Status of Zinc and Copper Concentrations in Seminal Plasma of Male Infertility and Their Correlation with Various Sperm Parameters

Basil Oied Mohammed Saleh*, Nawal Khiry Hussain**, Ali Yakub Majid ***, Ban Thabet****, Khazraj Abbas Fadhil****

ABSTRACT:

BACKGROUND:

Human semen contains high concentrations of zinc (Zn) and copper (Cu) in bound and ionic forms .The presence of abnormal levels of these trace elements may affect spermatogenesis with regard to production , maturation , motility , and fertilizing capacity of the spermatozoa. The aim of this study is to evaluate the levels of Zn and Cu in seminal plasma in different groups of male infertility and to correlate their concentrations with various semen parameters.

METHODS:

Forty primary infertile male individuals ,who had regular unprotected intercourse for at least one year without conception with their partners ,aged 25-40 years were involved in the present study .After seminal plasma fluid analyses they were grouped as, azoospermic (n=12), oligoasthenozoospermic (n=16) ,and teratozoospermic (n=12) .Twelve fertile males selected from general population and after seminal fluid investigation were taken as normospermic control group .Zinc and Copper concentrations in separated seminal plasma of each infertile male and fertile control subject were determined by atomic absorption spectrophotometer.

RESULTS:

This study showed significant decrease of seminal plasma Zn mean (\pm SEM) value in oligoasthenozoospermic infertile males than in fertile male controls (p<0.037) .In azoospermic males, the mean (\pm SEM) value of seminal plasma Zn levels was decreased (but still beyond the significant level,p=0.08) when compared with that of fertile males. With regard to seminal plasma copper level there was insignificant decrease in its level in azoospermic males and insignificant increase in its concentration in oligoasthenozoospermic and in teratozoospermic males when compared with that of healthy fertile males. In addition, there was a significant decrease in seminal plasma copper concentration in azoospermic males than in oligoasthenozoospermic males (p<0.035). The results also revealed a significant positive correlation between the sperm motility values and the seminal plasma zinc levels in oligoasthenozoospermic group(r=0.68, p<0.022) along with a significant negative correlation between sperm morphology values and zinc seminal plasma levels in teratozoospermic group (r=-0.63,p<0.049). **CONCLUSION:**

On the basis of the findings of this study, it seems that the estimation of seminal plasma levels of zinc and copper may aid in investigation and treatment of infertile males.

KEYWORDS: infertility, azoospermic, oligoasthenozoospermic, seminal Zinc, seminal Copper.

INTRODUCTION:

Infertility is complex and has many causes and consequences depending on the gender, sexual

*Dept. of Physiological Chemistry, College of Medicine, Univ. of Baghdad.

**Institute of Emberyo Research and Infertility,Univ. of AL- Nahrain.

***Poisoning Consultation Center.

****Institute of Emberyo Research and Infertility,Univ. of AL- Nahrain.

*****Al-Mussaib Hospital

history, life style of society and cultural background of people it affect ⁽¹⁾.

Infertility affects approximately 8% to 12% of the world's population and in about half of cases male are either the single cause of or contribute the couple's infertility ⁽²⁾.More than 90% of male infertility cases are due to low sperm counts ,poor sperm quality ,or both .In 30-40% of cases of sperm abnormlities ,the cause is unknown .It may be the end result of one or more factors that include chronic illness ,malnutrition ,genetic defects ,structural

abnormalities ,and environmental factors⁽³⁾ Human semen contains high concentrations of trace elements like calcium (Ca) ,magnesium (Mg), copper (Cu) ,selenium (Se) ,and zinc (Zn) in bound and free (ionic) forms .These trace elements play very vital role in affecting various parameters of semen ⁽⁴⁾.

The testicular plasma ,that is ,the fluid composed of the secretions originating in the seminiferous tubules ,tubuli recti ,rete testis ,and ductuli efferents ,and the epididymal plasma serve as nutrient medium in which maturation of the developing spermatozoa takes place ^(5,6) .Trace elements have been shown to be essential for testicular development and spermatogenesis ⁽⁷⁾. Zinc in seminal plasma stabilizes the cell membrane and nuclear chromatin of spermatozoa ^(8, 9) .Zinc may also be the primary factor responsible for the antibacterial activity of the seminal plasma ⁽¹⁰⁾ and protect the male gonads against the degenerative changes ⁽¹¹⁾.

