The relationship between seminal plasma zinc levels and high molecular weight zinc binding protein and sperm motility in Iraqi infertile men

Omar F. Abdul-Rasheed, *MSc, PhD (Clinical Chemistry).*

ABSTRACT

الأهداف: لدراسة العلاقة بين مستوى الزنك في بلازما السائل المنوي ونسب البروتين الرابط للزنك عالي الوزن الجزيئي في الرجال الأصحاء والمصابين بالعقم.

الطريقة: أجريت هذه الدراسة في قسم الكيمياء والكيمياء الحياتية في كلية الطب – جامعة النهرين – بغداد – العراق، خلال الفترة مابين مارس 2005م وحتى فبراير 2006م. شملت الدراسة 60 مريضاً يعانون من حالات عقم مختلفة، والذين يراجعون مستشفى الكاظمية التعليمي ومركز أبحاث الأجنة وعلاج العقم في بغداد بشكل دوري. تم تقسيم المرضى اعتمادا على نتيجة فحص السائل المنوي إلى 32 مريضاً يعانون من عقم قلة النطف (53.3%)، 22 مريضاً من عقم فقد النطف (36.6%) و6 مرضى من عقم وهن النطف (10.0%)، أما المجموعة الضابطة فضمت 39 فرداً من الأصحاء بمتوسط عمر (31.87±3.76 عام) بهدف المقارنة، واللذين حصل لديهم إنجاب خلال عام قبل المشاركة بالدراسة.

النتائج: كان مستوى الزنك في الرجال المصابين بعقم قلة النطف هو 181.92±23.40µg/mL، أما في مجموعة عقم وهن النطف فيبلغ 195.33±13.00µg/mL، ولدى مجموعة عقم فقد النطف كان 178.50±18.61µg/mL، في حين كان التركيز في المجموعة الضابطة 184.66±21.31µg/mL.

خاتمة: إن نسبة البروتين الرابط للزنك عالي الوزن الجزيئي (HMW–Zn) وليس مستوى الزنك الكلي في بلازما السائل المنوي هو العامل المحدد لفعالية وحركية النطف في حالات العقم المختلفة.

Objectives: To evaluate the relationship between sperm motility and total seminal plasma zinc concentration and high molecular weight zinc bound protein values in infertile Iraqi men.

Methods: A case-control study was conducted at the Chemistry and Biochemistry Department, College of Medicine, Al-Nahrain University, Baghdad, Iraq between March 2005 to February 2006. The subjects for the study included 60 infertile male patients who were recruited Al-Kadhimiya Teaching Hospital, and Institute of Embryo Research and Infertility Treatment, Baghdad, Iraq. They were categorized according to their seminal parameters to oligozoospermia (n=32), azoospermia (n=22), and asthenozoospermia (n=6). Thirty nine fertile men (age range 31.87±3.76 years) were selected as controls, whose partners had conceived within the last year before participation with this study, and having normal spermiogram parameters. Seminal plasma zinc concentration and high molecular weight zinc binding proteins (HMW-Zn) were assayed in the ejaculates of fertile and infertile men.

Results: The seminal plasma zinc levels were 181.92 ± 23.40 µg/mL in the oligozoospermia group, 178.50 ± 18.61 µg/mL in the azoospermia group, 195.33 ± 13.00 µg/mL in the asthenozoospermia group, and 184.66 ± 21.31 µg/mL in the control group.

Conclusion: The HMW-Zn% is a good index of sperm function rather than the total seminal plasma zinc levels.

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From the Chemistry and Biochemistry Department, College of Medicine, Al-Nahrain University, Al-Kadhimiya, Baghdad, Iraq.

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Address correspondence and reprint request to: Dr. Omar F. Abdul-Rasheed, Chemistry and Biochemistry Department, College of Medicine, Al-Nahrain University, PO Box 70027, Al-Kadhimiya, Baghdad, Iraq. Tel. +964 (790) 1885283/(770) 3919680. E-mail: omar_rasheed39@yahoo.com

