

Assessment of Seminal Plasma Trace Elements among Infertile Sudanese Males in Khartoum State, 2019

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Abstract

Background: Male infertility is a multifaceted state and overlaps a lot of factors and affects infertility in about 8–5 % of the people in the world and the man is responsible for 40% of these cases. Dietary insufficiency of trace element, zinc and copper may play a role in male infertility as trace element plays an important role not only in normal testicular improvement, but also in spermatogenesis and sperm motility. **Objective:** The current study was intended to analyze the level of seminal plasma trace elements mainly zinc and copper amongst dissimilar groups of infertile men. **Method:** The concentrations of zinc and copper were measured in 160 semen samples from normozoospermic, oligoathenospermic, and azoospermic men using the atomic absorption spectroscopy, data was analyzed using the statistical software package SPSS version 17. **Result:** Outcomes showed that the mean values of seminal plasma zinc concentrations were significantly decreased in the two groups of infertile male subjects, azoospermic (P.value=0.000), and oligozoospermic (P.value=0.013) compared with fertile males, while there was significant decrease in seminal plasma copper concentration of azoospermic patients compared to control (P.value=0.000), and significant decrease in oligozoospermic patients compared to control (P.value=0.000).

Keywords: Seminal Plasma, Trace Elements Infertile Male.

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INTRODUCTION

Infertility is complicated and has manifold reasons and outcomes depending on the sex, sexual history, life style and cultural environment [1]. Infertility have an effect on about 8% to 12% of the world's population and in about half of cases, men are either the single reason or add to couple's infertility [2]. Seminal plasma is very vital for sperm metabolism, function, survival, and transport in the female genital tract. Cations such as Na, K, Ca, and Mg set up osmotic balance, as necessary trace elements are components of several essential enzymes in the seminal plasma [3]. The probable effect of the trace elements particularly Cu and Zn on male infertility is a topic of great interest [1], rising proof of a direct association of zinc was found with seminal parameters [4]. Zinc is the second major element, after iron, in seminal plasma. It maintains the cell membrane and nuclear chromatin of spermatozoa [5]. It may also have an antibacterial role [6], defends testis from the degenerative alterations [7].

It regulates mechanism of capacitation and acrosome reaction [8]. Zinc has a significant function in normal testicular growth, spermatogenesis and sperm motility [9]. It is a cofactor for a number of metalloenzymes in human semen, concerned in DNA transcription and protein production. Shortage of zinc in the reproductive system leads to hypogonadism and gonadal hypo function [6, 10, 11] reported that zinc in seminal plasma is implicated in nuclear chromatin decondensation and acrosin activity. Zinc insufficiency in the nucleus may destabilize the quaternary structure of chromatin; a characteristic essential for the fertilizing capability of the spermatozoa [12, 13] conducted an experiment in adult males and reported that production of testosterone b [14, 8]. Preceding study discussed the role of zinc in exchange of testosterone into its biologically active form 5 α -dihydrotestosterone (DHT) and mentioned that reduction of dietetic zinc May reduce semen volume and serum testosterone levels [15]. Zinc content in seminal plasma is mostly secreted by the prostate gland and may reflect prostatic function. Copper is an

imperative element for many metalloenzymes and metalloproteins that are concerned in energy metabolism. It works in diverse ways in order to preserve normal environment for spermatozoa for normal fertilization to occur [16]. Though, a higher level is toxic to a variety of cells, including human spermatozoa [16]. In *in vitro* studies, established that utilize of Cu in intrauterine devices stop conception [16]. The recent study was intended to assess seminal plasma levels of zinc and copper and to correlate their concentrations with a variety of semen parameters between fertile and infertile Sudanese male.

Rationale

The determination of spermatozoa concentration, morphology and motility remains the primary clinical means for the evaluation of male infertility. Nutritional deficiency of trace element, zinc and copper may engage in recreation a role in male infertility.

This study aimed to enrich data about the level of these vital trace elements between infertile Sudanese men.

OBJECTIVES

General objective

To assess the seminal plasma trace elements among infertile Sudanese males in Khartoum state.

Specific Objectives

- To estimate seminal plasma zinc & copper among infertile males.
- To compare between Azoospermia and Oligoasthenospermia in the level of seminal plasma trace elements.

MATERIALS AND METHODS

Study design

Case control hospital based study.

Study area

Reproductive care center in Al Muk Nemer Street in Khartoum state.

Study Duration

The study was carried out during the period from December 2016 to September 2019.

Study Population

Infertile Sudanese male referred to the study setting, by various fertility centers and hospitals in Khartoum state during study period.

Inclusion Criteria

Males with oligoasthenospermia and azoospermia as test group and normal males (age group 24 – 78 years) belong to the same socioeconomic status were selected as control group.

Exclusion Criteria

Infertile men under hormonal treatment and diabetic patients.

Sample Size and Study population

One hundred and sixty blood samples were collected in this study.

Case group: 80 infertile male patients (oligoasthenospermia and azoospermia).

Control group: 80 apparently healthy individuals.

Blood Sample Collection and Analysis

Before collection, a local antiseptic (70% alcohol) was used to clean the skin, venous blood (about 4 ml) was taken from each participant in lithium heparin container, then the centrifugation will be done at 3000 rpm for 3-5 minute to obtain plasma, used for measurement of zinc and copper.

