Zinc Sulfate Improves Progressive Sperm Motility in Subfertile Men with Abnormal Sperm Agglutination

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Abstract

Back ground: Agglutination of spermatozoa means that motile spermatozoa stick to each other in the ejaculate. It can be due to infection or antibodies that react with sperm. Sperm agglutination has a negative impact on fertility due to impeding of sperm function, inhibition of fertilization and implantation. Zinc is an essential trace element for male sex function. It is a cofactor in more than 300 enzymes influencing various organ functions. Zinc found in seminal fluid, increase sperm count and motility and blood testosterone levels. Zinc had direct effects on the production, maturation and function of leucocytes and can modulate antibody production.

Objective: The objective of this study to assess the effect of zinc sulfate treatment on sperm agglutination among subfertile patients with asthenospermia.

Design: Prospective study.

Setting: Infertility clinic and assisted reproduction unit at the Institute of Embryo Research and Infertility Treatment, Al-Nahrain University.

Patients: Fifty - three subfertile male patients.

Materials and Methods: Semen analysis was performed before zinc treatment using direct slide examination for fifty - three infertile patients. A daily dose of 440 mg of zinc sulfate supplementation was orally administered for a period of two weeks, three weeks, four weeks, and six weeks. After each period semen samples were obtained by masturbation after a recommended period of 3-5 days of sexual abstinence, and evaluated for determinations of semen volume, pH, concentration, motility, normal morphology, total progressive motile sperm count, round cells, and sperm agglutination, according to WHO recommendation.

Results: subfertile men demonstrated a significant ($P< 0.05$) decrease in round cells /HPF after third week of zinc treatment. Sperm agglutination was significantly ($P< 0.01$) decreased after second, third, fourth week, and ($P< 0.02$) at six weeks of zinc treatment compared to sperm agglutination before treatment. Also the study showed significant ($P< 0.05$) improvement in progressive sperm motility [grade –**A** after three week and grade –**B** after three and four week of zinc treatment], significant ($P< 0.01$) increase of total progressive motile sperm count /ejaculate, and of morphologically normal sperm after third week of oral zinc sulfate supplementation.

Conclusions: It was concluded from the results of the work that the oral zinc sulfate supplementation is effective in decreasing of abnormal sperm agglutination and round cells, with subsequent improvement of progressive sperm motility.

Key words: Round cells, sperm agglutination, progressive sperm motility, zinc.

الخلاصة

تمهيد: التصاق النطف المتحركة ببعضها في السائل المنوي قد ينجم عن التهاب او خمج السائل المنوي او تكون اجسام مضادة لغلاف النطف.ان لالتصاق النطف اثارا سلبية على الخصوبة من خلال التاثير السلبي على وظائف النطف،الاخصاب وانغراس الجنين بالرحم.

