ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research

Vol. 13, Issue, 02, pp. 61630-61633, February, 2023

https://doi.org/10.37118/ijdr.26297.02.2023

RESEARCH ARTICLE **OPEN ACCESS**

ASSESSMENT OF SEMINAL PLASMA ZINC AND COPPER AMONG INFERTILE SUDANESE MALES IN KHARTOUM STATE

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ARTICLE INFO

Article History:

Received 20th January, 2023
Received in revised form
28th January, 2023
Accepted 06th February, 2023
Published online 25th February, 2023

KeyWords:

Infertile Male, Seminal Plasma, Zinc, Copper, Sudanese.

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ABSTRACT

Background: Male infertility is a complex condition and overlaps many factors and affects infertility in about 5-8% of the population in the world and the man is responsible for 40% of these cases. Nutritional deficiency 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 development, but also in spermatogenesis and sperm motility. ***Objective:*** The current study was intended to analyze the level of seminal plasma trace elements (zinc and copper) amongst dissimilar groups of infertile men. ***Materials and method:*** This is a facility based study, conducted in Reproductive Care Center in AlmukNemer Street in Khartoum state. Which include 160 semen samples collected from normozoospermic, oligoathenospermic, and azoospermic men and clinical information collected by the use of questionnaire. The concentrations of zinc and copper were measured by using the atomic absorption spectrophotometer, then the data was analyzed using the statistical software package SPSS version 17. ***Result:*** The results showed that the mean values of seminal plasma copper concentrations were significantly decreased in the two groups of infertile male subjects, azoospermic (p. value 0.000), and oligozoospermic (p. value 0.000) compared with fertile males, while there was significant decrease in seminal plasma zinc concentration of azoospermic patients compared to control (p. value 0.000), and insignificant decrease in oligozoospermic patients compared to control (p. value 0.130). The study showed statistically insignificant deferent in the seminal plasma level of copper between azoospermia and oligoathenospermia, the p. value is (0.474), and statistically significant decrease in the seminal plasma level of zinc in azoospermia when compared with oligoathenospermia, the p. value is (0.006). ***Conclusion:*** The seminal plasma zinc and copper is significant decreased in patients with azoospermia as compared with control group. And significant decrease in the seminal plasma copper in patients with oligoasthenospermia as compared with control group, and insignificant decrease in the seminal plasma zinc in patients with oligoasthenospermia as compared with control group.

Citation: ***Abdelrhman Mohamed Sid Ahmed, Salman Taha Ahmed Elmukashfi, Mohamed Elamin Almahy, Rasha Alsamani, Mohamed Abdelrhman Eltahir and Elhussein M. Ibrahim. 2023.*** "Assessment of seminal plasma zinc and copper among infertile sudanese males in Khartoum State*", International Journal of Development Research*, 13, (2), 61630-61633.

INTRODUCTION

Infertility is complicated and has manifold reasons and outcomes depending on the sex, sexual history, life style and cultural environment (Gurunath, 2011). 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 (Barnett, 2003). 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 elementsare components of several essential enzymes in the seminal plasma (Meizel, 1997). The probable effect of the trace elements particularly Cu and Zn on male infertility is a topic of great interest (Gurunath *et al*., 2011), rising proof of a direct association of zinc was found with seminal parameters (Stanwell-Smith, 1983). Zinc is the second major element, after iron, in seminal plasma. It maintains the cell membrane and nuclear chromatin of spermatozoa (Kvist, 1980). It may also have an antibacterial role (Langley, 1987), defends testis from the degenerative alterations (Batra *et al*., 2004). It regulates mechanism of capacitation and acrosome reaction (Wong *et al*., 2011). Zinc has a significant function in normal testicular growth, spermatogenesis and sperm motility (Ebisch, 2007). 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 (Langley *et al*., 1987; Sandstead *et al*., 1967 and Steven *et al*., 1982), 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 (Prasad, 1991 and Netter, 1981) conducted an experiment in adult males and reported that production of testosterone b (Hunt, 1992 and Wong, 2001). Preceding study discussed the role of zinc in exchange of testosterone into its biologically active form 5α-dihydrotestosterone (DHT) and mentioned that reduction of dietetic zinc May reduce semen volume and serum testosterone levels (Skandhan, 1992). 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. Though, a higher level is toxic to a variety of cells, including human spermatozoa. In vitro studies, established that utilize of Cu in intrauterine devices stop conception (Roblero *et al*., 1996). The recent study was intended to assess seminal plasma levels of zinc and copper among infertile Sudanese male.

MATERIALS AND METHODS

Study design: This is a facility based study.

Study area: Reproductive Care Center in AlmukNemer 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: Male with oligoasthenospermia and azoospermia as test group and normal males (age group 24 – 78 years) belonging to the same socioeconomic status were selected as control group.

Exclusion criteria: Infertile male under hormonal treatment and diabetic patients.

Sample size: One hundred and sixty samples were collected in this study. Which is divided into two groups, a 80 samples collected from infertile male patients (oligoasthenospermia and azoospermia) as case group, and the rest of samples (80 samples) collected from apparently healthy individuals as control group.

Collection of semen specimens 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.

