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Original Article

Physiology Section

Zinc Levels in Seminal Fluid in Infertile Males and its Relation with Serum Free Testosterone

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ABSTRACT

Introduction: The role of zinc is critical to reproduction potential. Seminal zinc is thought to be derived almost exclusively from prostatic secretions. Sperm motility is significantly influenced by zinc. Zinc deficiency has been linked with male sterility and subfertility.

Aim: To assess the influence of seminal plasma zinc on seminogram characteristics and whether endogenous testosterone affects the seminal levels of zinc.

Materials and Methods: The semen samples were obtained from 150 male partners of infertile couples who attended the Reproductive Biology Unit of the Department of Physiology, within the age 21-50 years and semen samples were analysed for the routine seminogram parameters. All the subjects were classified into two main groups, A- the subjects with normal ejaculates (n=62) and B- the subjects with abnormal ejaculates, who were further sub divided into the following groups: i) Asthenoteratozoospermics (n=43); ii) Oligoasthenoteratozoospermics (n=24); and iii) Azoospermics (n=21). The seminal plasma zinc was measured spectrophotometrically. The sample for serum free testosterone was sent to Thyrocare laboratory.

Results: The seminal plasma zinc was found to be significantly lower in the abnormal ejaculates than in the normal ejaculates. A statistically significant positive correlation was observed between the seminal plasma zinc and serum free testosterone ($p<0.05$, $r=0.449$). Statistically significant correlation was also found between seminal plasma zinc and all the seminogram parameters such as the sperm concentration, sperm motility and sperm morphology ($p<0.05$, r= 0.86, 0.87 and 0.86 respectively).

Conclusion: Low seminal plasma zinc might be a significant causative factor in impairing sperm functions and its dependence on endogenous free testosterone, was observed from a positive correlation between the two.

Keywords: Male infertility, Seminal zinc, Seminogram, Spectrophotometric method

INTRODUCTION

The biochemical composition of seminal fluid is very precisely maintained within the physiological range. These biochemicals are contributed by the various accessory sex glands, the secretory activity of which is mainly under the control of testosterone. Zinc is one of the most important compound of seminal fluid contributed by prostate gland. Zinc in the body plays an important role in normal testicular development, spermatogenesis, and sperm motility [1]. Zinc in seminal plasma stabilizes the cell membrane and nuclear chromatin of spermatozoa [2]. Sperm motility is significantly influenced by zinc. There occurs stiffening of the outer dense fibres by formation of disulfide bridges during epididymal sperm maturation, which seems to be an essential physiologic step for the generation of sperm motility; especially progressive motility [3].

Zinc copes up with excessive amount of superoxide anions, thus adequate amount of zinc in seminal plasma exerts protective effect by virtue of this antioxidant activity [4]. Zinc appears to protect sperm from bacteria and chromosomal damage [5]. It may also play a regulatory role in the process of capacitation and acrosome reaction [6,7]. Insufficient development of secondary sex characteristics and hypogonadism has been linked to zinc deficiency [8].

AIM

The aim of the present study was to assess the influence of seminal plasma zinc on seminogram characteristics and whether endogenous testosterone affects the seminal levels of zinc. We could not find any direct study in literature elaborating the correlation between serum free testosterone and seminal plasma zinc.

MATERIALS AND METHODS

The present study was carried out from December 2012 to June 2014 in the Reproductive Biology Unit (Infertility Clinic) in the Department of Physiology. The patients were referred from Gynaecology & Surgery Department.

The semen samples were obtained from 150 male partners of infertile couples. Cases of both primary as well as secondary infertility were included in the study. Detailed history of present and past illness as well as medical and surgical intervention was taken. The clinical examination of external genitalia was done at Surgery OPD.

Inclusion Criteria

Subjects belonging to reproductive age group (primary or secondary infertile men aged 21-50 years) who have not conceived after one year of regular, unprotected intercourse.

