59	11720.50 369.50	32.50	-3.406	0.001	0.001
8	Oligo vs Astheno	82 19	48.07 63.66	3941.50 1209.50	538.50	-2.092	0.036	0.036
9	Oligo vs Azoo	82 11	50.02 24.45	4102.00 269.00	203.00	-2.953	0.003	0.003
10	Astheno vs Azoo	19 11	19.34 8.86	367.50 97.50	31.50	-3.147	0.001	0.001

Relationship between Zinc levels and sperm quality parameters

Table 7

Spearman's coefficient of correlations for relationships between sperm quality parameters and Zinc levels in blood, Semen and testosterone

	Semen Zinc –C (µg/ml)		**Blood serum Blood Zinc (µg/ml)**		**Blood Testosterone (ng/ml)**	
Parameter	r-value	p-value	r-value	p-value	r-value	p-value
Normal morphology%	0.198	0.013	-0.063	0.435	0.168	0.036
Abnormal morphology%	-0.185	0.021	0.047	0.558	-0.980	0.081
Total count (millions/ejaculate)	-0.083	0.303	-0.098	0.222	0.145	0.078
Count concentration (millions/ml)	-0.083	0.301	0.168	0.035	0.231	0.004
Motility ≥50%	0.186	0.020	-0.067	0.403	0.135	0.093
Rapid movement%	0.145	0.071	-0.111	0.166	0.209	0.008
Slow movement%	0.108	0.179	-0.125	0.118	0.109	0.197
Immotile %	-0.146	0.068	0.088	0.275	0.110	0.172
Sperm volume (ml)	0.028	0.730	0.061	0.445	0.057	0.475

The relationship between Zinc –C (μg/ml) and the sperm quality parameters

There was a positive correlation between Zinc- C and normal sperm morphology (r = 0.198, *p* = 0 .013) motility (r = 0 .186, *p* = 0 .020) and negative correlation to abnormal sperm morphology (r = -0.185, *p*= .021).

The relationship between Blood Zinc (μg/ml) and the sperm quality parameters

The Blood Serum Zinc levels and sperm concentration showed positive correlation between them (r = 0.168, *p* = 0.035).

Blood testosterone levels and the relationship with the sperm quality among the study population

Positive correlation was observed between Blood testosterone levels and sperm rapid movement (r = 0.209, *p* = 0.008), normal morphology of sperms (r =0.168 *p*= 0.036), sperm concentration (r =0.231, *p* = 0 .004).

DISCUSSION

The average seminal plasma Zinc Concentration (Zn-C) of 124.83 μg/ml in this study, is comparable to various studies from other parts of the world, for example a study by Kruse et al, (123 μg/ml). [1]

The levels of both Zn-C and Zn-T were lower for pathozoospermia (Astheno-, Oligo- , Terato- and Azoospermia) compared to the control (Normozoospermia).This is consistent with other studies which indicated that pathozoospermia is associated with low-seminal plasma levels.[2], low Zinc in Oligozoospermia, oligoastheno-zoospermia and Azoospermia.[3] .The low Seminal Zinc level in pathozoospermia can be associated to various factors like chronic inflammation of the prostate.[1], accumulation of heavy metals like lead and cadmium in the testicular tissues[4,5] and micronutrient malnutrition among others. The low seminal Zinc level may affect the sperm quality by reducing the antioxidant capacity, increased buildup of heavy metals in the testicular tissues, reduced active progressive motility and acrosome reaction of sperms which is a very significant element in male fertility [4,5].

This study showed that levels of zinc in both blood and semen have positive correlation with sperm motility, normal morphology, count (concentration), and volume. Blood Zinc levels and sperm concentration (r = 0.168, p = 0.035). Zinc-C and normal sperm morphology (r = 0.198, P = 0 .013), sperm motility (r = 0.186 P = 0.020) and negative correlation to abnormal sperm morphology (r = -0.185, P = -0 .021). There was equally a positive correlation between Zinc-T and sperm volume (r = 0.387, p = 0.000), sperm motility (r = 0.162, p = 0.043) and normal sperm morphology (r = 0.152, p = 0.050).

A number of studies elsewhere in the world concur with these findings, for example in Nigeria a study carried out at Calabar University Hospital found that there was a positive correlation between Zinc and percentage motility in oligospermic group [6]. Studies in Srilanka and Pakistan concluded that the sperm characteristics that were sensitive to seminal plasma Zinc include motility and viability [2.3].

Zinc has an influence on the formation of disulfide bridges that result in the stiffening of the outer dense fiber during maturation of sperms in the epididymis which is an essential physiological process that affects sperm motility [7]. The enzyme estrogen synthetase (aromatase) found in a number of body tissues catalyses the hydroxylation of testosterone to form estrogen which can lower the levels of blood testosterone. Zinc inhibits this enzyme estrogen synthetase (aromatase) hence helps in ensuring optimal level of testosterone is maintained and by extension optimal spermatogenesis [8].

However, a few studies contrast these findings by indicating that there is no strong correlation between levels of zinc in semen and sperm quality parameters, and that infertile males had normal levels of Zinc in the seminal plasma [9,10].

CONCLUSION

Zinc assay on semen and blood should be strongly considered as routine tests in management of male infertility especially where abnormalities are on motility and morphology. Secondly Kenya as a country needs to pay special attention to micronutrient malnutrition especially micronutrient Zinc among the adult males. Finally, there should be a larger study on this subject in Kenya to help authenticate these findings, help set up a national reference value on Zinc levels among adult males that will in turn guide similar studies.

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