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MAGNESIUM CONTENT IN SEMINAL FLUID AS AN INDICATOR OF CHRONIC PROSTATITIS

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Abstract - Magnesium and zinc are both involved in a high number of enzymic activities vital for mammals. They are found in prostate in remarkably high concentrations and released into seminal fluid. Furthermore, drastic reduction of Zn and Mg concentrations in the semen fluid may lead to disorders in male fertility. We aimed to analyse the differences in Mg and Zn levels in the seminal plasma of 213 males including 48 normozoospermic, 30 azoospermic, 28 oligoasthenozoospermic, 22 asthenozoospermic and 85 chronic prostatitis. Mg and Zn concentrations were measured using an atomic absorption spectrophotometer. While zinc levels did not show correlation either with the volume of the sperm or the percentage of pathological forms, magnesium concentrations in seminal plasma were significantly decreased in chronic prostatitis patients as compared to other groups or normozoospermic patients ($p<0.001$) We propose therefore magnesium as a marker of prostatitis.

Key words: Magnesium, zinc, seminal plasma, male infertility, prostatitis

INTRODUCTION

Trace elements are essential for cellular homeostasis and physiological functions. Their imbalance may be indicative of some specific disorders such as bone disease, cancer and male infertility. Cell concentrations of micronutrients usually ranges from 1×10^{-4} to 1×10^{-12} M, existing at optimal concentration for enzymic catalysis. It is known that magnesium and zinc concentrations in seminal fluid of patients help to indicate some specific forms of male infertility, when their measure is combined with cytological data like spermocytograms (31,37). It has been shown that the presence of abnormal levels of Ca^{++}, Mg^{++}, Zn^{++} and Cu^{++}, may affect spermatogenesis with regard to production, maturation, motility and fertilizing capacity of the spermatozoa (18,37,43).

It has been stated that zinc plays a key role in reproductive functions (4,7,17,28,). In men, zinc is found mainly in muscles (60%), bones (30%) and in prostate. Zinc levels are high in prostate glands, prostate secretions and spermatozoa (*i.e.* approximately 2.0 mM), but the exact role of zinc in reproduction still remains to be elucidated (43). Zinc in the seminal liquid seems to be secreted by the prostate gland as zinc citrate or bound to glycoproteins of the sperm vesicles (22). Zinc influences oxygen consumption of the spermatozoa in seminal plasma (9,19,40), coordination between head and flagella (5,17) and acrosin activity (39), and it stabilizes cell membrane and nuclear chromatin decondensation in spermatozoa (21,24). On a biochemical level, zinc is a cofactor for more than 200 metalloenzymes in a variety of animal species (12). Zinc finger proteins are involved, among others, in the genetic expression of steroid receptors (10).

As for magnesium, it plays a major role among plastic and catalytic micronutrients, intervening in the physiological equilibrium of the organism through two mechanisms: a) chelation of intercellular anionic ligands (mainly ATP), and b) competition with calcium (41). Mg^{++} ions are cofactors for more than 300 enzymes in glycolysis, phosporylation, membrane transfer of cations, DNA, RNA and protein synthesis. About 60% of Mg^{++} is found in skeleton, 20% in skeletal muscles, 19% in other cells and

1% in extra-cellular spaces (33). Magnesium bears two distinct forms in the blood:
- a diffusible form, including ionised free Mg^{++} (68%) and a complexed one (7%),
- a non-diffusible form, corresponding to Mg^{++} attached to plasma proteins (25%).

Several methods have been used to determine micronutrients in biological fluids, namely: spectro-colorimetry using ortho-cresol-phtalein complexes, electrochemistry, mass spectrometry, fluorescence analysis, and atomic absorption spectrophotometry with or without flame. The flame atomic absorption spectro-photometry has been preferred in our investigation since it ensures more reliable results even for slight physiological and pathological variations of metal ions in fluids in a short time interval. We focused our investigation on male patients complaining of infertility. The goal of the present work is to determine the magnesium and zinc concentrations in the seminal liquid of healthy men and to compare to patients examined for sterility in a reproductive unit of Central Hospital of Sofia (Laboratory of Biochemistry), Bulgaria.

MATERIALS AND METHODS

Patients and semen collection

The collection of the sperm was preceded by a 3-5 day sexual and alcohol abstinence. After hygienic cares - including hands washing and careful local cleaning - sperm was collected by masturbation into sterile propylene polymer tubes. An aliquot of the seminal plasma was separated from the spermatozoa after 10 min centrifugation at 8000 g at room temperature and then stored at -20°C until analysis.

The semen were collected from 213 men and were routinely analyzed for concentration, mobility, morphology of spermatozoa, as well as volume, pH, number of spermatozoa, viscosity, percentages of normokinesia, akinesia and number of leucocytes. According to the World Health Organization (44), a sperm count of $<20 \times 10^6$/ml (oligozoospermia), a motility decrease of more than 50% spermatozoa (asthenozoospermia) or a high proportion of abnormal morphology (>30%, signature of teratozoospermia) in the ejaculate sperms is regarded as abnormal semen. Furthermore, patients with clinical evidence of prostatitis were included in the trial. Finally, 5 groups of patients were obtained in our investigation as shown in Table 1.