Exclusion Criteria

Subjects with hydrocele, hernia, undescended testes, varicocele, or any surgical history of genitourinary tract which may interfere with male infertility were not included in the study. Subjects with any acute febrile illness or treatment history which may suppress the spermatogenesis were also excluded from the study.

Study was conducted with permission of ethics committee of the institute and written consent was taken from each subject included in the study. The subject had to observe three days of abstinence and semen samples were delivered on the fourth day. Semen sample were collected by masturbation. After complete liquefaction at room temperature, each sample was tested.

Following parameters were studied:

1) Routine Semen Analysis

Routine semen analysis was done by SQA II C-P (Sperm Quality Analyser) {Medical Electronic System Ltd. Israel} for sperm concentration (millions/ml), sperm motility, sperm morphology.

According to WHO guideline [9,10] subjects were grouped into four categories with following criteria:

i) Normozoospermics

- Persons with sperm concentration of 15 millions/ml or more.
- Sperm motility (progressive + non progressive) being 40 % or more.
- Normal sperm morphology in 30% cells or more.

ii) Oligoasthenoteratozoospermics:

- Sperm concentration less than 15 millions/ml.
- Sperm motility (progressive + nonprogressive) below 40%.
- Normal sperm morphology in less than 30% of sperms.

iii) Asthenoteratozoospermics:

- Persons with sperm concentration of 15 millions/ml or more.
- Sperm motility (progressive + nonprogressive) below 40%
- Normal sperm morphology in less than 30% of sperms.

iv) Azoospermics:

- Total absence of spermatozoa in semen (even after centrifugation).

Except for the normozoospermic, the other subjects with abnormal seminogram pattern were asked to come for repeat semen analysis after one month. If the second report was also abnormal then they were included in the abnormal groups and semen sample were utilized for further physiochemical assessment

2) Estimation of serum free testosterone:

Blood samples were collected in the hospital (between 9:00-10:00 a.m.) and samples were sent to Thyrocare laboratory for measurement of serum free testosterone by Radioimmunoassay technique. The report was collected after 3 days.

3) Estimation of Zinc [11]

After evaluation of physical parameters, whole semen sample was centrifuged at 3000 rpm for 10 minutes. Then, without disturbing the pellet at the bottom, supernatant seminal plasma was taken for zinc assay.

Zinc was estimated by spectrophotometric method (kit was supplied by Coral Clinical systems, Verna Industrial Estate, Verna, Goa).

Principle

Zinc in an alkaline medium reacts with Nitro-PAPS to form a purple coloured complex. Intensity of the complex formed is directly proportional to the amount of Zinc present in the sample.

$$\text{Zinc + Nitro - PAPS} \xrightarrow{\text{Alkaline Medium}} \text{Purple Coloured Complex}$$

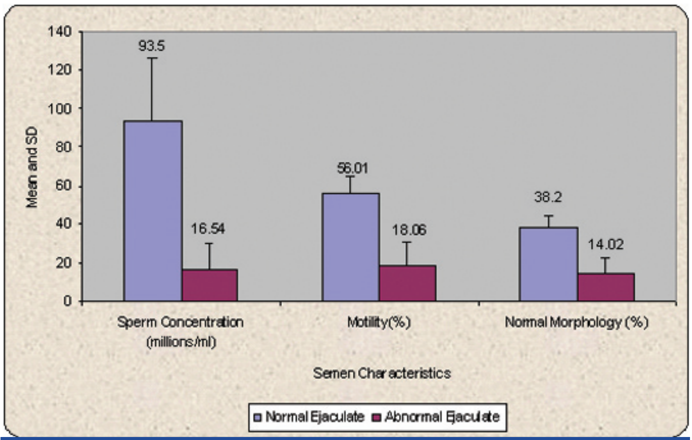
The absorbance of the standard and the test sample were taken on spectrophotometer at 570 nm against reagent blank.