It may also play a regulatory role in the process of capacitation and acrosome reaction $^{(4, 12)}$.Low content of this metal (Zn) has been suggested to diminish fertility potential in males $^{(13)}$.

Zinc deficiency leads to gonadal dysfunction, decreases testicular weight, and causes shrinkage of seminiferous tubules ⁽¹⁴⁾. Copper is an important element for numerous metalloenzymes and metalloproteins that are involved in energy or antioxidant metabolism. However, in its ionic form (Cu²⁺) and at high level, this trace element rapidly becomes toxic to a variety of cells, including human spermatozoa ⁽⁶⁾.

It has been identified that Cu is highly toxic for sperm ⁽¹⁵⁾. In vitro studies, it has been demonstrated the effect of Cu in intrauterine devices preventing conception ⁽¹⁶⁾. The present study was designed to evaluate seminal plasma levels of zinc and copper and to correlate their concentractions with various semen parameters among fertile and infertile male subjects.

SUBJECTS AND METHODS:

This study was carried out at the Institute of Emberyo Research and Infertility, Univ. of AL- Nahrain, Baghdad, during the period from Feb.2006 to August 2006. Forty primary infertile male subjects ,who had regular unprotected intercourse for at least one year without conception with their partners , aged 25-40 years were included in this study .

Patients had no external ,infections ,traumatic abnormalities which could be implicated in the development of infertility . At first clinic attendance,

a detailed background history and physical examination were done on both husband and wife. Semen specimens from all infertile patients were collected into sterile polystyrene jars after an abstinence period of 3 to 5 days .A portion of each semen sample was examined for sperm count ,motility and morphologic features .Infertile male patients were then divided into the following three groups [according to their sperm count /motility and /or morphology ,WHO criteria ,1992⁽¹⁷⁾]

Group I: Azoospermic (sperm count =zero, n=12), **Group II** :Oligoasthenozoospermic (sperm count $<20\times10^6/m1$, motility<50%, n=16), and

Group III: Teratozoospermic (sperm count > 20×10^6 /ml, motility >50%, morphology<50%, n=12). Twelve fertile males whose partners had conceived within one year and having sperm count more than 20 million /m1 with motility and morphology more than >50% were selected from general population and taken as normospermic control group .

After liquefaction ,the seminal plasma was collected after centrifugation at 2000 rpm for 15-20 minutes .Supernatant ,the seminal plasma ,was transferred in fresh tubes and stored frozen ,at-20°C until assay .The concentrations of zinc and copper in separated seminal plasma of each infertile patient and fertile control were determined by atomic absorption spectrophotometer, Buck Scientific, model 210 VGP. **RESULTS:**

Results are expressed as mean $(\pm$ SEM) form each parameter. Table 1 summarizes the mean $(\pm$ SEM) value on seminal plasma levels of Zn and Cu elements in the three groups of infertile male subjects (azoospermia ,oligoasthenozoospermia and teratozoospermia) and in fertile control group.

The mean (+SEM) value of seminal plasma Zn concentrations was significantly decreased in oligoasthenozoospermic infertile males than in fertile male controls (2.31+0.59 mg/dl, 3.85+0.59 mg/dl, respectively, p<0.037, Table 1). In azoospermic patients ,the mean (+SEM) value of seminal plasma Zn level was decreased in comparison with that of controls ,but still beyond the significant value (p=0.08) .There was no significant differences in seminal plasma Zn concentration between the teratoazoospermic patients and fertile controls as well as among the different groups of infertile males (Table 1).Table 1 also shows that the mean (+SEM) value of seminal plasma Cu levels was insignificantly decreased in azoospermic patients when compared with that of fertile control group.

Furthermore ,this element ,Cu ,was insignificantly increased in oligoasthenozoospermic infertile males and in teratozoospermic infertile males than in fertile control subjects (Table 1).

