Assessment of Zinc and Copper level in Seminal plasma of Infertile Male

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Abstract: Background: Human semen contains high concentrations of zinc (Zn) and copper (Cu). The presence of abnormal levels of these trace elements may affect sperm production, maturation, motility and fertilizing ability. **Objective:** Evaluate seminal plasma levels of zinc and copper and to correlate their concentrations with various semen parameters among fertile and infertile male subjects. **Subjects and Methods:** This is a case control study done on 50 infertile men who were attended to Damietta University Hospital seeking for infertility medical advice, during the period from February to September 2016. Another 50 fertile males matched for ages were included in the study as a control group. An informed consent was taken from all subjects participating in this study. All subjects included in this study were subjected to: Full history taking, Clinical examination and Laboratory investigation in the form of (Semen analysis by conventional method and Computer Assisted Semen Analysis (CASA), Estimation of zinc and copper level in seminal plasma by spectrophotometric method. **Results**: There were high statistical significant differences between infertile and control groups as regard to normal morphology of sperms % ($p<0.001$), less statistical significant differences as regard to total motility(PR+NP) % ($p=0.015$) and no statistical significant differences as regard to volume of seminal fluid (ml) nor count of sperms (million/ml). The control group has higher level of seminal Zinc than infertile group with statistical significant differences between them (P value < 0.048). The control group has lower level of seminal Copper than infertile group with statistical significant differences between them (P value < 0.026). The control group has higher level of seminal Zn/Cu Ratio than infertile group with high statistical significant differences between them (P value < 0.001). **Conclusions**: Adequate seminal plasma concentration of Zn and Cu are required for normal sperm function and that high toxic concentration of these elements in seminal plasma is apparently related to defective morphology and /or motility in infertile males.
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1. Introduction

Infertility is defined as no conception after at least 12 months of unprotected intercourse. Infertility affects approximately 15% of couples trying to conceive, and a male factor contributes to roughly half of these cases (**Agarwal et al., 2014**). Infertility is complex and has multiple causes and consequences depending on the gender, sexual history, life style and cultural background (**Gurunath et al., 2011**). This problem has been worsening due to the deterioration of the human semen quality by as much as 3% per year suggesting that male reproductive problems may be increasing over time (**Abed and Jarad, 2014**). Seminal plasma is very important for sperm metabolism, function, survival, and transport in the female genital tract. Cations such as Na, K, Ca, and Mg establish osmotic balance, while essential trace elements are components of many important enzymes in the seminal plasma (**Arrier Battut et al., 2002**). The possible influence of the trace elements especially Cu and Zn on male infertility is matter of great interest (**Gurunath et al., 2011**). Increasing evidence of a direct relationship of zinc was found with seminal parameters. Levels of metal ions in human semen appear to be significantly correlated with male infertility suggesting that trace elements (Zn and Cu) in human seminal plasma are important factors in male reproductive function (**Altaher and Abdrabo, 2015**). The Zn which is second only to iron as the most abundant element in the body is found in chicken, nuts, meat, fish, milk, and legumes. Despite this, the World Health Organization (WHO) estimate that one-third of world population is deficient in Zn. Zn is critical to reproduction potential. The Zn content of semen is 87 times that in the blood and has been reported to protect sperm from bacteria and chromosomes damage (**Judith, 2008**). Zinc content in seminal plasma is predominantly secreted by the prostate gland and may reflect prostatic function (**Altaher and Abdrabo, 2015**). Zn plays an important role not only in normal testicular development, but also in spermatogenesis and sperm motility. Deficiency of Zn is associated with hypogonadism and insufficient development of secondary sex characteristics (**Khan et al., 2011**). It is a cofactor for a number of metalloenzymes in human semen,

(including many so-called zinc-finger proteins) involved in DNA transcription and protein synthesis. Deficiency of zinc in the reproductive system causes hypogonadism and gonadal hypofunction (**Amidu et al., 2012**). Copper is an important element for numerous metallo enzymes and metalloproteins that are involved in energy metabolism. Copper works in different ways in order to maintain normal environment for spermatozoa for normal fertilization to occur. However, a higher level is toxic to a variety of cells, including human spermatozoa (**Altaher and Abdrabo, 2015**).

The aim of the study was to evaluate seminal plasma levels of zinc and copper and to correlate their concentrations with various semen parameters among fertile and infertile male subjects.

