

Impact of Seminal Plasma Trace Elements on Human Sperm Motility Parameters

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Introduction. Human seminal plasma contains a variety of macro and trace elements including magnesium (Mg), copper (Cu), zinc (Zn), and iron (Fe) that have essential roles in normal functioning of semen and its quality. The imbalance of these elements has been reported in several pathologic and male infertility disorders. Therefore, this study aimed to determine the levels of these elements in seminal plasma samples, their relationships with each other and their impact on sperm motility.

Methods. Overall, 192 males (96 normospermic and 96 asthenospermic males) were enrolled in the study. Semen samples were collected by masturbation and computer-assisted/aided semen analysis of sperm motility was performed. The samples were centrifuged and seminal levels of Mg, Cu, Zn and Fe were measured using atomic absorption spectroscopy.

Results. The levels of Zn did not differ between the two groups, while the levels of Mg, Cu, and Fe were significantly higher in normospermic males. Fe showed a positive correlation with Mg and Cu in asthenospermic group. However, a negative relationship was found between Mg and Fe levels and between Mg and sperm concentration in the normospermic group. Fe levels were higher in the normospermic group compared to the asthenospermic group. Nevertheless, increased Fe levels caused a decrease in most of sperm motility fractions.

Conclusion: Elements play major roles in male fertility and directly affect sperm quality. According to the results of this study, the levels of Zn do not affect the sperm quality and motility, while Fe, Cu and Mg are decreased in males with sperm motility problems. Nevertheless, Fe levels can adversely affect sperm motility in normospermic men.

Key words: sperm motility; magnesium; copper; zinc; iron.

INTRODUCTION

Human seminal plasma contains a variety of macro and trace elements including magnesium (Mg), copper (Cu), zinc (Zn) and iron (Fe) that have essential roles in normal functioning of semen and its quality [1-4]. Mg is required as the main cofactor for kinase enzymes [5] and acts as an intracellular calcium (Ca) antagonist [6]. Increased Mg levels compared to Ca improve erection and ejaculation processes [7]. Cu is an important component of many metalloenzymes such as superoxide dismutase (SOD), ceruloplasmin and lysine oxidase that are crucial in energy metabolism pathways and antioxidant processes [8]. However, it can be toxic to a variety of cells even spermatozoa [9].

Zn has an important role in sperm motility and directly affects its morphology [10, 11]. It exists in spermatozoa and seminal plasma owning the highest concentration in compare to any other body fluid [12]. Zn cooperates with Fe in oxidation-

reduction processes and is involved in antioxidant reactions as the cofactor of Cu-Zn SOD [13]. However, some studies showed no significant relationship between concentration of Zn in seminal plasma and sperm count or motility [14]. On the other hand, Fe is suspected to induce and catalyze lipid peroxidation that reduces semen quality and sperm motility [15]. Therefore, this study aimed to determine the seminal plasma levels of Mg, Cu, Zn and Fe in normozoospermic and asthenozoospermic men, and compare their levels to evaluate the effects of each element on sperm motility characteristics by computer-assisted/aided semen analysis (CASA).

MATERIAL AND METHODS

STUDY POPULATION

This study was conducted on 192 men referred to clinical laboratories in Gorgan (northern Iran) for routine semen analysis as the primary

approach for men fertility testing. The subjects were equally divided into two groups of cases and controls according to semen analysis results. The control group included normozoospermic subjects (producing spermatozoa normal in number, morphology, and motility) with total motility rate of $\geq 40\%$. The case group included asthenozoospermic subjects with total motility rate of $< 40\%$ and normal sperm morphology and concentration.

SAMPLE COLLECTION

Semen samples were collected in sterile containers by masturbation after 3-7 days of sexual abstinence. The samples with volume of less than 2 mL and sperm concentration of less than $20 \times 10^6/\text{mL}$ were excluded. After 15-60 minutes of liquefaction, volume and pH of samples were determined and manually analyzed. CASA was performed according to the WHO laboratory manual for the examination and processing of human semen. Total sperm motility and its subgroups were defined for each sample according to the categorization of WHO: motility type A (rapid progressive motile), motility type B (slow or sluggish progressive motile), motility type C (non-progressive motile) and motility type D (immotile).

