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Effect of a significant deficiency in some antioxidants on semen parameters in cases of male infertility in Diyala Province, Iraq

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Abstract

***Background & Aims*:** Scientific evidence indicates that oxidative stress (OS) is a major factor in the etiology of male infertility. OS occurs when there is an imbalance between reactive oxygen species (ROS) and protective antioxidants. Current study aimed to evaluate some antioxidants and their effects on semen parameters in infertile Iraqi men.

***Materials & Methods*:** In this study, 47 infertile males and 21 healthy people as the control group were enrolled. parameters for seminal fluid were including count, motility and morphology for assessment of semen quality. Glutathione, Vitamin E, Zinc and Selenium were also measured.

***Results*:** Findings showed that sperm counts, percent of sperm motility and percent of normal morphology were significantly lower in the infertile male group compared to healthy group ($p<0.001$); whereas, abnormal motility and morphology in infertile male group were considerably higher than healthy group ($p<0.001$). Moreover, Results showed significant decrease in levels of Glutathione, Vitamin E, Zinc and Selenium in the infertile male group than the healthy group ($p<0.001$). Additionally, study reported a non-significant correlation between glutathione, vitamin E, zinc and selenium with semen parameters ($P>0.05$), except zinc level which showed significant correlation with the normal morphology of sperm ($P<0.05$). The results also showed a significant decrease in Vitamin E, Zinc and selenium levels in abnormal infertile groups compared to healthy group.

***Conclusion*:** We believe that male infertility may be related with low antioxidant levels. Therefore, future studies should concentrate on enzymatic and non-enzymatic antioxidants, as well as genetic susceptibility and its effects on sperm quality.

***Keywords*:** Glutathione, Male Infertility, Vitamin E, Selenium, Zinc

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Introduction

The inability to achieve spontaneous pregnancy after 12 months or more of regular unprotected sexual contact is known as infertility. Infertility affects couples on a variety of levels; recent studies showed that infertility is affecting about 15% of couples worldwide and male factor is responsible for almost 50% of infertility cases (1). According to the International Classification of Diseases, 11th Revision (ICD-11) (Geneva: WHO 2018) male infertility is frequently brought on by changes in semen ejection, a lack of sperm or sperm levels, or changes in sperm morphology and motility (2). Male subfertility's idiopathic causes have only recently been the subject of clinical and laboratory research efforts. The significant role of oxidative stress at the cellular level has been highlighted by researchers (3). It is estimated that half of all infertility cases between 25% and 87% of male subfertility is caused by the harmful effects of oxidative stress on sperm, and 1 in 20 man will be affected by subfertility (4). The overwhelming scientific evidence indicates that oxidative stress (OS) is a major factor in the etiology of male infertility (5). OS can occur when protective antioxidants and reactive oxygen species (ROS) are not balanced. OS can result in abnormal sperm parameters, significant sperm DNA damage, and even apoptosis. (6,7).

The term "male oxidative stress infertility" (MOSI) has recently been proposed, pointing out that between 30% and 80% of infertile men have elevated ROS in their sperm, affecting about 37.2 million infertile men (8). Exogenous or endogenous factors may be to blame for elevated ROS levels. The most frequent exogenous causes of oxidative stress in reproductive cells include obesity, poor nutrition, smoking, alcohol use, and environmental pollution. Additional causes recognized as endogenous are infections, autoimmune diseases, and chronic illnesses (9). Therefore, maintaining the redox potential balance, or balancing ROS by antioxidants, is necessary to maintain the optimal functioning of sperm cells (10).