Metals analysis of Mg and Zn in seminal liquid

Magnesium and zinc levels were determined using a flame atomic absorption spectrophotometer (Perkin Elmer, Model 460). The equipment was calibrated according to the manufacturer's instructions as follows: 213.9 nm for Zn and 285.2 nm for Mg (16). Calibration solutions were sequentially analyzed for Mg, 3.2 µmol/l and 6.4 µmol/l and for Zn, 6.12 µmol/l. Seminal fluids and calibration solution were all diluted to 1/200 with a 0.5% lanthane solution.

Table 1 Studied groups according to the routine analysis of semen

Diagnosis	Number
Chronic prostatitis	85
Normozoospermia	48
Oligoasthenozoospermia	28
Asthenozoospermia	22
Azoospermia	30

Statistical analysis

The results are expressed as mean ± SD values and analyzed using Student *t*-test to assess the statistical significance for $p<0.001$.

RESULTS AND DISCUSSION

Both magnesium and zinc, dosed in the seminal fluid, displayed a Gaussian curve in all groups as exemplified in oligoasthenozoospermia and asthenozoospermia series (Fig. 1 and 2) except in patients with chronic prostatitis. In Table 2 we present data of the analyzed elements in patients with oligoasthenozoospermia, azoospermia, asthenozoo-spermia and chronic prostatitis compared to normo-zoospermic patients.

The results showed that, among the two micronutrients analyzed in the seminal plasma of patients with pathological figures in their sperm counts, only magnesium concentration was significantly decreased in the chronic prostatitis group ($p<0.001$, Table 2). This was probably due to the inflammatory state of the prostate as suggested previously by Eliasson and Lindholmer (8). Furthermore we have established a strong correlation between magnesium and zinc concentrations in the seminal plasma of patients suffering from oligoasthenozoospermia and asthenozoospermia (Fig. 1 and 2). However, no correlation for the other groups could be observed (data not shown). Noteworthy, Saaranen *et al.* (34) have also reported a positive correlation between magnesium and zinc concentrations in the sperm of certain infertile patients.

Zinc concentration is, on the contrary, invariable for all groups with abnormal sperm counts as compared to the control group (Table 2). These results are in agreement with those observed by Saaranen *et al*. (34) and Stanwell-Smith *et al*. (38). However unlike Madding *et al.* (26), we did not find any correlation between zinc concentration and the volume of seminal liquid or the number of spermatozoa. The zinc concentration observed in the seminal liquid of healthy individuals with normozoospermia (2541 ± 1531 µmol/l) is close to that found by Saaranen *et al*. (34), namely 2264 ± 1148 µmol/l.

Daily needs of zinc are for men 15 mg for adults, and 25 mg for breast feeding women, and they vary with age. These are normally covered by diet. The syndrome of endemic zinc deficiency has been described in Iran and Egypt (30). The most obvious signs of zinc deficiency include dermatitis, retarded growth, hypo-secretion of growth hormones in response to stimulation (3), hypogonadism (35), delayed sexual development,