الخارصين يفرز من غدة البروستات و يعتبر احد المعادن المهمة للوظيفة التناسلية عند الذكور، يتواجد الخارصين في السائل المنوي و يلعب دورا في زيادة مستوى الهرمون الذكري و زيادة عدد و حركة النطف . يوصف الخارصين بانه مضاد للتاكسد و بقدرته على المحافظة على استقرار الاغشية.
للخارصين اهميته في انتاج و انضاج ووظيفة كريات الدم البيضاء والخلايا الابتلاعية و في تطور المناعة الخلوية و المكتسبة ، و يعتبر الخارصين معدل و منظم لوظيفة الجهاز المناعي ونقص الخارصين يجعل المصابين به عرضة للاصابة بمختلف الامراض.
الاهداف:معرفة مدى تاثير جرع سلفات الخارصين الفموي على الذين يعانون من وهن النطف والتصاق النطف المتحركة ببعضها داخل السائل المنوي.
الطرق: دراسة مستقبلية لثلاثة و خمسون مريضا يعانون من عدم القدرة على الانجاب بسبب وهن النطف والتصاق النطف المتحركة ببعضها داخل السائل المنوي.تم اجراءالفحص المختبري للسائل المنوي قبل و بعد اعطاء سلفات الخارصين الفموي ولفترة اسبوعين، ثلاثة اسابيع، اربعة اسابيع،وستة اسابيع للتقصي عن المتغيرات في معالم السائل المنوي.
النتائج: اظهرت الدراسة انخفاض عدد كريات الدم البيضاء و نسبةالتصاق النطف المتحركة ببعضها وبمدلول احصائي بعد الاسبوع الثالث من العلاج بالخارصين بالنسبة لكريات الدم البيضاء .بعد الاسبوع الثاني، الاسبوع الثالث، الاسبوع الرابع،و الاسبوع السادس من العلاج اظهر انخفاضا وبمدلول احصائي بنسبةالتصاق النطف المتحركة ببعضها.و اظهرت الدراسة تحسن وبمدلول احصائي بنسبة شكلياء النطف بعد الاسبوع الثالث ونسبة حركة النطف التقدمية وعدد النطف ذات الحركة التقدمية الكلي بعد الاسبوع الثالث و الرابع من العلاج بالخارصين.
الاستنتاجات: للخارصين دورا بالمساعدة بخفض عدد كريات الدم البيضاء و خفض نسبةالتصاق النطف المتحركة ببعضها و دورا في تحسن و زيادة نسبة حركة النطف التقدمية والعدد الكلي النطف ذات الحركة التقدمية.
الكلمات الدليلية: الخارصين، التصاق النطف، النطف ذات الحركة التقدمية.

Introduction

Seminal infection induces leukocytospemia and this in turn results in production of antigen, reactive oxygen species(ROS), complement proteins, neuropeptides, lipoproteins and inflammatory mediators' production, all these might be harmful for sperm cell function, inducing alteration of sperm motility, inhibition of acrosomal reaction, oxidative stress, or sperm DNA damage[1,2].

Sperm agglutination is clumping or sticking of motile sperm to each other in the ejaculate. It can be due to infection or antibodies that react with sperm. Sperm agglutination is the only semen alteration related to the presence of antisperm antibodies (ASAs).There are two different types of agglutination depending on the factors that alter the membrane's electrical charge- chemical agglutination and immunologic agglutination [3,4].
Immunological agglutination is very specific. It is based on specific antibodies that are formed against components of the sperm membrane. Known causes of ASAs include ductal obstruction, infection, testicular torsion, cryptorchidism, testicular or spermatic cord trauma, or varicocele [5]. It is proposed that immunologic infertility is the consequence of the combined actions of multiple antisperm antibodies (ASAs) in immobilizing and/or agglutinating spermatozoa, impeding of sperm function by altering of motility, decreased ability to penetrate the cervical mucus [6], premature acrosome reaction, and decreased ability to bind to the zona pellucida, blocking sperm egg interaction, inhibit fertilization and preventing implantation and/or arresting embryo development [7, 8]. An observation of $\geq$ 5 % agglutination in sample is considered abnormal and when more than twenty-five percent of sperm are agglutinated, fertility is very unlikely [9]. Sperm agglutination has a negative impact on fertility by decreasing pregnancy rates in intrauterine insemination cycles [10].

There are many who advocate the use vitamin E and C to help decrease the sperm clumping. This is a low risk treatment and may help[11]. Other relatively simple

treatment for several factors that influence fertility is the zinc. Zinc is an essential trace element for all organisms. It is a cofactor in more than 300 enzymes influencing various organ functions. It is secreted predominantly by the prostate and is present in high concentrations in the seminal fluid, and in the maturing spermatozoa. Zinc plays an important role in normal testicular development. Spermatogenesis and sperm motility [12, 13, 14]. Zinc is thought to help to extend the functional life span of the ejaculated spermatozoa [15].

The nervous, reproductive and immune systems are particularly influenced by zinc deficiencies [16]. Zinc deficiency first impairs angiotensin converting enzyme (ACE) activity, and this in turn leads to depletion of testosterone and inhibition of spermatogenesis [15].