Among infertile male groups there was a significant decrease in plasma Cu level in azoospermic patients than in oligoasthenozoospermic infertile group $(0.30\pm0.14 \text{ mg/dl}, 0.70\pm0.13 \text{ mg/dl};$ respectively p<0.035, Table 1).

 Table 1:Zinc and Copper Concentrations in Seminal Plasma in Three Groups of Infertile Males and Fertile

 Control Group .The Values are Expressed as Mean(<u>+</u> SEM).

Parameters	Azoospermic (n=12)	Oligoastheno- zoospermic (n=16)	Teratozoos- permic (n=12)	Normospermic (n=12)
Seminal Plasma Zn concentration (mg/dl)	2.40 <u>+</u> 0.64	2.31 <u>+</u> 0.59 *	2.97 <u>+</u> 0.61	3.85 <u>+</u> 0.59
Seminal Plasma Cu concentration (mg/dl)	0.30 <u>+</u> 0.14 **	0.70 <u>+</u> 0.13	0.42 <u>+</u> 0.14	0.41 <u>+</u> 0.14

p<0.05 when oligoasthenozoospermic group is compared with normospermic group.

**p<0.05 when azoospermic group is compared with oligoasthenozoospermic group.

Correlation coefficient of seminal plasma concentration of Zn and Cu with various seminal parameters in azoospermic ,oligoasthenozoospermic and teratozoospermic males are showed in Table 2.

There was a significant positive correlation between seminal plasma Zn concentrations and sperm motility in oligoasthenozoospermic group (r=0.68; p < 0.022) as well as a significant negative correlation between Zn seminal plasma levels and sperm morphology in treatozoospermic group (r = -0.63; p < 0.049, Table 2).

Table 2: Correlation Coefficient (r) of Seminal Plasma Zinc and Copper Concentrations with Semen Parameters.

Parameters	Azoospermic(n=12) Zn (r:p- value) ;Cu (r:p- value)	Oligoastheno- zoospermic (n=16) Zn (r:p-value) ;Cu (r:p-value)	Teratoazoos- permic (n=12) Zn (r:p-value) ;Cu (r:p-value)
Sperm count	(0:0);(0:0)	(-0.31 :0.35);	(0.19:0.60);
(10 ⁶ /ml)		(0.014:0.96)	(0.53:0.15)
Sperm	(0:0);(0:0)	(-0.095 : 0.78);	(-0.63 : 0.049 *);
Morphology (%)		(-0.29:0.37)	(0.005 : 0.99)
Motility (%)	(0:0); $(0:0)$	(0.68 : 0.02 *); (-0.41 : 0.18)	(0.07 : 0.84) ; (-0.07 : 0.86)

• P-value < 0.05 was considered significant.

DISCUSSION:

The results of the present study showed that there was significant low concentration of seminal plasma Zn in oligoasthenozoospermic males when compared with that of fertile controls. Zinc level was also decreased in azoospermic infertile males in comparsion with fertile healthy controls, but does not reachs the significant level. With regard to plasma seminal Cu level, it was significantly reduced in azoospermic male patients than in

oligoasthenozoospermic infertile males. Ali and associates, 2005 ⁽¹⁸⁾ and Ali et al, 2007 ⁽⁴⁾ observed significant decrease in seminal plasma Zn in oligoospermic and azoospermic infertile males. These authors concluded 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 , 1999 ⁽¹⁹⁾ have found no significant differences in the

