Levels of Zinc and Copper in Seminal Plasma of Sudanese Infertile Males

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Authors’ contributions

This work was carried out in collaboration between both authors. Author AAA designed the study, performed the statistical analysis, wrote the protocol, and correct the last draft of the manuscript. Author YMA managed the analyses of the study. Both authors read and approved the final manuscript.

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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: To evaluate the levels of Zn and Cu in seminal plasma of Sudanese infertile male and their effect on reproductive capacity.

Methods: The concentrations of Zn and Cu were measured in 150 semen samples from normozoospermic, oligozoospermic, and azoospermic men using the atomic absorption spectrometry and data was analyzed using the statistical software package SPSS version 17.

Results: Results showed that the mean values of seminal plasma Zn concentrations were significantly decreased in the two groups of infertile male subjects, azoospermic (p=0.02), and oligozoospermic (p=0.03) compared with fertile males, while there was insignificant decrease in seminal plasma Cu concentration of azoospermic patients compared to control (p=0.21), and insignificant increase in oligozoospermic patients compared to control (p=0.06).

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Conclusion: On the basis of the observations of the present study, seminal zinc may contribute to fertility through its effects on semen parameters.

Keywords: Male infertility; zinc; copper; oligozoospermia; azoospermia.

1. INTRODUCTION

Infertility is complex and has multiple causes and consequences depending on the gender, sexual history, lifestyle, and cultural background [1]. Infertility affects about 8% to 12% of the world’s population and in about half of cases, men are either the single cause or contribute to couple’s infertility [2]. 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 [3-4]. The possible influence of the trace elements especially Cu and Zn on male infertility is of great interest [1], increasing evidence of a direct relationship of zinc was found with seminal parameters [5]. 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 [6]. Zinc is the second main element, after iron, in seminal plasma. It stabilizes the cell membrane and nuclear chromatin of spermatozoa [7,8]. It may also have an antibacterial function [9] protects testis against the degenerative changes [10]. It regulates process of capacitation and acrosome reaction [11]. Zinc plays an important role in normal testicular development, spermatogenesis and sperm motility [12]. 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 [9,13]. Steven et al. [14] reported that zinc in seminal plasma is involved in nuclear chromatin decondensation and acrosin activity. Zinc deficiency in the nucleus may destabilize the quaternary structure of chromatin, a feature important for the fertilizing capacity of the spermatozoa. Kvist et al. [15] and Prasad [16] conducted an experiment in adult males and reported that synthesis of testosterone by Leydig cells depends on adequate dietary zinc [17,18].

2. MATERIALS AND METHODS

This study was carried out on patients attending fertility clinic for routine infertility medical check-up. During the period from January 2014 to April 2014, hundred infertile male subjects, those who had regular sexual intercourse for at least one year without conception, age range between 22-55 years. The study was approved by Alneelain University Ethics Committee and all subjects gave informed consent (Based on Helsinki Declaration), the informed consent was signed by them. Patient’s information were collected by a structured questionnaire. Semen specimens were collected in sterile polystyrene semen containers through masturbation after 3 days of abstinence. Semen samples were incubated for 30 mins at 37°C for liquefaction. A routine semen analysis was performed upon liquefaction according to WHO criteria to measure volume, pH, sperm concentration, motility and morphology [21]. Specimens from all infertile males were then divided into the following three groups according to their sperm count, motility, and morphology.

Group I: Azoospermic (sperm count = zero, n=50), Group II: Oligozoospermic (sperm count <33 million/ejaculate, n=50), and 50 fertile males whose partners had conceived within one year and having sperm count 33-46 million/ejaculate.
with normal motility and morphology taken as normozoospermic control group.

After liquefaction, the seminal plasma was collected after centrifugation at 2000g for 15-20 minutes. Supernatant (the seminal plasma) was transferred into fresh tubes and stored frozen, at -20°C.