Zinc influences the fluidity of lipids, and thus the stability of biological membranes, the appropriate balance between superoxide radical generation and superoxide dismutase (SOD) activity is decisive for the functional status of sperm, the cofactors of this enzyme (SOD) are zinc and copper[17,18]. Exogenous addition of SOD and catalase enhances the motility, acrosome integrity and fertility of ram spermatozoa [19].

Zinc specifically interacts with components of the immune system, had direct effects on the production, maturation and function of leucocytes[16]. The effect of zinc on the immune system is well known as deficiency in zinc causes lymphopenia, a 50% reduction in leucocytes and 40–70% reduction in antibody-mediated and cell-mediated immunity and reduced immune capacity among affected humans[20, 21, 22]. Zinc deficiency has adverse effects on macrophage and has been associated with reduced resistance to several microorganism, the addition of a of zinc to a culture system can cause a polyclonal activation of lymphocytes[6] for these reasons, zinc is considered to be an immunomodulator and has been used successfully to treat many diseases with altered immune response [6,23]. The proportion absorbed is thought to be inversely related to the amount ingested[24]. The dosage prescribed must be based on the amount of elemental zinc present in the preparation, which varies from one compound to another. For example, a standard capsule of 220 mg of zinc sulphate contains approximately 50 mg of elemental zinc. The adverse effects were mild and include nausea and vomiting. Pharmacological doses in the range of 4–12 mg/ kg of elemental zinc per day can lead to gastroenteritis, gastrointestinal bleeding, hypocupraemia, microcytosis, relative neutropenia and hypoceruloplasminaemia[25].

Previous studies have shown that secondary zinc deficiency might be due to infection, including bacterial, viral or parasitic[26] and people with low or, marginal serum zinc levels are at increased susceptibility to infection[20].

Zinc has been suggested as an important anti-inflammatory factor, antiviral activity[26], and the antimicrobial spectrum of zinc has been suggested to include many potential genitourinary pathogens gram-positive and gram-negative bacteria. *Trichomonas vaginalis*, *Candida*, and *Chlamydia trachomatis*[27].

Zinc therapy reduces asthenozoospermia through several mechanisms such as prevention of oxidative stress, apoptosis and sperm DNA fragmentation[28]. Also zinc treatment has a role in improving sperm parameters in men with asthenozoospermia, probably through its membrane stabilizing effect as an antioxidant and its effect on cellular and humoral immunity by reducing the levels of antisperm antibodies and tumor necrosis factor (TNF) α and increasing that of interleukin-4[29].

The present work was done to assess the effect of zinc sulfate treatment on sperm agglutination among subfertile patients with asthenospermia.

Materials and Methods

Patients

Our prospective study enrolled a total of 53 subfertile male partners from May 2005 to July 2006 from couple who had consulted the infertility clinic of institute for Embryo Research and Infertility Treatment Al-Nahrain University.

A detailed medical history was taken and physical examination was performed. The inclusion criteria were the presence of asthenospermia, asymptomatic leukocytospemia and sperm agglutination in two semen samples taken before the start of the treatment, and absence of endocrinopathy, varicocele, and absence of female factor infertility.

Semen analyses

Before and after zinc sulfate supplementation, semen samples were obtained by masturbation after a recommended period of 3 days of sexual abstinence. After liquefaction (at room temperature) routine semen analyses including semen volume, pH, concentration , sperm motility, normal sperm morphology, total progressive motile sperm count/ejaculate , round cells , and sperm agglutination, were performed according to 1999 WHO recommendation[9].

Agglutination of spermatozoa means that motile spermatozoa stick to each other-head to head, tail to tail, midpiece to midpiece, head to tail, midpiece to head agglutination, midpiece to tail, or mixed sperm agglutination. The specimen was observed and assessed for sperm agglutination by preparing a drop (50 μL) of semen into a warm microscopic slide, covered by a cover slip under microscope(x400 magnification). The presence of sperm agglutination with shaky head was suggestive of the existence of an immunological cause of infertility. The presence of only a few groups of small numbers of agglutinated spermatozoa was also recorded.