mean values of seminal plasma Zn levels between the entire group of samples from infertile individuals and those from fertile individuals .However, the latter authors observed that Zn concentration of the seminal plasma in azoospermic and oligoasthenozoospermic patients was significantly lower than that in other groups of male infertility .The authors concluded that adequate seminal concentration of the Zn is required for normal sperm function. It has been demonstrated that Zn in human semen is derived from the prostate ⁽¹⁹⁾. One important function of the Zn seems to be its regulatory role in the level of dihydrotestosterone by modulating the 5 α - reductase enzyme activity ,which is located in the microsomal and nuclear fraction of the prostatic cell ⁽²⁰⁾. Other important roles suggested for the Zn include maintenance of the structural integrity of macromolecules ⁽²¹⁾, regulation of protein and nucleic acid metabolism (22), and activation of mitochondrial function and ATP production ⁽²³⁾. A clinical study demonstrated that adult males experimentally deprived of Zn showed that the Leydig cell synthesis of testosterone was disturbed ⁽²⁴⁾. Zinc appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa and /or leukocytes in human semen after ejaculation ⁽²⁵⁾. Thus, it seems that seminal plasma, due to its adequate content of Zn, will exert protective, antioxidant - like activity sufficient to cope with the excessive amount of superoxide anions ⁽²⁶⁾. The abnormal spermatozoa that infertile men have a higher percentage of it would be a source of superoxide anions that bind with Zn present in the seminal plasma and thus reduce the Zn levels (7). Omu and associates, 1998 ⁽²⁷⁾ have demonstated that Zn therapy results in significant improvement in sperm quality with increases in sperm density, progressive motility, and improved conception and pregnancy outcome.

Our study also demonstrated that seminal plasma Zn concentration was significantly correlated with sperm motility. This observation is in accordance with results reported by other authors ^(7, 19) and differs from the results of other studies ⁽⁴⁾. Human prostatic fluid causes the immotile sperm in vesicular fluid to become motile and this change may be induced by Zn in prostatic fluid ⁽²⁸⁾. Zinc has been detected in human sperm and this suggested its role in motility ⁽²⁹⁾. The results of the present study also showed that Cu concentration was insignificantly increased in oligoasthenozoospermic and in teratozoospermic males than in fertile male controls. This result is in

compatible with that observed by Wong et al, 2001 ⁽⁶⁾. Copper is an essential trace element that plays an important role in several enzymes such as superoxide dismutase. 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. Superoxide dismutase (Cu-metalloenzyme) protects human spermatozoa from this peroxidative damage ⁽⁶⁾.

However, this element in its ionic form (Cu²⁺), particularly, at high concentration rapidly become toxic to a variety of cells, including human spermatozoa⁽⁶⁾. In vitro studies, Roblero et al ⁽¹⁶⁾ have demonstrated the effect of Cu in intrauterine devise in preventing conception.

CONCLUSION:

On the basis of the observations of the present study and other reports, seminal zinc and copper may contribute to fertility through their effects on various semen parameters. Adequate seminal plasma concentration of Zn and Cu are required for normal sperm function and that high toxic concentration of these elements in seminal plasma is apparently related to defective morphology and /or motility in infertile males. It seems that the estimation of seminal plasma Zn and Cu may help in investigation and treatment of infertile males.

REFERENCES:

- **1.** Kerry LW. Defining infertility. Family Health International. 2003 ;23 :4-8
- **2.** Barbara B. Men contribute to and suffer from infertility. Family Health International. 2003 ;23 :17-20
- **3.** Haymond S and Gronowski A M. Reproductive related disorders: In Tietz Textbook of Clinical Chemistry and Molecular Diagnostics edited by Burtis CA, Ashwood ER and Bruns DE, Hsevier Saunders, 2006; 2079-2152.
- **4.** Ali H, Ahmed M, Baig M, and Ali M. Relationship of zinc concentrations in blood and seminal plasma with various semen parameters in infertile subjects. Pakistan Journal of Medical Sciences. 2007; 23: 111-114.
- 5. Mann T. The biochemistry of semen and of the male reproductive tract. London: Methuen & Co LTD, 1964.

