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Zinc, Magnesium and Gamma Glutamyltransferase levels in Human Seminal fluid

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Objectives: The purpose of this study was to determine the levels of Zn, Mg and γ-GT in seminal fluids as an indirect method for the evaluation of the excretory function of the prostate gland.

Subjects: Seminal fluid specimens were collected from 167 Jordanian males referred for infertility investigation.

Methods: Descriptive seminal fluid analyses were performed on all samples according to WHO standards, and were divided into four groups: normospermia (n=70); oligospermia (n=50), azoospermia (n=22) and asthenospermia (n=25). Zn and Mg were determined in the seminal plasma of each group by atomic absorption spectrophotometery and γ-GT was determined by enzymatic method.

Results: Compared with the other groups, oligospermic group had the highest proportion of specimens with abnormal liquefaction (26.0%) and leukocyte counts (32.0%). Both oligospermic and asthenospermic groups had comparable sperm motility, which was lower than that in normospermia ($p < 0.05$). The mean levels of γ-GT and Mg in oligospermia were significantly lower than that in all other groups. The mean Zn levels in oligospermia and azoospermia were lower than that in the other two groups. Significant positive correlation was observed between γ-GT, Zn and Mg in all four groups. The mean levels of γ-GT, Zn and Mg correlated positively with sperm count and motility only in the normospermic group.

Conclusion: The presence of a high ratio of leukocytes in association with decreased levels of Zn, Mg and γ-GT in oligospermia, suggests disturbed function of the prostate gland, most probably due to infection. Measurement of these parameters may shed some light on the treatment and management of oligospermic infertile men.

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Despite the fact that the diagnosis of male infertility is a rapidly developing field of investigation, descriptive seminal fluid analysis remains the major tool for the evaluation of semen quality and its possible contribution to male infertility. In the last two decades, numerous biochemical and biophysical assays were performed on normal and abnormal semen qualities in an attempt to elucidate factors that contribute to male infertility. The levels of trace elements and enzymes in seminal fluid were mostly investigated in fertile and infertile men, mainly for the purpose of evaluating the functional condition of the genital accessory glands[1-4]. Zinc (Zn) and magnesium (Mg) are among the major cations secreted by the prostate gland[5-7], and their relationship to male infertility is not clear. However, there is evidence that altered seminal levels of Zn and Mg have been correlated with decreased fertility potential; low levels of these cations in seminal fluid have been observed in infertile men suffering from poor seminal fluid quality such as oligospermia, asthenospermia, and prostatitis[8-13]. On the other hand, high levels of these cations in seminal fluid have been associated with asthenospermia and with other seminal fluid qualities[14-16]. Gamma Glutamyltransferase (γ-GT) in seminal fluid is secreted mainly from the prostate gland, and is approximately 200 times higher than that of blood[17-19]. Its contribution to male infertility is not well defined, and its levels in normal and abnormal seminal fluids are conflicting[17, 19,20]. Recent studies have demonstrated that the enzyme γ-GT itself is not necessary for reproductive function, but plays a major role in the glutathione system that is involved in the protection of spermatozoa against oxygen radicals[21-23].

Literature relevant to seminal fluid quality and its possible contribution to male infertility in Jordanian men is very scarce, if at all available. The aim of this study is to determine the levels of Zn, Mg and γ-GT in the seminal plasma and to correlate them with semen quality in Jordanian males attending infertility clinics.

METHODS

A total of 167 men attending infertility clinics were referred to Modern Medical Laboratories (Amman, Jordan) for seminal fluid analysis. The mean age of investigated patients was 27 $\pm$ 7 years. Samples were collected by masturbation in wide mouth sterile container after 3 days of abstinence. Shortly after collection, semen samples were analyzed for liquefaction time, volume, leukocyte count, motility, morphology and count of spermatozoa according to the World Health Organization (WHO) standards[24]. Accordingly, samples were divided into four groups: normospermia (n = 70) indicated by sperm count $\geq$ 20 millions/ml, motility $\geq$ 50% and normal morphology > 40%; oligospermia (n = 50) indicated by sperm count <20 millions/ml irrespective of motility or morphology; azoospermia (n = 22) indicated by the complete absence of spermatozoa; and asthenospermia (n = 25) indicated by sperm count $\geq$20 millions/ml and motility <50% irrespective of morphology. All samples were centrifuged at 4000 rpm for 15 minutes and seminal plasma was separated and kept in trace elements-free containers at -70^{o}C until analyses. γ-GT enzyme levels were determined by spectrophotometer at 37^{o}C using commercially available kit (Boehringer Mannheim, GMPH). Zinc and magnesium levels were determined by Varian atomic absorption spectrophotometer.

Data analysis was carried out by means of one-way analysis of variance (ANOVA) and by multiple comparison using computer statistical analysis software

(STATISTICA for Windows (1995), Stat Soft Inc, OK, USA). Data are expressed as the mean ± SD and $P < 0.05$ were considered statistically significant.