Sperm agglutination was graded as follow[4, 9].

Degree of sperm agglutination	Percent of sperm agglutination
Normal	< 5
Mild sperm agglutination	5-9%
Moderate sperm agglutination	10-50%
Large sperm agglutination	> 50 %
Gross sperm agglutination	Complete sperm agglutination

The 53 infertile patients received 440 mg of 7H2O zinc sulfate (equivalent to 100 mg elemental zinc) daily for a period of two weeks, if no significant decline in percentage of sperm agglutination was happened then treatment was continued with same dose for one week (i.e. 3weeks), semen analysis was done and if no decrease in sperm agglutination, other course of 440 mg zinc sulfate supplementation was given for one week (i.e. 4weeks), those patients who still did not showed decline in percentage of sperm agglutination, the zinc therapy continued for further two weeks(i.e. 6 week).

The present work was done to assess whether oral zinc sulfate was effective in treating asthenozoospermia associated with abnormal sperm agglutination.

Statistical Analysis

All parameters of semen before and after zinc therapy were analyzed. Calculations were performed using the Statistical Package of Social Sciences (SPSS)

version 10 (Inc, Chicago, IL, USA). Student's t-test was used to carry out statistical comparison. P value of less than 0.05 was considered to indicate statistical significance.

Results

The present study was undertaken to show the effect of zinc among subfertile patients with clinically asymptomatic genital tract infection, abnormal sperm agglutination, asthenospermia.

In the present study, testing was performed under standard conditions; all ejaculates were obtained in the institute after a recommended period of sexual abstinence of 3 days, and were examined directly after liquefaction. None of the men was treated with antibiotics or corticosteroids during the time of the study.The mean age ± SEM of 53 subfertile male partners was 33.67±1.29 years (ranged 21-55 years). Table 1 showed that the majority of subfertile male patients (86.7%) had moderate sperm agglutination before zinc therapy.

Sperm agglutination percentage dropped from 34.7 ±2.2 % before treatment to 6.25±1.09 % at six weeks of zinc therapy. The mean ± SEM [before and after various periods (2, 3, 4, and 6 weeks) with oral zinc sulfate supplementation] of semen characteristics, semen volume, and sperm concentration did not vary significantly.Subfertile men demonstrated a significant ($P<0.05$) decrease in round cells /HPF from (12.08±1.83 to 7.86±0.81) after third week of oral zinc treatment. Sperm agglutination was significantly ($P<0.01$) decreased (from 34.7 ±2.2 to 18.92±1.78) after second week, (from 38.91±2.83 to15.18±1.60) at third week, (from 41.90±3.92 to 10.80±1.21) after fourth week, and significantly dropped ($P<0.02$) from (14.50±0.94 to 6.25±1.09) at six weeks of zinc treatment compared to sperm agglutination before treatment. Also the study showed significant ($P<0.05$) improvement in progressive sperm motility-grade –**A** from (9.05 ± 1.34 to 11.71±1.43) after two weeks and significant increase ($P< 0.03$) from (9.29 ±1.74 to 15.54±1.42) at three weeks, significant ($P< 0.03$) improvement in progressive sperm motility-grade –**B** from(21.6±2.13 to 25.43±1.64) after three week and significant increase ($P< 0.02$) from (16.8±2.42 to 22.33±1.53) at four week of zinc treatment. Also the study demonstrated a significant ($P< 0.01$) increase from (38.9 ±2.83 to 75.65±12.24) of total progressive motile sperm count/ejaculate after treatment compared to that before treatment at three week.