2. Subjects and Methods

This is a case control study done on 50 infertile men who were attended to Damietta University Hospital seeking for infertility medical advice, during the period from February to September 2016. Another 50 fertile males matched for ages were included in the study as a control group.

An informed consent was taken from all subjects participating in this study.

Inclusion criteria:

Include infertile males with no conception for at least one year with unprotected intercourse, and their semen analysis presented by abnormalities in the form of oligozoospermia, asthenozoospermia, oligoasthenozoospermia, oligoastheno teratozoospermia.

Exclusion criteria:

Patients with one or more of the following criteria were excluded from the study: 1) History of chronic diseases e.g. hepatic, renal, etc. 2) Patients with testicular varicocele, genital infection, leukocytospermia. 3) Patients on treatment with drugs which affect level of Zn and Cu. 4) History of protected sexual life. 5) Patients having history of similar condition in their families.

All subjects participating in this study were analyzed for: 1) Full history taking including personal history, complaint, present and past history. 2) Clinical examination including general and local examination. 3) Laboratory investigations including: a) Semen analysis by conventional method and computer assisted semen analysis (CASA). b) Estimation of zinc and copper level in seminal plasma by spectrophotometric method.

Statistical presentation and analysis of the present study was conducted using the mean, standard deviation, student t- test, linear correlation coefficient tests by SPSS V17 (statistical program for social science). Description of quantitative variables by mean, SD, rang and description of qualitative variables by number and percentage. A value of $P \leq 0.05$ was considered significant.

3. Results

Comparison between infertile and control groups as regard to age, seminal fluid examination parameters in the form of (volume of seminal fluid, count, % of total motility and % of normal morphology of sperms), seminal Zn level, seminal Cu level and Zn/Cu ratio were done.

Table (1): Comparison between infertile and control groups as regard to age, seminal fluid examination parameters, seminal Zn, seminal Cu and seminal Zn/Cu ratio.

Parameters		Groups						P-value	Sig.
		Infertile group			Control group				
Age (Years)	Mean ±SD	33.4	±	4.3	30.76	±	4.7	0.354	N.S.
Volume of seminal fluid (ml)	Mean ±SD	2.98	±	1.25	3.35	±	0.842	0.106	N.S.
Count of sperms (million/ml)	Mean ±SD	19.12	±	18.08	49.7	±	19.35	0.337	N.S.
Total motility (PR+NP) %	Mean ±SD	23.04	±	12.6	76.01	±	8.39	0.015*	↓ H.S.
Normal Morphology of sperms %	Mean ±SD	10.26	±	7.87	30.89	±	11.88	<0.001*	↓ V.H.S
Seminal Zinc (mg/dl)	Mean ±SD	2.03	±	0.222	3.95	±	0.3	0.048*	↓ S
Seminal Copper (mg/dl)	Mean ±SD	0.66	±	0.134	0.45	±	0.086	0.026*	↑ S
Seminal Zn/Cu ratio	Mean ±SD	3.36	±	0.985	8.74	±	2.17	<0.001*	↓ V.H.S

*P-value<0.05 is significant

The results of present study showed no statistical significant differences between infertile and control groups as regard to age, volume of seminal fluid (ml) and count of sperms (million/ml). There was high statistical significant decrease between infertile and control groups as regard to normal morphology of sperms % ($p<0.001$), less statistical significant decrease as regard to total motility (PR+NP) % ($p=0.015$).

Also there was statistical significant decrease between infertile and control groups as regard to seminal Zn level ($p=0.048$). Seminal plasma Cu level was statistical significant decrease between infertile and control groups ($p=0.026$).

High statistical significant decrease in Zn/Cu ratio also observed in study group when compared

with control (p<0.001) (Table 1).

There was a significant negative correlation between Zn/Cu ratio and count of sperms r=-0.326 p=0.021. Also there was a highly significant negative correlation between Zn/Cu ratio and normal morphology of sperms r=-0.369 p=0.008 among study group.

Table (2): Correlation between seminal Zn/Cu ratio and other parameters.

