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**Original research**

# Association of seminal zinc and copper level with infertility in Sudanese male in Shendi town - Northern Sudan

Hanan Ahmed Abedraman [(1)] , *Mosab .O. Khalid [(1)], and HaghamadAllzain [(2)]

[1] Department of Clinical Chemistry- Faculty of Laboratory Sciences- Shendi University-Sudan

[2] Department of Biochemistry- Faculty of Medicine- Shendi University- = Department of Biochemistry- Faculty of Medicine-Elsheikh Abdallah Elbadri University (EAEU) – Sudan.
**ORCID** : haghamad Bulla----- https://orcid.org/**0000-0002-2812-553X** Web of Science Researcher ID: **ADP-4770-2022**

## Abstract

**Background:** Male fertility is the essence for human race continuity**.** The presence of abnormal levels of zinc (Zn) and copper (Cu) concentrations in human semen may affect sperm production, maturation, motility and fertilizing ability. **Objective:** To evaluate the levels of zinc and copper in seminal plasma and to correlate their concentrations with various semen parameters among fertile and infertile Sudanese male subjects in Shendi town- River Nile State - Northern Sudan. **Methods:** The study was conducted from September 2021 to January 2022.The concentrations of Zn and Cu were measured in fifty (50) semen samples ، thirty five (35)of them had abnormal semen analysis ( oligozoospermic, azoospermic and asthenozoospermic ) as case study and fifteen (15) were normal semen analysis as control. **Results:** Results showed that the mean value of seminal plasma Zn concentration was significantly decreased in the case group compared to control group. The mean value of seminal plasma Zn concentrations was significantly decreased in the groups azospermic with P. value of (0.007)and oligozoospermic with P. value of (0.011) and no effect of asthenozopermic on seminal Zn with P. value of (0.713). While the mean value of seminal plasma Cu concentration were significantly increase in the case group compared to control group, with no effect as regard to azoospermic with P. value of (0.652), asthenozopermic with P. value of (0.329) and oligozoospermic with P. value of (0.122) .There is no correlation between body mass index and level of zinc with P. value of (0.458) and copper with P. value of (0.209).

***Keywords: Infertility, Semen ,Zinc (Zn), Copper (Cu), oligozoospermia, asthenozopermia. Azoospermia, Sudanese.***

## Introduction

Men in about fifty percent of cases are either the single cause or contribute to couple's infertility , which affects about (8%) to (12% ) of the world's population Seminal plasma is very important for sperm metabolism, function, survival, and transport in the female genital tract.[ 1] Cations such as Na, K, Ca, and Mg establish osmotic balance,. Increasing evidence of a direct relationship of zinc which represent prostatic secretary function was found with seminal parameters.[1]

The decrease in the seminal plasma zinc concentration may result from inadequate intake which is commonest cause, reduced absorption, increased losses, or increased demand. [2]

Copper is an important element for numerous metalloenzymes and metalloproteins, involved in energy metabolism. It,s higher level is toxic to human spermatozoa . The use of Cu in intrauterine devices prevents conception .[3]

According to the World Health Organization (WHO) guidelines semen analysis include volume, pH, vitality, sperm concentration, motility and morphology .Sperm concentration was determined by a Neubauer counting chamber, The samples were divided into 4 groups of; normospermic, azospermic (no sperm in semen), oligozoospermic (sperm concentration fewer than 20×106/ml) and asthenozoospermic (fewer than 50% spermatozoa with forward progression) groups. [4]

Zn is referred to as a trace element, as its plasma concentration is only (12–16 μM), In the serum Zn is predominantly bound to albumin (60 %, low-affinity), α2-macroglobulin (30 %, high-affinity) and transferrin (10 %; )[5].

Zn has important role as inhibitor of oxidative damage[6].Evidence suggests that seminal Zn has an important role in the physiologic functions of the sperm and that its reduced levels result in low seminal quality and subsequent chances of fertilization[7].

Zn therapy improves sperm quality with increases in sperm density, progressive motility and improved conception and pregnancy outcome. Zn plays an important role in membrane-stabilizing and antioxidant activity and maintains sperm viability by inhibiting DNases [7].

Copper is an essential trace element for humans and exist mostly in second oxidation form [8]. Copper plays an important role in our metabolism, can act as both anti oxidant and pro-oxidant [9]. Copper deficiency is more commonly an acquired condition, attributable to nutritional deficiency, and may be seen in malnourished low-birth-weight infants, newborns, and small infants, also after gastrointestinal surgery, intractable diarrhea, and prolonged parenteral or enteral nutrition[10,11,12] .Copper depletion affects male reproduction in different species , the copper chelation was involved in suppression of spermatogenesis. On the other hand, it can be toxic at elevated concentrations [4].