Table 1. Percentage of sperm agglutination before and after oral Zinc treatment

Sperm agglutination %	2 Weeks Zinc treatment		3 Weeks Zinc treatment		4 Weeks Zinc treatment		6 Weeks Zinc	
	Before Therapy	After Therapy	Before Therapy	After Therapy	Before Therapy	After Therapy	Before Therapy	After Treatment
0 – 4%	0	8 (15.09%)	0	5 (13.51%)	0	0	0	2 (25%)
5 - 9%	0	4 (7.54%)	0	4 (10.81%)	0	9 (42.85%)	0	5 (62.50%)
10 - 15%	4 (7.54%)	16 (30.18%)	12 (32.43%)	16 (43.24%)	11 (52.38%)	9 (42.85%)	7 (87.50%)	1 (12.5%)
16 - 20%	10 (18.86%)	7 (13.21%)	6 (16.21%)	5 (13.51%)	7 (33.33%)	3 (14.28%)	1 (12.50%)	0
21 - 25%	11 (20.75%)	6 (11.32%)	7 (18.91%)	2 (5.40%)	0	0	0	0
26 - 30%	5 (9. 43%)	5 (9. 43%)	5 (13.51%)	3 (8.11%)	2 (9.52%)	0	0	0
31 - 35%	4 (7.54%)	0	0	1 (2.70%)	1 (4.76%)	0	0	0
36- 40%	4 (7.54%)	4 (7.54%)	4 (10.81%)	1 (2.70%)	0	0	0	0
41 - 45%	5 (9. 43%)	1 (1.88%)	1 (2.70%)	0	0	0	0	0
46- 50%	3 (5.66%)	2 (3.77%)	2 (5.40%)	0	0	0	0	0
51 - 55%	0	0	0	0	0	0	0	0
56 - 60%	3 (5.66%)	0	0	0	0	0	0	0
61- 65%	1 (1.88%)	0	0	0	0	0	0	0
66 - 70%	1 (1.88%)	0	0	0	0	0	0	0
71 - 75%	0	0	0	0	0	0	0	0
76 - 80%	2 (3.77%)	0	0	0	0	0	0	0

Morphologically normal sperm cells increased significantly ($P< 0.02$) from (43.35±2.72 to46.16±2.54) at third week of oral zinc sulphate supplementation (Table- 2)

Table 2. Semen characteristics before and after oral Zinc treatment

Variable of semen analysis		2 Weeks Zinc Treatment			3 Weeks Zinc Treatment			4 Weeks Zinc Treatment			6 Weeks Zinc Treatment		
		Before Therapy M± SEM n=53	After Therapy M± SEM *n=53*	*P*	Before Therapy M± SEM *n=37*	After Therapy M± SEM *n=37*	*P*	Before Therapy M± S *n*	After Therapy M± SEM *n=21*	*P*	Before Therapy M± SEM *n=8*	After Therapy M± SEM *n=8*	*P*
Volume of semen(mL))		3.02 ± 0.20	2.84 ± 0.16	0.31	3.05 ± 0.25	2.82± 0.17	0.3	2.99± 0.34	2.84± 0.16	0.63	3.03± 0.14	3.19± 0.15	0.2
Sperm count (10^6/ mL)		58.83 ± 7.34	58.16 ± 7.37	0.87	70.10± 9.57	63.62± 6.4	0.2	65.47± 14.88	52.23± 8.71	0.1	64.12± 17.66	6.78± 18.79	0.07
Sperm Motility %	Grade-A	9.05 ± 1.34	11.71 ± 1.43	0.05	9.29 ± 1.74	15.54± 1.42	٠.٠	8.95 ± 2.63	11.42± 1.61	0.2	11.87± 2.68	12.50± 3.91	0.6
	Grade-B	20.50 ± 1.76	23.01 ± 1.64	0.13	21.6 ± 2.13	25.43± 1.64	0.03	16.8± 2.42	22.33± 1.53	0.02	22.25± 2.87	23.37± 3.08	0.1
	Grade-C	26.33 ± 1.79	24.20 ± 1.39	0.31	26.9 ± 2.01	24.62± 1.22	0.03	24.1 ± 2.09	25.95 ± 1.44	0.43	25.50± 3.15	2.50± 2.580	0.7
	Grade-D	43.92± 2.88	41.88 ± 2.26	0.34	41.9 ± 3.32	35.05± 1.95	0.04	49.57 ± 4.21	40.28 ± 3.06	0.02	40.37± 6.57	38.50± 7.07	0.2
TPMSC / ejaculate		60.35± 13.26	50.58 ± 6.88	0.34	38.9 ±2.83	75.65± 12.24	0.0	72.13 ± 31.56	55.83 ± 12.77	0.45	75.83± 29.19	97.31± 48.21	0.3
Normal sperm morphology %		41.98 ± 2.3	41.9 ± 2.41	0.98	43.35 ± 2.72	46.16± 2.54	0.02	41.23 ± 3.67	40.85 ± 3.43	0.92	39.75± 6.77	39.62± 7.243	0.9
Round cells / HPF		11.13 ± 1.33	9.64 ± 1.6	0.4	12.08 ± 1.83	7.86± 0.81	0.03	12.35 ± 2.97	7.65 ± 0.744	0.15	8.00± 1.06	8.87± 1.24	0.4
Sperm agglutination %		34.7 ± 2.2	18.92 ± 1.78	0.0	38.91 ± 2.83	15.18± 1.60	0.0	41.90 ± 3.92	10.80 ± 1.21	0.0	14.50± 0.94	6.25± 1.0.9	0.02