RESULTS

Presented in table 1 are the spermogram results for the four groups of seminal fluid samples classified according to WHO standards. As expected, the mean sperm count in oligospermia was significantly lower than that in the other groups and the sperm motility in asthenospermia was lower than that in normospermia. No significant difference was observed regarding the volume of ejaculate and sperm morphology among the four groups. The table demonstrates that patients with oligospermia, compared with the other groups, constitute the highest percentages of ejaculates with abnormal liquefaction (26.0%) and leukocyte counts (32.0%). Additionally, the table shows that sperm motility in oligospermia is comparable with that in asthenospermia, but significantly lower than that in normospermia ($p < 0.05$).

Table 1. **Classification and seminal fluid analysis of patients referred for infertility investigation**.

Parameter	**Normospermia**	**Oligospermia**	**Azoospermia**	**Asthenospermia**
n (%)	70 (41.9)	50 (29.9)	22 (13.2)	25 (15.0)
Volume (ml)*	2.9 ± 1.0	2.7 ± 0.9	3.1 ± 1.2	3.2 ± 1.2
Count (millions/ml)*	77.4 ± 55.6	6.8 ± 5.8	-----	89.7 ± 54.0
Motility (%)*	65.0 ± 7.0	33.1 ± 20.0	------	31.1 ± 11.3
Normal Morphology (%)*	70.4 ± 9.9	51.1 ± 16.0	------	56.5 ± 12.5
Abnormal Liquefaction, n (%)	12 (17.1)	13 (26.0)	4 (18.2)	4 (16.0)
Leukocytes, >1million/ml, n (%)	5 (7.0)	16 (32.0)	2 (9.0)	5 (20.0)

**Results expressed as mean ± SD*

Table 2 shows the mean levels of the biochemical parameters in the four groups of semen specimens. The table reveals that patients with oligospermia have significantly lower mean levels of γ-GT than that in the other groups ($p < 0.05$). No significant difference was observed among the other groups regarding the levels of γ-GT. Patients with oligospermia and azoospermia had lower mean Zn levels than that in both normospermia and asthenospermia ($p<0.01$). The lowest mean Zn levels were observed in the azoospermia group while the highest was in asthenospermia. Magnesium mean levels were significantly lower in patients with oligospermia than that in the other groups ($p<0.05$). No significant difference was observed regarding Mg in the other groups. Statistically significant positive correlation was observed between the mean levels of γ-GT, Zn and Mg in all four groups ($p<0.001$). However, the mean levels of γ-GT, Zn and Mg correlated positively with sperm count and motility only in normospermic group ($p<0.05$). No significant correlation was observed between the measured parameters and the spermogram of the other groups.

Table 2. **Seminal fluid biochemical parameters in the four groups of patients.**[*]

Parameter	Normospermia	Oligospermia	Azoospermia	Asthenospermia
γ-GT (U/L)	10470 ± 4656	7125 ± 4105[a]	9315 ± 3674	9835 ± 5543
Zn (mg/dl)	22.5 ± 7.3[b]	17.4 ± 5.8	14.8 ± 3.5	23.3 ± 8.5[b]
Mg (mg/dl)	8.3 ± 4.3	6.4 ± 3.9[c]	8.9 ± 4.1	8.6 ± 3.8

[*] *Results expressed as mean ± SD*
[a] *Significantly lower than that in the other groups (p < 0.05)*
[b] *Significantly higher than that in the other groups (p < 0.01)*
[c] *Significantly lower than that in the other groups (p < 0.05)*

DISCUSSION

The present study was conducted on 167 Jordanian males referred to the laboratory for seminal fluid analysis. The specimens were classified into four groups as indicated earlier. The results demonstrate (Table 1) that 41.9% of specimens were normal (normospermia group) while the others were of abnormal qualities. Within the specimens with poor seminal fluid qualities, the oligospermic group was the predominant one (29.9%), with the highest proportion of specimens with abnormal liquefaction (26%), increased leukocytes count (32.0%), and decreased sperm motility. Sperm motility in the oligospermic group was comparable to that of the asthenospermic group and is attributed to the presence of 34 specimens (68.0%) with poor sperm motility among the oligospermic group. If this number is added to that in the asthenospermic group, this would indicate that poor sperm motility is a major abnormality among referred patients.
Previous reports[25,26] have demonstrated that the presence of leukocytes in a concentration greater than 1 million/ml of ejaculate might be caused by accessory gland infection that would be associated with poor seminal quality such as decreased sperm count and motility. Results obtained by this study are in close agreement with these reports and may suggest that abnormalities observed in oligospermia, and probably in asthenospermia, are likely to be caused by infection in the genital accessory glands.

The role of trace elements and enzymes secreted by excretory glands into ejaculates and their contribution to male infertility is poorly understood. However, many biochemical investigations were previously, and are still being, conducted on normal and abnormal seminal fluids in an attempt to correlate certain parameters with poor seminal fluid quality that may contribute to male infertility. Since Zinc, Mg and γ-GT are excreted mainly from the prostate gland, their measurement in the seminal fluid could be considered as markers for the evaluation of the function of this gland.
The positive correlation observed between Zn, Mg and γ-GT in the four types of seminal fluid specimens were in agreement with previous reports[2, 5,8,10,27]. This is in fact an expected finding because the origin of all of these parameters is the prostate gland.