## Objectives

The general objective of this study is to evaluate semen zinc and copper level among abnormal semen Sudanese male, while the specific objectives are to associate semen zinc and copper with azoospermia, oligospermia, asthenozoospermia, in infertile Sudanese males.

## Material and methods

It is case control study, conducted from September 2021 to January 2022 , in Shendi town. It included fifty (50) males , thirty five (35) of them who had regular sexual intercourse for at least one year without conception, and their age is between( 25- 55) years old, with abnormal semen analysis parameters and fifteen

(15) of them males are with normal semen analysis parameters. The study included all willing patients attending fertility clinic for routine infertility medical check- up Exclusion from the study is based on clinical assessment, those with a history of sexually transmitted diseases, mumps-related orchitis ,diabetes mellitus ,testicular injury , small testicles (<10mL), and varicocele . Also excluded those with past history of excessive alcohol intake, abnormalities of reproductive endocrine system and fever including typhoid fever in the past three (3) months duration.

**Collection of samples and preparation**

Patient's information was collected by a structured questionnaire.

Semen specimens were collected in polystyrene semen containers through masturbation after three (3) days of abstinence. The samples were centrifuged 3000 at (15-20 m). The supernatant was frozen at -20 for further analysis.

For examination of sperms motility, one drop of well mixed, liquefied semen was placed on a clean dry slide and covered with a cover slip. Using x40 objective, with the condenser iris closed sufficiently to give a good contrast, several fields were examined formotile spermatozoa. Then the approximate percentage of actively motile spermatozoa was reported. The. Sperm Cell Count Using a graduated tube, the semen was diluted 1 in 20as follows: The tube was filled to the 1ml mark with the well-mixed liquefied semen, then sodium bicarbonate- formalin diluting fluid was added to the 20 ml mark and then mixed very well. Using a Pasteur pipette, an improved neubauer countingchamber was charged with the diluted semen and allowed to stand for 3-5 minutes for the spermatozoa to settle. Using x10 objective lens, with the condenser iris closed sufficiently to give a good contrast,.to. Morphology The morphological examination of the semen was doneas follows: A thin smear of the liquefied well mixed semen was made on a slide while the samples were still wet; the smear was fixed with 95%v/v ethanol for 5-10

minutes and then allowed to air dry. The smear was washed with sodium bicarbonate-formalinsolution toremove any mucus which may be present and then rinsed with several changes of water. The smear was covered with dilute carbolfuchsin (1 in 20) and allowed to stain for 3 minutes, and the stain was then washed off. This was counterstained with dilute Loeffler's methylene blue for 2 minutes and then washed off with water, drained and allowed to air dry. Using the oil and x 40 objective and the condenser iris closed gently to give a good contrast, the slide was examined for normal and abnormal spermatozoa. Seminal plasma were diluted 1:10 with 0.5% v/v $HNO_3$ to determine the concentrations of zinc and copper using flame atomic absorption spectrophotometer (Buck Scientific, model 210 VGP).

**Statistical Analysis**

The data was analyzed using Statistical Package of Social Science (SPSS) version (22) for windows. Frequencies, Means, SD, Independent t-test and Pearson's correlation have been used to compare and correlate between parameters and study variables.

**Ethical Approval**

The study was approved by the Board of Faculty of Graduate Studies in Shendi University. Verbal informed consent was obtained from all participant after explanation of the study aims .

## Results

**Table (1): Zinc concentration in seminal plasma in case and control groups.**

| Group | N | Mean concentration (mg/l) | Std. deviation | P. value |
|---|---|---|---|---|
| Case | 35 | 12.62 | 4.44 | 0.000 |
| Control | 15 | 20.66 | 2.52 | |

**Table (2) : Copper concentration in seminal plasma in case and control groups.**

| Group | N | Mean concentration (mg/l) | Std. deviation | P. value |
|---|---|---|---|---|
| Case | 35 | 0.23 | 0.11 | 0.000 |
| Control | 15 | 0.150 | 0.03 | |

**Table (3): Zinc and copper concentration in seminal plasma in oligospermia:**

| Group | | N | Mean concentration (mg/l) | Std . deviation | P. value |
|---|---|---|---|---|---|
| Zn | Oligospermia | 23 | 15.56 | 5.13 | 0.011 |
| | Other abnormalities | 12 | 18.65 | 1.27 | |
| Cu | Oligospermia | 23 | 0.25 | 0.12 | 0.122 |
| | Other abnormalities | 12 | 0.19 | 0.09 | |