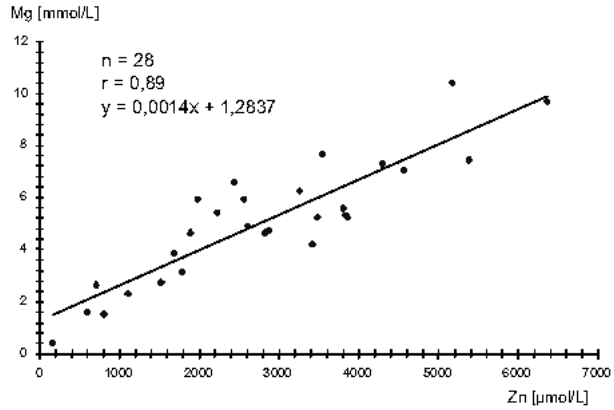

Fig. 1 Correlation in Zn and Mg levels in oligoasthenozoospermia

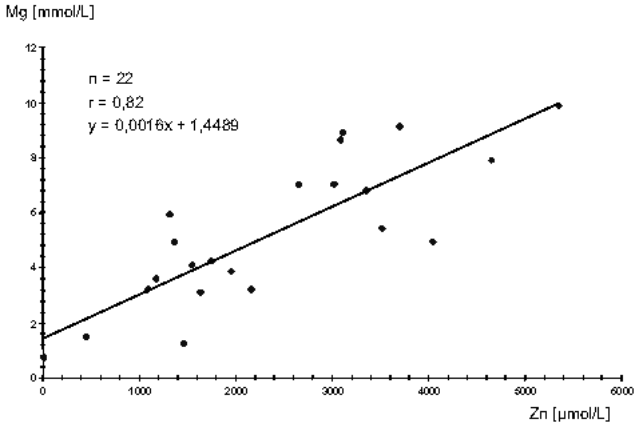

Fig. 2 Correlation in Zn and Mg levels in asthenozoospermia

pernicious anaemia (29), hepatosplenomegaly and hyperpigmentation. Zinc supplementation in the diet (30 mg/day) quickly reverses those effects (30). The zinc concentration found in the seminal liquid is many times higher than the concentration in other tissues or body fluids (11,45). Xu *et al.* (45) found that the mean concentration of zinc in seminal plasma was about 30 times higher than in blood and positive correlation in zinc content of seminal plasma and sperm density in normozoospermic men but not in oligozoospermic men. It seems therefore that zinc is an important factor playing a major role in the regulation of fertility (6,7,27,42), even if the mechanism in which it participates is not yet completely clear. Mohan *et al*. (28) and Huang *et al*. (20) reported that levels of zinc in serum and semen were significantly lower in infertile patients as compared to those of fertile males. In the study of Huang *et al*. (20), the zinc concentrations in both oligozoospermia and asthenozoospermia were lower than in controls. On the other hand, Lewis-Jones *et al*. (23) demonstrated that zinc concentrations were not influenced by the motile sperm concentrations (r = 0.062) and so concluded that seminal plasma zinc level is an unreliable marker of spermatogenesis, as it was shown in our study. In contrast, Henkel *et al*. (17) reported that the mean zinc concentration in the seminal plasma (144.3 mg/l) and in the whole ejaculate (146.9 mg/l) were significantly correlated with motility endpoints. The same authors observed that sperm heads contained only 6.7% of the zinc that was present in the whole spermatozoon. On the other hand, the zinc concentration in the flagella was negatively correlated with sperm motility and velocity, and positively correlated with the percentage of abnormally blue-stained flagella and the age of the patients. Semen zinc levels in normozoospermic men were significantly increased as compared to oligozoospermic men according to the study of Robak-Cholubek *et al*. (32). At the molecular level, zinc

Table 2 Concentration of Mg and Zn in the seminal plasma of patients with respectively normozoospermia, oligoasthenozoospermia, azoospermia, asthenozoospermia and chronic prostatitis

Elements	Normo zoospermia			Oligoastheno-zoospermia			Azoospermia			Astheno-zoospermia			Chronic Prostatitis		
	n	$\bar{x}$	sd	n	$\bar{x}$	sd	n	$\bar{x}$	sd	n	$\bar{x}$	sd	n	$\bar{x}$	sd
Mg (mmol/l)	48	5.4	2.96	28	5.2	3	30	5.4	2.6	22	5.6	3.3	85	1.3	0.54
Zn (µmol/l)	48	2541	1531	28	2826	1671	28	2596	1392	22	2404	1288	83	2205	1314
Student test Mg					NS			NS			NS			p<0.001	
Zn					NS			NS			NS			NS	

NS: not significant; n: number of patients; $\bar{x}$: mean; sd: standard deviation

may elicit an inhibition of both superoxide anion production and SOD-like activity in human spermatozoa. The existence of novel zinc-related mechanism(s) decreasing oxidative events occurring after ejaculation may be supposed (13,14). Indeed male germ cells generate reactive oxygen metabolites due to their energy consumption for motility, and zinc may reduce the effects of oxidative stress (2).

Like zinc, magnesium in seminal plasma is mainly released from prostate and its concentration reflects prostate function (8,25). During urological infection and prostatitis the level of Mg (as it was shown in our study) and Zn in the seminal plasma were lowered, in accordance with the prostatic origin of these ions. So the determination of the concentration of zinc and magnesium in the seminal plasma may be useful in evaluating the secretory capacity of prostate gland. However, we were unable to confirm in our study the decrease of Zn content in seminal fluid of patients with prostatitis.

Our results showed a significant relation of low concentration of magnesium in seminal plasma in patients with chronic prostatitis but not with fertility troubles. We suggest therefore that the seminal magnesium concentration may serve as a biomarker of prostatitis.

CONCLUSION

Two parameters (genetic and environmental) are involved in male infertility. At the genetic level, infertility is related to mutations of the Y chromosome which is know to present specially high rates of spontaneous DNA damage (1,15). However male infertility is also in relation with nutritional and environmental factors as it has been confirmed that a) male sperm counts are declining, and b) environmental factors, such as pesticides, exogenous estrogens, but also metals may negatively impact on spermatogenesis (36).

Thus a multi-faceted therapeutic approach is needed to improve male fertility with the identification of harmful environmental or occupational risk factors (like 2-butoxyethanol) and with the correction of underlying nutritional imbalances. Both actions may increase sperm production and function. Among those environmental factors are those at the origin of chronic prostatitis that lower magnesium concentration in the seminal plasma. We suggest that such analysis may serve as criteria to differentiate prostatitis from pure infertility problems.

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