

Evaluation of testosterone hormone and zinc Levels among infertile males in Kirkuk province/ Iraq

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Abstract

Background and objectives: Male Infertility is often caused by problems with sperm production or motility. Zinc in human semen seems to play an important role in the physiology of spermatozoa. This study was designed to demonstrate the relationships between concentrations of zinc and testosterone in serum and seminal plasma and sperm quality among infertile men.

Methods: One hundred four infertile males, aged (19-44) years, were selected from Infertile Clinic-Azadi Teaching Hospital- Kirkuk Province. Forty known fertile males were selected as normospermic control group. Semen samples were analyzed according to WHO criteria. Serum and seminal plasma zinc concentrations were estimated by atomic absorption technique. Serum testosterone was measured by MiniVIDAS apparatus.

Results: The mean value of serum testosterone was significantly lower in infertile males (4.87 ± 0.15 ng/ml) as compared to control group (6.41 ± 0.16 ng/ml); ($P < 0.01$), significant correlations were observed between serum testosterone with seminal plasma zinc level in oligospermic subjects ($r=0.44$) and with serum zinc level in azospermic subjects ($r=0.37$), ($P < 0.01$); ($P < 0.05$) respectively. Serum and seminal plasma zinc levels were lower in infertile men (7.75 ± 0.18 $\mu\text{mol/L}$); (0.83 ± 0.02 mmol/L) when compared with normospermic control group (14.09 ± 0.27 $\mu\text{mol/L}$); (1.41 ± 0.01 mmol/L) respectively ($p < 0.01$).

Conclusion: Zinc may contribute to fertility through its positive effect on spermatogenesis. Also there was significant decrease in serum and seminal plasma zinc levels in oligospermic and azospermic infertile males with significantly low androgen. It indicates that the zinc may have a role for steroidogenesis.

Key words: Male infertility, testosterone, serum and seminal plasma zinc

Introduction

Infertility is a global reproductive health issue and its social and psychological consequences simply cannot be ignored¹. It is important to note that infertility can occur just as equally in men as in women, with 30 % of infertility attributable to men and 30 % to women, while another 30 % is attributed to both partners and the remaining 10 % is related to unknown factors^{2,3}. One supplement that should be considered absolutely essential for a healthy sperm is zinc. This busy mineral is involved in almost every

aspect of male reproduction, including sperm formation and motility⁴. Zinc is the second most abundant trace element in the human body, totaling nearly 2 g. Found in more than 300 enzymes, zinc is a cofactor for multiple biologic processes including DNA, RNA, and protein synthesis⁵.

Methods

1-Subjects: One hundred four infertile males, without any treatment, who had regular unprotected intercourse for at least one year without conception with their

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partners, aged (19-44) years, were selected from Infertile Clinic-Azadi Teaching Hospital- Kirkuk Province from October (2008) to September (2009). Microscopical examination of the seminal fluid showed isolated or combined abnormalities of the sperms in regard to their number, motility and their morphology. Thirty eight patients were oligospermic, 39 patients, asthenospermic, 16 patients azoospermic and 11 patients were oligoasthenoteratospermia (OTA). Forty known fertile males were selected as normospermic control group (their wives had given birth to a sample child within one year).

2-Sampling: Semen samples were obtained by masturbation into (50) ml sterile polystyrene jars after an abstinence period of (3-4) days, in a room near the laboratory. The samples were placed in the incubator at 37 °C to allow the semen liquefaction⁶, because of the fluctuation of semen parameters, each patient had at least two seminal fluid analyses, at least one month period in between. Semen samples analyzed according to WHO blood criteria⁷.

3-Blood sampling for zinc and testosterone analysis: A 5 ml of blood samples were taken from each subject in the morning, transferred to labeled test tube, allowed to clot, then centrifuged at (2500) rpm for 8 minutes in Hitachi centrifuge at room temp. one and half ml of the serum was kept frozen at (-20) °C until later used for zinc assay. Serum concentration of testosterone hormone was measured directly without freezing using the ELFA technique (Enzyme Analysis Fluorescent Assay), MiniVIDAS apparatus.

4-Analysis of zinc in serum and seminal plasma: The seminal plasma was collected after centrifugation at (3000) rpm for (15) minutes. Supernatant was transferred in labeled test tube and stored at (-20) °C until later used for zinc assay. Serum and seminal plasma were diluted (2), and (100) times with double distilled water respectively. Estimation of zinc in diluted serum and seminal plasma was carried out

at (213.9) nm (wave length) using fast sequential atomic absorption spectroscopy (Varian, USA 2005).