Collection of Semen Samples and analysis

Semen was collected by masturbation into a sterile plastic specimen container at the hospital. Subjects were instructed to abstain from ejaculation for at least 72 hours prior to producing the semen sample. The sample was liquefied for at least 20 minutes, but no longer than 1 hour prior to performing a routine semen analysis, which included measurements of volume, pH, sperm concentration, sperm motility and morphology and direct microscopic examination.

Estimation of sperm counting will be done using the Neubauer chamber. Sperm analysis was carried out according to the World Health Organization guidelines, based on the sperm concentration the infertile subjects were classified as follows:

- Normozoospermia (> 20 million sperm /ml and normal semen profile)
- Oligoasthenospermia (<20 million sperm/ml and motility grade C or D)
- Azoospermia (no spermatozoa)

In proven fertile controls, the sperm count ranged from 20 – 120 million sperm /ml

Seminal Plasma Collection and Analysis

Sample Preparation

Seminal plasma specimens were diluted 1:10 with 0.5% v/v HNO₃ to determine the concentrations of zinc and copper.

Instrument

Atomic absorption spectrophotometer (*Buck Scientific, model 210 VGP*).

Principle of Atomic Absorption Spectrophotometer

Atomic absorption spectrophotometer utilizes the phenomenon that atoms absorb, radiation of particular wavelength. When the light from a hollow cathode lamp shines into the flame, ground state atoms of the element wanted to be measured absorb some of the light resulting in a decrease of the light (atomic absorption).

Data Collection

Direct questionnaire was done to obtain clinical data for each participant and sample.

Data Analysis

Data was analyzed using Statistical Package for Social Science Software (SPSS).

Ethical Considerations

This study was approved by the research committee – College of Medical Laboratory Sciences - Shendi University. Informed consent was obtained from each participant before taking the samples.

RESULTS

This is a case control hospital based study conducted in Khartoum state in the Reproductive Care Center during the period from December 2016 to September 2019. This study included 160 samples, 80 from these samples were collected from infertile males as case group (40 of them collected from Azoospermia 25% and the rest from Oligoasthenospermia 25%) and the rest of the samples collected from normal male (Normozoospermia 50%) as control group. The results of the study were presented in texts and tables.

Table-1: Independent sample T.test showed mean and std. deviation of seminal plasma Copper among case group (Azoospermia and Oligoasthenospermia) and Control group

	Azoospermia		Oligoasthenospermia	
	Case	Control	Case	Control
No	40	80	40	80
Mean (mg/dl)	0.05	0.13	0.057	0.123
Std. Dev	0.038	0.037	0.035	0.037
P. Value	0.000		0.000	

P. value \leq 0.05 is considered significant.

Table-2: Independent sample T. test showed mean and std. deviation of seminal plasma Zinc among case (Azoospermia and Oligoasthenospermia) group and Control group

	Azoospermia		Oligoasthenospermia	
	Case	Control	Case	Control
No	40	80	40	80
Mean (mg/dl)	16.0	22.1	20.7	22.1
Std. Dev	6.55	4.44	5.76	4.44
P. Value	0.000		0.13	

P. value \leq 0.05 is considered significant.

Table-3: Paired sample T. test showed Mean of seminal Trace elements among (Oligoasthenospermia) and Azoospermia

Paired Group	Mean (mg/dl)	N	Std. Deviation	P. Value
Copper level AZO	0.05	40	0.038	0.474
Copper level OAS	0.056	40	0.035	
Zinc level AZO	16.0	40	6.55	0.006
Zinc level OAS	20.6	40	5.76	

P. value \leq 0.05 is considered significant.

DISCUSSION

Statistical analysis of the gathered data shows significant decrease in mean of seminal plasma Copper among case (Azoospermia) group compared to Control group with P. Value (0.000). The results revealed a marked decrease in seminal plasma copper in case (oligoasthenospermia) group, mean (0.057 mg/l) while it is (0.123mg/l) in control group. Agree with [17, 18]

The laboratory analysis for seminal plasma samples demonstrate very low level of zinc in patients with azoospermia compared to control group with mean of (16.0 mg/l) and (22.1) respectively. also the results show insignificant decrease in seminal plasma zinc level among patients with oligoasthenospermia compared to control group, with mean of (20.7 mg/l) and (22.1mg/l) respectively. This result agrees with Yosra M et al 2015 and Mohammad Shoaib Khan 2011.

The trace elements Zn and magnesium (Mg) found in seminal plasma originate primarily from the prostate gland and may reflect prostatic secretory function. Studies have suggested that Mg may play a role in spermatogenesis, particularly in sperm motility [19]. The same is true for Zn, although the results of several studies are still contradictory [20]. Zn, B complex vitamins (B6, B12 and folic acid), vitamin C, and antioxidants are critical nutrients in the male reproductive system for proper hormone metabolism, sperm formation, and motility [21]. Moreover, a positive correlation has been observed between the sperm count and seminal plasma Zn concentration in oligozoospermic and azoospermic patients [22].

CONCLUSION

According to our result, we conclude that there was significant decrease in seminal zinc and copper in patients with azoospermia and oligoasthenospermia as compared with control group.

RECOMMENDATIONS

According to our outcomes we recommend:

- Molecular based study to confirm effect of zinc deficiency on the spermatozoa integrity.
- Experimental supplementation of zinc & copper for patients with Azoospermia and Oligoasthenospermia.
- Health education plan should be achieved to aware population for the significance of micronutrient.

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