2.1 Specimen Preparation

Seminal plasma were diluted 1:10 with 0.5% v/v HNO3 to determine the concentrations of zinc and copper using flame atomic absorption spectrophotometer (Buck Scientific, model 210 VGP) at Academy of Science and Technology Khartoum-Sudan. The data was analyzed using the statistical software package SPSS, version 17 (SPSS Inc., Chicago, IL). Result were expressed in mean and standard deviation (M±SD). Normal distribution of the studied variables was examined using Kolmogorov-Smirnova and Shapiro-Wilk tests. Unpaired T test and Mann-Whitney U test were used to assess significant difference in the means of the studied variables in different groups, P<0.05 was considered statistically significant.

3. RESULTS

The results of present study showed significant decrease in seminal plasma Zn in oligozoospermic infertile males (M±SD) = (18.0±0.94 mg/dl) compared to control (21.1±0.66 mg/dl), p = (0.03).

Also there was significant decrease in seminal plasma Zn level in azoospermic males (17.1±1.16 mg/dl) compared to control (21.1±0.66 mg/dl), p = 0.02. Seminal plasma copper level was insignificant increase in oligozoospermic male (0.40±0.21mg/dl) compared to control (0.30±0.17 mg/dl), p=0.06. Insignificant decrease in level of copper was also observed in azoospermic males (0.24±0.11 mg/dl) when compared with control (0.30±0.17 mg/dl), p=0.22 Table 1.

4. DISCUSSION

The results of the present study showed that there were significant lower levels of seminal plasma zinc in oligozoospermic and azoospermic infertile males compared to fertile male control. This finding is in agreement with what reported by Hasan et al. [22] and Ali et al. [23] who observed significant decreases in seminal plasma Zn in oligozoospermic and azoospermic infertile males. Hasan et al. [22] 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. [24] found no significant difference in the mean value of seminal plasma Zn between infertile individuals compared to fertile individuals. The result of seminal plasma Zn of the current study was not in accordance with what was reported by Akinloye O et al. [25], who observed inverse correlation between seminal Zn and sperm count.

Zinc is important element for conception, successful implantation, and pregnancy stability [26,27]. 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 [28]. It has been suggested that Zn is an important anti-inflammatory factor, and also involved in sperm oxidative metabolism [29,30]. Alsalman AS et al. [31] reported that zinc plays a vital 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. The authors concluded that adequate seminal concentration of the Zn is required for normal sperm function [25].

Omu and associates (1998) had demonstrated that Zn therapy results in significant improvement in sperm quality with increases in sperm density, progressive motility, and improved conception and pregnancy outcome [32].

This study showed that Cu concentration was insignificantly decreased in seminal plasma of azoospermic patients and insignificantly increased in oligozoospermic when compared to fertile control males. The current results were comparable with the findings of Saleh et al. [33].

Copper is an essential trace element that plays an important role in several enzymes such as superoxide dismutase cytochrome oxidase, ferroxidase, spermin oxidase. 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.
Table 1. Seminal plasma zinc and copper concentrations in study population

| Parameter                   | Study population (n=150) | P. value |
|-----------------------------|--------------------------|----------|
|                             | Oligozoospermia (n=50) M±SD | Azoospermia (n=50) M±SD | Control (n=50) M±SD | Oligo. vs control | Azo. vs control |
| Zinc (mg/dl)                | 18.0±0.94                | 17.1±1.16 | 21.1±0.66 | 0.03             | 0.02             |
| Copper (mg/dl)              | 0.40±0.21                | 0.24±0.11 | 0.30±0.17 | 0.06             | 0.21             |

Superoxide dismutase (Cu-metalloenzyme) protects human spermatozoa from this peroxidative damage [34]. However, this element in its ionic form (Cu2+), particularly at high concentration has a rapid toxic effect to a variety of cells, including human spermatozoa [33]. In vitro studies carried out by Roblero et al. [20] have demonstrated the effect of Cu in intrauterine device which prevents conception.

5. CONCLUSION

On the basis of the finding of the present study, Zn may be considered a useful tool in addition to other parameters in assessing male infertility, while Cu has no significant role.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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