Results

The mean value of serum testosterone in infertile patients (4.87 ± 0.15 ng/ml) was found to be significantly lower than the mean value of control group (6.41 ± 0.16 ng/ml); ($P < 0.01$). Mean serum testosterone levels among different subject groups were given in Figure 1. The mean value of testosterone was significantly low in azoospermic (3.65 ± 0.31 ng/ml), oligospermic (4.43 ± 0.17 ng/ml) and OTA- syndrome (4.16 ± 0.23 ng/ml) as compared to normospermic control group (6.41 ± 0.16 ng/ml); ($P < 0.01$). No significant difference was found between the mean value of serum testosterone in asthenospermic subject group (6.0 ± 0.26 ng/ml) and the mean value of control group. The mean value of serum zinc was (7.75 ± 0.18 $\mu\text{mol/L}$) in infertile patients, this value is significantly less than that obtained in control group (14.09 ± 0.27 $\mu\text{mol/L}$); ($P < 0.01$). The mean value of zinc in seminal plasma for infertile men was (0.83 ± 0.02 mmol/L), this value also found to be significantly less than that obtained in control group (1.41 ± 0.01 mmol/L); ($P < 0.01$). Mean zinc levels in serum and seminal plasma among different subject groups in the study population were given in (Table 1). Mean zinc levels in both serum and seminal plasma were lower in azoospermic than in other subject groups, males with normal sperm counts had higher levels than in oligospermic. A similar trend was observed when zinc levels were compared between asthenospermic group and those having normal motility. All these changes were statistically significant ($P < 0.01$). Correlation between zinc levels in serum and seminal plasma with sperm count, motility, morphology and volume was given in (Table 2). Positive correlations were observed between zinc levels in serum with sperm count ($r=0.66$); ($P < 0.01$), and motility ($r=0.39$); ($P < 0.05$).

However, the positive correlation between zinc in serum with sperm morphology ($r=0.09$) were statistically not significant. No significant negative correlation was found with semen volume. Positive correlations were observed between the seminal plasma zinc levels with sperm count ($r=0.56$); ($P < 0.01$), and motility ($r=0.34$); ($P < 0.05$). However, the positive correlation between zinc in seminal plasma with sperm morphology ($r=0.01$) was statistically not significant. No significant negative correlation was found with semen volume.

The correlation between serum testosterone with seminal plasma zinc level in oligospermic subjects was ($r=0.44$) and with serum zinc in azospermic subjects was ($r=0.37$). These correlations are statistically significant ($P < 0.01$, $p < 0.05$) respectively.

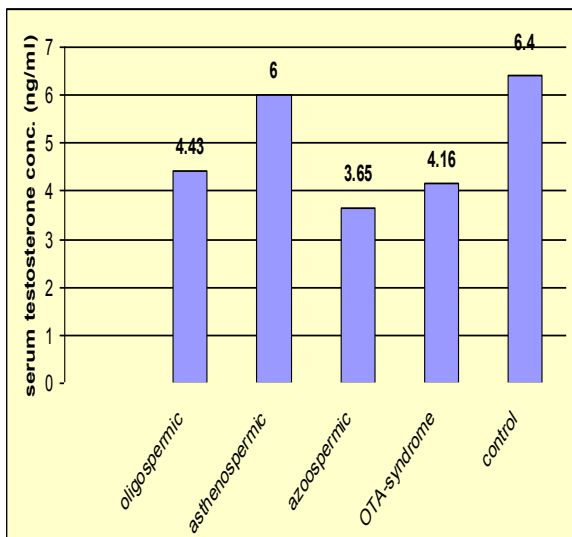


Figure 1: Mean serum testosterone concentration among different subject groups in the study population.

Table 1: Mean \pm SEM of zinc levels in serum and seminal plasma among different subject groups in the study population.

Semen category	Serum zinc conc. $\mu\text{mol/L}$ (Mean \pm SEM)	Seminal plasma zinc conc. mmol/L (Mean \pm SEM)
Oligospermia	7.69 \pm 0.28	0.87 \pm 0.03
Asthenospermia	8.03 \pm 0.33	0.83 \pm 0.03
Azoospermia	7.29 \pm 0.43	0.76 \pm 0.43
OTA-syndrome	7.61 \pm 0.4	0.79 \pm 0.44
Control group	14.09 \pm 0.27	1.41 \pm 0.01

Table 2: Correlation coefficient (r) of serum and seminal plasma zinc values with semen parameters of infertile subjects.

Parameters	Correlation coefficient of serum	Correlation Coefficient of seminal plasma
Count	0.66	0.56
Motility	0.39	0.34
Morphology	0.09	0.01
Volume	- 0.12	- 0.1

Discussion

Male fertility depends upon an intact hypothalamo pituitary- testicular axis to initiate and maintain quantitatively and qualitatively normal spermatogenesis, maintain normal secondary sex glands and sexual functions⁸. Thus, it is surprising how infrequent infertile males have a recognizable endocrinopathy, even though up to 20% of male infertility can be attributable to endocrinopathy. In fact, endocrine disorders which may be associated with significant medical pathology remain an important factor to consider in the etiology of male infertility because they can be amenable to