Data presented as M ± SEM.
M = Mean.
SEM = Standard error of mean.
n = Number of patients.
P <0.05 was considered significant
TPMSC = Total progressive motile sperm count / ejaculate.
HPF = High power field.

Discussion

Sperm agglutination is common and prevents the sperm from being viable .It may be related to the presence of seminal infection or antisperm antibody[3, 4, 30].

Certain properties of zinc sulfate might explain the effects we observed during our study. Zinc is essential for spermatogenesis, hormone metabolism and maintenance of normal testosterone levels[12, 13, 14, 15]. Zinc sulfate has anti-inflammatory[26], broad spectrum antimicrobial activity[27].

Zinc can affect the immune system of individuals whose serum level of zinc is low and also those individuals whose serum level of zinc is normal or marginal[20] through its effect on cellular and humoral immunity by reducing the levels of antisperm antibodies and elevation of Th-2 cytokines and down regulation of Th-1 cytokines[29]. For this reason, zinc has been used as an

immunomodulator for the treatment of many diseases[6, 23].

The adverse effects were mild and did not necessitate stoppage of treatment. These were nausea (80%), vomiting (8·3%) and mild epigastric pain (11·1%).

In our study there is reduction in round cells /HPF after third week of oral zinc treatment, and this might be attributed to zinc' antimicrobial activity[31, 32, 33] and to an increase in the development and activation of T lymphocytes which fight the infection, and speeding recovery from infection[34] resulting in minimizing link of specific sugar to sperm membrane[30] and consequently decrease in the sperm agglutination. Also dropping in sperm agglutination, which might be attributed to immunomodulatory effect of zinc and it's action on cellular and humoral immunity by reducing the levels of antisperm antibodies[29].

Improvement in progressive motility, total progressive sperm motility/ejaculate and morphologically normal sperm might be attributed to diminution of round cells/HPF and dropping of sperm agglutination, and probably through membrane stabilizing activity and prevention of oxidative stress, sperm apoptosis and sperm DNA fragmentation[28]. The result showed that zinc can improve sperm motility in men with decreased motility and this differs from the result of several studies[14, 35] and are in accordance with similar results reported elsewhere[28, 29].

Conclusion

Zinc sulfate can reduce leukocytospemia, sperm agglutination, and improves progressive sperm motility.

Recommendations

1. Measurements, before and after zinc treatment, of seminal plasma zinc, confirm the suggested mechanism of zinc
2. Immunocytochemical round cell differentiation to determine leukocyte counts and ratios, and peroxidase staining to detect polymorph nuclear leukocytes.
3. How ever, to see whether the improvement in sperm motility observed after administration of zinc will lead to an increase in pregnancy rates remains to be established.

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