Sample preparation: Seminal plasma were diluted 1:10 with 0.5% v/v HNO_3 to determine the concentrations of zinc and copper.

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

Data collection: Direct questionnaire was done to obtain clinical data for each participant and seminal specimens was collected.

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 specimens. Also verbal consent was obtained fromadministrative in reproductive care center.

RESULTS

This is a facility 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 oligoathenospermia 25%) and the rest of the samples collected from normal male (normozoospermia 50%) as control group, to assess the seminal plasma trace elements (zinc and copper) among infertile Sudanese males in Khartoum state. The data collected by the use of questionnaire and semen specimens, and the levels of the trace elements is measured by Atomic absorption spectrophotometer (Buck Scientific, model 210 VGP). Then the collected data is analyzed by the use of SPSS, and the results is presented in tables. The results of the study showed statistically significant decrease in the seminal plasma level of copper in case group (azoospermia and oligoathenospermia) when compared with control group, the means of copper in case group is (azoospermia: 0.050 and oligoathenospermia: 0.057) and in control group is (0.13) with p. value (0.000) respectively, that illustrated in Table 3.1. And showed statistically significant decrease in the seminal plasma level of zinc in azoospermia and insignificant decrease of zinc in oligoathenospermia and oligoathenospermia is (16.0 and 20.7) and in control group is (22.1) with p. value (azoospermia: 0.000 and oligoathenospermia: 0.130) respectively, that illustrated in Table 3.2. The study showed statistically insignificant deferent in the seminal plasma level of copper between azoospermia and oligoathenospermia, the mean is (0.050 and 0.056) with p. value (0.474) respectively. And statistically significant decrease in the seminal plasma level of zinc in azoospermia when compared with oligoathenospermia, the mean is (16.0 and 20.6) with p. value (0.006) respectively that illustrated in Table 3.3.

Table 3.1. Independent sample T. test showed the mean and std. deviation of seminal plasma copper among case group (azoospermia and oligoathenospermia) and control group

Study groups		No	Mean (mg/dl)	Std. Dev	P.Value
Azoospermia	Case	40	0.050	0.038	0.000
	Control	80	0.13	0.037	
Oligoathenospermia	Case	40	0.057	0.035	0.000
	Control	80	0.13	0.037	

P. value ≤ 0.05 is considered significant

Table 3.2. Independent sample T. test showed the mean and std. deviation of seminal plasma zinc among case (azoospermia and oligoathenospermia) group and control group

Study groups		No	Mean (mg/dl)	Std. Dev	P.Value
Azoospermia	Case	40	16.0	6.55	0.000
	Control	80	22.1	4.44	
Oligoathenospermia	Case	40	20.7	5.76	0.130
	Control	80	22.1	4.44	

P. value ≤ 0.05 is considered significant

Table 3.3. Paired sample T. test showed the mean of seminal trace elements among oligoathenospermia and azoospermia

Paired Group	Mean (mg/dl)	N	Std. Deviation	P.Value
Copper level AZO	0.050	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	

DISCUSSION

Statistical analysis of the gathered data shows significant decrease in the level of seminal plasma copper in azoospermia compared to control group, the mean of seminal plasma copper in azoospermia is (0.050) and in control group is (0.13) with P. value (0.000), and showed significantly decrease in the level of seminal plasma copper in oligoathenospermia compared to control group, the mean of seminal plasma copper in oligoathenospermia is (0.057) and in control group is (0.13) with P. value (0.000), that illustrated in table 3.1. This agree with (Altaher, Y.M. and Abdrabo, A.A., 2015 and Khan, M.S., *et al*, 2011) [17 & 18]. The laboratory analysis demonstrate statistically significant decrease in the level of zinc in seminal plasma samples in patients with azoospermia compared to control group, the mean of seminal plasma zinc in azoospermia is (16.0) and in control group is (22.1) with P. value (0.000), also the results show insignificant decrease in seminal plasma zinc level among patients with oligoathenospermia compared to control group, the mean of seminal plasma zinc in oligoathenospermia is (20.7) and in control group is (22.1) with P. value (0.130), that illustrated in table 3.2. This result agree with (Altaher, Y.M. and Abdrabo, A.A., 2015 and Khan, M.S., *et al*, 2011) [17 & 18]. The trace elements zinc and magnesium (Mg) found in seminal plasma originate primarily from the prostate gland and may reflect prostatic secretary function. Studies have suggested that Mg may play a role in spermatogenesis, particularly in sperm motility (Abou-Shakra, F.R., *et al*, 1989) [19]. The same is true for zinc, although the results of several studies are still contradictory (Lewis-Jones, D.I., *et al*, 1996) [20]. Zinc, 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 (Czeizel, A.E., 1998) [21]. Moreover, a positive correlation has been observed between the sperm count and seminal plasma zinc concentration in oligozoospermic and azoospermic patients (Ali, H., *et al*, 2007) [22].

CONCLUSION

From the results of this study we conclude that there was a significant decrease in the seminal plasma zinc and copper in patients with azoospermia as compared with control group. And significant decrease in the seminal plasma copper in patients with oligoasthenospermia as compared with control group, and insignificant decrease in the seminal plasma zinc in patients with oligoasthenospermia as compared with control group.

Competing interests: Authors have declared that no competing interests exist.

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