Calculations:

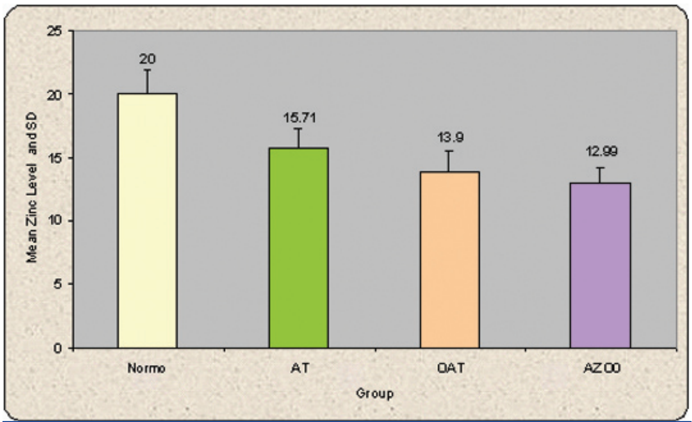
$$\text{Zinc in mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200 \times 100 \text{ (dilution factor)}$$

STATISTICAL ANALYSIS

All the statistical analysis was done by using descriptive and inferential statistics using Z-test, One-way ANOVA and Pearson's Correlation Coefficient using SPSS (version 17.0) and Graph Pad Prism (version 5.0) under the guidance of statistician. The p<0.05 was considered as level of significance.



[Table/Fig-1]: Semen characteristics in normal ejaculates and abnormal ejaculate group.



[Table/Fig-2]: Zinc levels (mg/dl) of seminal plasma in normal ejaculate and abnormal ejaculate group.
Normo- Normozoospermics, AT- Asthenoteratozoospermics
OAT-Oligoasthenoteratozoospermics, AZOO- Azoospermics

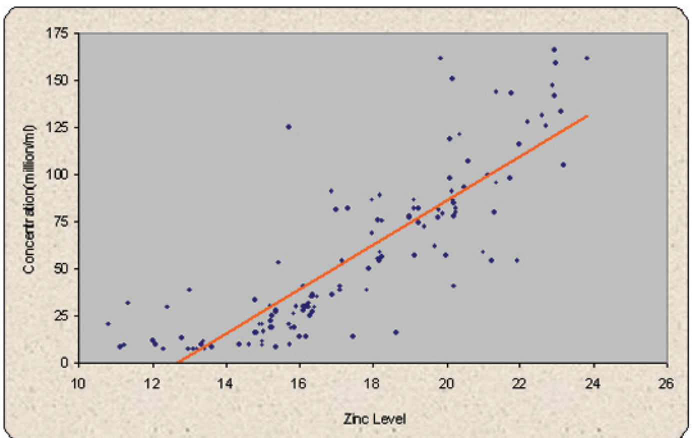
RESULTS

The mean values of the different seminogram parameters and the values of seminal plasma zinc in the subjects with normal and abnormal ejaculates have been shown in [Table/Fig-1,2].

The mean Zinc level (mg/dl) of seminal plasma was found to be highest in Normozoospermics, followed by

Parameters	Mean	Std. Deviation	N	Correlation 'r'	p-value
Zinc Level	16.82	3.30	150	-	-
Concentration	48.35	44.61	150	0.86	0.000,S,p<0.05
Motility	33.75	22.02	150	0.87	0.000,S,p<0.05
Morphology	24.02	14.15	150	0.86	0.000,S,p<0.05