treatment⁹. In clinical practice hormonal evaluation is usually done in patients with severe abnormality in their sperm count (azoospermia and severe oligospermia (sperm count < 5 million/ml)) because endocrinopathy is rare in patients with sperm count above 5 million/ml^{9, 10}. Out of the (104) patients evaluated, 10.57% were found to have azoospermia and 14.42% were found to have severe oligospermia, which was similar to the results obtained by Yeboah *et al* 1992. In our study testosterone evaluation is done for all subjects in the study population, the mean serum testosterone levels of infertile men (4.87±0.15 ng/ml) which was significantly lower as compared to fertile men (6.41±0.16 ng/ml); (P< 0.01). In various studies similar observations were observed^{12, 13}. In the present study there were significantly low serum testosterone in azoospermic (3.65±0.31 ng/ml) and oligospermic groups (4.43±0.17 ng/ml) when compared to normospermic (6.41±0.16) control group (p<0.01). Our results are in agreement with other studies conducted by Ali H. *et al* 2005 and Mubashir *et al* 2005. Several trace elements have been shown to be essential for testicular development and spermatogenesis. Zinc plays an important role in male reproduction as it plays a role in sperm production and/or viability, in the prevention of the spermatozoa degradation, and in sperm membrane stabilization. Zinc deficiency leads to gonadal dysfunction, decreases testicular weight. The gonads are the most rapidly growing tissues in the body, and some of the vital enzymes involved in nucleic acid and protein synthesis are zinc metalloenzymes¹⁶. Male fertility is influenced by zinc in several different ways. Low zinc levels have a negative effect on serum testosterone concentration¹⁷. Seminal plasma zinc concentration has been significantly correlated with sperm density, possibly contributing a positive effect on spermatogenesis¹⁸. Other studies have shown the effects of zinc on sperm motility¹⁹, emphasizing the mineral's role in flagella function.

Infertile males have been shown to have lower levels of seminal plasma zinc that have been associated with reduced levels of zinc in their blood²⁰. In the present study, there was a significant difference between the mean serum zinc concentration of the fertile (14.09±0.27 µmol/L) and infertile groups (7.75±0.18 µmol/L); (P< 0.01). Similar results have been reported elsewhere Chia *et al*¹⁸, Mohan *et al*²⁰, Koca *et al*²¹. There was a significant difference between the mean seminal plasma zinc concentrations of the fertile (1.41±0.01 mmol/L) and infertile groups (0.83±0.02 mmol/L); (P< 0.01). Chia *et al*¹⁸ compared zinc levels between fertile and infertile groups and found geometric means of the seminal plasma zinc concentrations were significantly lower in the infertile group compared with those of the fertile group. This observation is also supported by other study²². Studies have demonstrated that zinc therapy results in significant improvement in sperm quality with increases in sperm density, progressive motility, and improved conception and pregnancy outcome^{23, 24, 25}. Zinc has been shown to have antioxidant activity and to maintain sperm viability by inhibiting DNA ases²⁶. Zinc appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa and/or leukocytes in human semen after ejaculation. Thus, it seems that seminal plasma, because of its high content of zinc, exerts protective, antioxidant-like activity sufficient to cope with the excessive amount of superoxide anions²⁷. In the present study significant positive correlations were observed between zinc levels in serum with sperm count (r=0.66) and sperm motility (r=0.39); (p<0.01), (p<0.05) respectively. Carreras and Mendoza²⁸ have reported that zinc in blood was significantly correlated with sperm count and sperm motility. Our study demonstrated that seminal plasma zinc concentrations were significantly correlated with sperm density (r=0.56) and motility (r=0.34); (p<0.01), (p<0.05) respectively. These observations were supported by

other studies^{13,29}. Abasalt *et al*²² found zinc concentrations to increase with increasing sperm density. Fuse *et al*³⁰ reported a positive correlation between zinc levels and sperm motility. It is expected that zinc might be responsible for antioxidant defense system; therefore lower zinc levels would cause oxidative damage. This would generate free radicals that could lead to defective sperm motility. There was no significant correlation of sperm morphology with seminal plasma zinc concentration. This result is comparable with other studies^{30,31}. In the present study volume of semen was found to be negatively correlated with the seminal plasma zinc in infertile subjects. These results are in accordance with several studies^{32,30}. In the present study significant positive correlation of serum testosterone was found with seminal plasma zinc level in oligospermic subjects ($r=0.44$, $p<0.01$). Therefore seminal plasma zinc level may affect the sperm count by decreasing the production of testosterone. These results are consistent with the results of other studies^{17,33}. In azospermic subjects significant positive correlation of serum testosterone was found with serum zinc ($r=0.37$, $p<0.05$). These results are in agreement with the results of other studies¹⁴.

Conclusion

Low levels of zinc in serum and/or seminal plasma could be an indicator of male infertility. Serum and seminal plasma levels of zinc are directly correlated with sperm count and motility. There is a direct correlation between zinc levels in seminal plasma and serum testosterone in oligospermic subjects also there is a direct correlation between serum zinc levels and serum testosterone in azospermic subjects. Zinc supplementation probably warranted in the treatment of male infertility, especially in cases of low sperm count or decreased testosterone levels.

Recommendations:

We recommended that a larger study may be necessary to be carried out considering confounding factors such as smoking and nutritional status. Trace elements analysis like selenium, copper, manganese and zinc should be introduced as a method for search of infertility causes.

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