Infertility is defined as the failure of a couple to achieve a pregnancy after at least one year of frequent unprotected intercourse.[1] It has been reported that the male partner contributes in 40% of the cases of infertility. Globally, the incidence of fertility is estimated to be about 13-18%.[2] Zinc is second to iron, as the most abundant trace element in the body, with 1.4-2.3 grams being present in a 70 kg adult.[3] Tissue and fluids especially rich in zinc are prostate, semen, liver, kidney, retina, bone, and muscle.[3] Zinc is an ubiquitous trace element, which plays an important role in a number of different physiological processes,[4] and is the most

critical trace element for male sexual function.[5] The high amount of zinc, which is 100 times higher in the ejaculates than in blood serum, has also been of interest in connection with fertility.[4] Zinc is involved in normal testicular development, spermatogenesis, spermatozoa motility, and prevents degradation of spermatozoa, as well as, maintains viability by inhibiting DNAases'. It has a fundamental role in the antibacterial activity of seminal plasma.[6-9] Human sperm properties may be influenced by interactions among the sex accessory gland secretions.[10] One of the biochemical processes related to the genital fluid mixing is the regulation of the free seminal zinc fraction, which can interact with spermatozoa. Zinc is first secreted in prostatic fluid in 2 forms available for sperm cells (free zinc and zinc-citrate complex). During ejaculation, a partial redistribution of the ion from citrate to very high affinity vesicular ligands reduces the unbound zinc fraction.[11-13] Therefore, the amount of zinc bound to vesicular high molecular weight proteins is considered to be an index of seminal plasma chelating capacity, and it is a measure of zinc bio-availability.[14] This work was carried out to identify the possible relationship between seminal plasma zinc level and high molecular weight zinc (HMW-Zn) bound protein levels with sperm progressive motility percents in Iraqi fertile and infertile men.

Methods. A case-control study was performed at the laboratories of the Chemistry and Biochemistry Department, College of Medicine, Al-Nahrain University, Baghdad, Iraq, between March 2005 to February 2006. The approval of the institutional research ethics committee, and written consent of every patient included in the study were obtained. Sixty primary infertile males aged 33.01 ± 4.20 years, without any treatment, who had regular unprotected intercourse for at least 12 months without conception with their partners, were selected to participate in this study. Wives of the infertile subjects included had no obvious cause of infertility, such as tubal blockage or ovulation disorder. At first attendance, a detailed background history and physical examination were carried out on both husband and wife. The sperm count was used to group the infertile subjects according to World Health Organization (WHO)[15] as oligozoospermic (n=32), azoospermic (n=22), and asthenozoospermic (n=6). The subjects having sperm count less than 20 million/ml were taken as oligozoospermic, asthenozoospermic having progression score of less than 2, while azoospermic having zero sperm count. Thirty-nine fertile males whose partners had conceived without any infertility treatment within a year, with sperm count more than 20 million/ml with >50% forward progressive motility (categories a and b; where a is a rapid progressive motile sperms percent, and b is a slow or sluggish progressive motility percent) were selected from the general population, and taken as the normozoospermic control group. Individuals having diabetes, thyroid disease, and patients who were on antipsychotic or antihypertensive drugs, or consuming alcohol, nicotine, vitamin and mineral supplementation were excluded from the study. All individuals signed a consent form to allow the use of their semen samples in this study.

Semen sampling. Semen samples were obtained by masturbation into a 50 ml sterile polystyrene jars after an abstinence period of 3-5 days. Samples were left at 37^{0}C for 30 minutes to complete the liquefaction process, and then samples were processed by conventional analysis to determine the volume, pH, sperm count, sperm motility, and sperm morphology according to WHO criteria.[15] The seminal plasma was collected after centrifugation at 2000 rpm for 20 minutes. Supernatants were transferred in fresh tubes and stored at -20^{o}C until used for zinc and HMW-Zn% assays.

Zinc assay. Zinc analysis was performed by atomic absorption spectrophotometry (AAS [Atomic Absorption Spectrophotometer Model AA-646]) (Shimadzu Corporation, Kyoto, Japan) using flame technique. An air-acetylene flame with an air flow rate of 10 liter/minute, and an acetylene flow rate of 2.4 liter/minute was used. The measurement was conducted at 213.8 nm wavelength, and with 0.7 nm slit width. The standard curve ranged from 10-40 µg/dl aqueous zinc standard, which corresponding to plasma zinc concentration of 50-200 µg/dl.[16] Seminal plasma zinc analysis was carried out according to Pleban and Mei.[17] The assay was performed on a 1000-fold sample dilution.

Sephadex G-75 gel chromatography. For determination of the amount of zinc bound to HMW-Zn %, the gel filtration of the seminal plasma on Sephadex G-75 was performed.[12] The gel was packed in a 1.8x30 cm glass column, equilibrated, and eluted with 0.05 M Tris buffer containing 0.15 M sodium chloride, and pH 7.4. The eluate was collected at 25^{o}C with a flow rate of 6.0 ml/minute in 50 fractions. The column void volume was measured using blue dextran-2000 by monitoring the absorbance of a blue color in eluted fractions at 650 nm, whereas all the fractions of seminal plasma samples were investigated for protein (A280 nm), and for zinc concentration by AAS. The HMW-Zn% was calculated from the zinc concentration in all fractions eluted, and expressed as a percentage of the total zinc. The A280nm and HMW-Zn% values were plotted against fraction number eluted from the column.