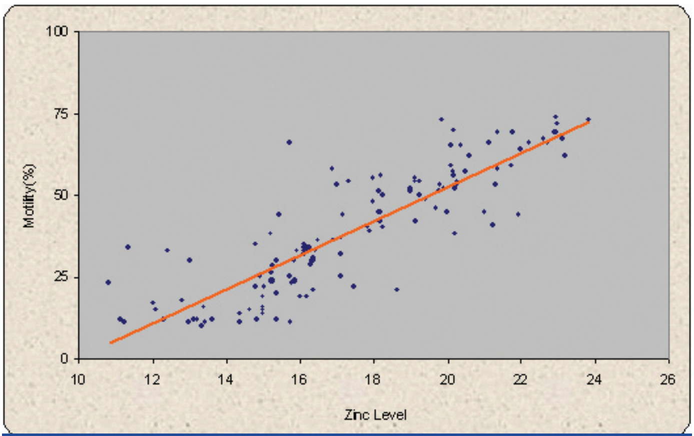
[Table/Fig-3]: Correlation of Zinc level (mg/dl) with sperm concentration (millions/ml), total % motility and % normal morphology
Pearson's Correlation Coefficient



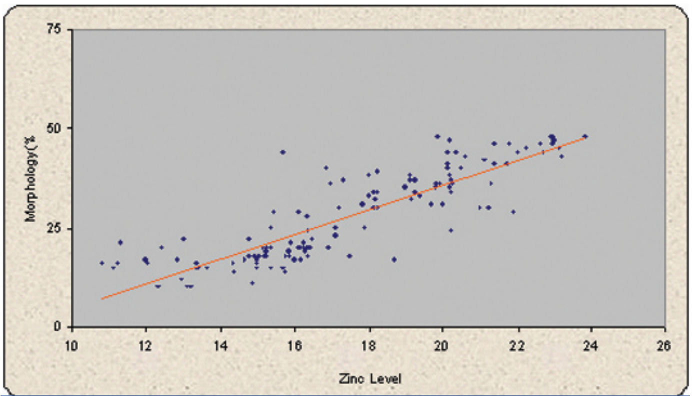
[Table/Fig-4]: Correlation of Zinc level (mg/dl) with sperm concentration (millions/ml).

Asthenoteratozoospermics, Oligoasthenoteratozoospermics and Azoospermics. Statistically significant variation was found in mean zinc levels of four groups (F=134.06, p<0.05).

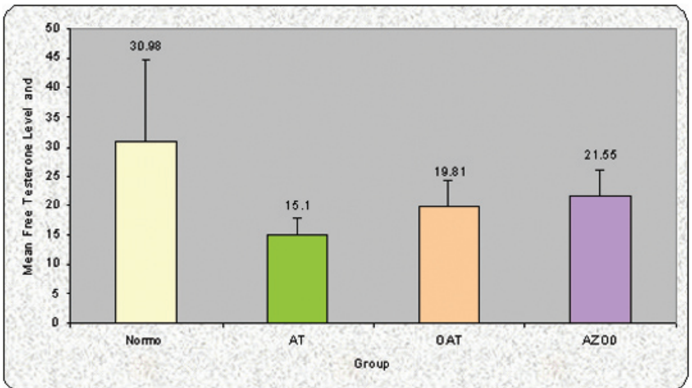
Seminogram parameters including sperm concentration, motility, normal morphology were found to be positively correlated with seminal zinc levels (r=0.86, 0.87, 0.86 respectively, p<0.05) [Table/Fig-3-6].



[Table/Fig-5]: Correlation of Zinc level (mg/dl) with sperm motility (total %).



[Table/Fig-6]: Correlation of Zinc level (mg/dl) with sperm normal morphology %.

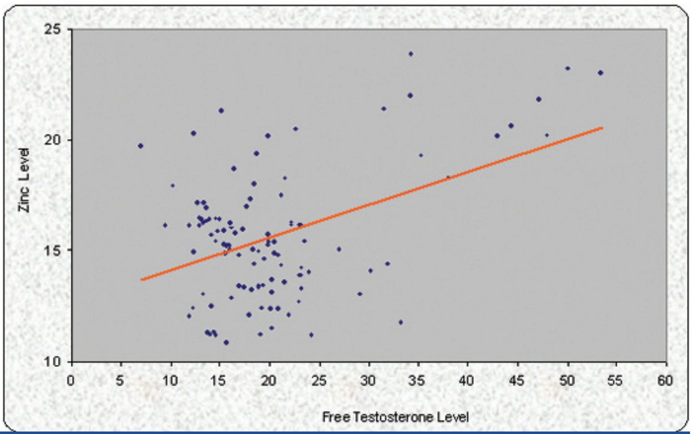


[Table/Fig-7]: Serum free Testosterone levels (pg/dl) in normal ejaculate and abnormal ejaculate group.