The function of γ-GT in seminal fluid is not well defined. However, it has been demonstrated that it affects production and viability of spermatozoa since it plays a role in the glutathione system in preventive oxidative stress on spermatozoa[21-23].

Previous studies reported low levels of seminal fluid γ-GT in association with various conditions such as prostate gland infection[28], oligospermia[3] and in ejaculates with abnormal liquefaction[20]. Others[29] observed higher levels in patients with excretory azoospermia. Although some investigators reported no significant correlations between γ-GT levels and volume, sperm density or motility[19,29], others however, reported positive correlation of γ-GT with sperm density only[20]. Results presented in the present study indicate that the levels of γ-GT were significantly lower in oligospermia than all other groups, and positively correlated with sperm count in the normospermia group, which is in agreement with some previous reports[3,19,20,28,29]. The observed low levels of γ-GT in the oligospermic group might be attributed to disturbances in the excretory function of the prostate gland. Previous reports have demonstrated that low level of γ-GT in seminal fluid is a good marker for the detection of infection in the accessory genital glands[28]. The finding that 32% of oligospermic specimens reported in this study had leukocytes concentration greater than 1 million/ml, suggests the presence of infection to the genital glands, most probably the prostate, which may lead to decreased excretion of γ-GT. Based on our observations, and of others, it can be suggested that decreased levels of γ-GT in association with infection, may lead to decreased production and viability of spermatozoa.

Various studies have suggested that Zn plays an essential role in the physiology and development of gonads and spermatozoa[30,31]. It is important in sperm production and/or viability, and is important in he prevention of spermatozoa degradation[31]. In addition to its antibacterial activity in seminal fluid[14], previous studies revealed that sperm motility was improved after the addition of zinc citrate to fresh semen specimens[32] and after treatment of patients suffering from asthenospermia with zinc sulfate[33]. Despite these facts, reports related to Zn levels in seminal plasma and its possible association with male infertility are conflicting. Earlier studies reported low Zn levels in seminal plasma in association with various conditions such as prostate infection[8], oligospermia[11, 12], oligoasthenospermia and azoospermia[15], and in infertile men regardless of semen quality[7, 13]. Moreover, positive correlation of seminal fluid Zn levels with sperm density and motility, have been reported by some investigators[13,15,34,35], while others reported no correlation with semen quality or with male infertility[4,6,27,36]. Furthermore, high levels of Zn were observed in some patients with asthenospermia and in ejaculates with poor sperm progressive motility[14-16]. Although no statistically significant correlation was observed between the low Zn levels and poor sperm motility in the oligospermic group, the results however, were consistent with previous reports[11,12,15]. The low Zn levels in the oligospermic group, that might be attributed to disorders in the prostate excretory function, or possibly due to asymptomatic prostate infection, may contribute to poor spermatogenesis and poor motility observed in these patients. The finding that patients with azoospermia had the lowest Zn levels among the other groups may be explained by the fact that due to the complete absence of sperms in this group, requirement of Zn as an essential element for spermatogenesis or motility is expected to be minimal. On the other hand, patients with asthenospermia had the highest levels of Zn, which were slightly higher than that in normospermia, is also in agreement with previous reports[14,15]. The observation of higher Zn levels in asthenospermia indicate an increased demand for Zn to enhance motility, and suggesting the presence of other factor that might contribute to poor motility.

Similar to Zn, the role of Mg in seminal fluid and its contribution to male infertility is not well defined. Reports pertaining to the levels of Mg in various seminal qualities are also conflicting. Few studies have reported low Mg levels in ejaculates associated with oligospermia and infection to the accessory genital glands[1,8-10], while others reported no significant association with poor semen quality or male infertility[2,4,27,32]. Treatment with magnesium sulfate has been reported to improve sperm motility and density in patients suffering from oligospermia[37]. Some workers suggested the measurement of magnesium, rather than Zinc, as markers for infection because it was found that Mg levels were markedly decreased during prostate infection[10]. Our results revealed positive correlation of Mg with sperm count and motility only in normospermia. This finding suggests that this group of patients have normal excretory function of the prostate gland. On the other hand, the observed low Mg levels in ejaculates with oligospermia, which is in agreement with some previous reports[8-10], suggest that this group of patients may suffer from disorder of the excretory function of the prostate gland, most probably infection.

CONCLUSIONS

The main outcome of this study is that poor sperm motility is a major abnormality among the studied patients. Poor quality of seminal fluid is associated with an altered levels of some of the trace elements and enzymes excreted by the prostate gland such as Zn, Mg and γ-GT. Measurement of these parameters in the seminal fluid, mainly in patients with oligospermia, may aid in the evaluation and treatment of male infertility.

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