The parameters assessed by CASA included: curvilinear velocity (VCL), straight-line/rectilinear velocity (VSL), average path velocity (VAP), mean angular displacement (MAD), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), linearity (LIN) defined as VSL/VCL , wobble (WOB) defined as VAP/VCL , and straightness (STR) defined as VSL/VAP .

After semen analysis, the samples were centrifuged at 2500 rpm for 10 minutes. The supernatant plasma was separated into 500 λ aliquots and stored at -70°C for future biochemical testing in less than a month.

SAMPLE PREPARATION

For biochemical measurements, the samples were thawed at room temperature. The samples were then deproteinized by equal volume of 10% tricarboxylic acid. The samples were centrifuged again and the supernatants were collected as the final sample with a 1:1 dilution ratio.

BIOCHEMICAL ANALYSIS

Seminal plasma levels of Mg, Cu, Zn and Fe were measured using flame atomic absorption spectrometry (YOUNGLIN AAS 8020, Hogye-

dong, Anyang, South Korea) at wavelengths of 285.20 nm, 324.70 nm, 213.90 nm, and 248.30 nm, respectively. Standard solutions (spectroECON-chemlab, Zedelgem, Belgium) were used to draw standard curves according to manufacturer's instruction. The prepared samples were diluted by high purity distilled water (DW) with 1:100 ratio for Mg and Zn measurement, 1:8 for Cu measurement, and 1:4 for Fe measurement. All measurements were done in duplicate. The spectrophotometer was calibrated after each series of assays and the lamp was adjusted. Standard solutions with concentrations of 1 $\mu\text{g}/\text{mL}$ (Mg and Zn), 0.1 $\mu\text{g}/\text{mL}$ (Cu) and 0.6 $\mu\text{g}/\text{mL}$ (Fe) were used for internal control. Results were reported as $\mu\text{g}/\text{mL}$.

STATISTICAL ANALYSIS

Analyses were performed using SPSS software version 21. Basic descriptive statistics including mean \pm standard deviation (SD) were calculated for the variables. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess normality of the data. Two independent samples Mann-Whitney U test was performed to compare the levels of variables between the two groups.

The values of 5th and 95th percentile of the levels of these elements were determined according to their values in control group. Then, each group was divided into three subgroups as follows:

Low: values lower or equal to 5th percentile, Normal: values higher than 5th percentile and lower or equal to 95th percentile, High: values higher than 95th percentile. Chi-square test was used for comparing the three subgroups of two groups. P-values of less than 0.05 were considered as statistically significant.

This study was approved by the local ethics committee of Golestan University of Medical Sciences.

RESULTS

The mean \pm standard deviation (SD) age of asthenozoospermic and normozoospermic men were 31.2 ± 5.9 years and 30.2 ± 5.3 years, respectively. The mean of sperm motility and all of its parameters were higher in the normozoospermic group (p -value < 0.01), while immotile fraction had higher percentages in the asthenozoospermic group as expected (Data are not shown).

The mean \pm SD of the studied elements are shown in Table 1. There was no significant difference in the level of Zn between the two groups, while Cu, Fe and Mg levels were significantly higher in the normozoospermic group than in the asthenozoospermic group (p -value $<$ 0.01). There was no intercorrelation between the

elements and sperm motion parameters in the asthenozoospermic group. However, there was a significant correlation between Fe levels and some of these parameters in the normozoospermic group (Table 2). Fe did not reveal any correlations with sperm motion parameters in asthenozoospermic patients.