Parameters	Mean	Std. Deviation	N	Correlation 'r'	p-value
Free Testosterone Level	20.34	8.81	108	-	-
Zinc Level	16.82	3.30	150	0.449	0.000,S,p<0.05

[Table/Fig-8]: Correlation of serum free testosterone Level (pg/ml) with seminal plasma Zinc level (mg/dl).
Pearson's Correlation Coefficient

The mean serum free testosterone level (pg/ml) was found to be highest in Normozoospermics. Statistically significant variation was found in mean free testosterone levels of four groups (F=25.02, p<0.05) [Table/Fig-7].



[Table/Fig-9]: Correlation of serum free testosterone Level (pg/dl) with seminal plasma Zinc Level (mg/dl).

Significant positive correlation was observed between serum free testosterone (pg/ml) and seminal plasma Zinc (mg/dl) (r=0.449, p<0.05) [Table/Fig-8,9].

DISCUSSION

The measurement of zinc in human seminal plasma is important in the evaluation of male infertility. In present study the seminal plasma zinc levels (in mg/dl) were found to be highest in Normozoospermics (Mean 20.00±1.93), followed by Asthenoteratozoospermics (Mean 15.71±1.63), Oligoasthenoteratozoospermics (Mean 13.90±1.62) and Azoospermics (Mean 12.99±1.25).

These findings of present study are in accordance with Mankad et al., and Doshi H et al., who reported lower seminal plasma zinc among azoospermics as compared to oligozoospermics and normozoospermics [12,13]. Awadallah et al., also reported lower mean zinc levels in seminal plasma of oligozoospermics and azoospermics when compared to normozoospermics and asthenozoospermics [14]. Ali et al., too found that seminal plasma zinc levels were low in oligospermic and azoospermic subjects when compared with normospermic control groups [15].

However, few studies have reported different results. Schoenfeld et al., could not find any statistically significant difference between zinc in seminal plasma of normozoospermic, oligozoospermic and azoospermic men [16]. Fuse et al., found highest seminal plasma zinc concentration in asthenoteratozoospermic males [17].

A positive correlation between seminal plasma zinc levels and sperm concentration, motility and normal morphology was also observed in our study. This was in accordance with the previous studies of Doshi et al., Hussain et al., Badade et al., Atig et al., and Abed [13,18-21]. Eliasson and Lindholme et al., in contrast could not find any correlation between zinc concentration and sperm density, motility or morphology [22].

Fuse et al., found a positive correlation of zinc and sperm concentration and motility but no correlation with sperm morphology was observed [17]. Mankad et al., found positive correlation between zinc and sperm count but no significant correlation was observed between zinc and sperm motility [12].

Thus it seems that zinc is important for semen quality. The low zinc levels in the infertile men in our study might be attributed to disorders in the prostate excretory function or possibly due to asymptomatic prostate infection.

Despite of extensive literature search we could not find any direct study elaborating the correlation between serum free testosterone and seminal zinc. Few related studies are discussed. Fuse et al., reported a correlation between zinc concentration and plasma testosterone concentration [17]. Ali et al., found weak correlation between seminal zinc level and testosterone in infertile oligospermic patients [15]. Abed showed that seminal plasma zinc levels are directly proportional to testosterone [21].

CONCLUSION

The present study concluded that seminal plasma zinc may have considerable role in improving male fertility potential. Adequate seminal plasma concentration of zinc is required for normal sperm function.

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