Statistical analysis. The statistical analysis was carried out using the Statistical Package for Social Sciences computer program version 10.8 for Windows (SPSS

Inc., Chicago, IL., USA). The means were compared using student t test, and one way analysis of variance (ANOVA) test, as needed. The statistical tests were considered to be significant at the $p \leq 0.05$ level.

Results. The characteristics of the study subjects with their seminal fluid parameters are shown in **Table 1**. There were no significant differences (p=0.122) between the age values of different patient groups and control group. There were highly significant differences in the sperm counts and progressive motility between infertile groups and the control group (p=0.005). The zinc concentrations in seminal plasma of the 4 groups studied are shown in **Table 2**. There were no significant differences in the seminal plasma zinc level between all infertility groups and the control group. At the same time, there is no significant correlation between seminal plasma zinc and progressive motility percents as shown in **Figure 1**. For determination of the amount of HMW-Zn%, the gel filtration of seminal plasma on Sephadex G-75 superfine was performed. The column volume was determined using blue-dextran 2000. The blue-dextran 2000 elution diagram was presented in **Figure 2**. From this Figure, it was found that the void volume of the 1.8x30 cm column used was 17 mL. The levels of HMW-Zn% obtained were summarized in **Table 2**. The HMW-Zn% values show that there were very high significant differences (p=0.001) between control group and different infertility groups. The HMW-Zn% values (mean ± SD) in seminal plasma of oligozoospermia, azoospermia, and asthenozoospermia were lower than in the controls ([p=0.001], 8.538 ± 1.888% azoospermia versus control [p=0.001], 6.378 ± 1.426% asthenozoospermia versus control [p=0.001]). The results obtained showed that there was a decrease in HMW-Zn percents in all infertility cases. The HMW-Zn% correlated in **Figure 3** positively and significantly (r=0.33, p=0.003) with progressive motility percents.

Discussion. Human sperm properties may be influenced by interactions among the gender accessory gland secretions.[10] One of the biochemical processes related to the genital fluid mixing is the regulation of the free seminal zinc fraction, which can interact with spermatozoa, such as, zinc and bioavailability. Zinc is first secreted in prostatic fluid in 2 forms available for sperm cells (free zinc and zinc-citrate complex). During ejaculation, however, a partial redistribution of the ion from citrate to very high affinity vesicular ligands reduces the unbound zinc fraction.[11-13,18] Therefore, the amount of zinc bound to vesicular HMW-Zn% is considered to be an index of seminal plasma chelating capacity, and it is a measure of zinc bioavailability.[14,19] Consequently, despite the routine measurement of total seminal zinc in the assessment of sperm activity, only HMW-Zn% values can be a suitable parameter in which to study the relationship between seminal zinc concentration and sperm function. Doshi et al[2] reported a positive correlation between seminal plasma zinc levels and sperm count, whereas in the present study, we could

Table 1 - Characteristics of patients and sperm parameters.

Variable	Azoospermia n=22	Oligozoospermia n=32	Asthenozoospermia n=6	Control n=39	ANOVA
Age, years	32.09 ± 4.51	30.90 ± 3.52	35.00 ± 3.84	31.87 ± 3.76	0.122
Seminal fluid volume, mL	3.75 ± 1.05	3.04 ± 1.23	3.66 ± 1.40	3.46 ± 1.72	0.308
Sperm count, million/mL	-	8.44 ± 5.13†	53.04 ± 11.10†	87.34 ± 38.72	0.000
Progressive motility, %	-	60.89 ± 9.40	38.22 ± 14.64*	64.06 ± 8.90	0.000
Morphology, %	-	75.34 ± 8.25	75.38 ± 7.48	78.29 ± 7.84	0.279

All values are expressed as mean ± SD, *p=0.01, †p=0.005

Table 2 - Seminal plasma zinc concentration and high molecular weight zinc binding proteins percent (HMW-Zn%) in different groups of infertility.