- 6. Wong W Y, Flik G, Groenen PMW, Swinkels DW, Thomas CMG, Copius –Peereboom JHJ, Merkus HMWM and Steegers-Theunissen RPM. The impact of calcium, magnesium, zinc and copper in blood and seminal plasma on semen parameters in men. Reproductive Toxicology. 2001; 15: 131-136.
- CHia S-E, Ong (C-N, Chua L-H, Ho L-M and Tay S-K. Comparison of zinc concentration in blood and seminal plasma and the various sperm parameters between fertile and infertile men. Journal of Andrology. 2000; 21,53-57.
- **8.** Chvapil M. New aspects in biological role of zinc. A stabilizer of marcromolecules and biological membrane. Life Science. 1973; 13, 1044-1049.
- **9.** Kvist U. Sperm nuclear chromatin decondensation ability. An in vitro study on ejaculated human spermatozoa. Acta physical Scand Suppl 1980; 486,1-24.
- **10.** Langley J, Goldsmid J, Davies N. Venereal trichomoniasis role in men. Genitourin Med. 1987; 63, 264-267.
- **11.** Batra N, Nehru B, Bansal MP. Reproductive potential of male Portan rats exposed to various levels of lead with regard to zinc status. Br J Nutr. 2004; 91, 387-391.
- **12.** Riffo M, Levia S, Astudillo J. Effect of zinc on human sperm motility and acrosome reaction. Inter J Androl. 1992; 15, 229-237.
- **13.** Marmar JL, Katz S, Praiss DE, Debenedicitis TJ. Semen zinc level in infertile and postvasectomy patients and patients with prostatitis . Fertility. Sterility. 1975; 26, 1057.
- **14.** Bedwal RS, Bahuguna A. Zinc, copper and selenium in reproduction. Experientia. 1994; 50, 626-640.
- **15.** SKandhan KP.Review on copper in male reproduction and contraception. Rev Fr Gynecol Obstet. 1992; 87: 594-598.
- **16.** Roblero L, Guadarrama A, Lopez T, and Zegers Hochschild F. Effect of copper ion on the motility, viability, acrosome reaction and fertilizing capacity of human spermatozoa in vitro . Reprod Fertil Dev. 1996; 8,871-874.
- World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen – cervical Mucus Interaction, 3 rd edn. Cambridge Univ. Press, Cambridge, UK 1992, 6.

- 18. Ali H, Baig m, Rana MF, Ali M, Qasim R, and Khem AK. Relationship of serum and seminal plasma zinc level and serum testosterone in oligospermic and azoospermic infertile men. J Coll physicians Surg Pak. 2005; 15, 671-673.
- **19.** Fuse H, Kazama T, Ohta S and Fujiuchi Y. Relationship between zinc concentrations in seminal plasma and various sperm parameters. Intern Urology and Nephrology. 1999; 31, 401-408.
- **20.** Leak AC, Chisholm GB, Habib FK. The effect of zinc on α reduction of testosterorie by the hyperplastic human prostate gland. J Steroid Biochem. 1984; 20, 651.
- **21.** Chvapil M. New aspects in the biological role of zinc .A stabilizer of macromolecules of biological membranes. Life Science. 1973; 13,1041.
- **22.** Bettger WJ, O Dell BL .A critical physiological role of zinc in the structure and function of biomembranes. Life Science. 1981; 28, 1425.
- **23.** Yamaguchi M, Kura ,M, Okada S. Role of zinc as an activator of mitochondrial function in rat liver. Biochem. Pharmcol. 1982; 31,1289.
- 24. Netter A, Hartoma R, Nahail K. Effects of zinc administration on plasma testosterone and dihydrotestosterone and sperm count. Arch Androl. 1981; 7, 69-73.
- **25.** Irvine DS. Glutathione as a treatment for male infertility. Rev Reprod. 1996; 1,6-12.
- **26.** Gavella M, Lipovac V. In vitro effect of zinc on oxidative changes in human semen. Andrologia. 1998; 30, 317-323.
- 27. Omu AE, Dashti H, Al-Othman S. Treatment of asthenozoospermic with zinc sulphate : andrological , immunological and obstetric outcome. Eur J Obstet Gynecol Reprod Biol. 1998; 79, 179-184.
- **28.** Lindholmer C.The importance of seminal plasma for human sperm motility. Biol. Reprod. 1974; 10,533.
- **29.** Baccetti B, Pallini V, Burrini A G. The accessory fibers of the sperm tail. I I. Their role in binding zinc in mammals and cephalopods. J Ultrastract .Res. 1976; 54,261.

THE IRAQI POSTGRADUATE MEDICAL JOURNAL 80