	Seminal Zn/Cu ratio	
	r	P-value
Age (Years)	-0.056	0.697
Volume of seminal fluid(ml)	0.155	0.282
Count of sperms (million/ml)	-0.326	0.021*
Total motility (PR+NP)	0.106	0.463
Normal Morphology of sperms	-0.369	0.008**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

Infertility is a major clinical problem, affecting people medically and psychologically. Approximately 15% of couples trying to conceive are infertile, in that about 30% cases are due to males only and in another 20% cases both partners have detectable abnormalities. Thus male factor plays an important role in 50% of infertile couples. Causes of infertility are anatomic defects, endocrinopathies, immunologic problems, gene mutation, radiation, chemotherapy, ejaculatory failures and environmental exposures (**Badade et al., 2012**).

Our study was done on 50 infertile men who were attended to Damietta University Hospital seeking for infertility medical advice; during a period from February to September 2016 with their age were 33.4±4.3 years.

Another 50 healthy fertile males matched for ages were included in the study as a control group.

All subjects included in this study were subjected to detailed history taking, full clinical examination, and laboratory investigations in the form of semen analysis by conventional method and Computer Assisted Semen Analysis (CASA), in addition to determination of levels of Zn and Cu in seminal plasma by spectrophotometric method**.**

In our study there was no statistical significant differences between infertile and control groups as regard to age, volume and spermatic count.

The result of seminal plasma Zn of the current study was not in accordance with what was reported by **Akinloye O et al. 2011** who observed inverse correlation between seminal Zn and sperm count.

Also there was highly statistical significant decrease between infertile and control groups as regard to % of total motility (P value 0.015). Also there was a very high statistical significant decrease between infertile and control groups as regard to % of normal morphology of sperms (P value <0.001). This finding is in agreement with what reported by **Amiri et al., 2006** who found that the mean of sperm motility of fertile males were significantly higher than that of infertile males.

This result reported that there were significant lower levels of seminal plasma Zinc in infertile males compared to fertile male control (P value 0.048). This finding is in agreement with what reported by **Altaher et al**. **2015**, **Hasan et al. 2007** and **Ali et al. 2005** they found significant lower levels of seminal plasma zinc in oligozoospermic and azoospermic infertile males compared to fertile male control. **Hasan et al. 2007** reported that Zn concentration in seminal plasma should be considered as one of the factors responsible for decreased testicular function in infertile male subjects. In contrast, **Fuse et al. 1999** found no significant difference in the mean value of seminal plasma Zn between infertile individuals compared to fertile individuals.

Omu and associates, 1998 have demonstrated that Zn therapy results in significant improvement in sperm quality with increases in sperm density, progressive motility, and improved conception and pregnancy outcome.

Alsalman AS et al. 2013 reported that zinc plays avital role in the physiology of spermatozoa and spermatogenesis and an essential nutritional component. A clinical study demonstrated that adult males experimentally deprived of zinc showed a disturbance of testosterone synthesis in the Leydig cell.

Zn is present at high concentrations in the 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 play a multifaceted role in sperm functional properties **(Mohammad et al. 2009).**

On the other hand our study showed that the infertile group has higher level of seminal Cu than control group with statistical differences between them (P value 0.026). This finding was comparable with the findings of **Saleh et al. 2008. T**hey showed that Cu concentration was insignificantly increased in oligoasthenozoospermic and in teratozoospermic males than in fertile male controls.

This result is incompatible with that observed by **Wong et al, 2001.**

Copper is an essential trace element that plays an important role in several enzymes such as superoxide dismutase. Human spermatozoa are particularly susceptible to peroxidative damage because they contain high concentrations of polyunsaturated fatty acids and also possess a significant ability to generate

a reactive oxygen species (ROS), mainly superoxide anion and hydrogen peroxide. Superoxide dismutase (Cu-metalloenzyme) protects human spermatozoa from this peroxidative damage **(khan et al 2001).**

Our results indicate that the diagnostic and prognostic capabilities of the seminal Zn and Cu levels are beyond those of conventional tests of sperm quality and function. It is strongly recommended that incorporating such tests into the routine Andrology workup as it an important step for the future of the male infertility practice.

Conclusions

This study suggests that seminal zinc and copper may contribute to fertility through their effects on various semen parameters. Adequate seminal plasma concentration of Zn and Cu are required for normal sperm function and that high toxic concentration of these elements in seminal plasma is apparently related to defective morphology and /or motility in infertile males. So, it seems that the estimation of seminal plasma Zn and Cu may help in investigation and treatment of infertile males.

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