Table 1
The levels of elements in the two study groups

Elements	Normozoospermic (mean \pm SD)	Asthenozoospermic (mean \pm SD)	p-value
Zn (mg/mL)	188.42 \pm 99.61	187.15 \pm 87.38	0.925
Cu (mg/mL)	1.29 \pm 0.58	1.05 \pm 0.28	$<$ 0.05
Fe (mg/mL)	2.02 \pm 0.74	1.56 \pm 0.48	$<$ 0.001
Mg (mg/mL)	550.12 \pm 282.51	130.94 \pm 96.75	$<$ 0.001

Table 2
Correlation between Fe levels and sperm motility types in the normozoospermic group

Motility type	r	p-value
Total motility	- 0.253	0.013
Progressive motility	- 0.215	0.035
Motility type A	- 0.303	0.003
Motility type B	0.236	0.021
Motility type C	- 0.249	0.014
Motility type D (immotile)	0.253	0.013
VCL	- 0.343	0.001
VSL	- 0.294	0.004
VAP	- 0.326	0.001
MAD	- 0.326	0.001
ALH	- 0.345	0.001
STR	- 0.213	0.037

The limits of normal ranges were defined as the 5th and 95th percentile for the elements according to normozoospermic group values equal to 44.6 and 357.5 μ g/mL for Zn, 0.752 and 1.615 μ g/mL for Cu, 0.96 and 3.658 μ g/mL for Fe and 106.3 and 1069.1 μ g/mL for Mg.

Considering these cutoffs, 45.8% (n = 44) of samples in the asthenozoospermic group had Mg levels lower than the 5th percentile, while only 4.2% (n = 4) of samples in the normozoospermic group had Mg levels lower than the 5th percentile (p -value $<$ 0.01). However, there was no significant difference in the level of other studied elements.

DISCUSSION

This study assessed the levels of four metal elements (Mg, Cu, Zn and Fe) in the seminal fluid of asthenozoospermic and normozoospermic men. These elements can improve sperm quality by

participating in key enzymatic reactions crucial for maintaining sperm normal physiology. However, these elements may impair sperm functions by inducing oxidative stress [15].

The results of this study showed significantly lower levels of Cu, Mg, and Fe in the seminal fluid of asthenozoospermic subjects in comparison to normozoospermic men. No statistically significant difference was observed in the levels of Zn between the two groups. Although Fe levels were higher in men with normal sperm motility, it adversely affected sperm motility in this group.

A significant difference in seminal plasma Cu levels was observed between the asthenozoospermic and normozoospermic groups. This is inconsistent with the results of Eidi *et al.* [16] and Yan Li *et al.* [17] that reported a negative correlation between semen quality and Cu levels. In the study of Li *et al.*, Cu was considered as an environmental chemical pollutant for the workers and staff of an industrial factory, while in the present study Cu was measured as a common metal element in

patients who were not exposed to poisonous amounts of Cu. In contrast to the findings of present study, Aydemir *et al.* showed higher levels of Cu in subfertile males compared to fertile males [18].

Meanwhile, Yuyan reported that levels of serum Cu have no impact on the sperm quality [19]. It is worth mentioning that serum samples were used in the mentioned study, while we analyzed seminal plasma samples. Moreover, it has been recently suggested that seminal Cu has higher potency as a sensitive indicator compared to seminal Cu [18].

It is thought that Fe may decrease sperm quality and motility through induction of lipid peroxidation and formation of hydroxyl radicals [20]. Although we found significantly higher levels of Fe in the normozoospermic group, Fe adversely affected most of sperm motility fractions in this group. These findings are partially in agreement with the results of Aydemir *et al.* that showed a positive correlation between serum levels of Fe and sperm motility while in another part of their results, they declared that seminal levels of Fe were higher in subfertile males than in fertile males [18]. In contrast to these results, Wróblewska *et al.* [21] reported the negative effect of Fe on sperm morphology. Kasperczyk reported that seminal levels of Fe increase in association to other metals [22]. We also observed an overall increase in three metals (Cu, Fe and Mg) levels in the normozoospermic group.

Fe is present in many enzymes and metallo-protein compounds and participates in oxidation-reduction processes [15]. The antagonistic relationship between Fe and other metal elements such as Zn and Cu in both absorption and metabolic functions causes some difficulty in interpretation of the role of Fe in male infertility.