**Table (4): Zinc and copper concentration in seminal__plasma in asthenospermia:**

| Group | | N | Mean concentration (mg/l) | Std. deviation | P. value |
|---|---|---|---|---|---|
| Zn | Asthenospermia | 15 | 16.29 | 5.00 | 0.713 |
| | Other abnormalities | 20 | 16.86 | 4.10 | |
| Cu | Asthenospermia | 15 | 0.25 | 137.5 | 0.329 |
| | Other abnormalities | 20 | 0.21 | 0.09 | |

**Table (5): Zinc and copper concentration in seminal plasma in azoospermia**

| Group | | N | Mean concentration (mg/l) | Std. deviation | P. value |
|---|---|---|---|---|---|
| Zn | Azoospermia | 7 | 12.70 | 4.97 | 0.007 |
| | Other abnormalities | 28 | 17.60 | 3.80 | |
| Cu | Azoospermia | 7 | 0.21 | 0.12 | 0.652 |
| | Other abnormalities | 28 | 0.24 | 0.11 | |

**Table (6): Correlation between zinc concentration (mg/l) in seminal plasma and body mass index (BMI) .**

| | N | Mean | Std. deviation | P. value | Person correlation – R |
|---|---|---|---|---|---|
| BMI | 35 | 28.91 | 5.25 | 0.458 | - 0.130 |
| Zn | 35 | 17.83 | 4.36 | | |

**Table (7): Correlation between copper concentration (mg/l) in seminal plasma and body mass index (BMI) .**

| | N | Mean | Std. deviation | P. value | Person correlation – R |
|---|---|---|---|---|---|
| BMI | 35 | 28.91 | 5.25 | 0.209 | - 0. 218 |
| Cu | 35 | 0.23 | 0.11 | | |

## Discussion

In this study, there was a significant low level of seminal plasma zinc in case group who had abnormal semen analysis ,with *P. value* (0.000) and mean of (16.6229) , compared to control with normal semen analysis; mean (20.6600). Similar findings have been reported by Hassan Ali, et al in (2007) [1] and other similar results have been reported by Yosra M. Altaher and AbdElkarim A. Abdrabo in (2014) [3] and also by Nawal .K. Hussain ,et al in (2011) [14] .On the other aspect ,Fuse. H, et al in (1999) [15] found no significant difference in the mean value of seminal plasma Zn between infertile individuals compared to fertile individuals .

In this study, there was significant decrease of zinc concentration in seminal plasma in oligospermic, with p. value of (0.0110) and mean of (15.5643), Azoospermic, with p .value (0.007) and mean of (12.7029) compare with other abnormal semen analysis and insignificant in asthenospermic,with p. value (0.713) and mean of (16.2960) . In odds with the findings obtained by Basil Oied ,et al. in (2008) [13] which point to insignificant level of Zn as regard to Azoospermia.

This study found that copper (Cu) concentration was significantly increased in seminal plasma in case group ,who had abnormal semen analysis ,with p. value of (0.000) and mean of (0.2363) compared to control group who had normal semen analysis, with mean of (0.1501) . The copper concentration was insignificantly increased between the sub groups of oligospermic, azospermic and asthenospermic with p. value (0.122) and mean (0.2583) ,p. value(0.652) and mean(0.2171). and p. value(0.329)mean(0.2587) respectively. The results of the current study were comparable with the findings of Yosra M. Altaher and AbdElkarim A. Abdrabo in (2014) and other result by Basil Oied ,et al. (2008)[13] and Nawal .K. Hussain ,et al (2011)[14] ,which showed significantly decrease copper concentration in asthenospermic and insignificant in oligospermia. Other study published by Hamad et al. in (2014) [16] reported significantly higher concentration of copper in infertile males. The findings of this study also support those obtained by Mathias Abiodun Emokpae and Muyiwa Adeleye Moronkeji in (2020) ) in Benin City, Nigeria as regard to the antagonistic interactions between copper and zinc concentration in seminal plasma as contributing factor in man fertility[17].

The association of higher copper concentration with infertility in men in this study provides supportive information regarding the effect of copper ion (Cu2+) released from intrauterine devices (IUD) in preventing pregnancy[18] .

In this study no correlation between body mass index and level of seminal plasma concentrations of zinc and copper.

## Conclusion

Based on the findings of this study and other reported results, there was a significant low level of seminal plasma zinc and increased concentration of copper in the infertile men. Therefore zinc and copper concentration in semen plasma contribute significantly to male fertility [19,20].

## Recommendations

On the basis of the results of this study and other recent ones findings, it is recommend that the concentration of zinc and copper in seminal plasma to be considered in laboratory investigation of infertility and zinc nutritional supplement to be offered to those who need it, as Zn deficiency may be an important risk factor for lowered semen quality [21,22,23] .

## Conflict of interests' declaration

All authors have no conflict of interests to be declared

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