Variable	Azoospermia n=22	Oligozoospermia n=32	Asthenozoospermia n=6	Control n=39
Seminal plasma zinc, g/mL	178.50 ± 18.61	181.92 ± 23.40	195.33 ± 13.00	184.66 ± 21.31*
HMW-Zn%	8.538 ± 1.888‡	6.797 ± 1.111‡	6.378 ± 1.426‡	12.555 ± 1.264†

All values are expressed as mean ± SD, *0.343 (ANOVA),
†$p \leq 0.0001$ (ANOVA), ‡significant difference from the control group at p=0.005

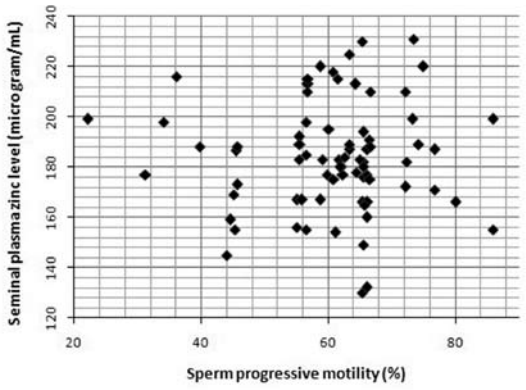

Figure 1 - Scatter plot for the correlation between seminal plasma zinc levels and sperm progressive motility percents (n=77, r=0.017, p=0.8).

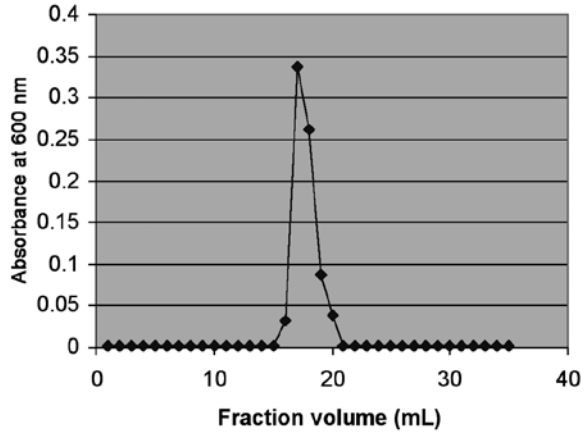

Figure 2 - Elution diagram of blue-dextran 2000 on Sephadex G-75 superfine.

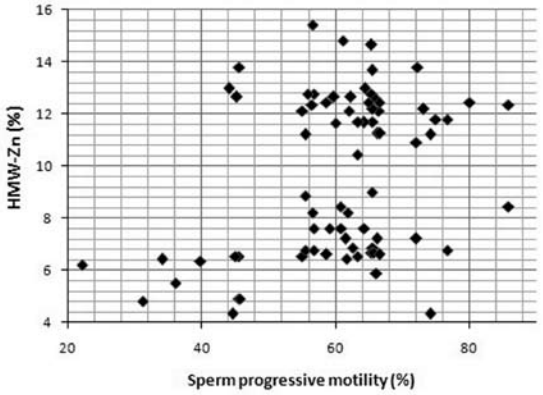

Figure 3 - Scatter plot for the correlation between high molecular weight zinc binding proteins percents and sperm progressive motility percents ((n=77, r=0.33, p=0.003).

not find any significant relationship between seminal plasma zinc level and sperm count, and this difference may be attributed to the fact that sperm count is a largely variable parameter, and is influenced by many factors, such as, past illnesses, smoking status, use of medication, and exact abstinence time. In the current study, abstinence time was kept constant, and special care was taken to ensure identical conditions during sampling, storage, and analysis. In addition, none of the participants suffered from any urogenital infection, and none were using any medication.[2] Conflicting clinical data concerning the influence of human seminal plasma zinc concentration on sperm motility have been reported.[20] Previous in vitro studies showed the inhibitory effect of free extracellular zinc concentrations on the motility of human spermatozoa.[21] Fuse et al[22] reported a positive correlation between seminal plasma zinc levels and sperm motility. However, in this study there was a weak positive correlation between seminal plasma zinc (r=0.017, p=0.885) and the progressive motility of human spermatozoa. Therefore, total seminal zinc concentration may not be a useful index of the zinc fraction interacting with sperm cells, and a more appropriate marker of the ion bio-availability should be used to evaluate its relationship to sperm function. We suspect that zinc might be responsible for antioxidant defense systems, therefore higher zinc levels would decrease oxidative damage. This would decrease the generation of free radicals that could lead to defective sperm motility. In the present study, free seminal zinc has been investigated by measuring the amount of zinc bound to vesicular HMW-Zn%, and it was found that HMW-Zn% correlated well significantly and positively with progressive motility (r=0.333, p=0.003). In fact, the unbound zinc fraction depends on a post-ejaculatory redistribution of the ion from prostatic to high affinity vesicular ligands.

As expected, total seminal zinc concentration was not a discriminating parameter among subjects with normal and low sperm motility, while HMW-Zn% was decreased in all infertility groups, especially asthenozoospermic subjects. This suggests an increase of the free extracellular zinc concentrations in their semen. A limitation of this study is the number of asthenozoospermic group patients. A prospective study with larger sample size is suggested with longer period of time for further evaluation of this infertility type.

In conclusion, HMW-Zn% is a good index of sperm function rather than the total seminal plasma zinc.

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