Seminal levels of Mg are often used to judge about premature ejaculation (PE) assessment [7]. El Aaleba and Aloosh showed similar results about the seminal levels of Mg levels in PE patients, indicating that PE patients had lower levels of seminal plasma Mg levels compared to normal men [7, 23]. Sorensen *et al.* reported no correlation between Mg, Zn and Ca levels, while we found significant positive and negative relationships between Mg and Fe levels in the asthenozoospermic and normozoospermic individuals, respectively. The decline in Mg levels could be due to the presence of an Mg diminishing factor like chelating agents in the semen

of the patients or a previous hypomagnesemia caused by low consumption of Mg [4].

There were no significant differences in the seminal levels of Zn between normozoospermic males and asthenozoospermic patients in the present study. However, Yuyan *et al.* showed that reduced serum levels of Zn may lead to an increased risk of asthenozoospermia [19]. It should be mentioned that Yuyan *et al.* analyzed serum levels of Zn while the current study was conducted on seminal samples which may define the observed variation between two studies. Since the present study was a retrospective analysis, we were not able to determine the risk of asthenozoospermia. Atig *et al.* and Saglam *et al.* found a significant difference in the seminal levels of Zn levels of normozoospermic men and patients with a pathologic spermogram [24-26]. The case group of the study of Atig *et al.* included males with all types of sperm impairments (asthenozoospermic, oligozoospermic, and teratozoospermic), while the present study only investigated patients with sperm motility problems (asthenozoospermic) [24]. Consistent with the findings of the present study, Wróblewska *et al.* and Camejo *et al.* reported no notable differences in seminal levels of Zn between normozoospermic males, varicocele patients, and patients with a pathologic spermogram [21, 27].

CONCLUSION

This study showed no significant relationship between seminal levels of Zn and sperm motility. Cu, Mg and Fe may affect sperm motility, because of their higher levels in normozoospermic males compared to asthenozoospermic males. Fe showed adverse effects on motility when analyzed within normozoospermic males group and its certain effect still remains as a mystery to be solved. Further studies are necessary to determine the exact effect of these elements on semen quality and sperm motility.

Acknowledgements. This study was financially supported by the deputy of research and technology of Golestan University of Medical Sciences and Health Services, Gorgan, Iran. The authors would like to thank the Department of Research at Golestan University of Medical Sciences for their financial support.

Conflict of Interest Disclosure. The authors declare that there are not conflicts of interest.

Introducere. Lichidul seminal conține o varietate de oligoelemente ca magneziu (Mg), cupru (Cu), zinc (Zn) și fier (Fe). Acestea au rol esențial în buna funcționare a spermei și influențează calitatea acesteia. Dezechilibrul acestor oligoelemente poate duce la infertilitate masculină. Studiul și-a propus să studieze asocierea dintre acest dezechilibru și impactul asupra parametrilor motilității spermei.

Metode. Au fost incluși 192 de bărbați (96 normospermici și 96 astenospermici). Sperma a fost recoltată după masturbare și a fost realizată analiza motilității. Probele au fost centrifugate și au fost analizate nivelurile de Mg, Cu, Zn și Fe prin spectrometrie de absorbție atomică.

Rezultate. Nivelurile de Zn nu au fost semnificativ diferite între cele două grupuri. Nivelurile de Mg, Cu și Fe au fost semnificativ mai mari la pacienții normospermici. Totuși, s-a observat o corelație negativă între nivelurile de Mg și Fe precum și între nivelurile de Mg și concentrația spermei în grupul de pacienți normospermici. Deși nivelurile de Fe au fost semnificativ mai mari la pacienții normospermici, nivelurile crescute ale Fe s-au asociat cu scăderea majorității parametrilor de motilitate.

Concluzii. Oligoelementele joacă un rol important și afectează calitatea spermei. Nivelurile de Zn nu afectează calitatea spermei însă nivelurile scăzute ale Fe, Mg și Cu duc la scăderea parametrilor de motilitate a pacienților.

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Received July 23, 2017