Antioxidants are classified into two types: enzymatic antioxidant systems and non-enzymatic antioxidant systems. The enzymatic system consists of ascorbate peroxidase, alpha-dioxygenase, dehydroascorbate reductases, catalase, glutathione reductase, glutathione peroxidase, NADPH oxidase, Glutathione-S-transferase, superoxide dismutase, and peroxiredoxin. On the other hand, the non-enzymatic system consists of ascorbate, vitamin A, bilirubin, uric acid, glutathione, fibrin, mycothiol, melatonin, serum albumin, and phenolics, in addition to a variety of ingredients that are ingested along with food, including dietary supplements such as vitamins (11-14). Although a connection between systemic antioxidant deficiency and male infertility has not yet been documented, it’s possible that some infertile men may be at the risk for antioxidant deficiency, especially those with certain lifestyles like smoking, drinking more alcohol, and dieting, who may also be at high risk for vitamin or antioxidant deficiencies (15). Nowadays, it is well known that a number of natural antioxidants, including alpha-lipoic acid, inositol, folate, zinc, selenium, coenzyme Q10, and vitamins are linked to an improvement in sperm quality by preventing OS (2). Due to the importance of the effect of antioxidants and their relationship to fertility, in our study, Glutathione, Vitamin E, Zinc and Selenium were chosen because of their important roles and significant effects in neutralizing the activity of free radicals and protecting sperm, as well as essential elements in the formation of sperm and testosterone biosynthesis (16). So, the present study aims to evaluate some antioxidants and effects on semen parameters in infertile Iraqi men.

Materials & Methods

Collection of seminal fluid:

The semen samples of forty-seven male infertile participants in this study and twenty-five healthy persons who recorded as the control group was collected after at least three days without having sex. Semen samples were collected in a sterile, clean, wide-mouthed, and labeled disposable plastic container by asking the groups to masturbate in the room next to the laboratory. Containers were closed and labeled (name, age, time of ejaculation, and duration of abstinence). To allow for liquefaction, the specimens were placed in an incubator at 37°C for 15–30 minutes. Following liquefaction and immediate processing, the following seminal fluid routine parameters were evaluated using the methods recommended by the World Health Organization (WHO) (17). After that, according to the guidelines of the WHO, the male group of infertile people were divided into three categories: azoospermia, oligozoospermia, and asthenozoospermia (18,19).

The parameters included sperm count, morphology, motility, viscosity, pH, and sperm appearance (18). Non-enzymatic antioxidant functions were evaluated using tests for Glutathione, Vitamin E, Zinc and Selenium after recording abnormal semen results. The participants' samples were taken between September 15, 2021, and August 20, 2022.

Collection of samples:

The samples were collected from different locations of Diyala Province, Iraq, from the healthy people as control group and infertile males as the case group from the auditors to consulting clinics and specialized laboratories in Baqubah City, Diyala Province, Iraq, for a purpose of diagnosis and treatment. The age ranged from 29 to 46 years.

After being informed about the study, each participant provided informed consent. As a result, the entire process of the investigation was based on the participants' agreed assent. In addition, all participants' weight and height were measured to calculate their Body Mass Index (BMI) using the equation = weight / (height)2 (20).

Six milliliters of venous blood were collected from the healthy and infertile groups and deposited in separate tubes. The serum samples were collected from the clotted blood using a centrifuge at 5000 rpm for 10 minutes. The serum was then stored in an eppendorf tube at -20°C. The enzyme linked immunosorbent assay (ELISA) was performed to evaluate the levels of Glutathione and Vitamin E (kits from Sunlong Biotech, China), whereas, Zinc and Selenium level were measured by atomic absorption.

Statistical analysis:

For the statistical analysis, SPSS (Statistical Package for Social Sciences) version 17.01 was used to examine statistical differences in parameter outcomes. In the study, an independent samples t-test was used to analyze the raw data and compare two groups' parameters; so there the mean and standard deviation were determined in study. Pearson's correlation test was used to investigate the relationship between non-enzymatic antioxidants and sperm parameters. Analysis of variance (ANOVA) were employed to compare non-enzymatic antioxidant levels in different groups. The threshold for statistical significance was set at a p-value of less than 0.01 or 0.05.

Results

As shown in the table 1, healthy and infertile male groups were similar in terms of age with significant difference ($p<0.05$). However, there was no statistically significant difference between both groups, in the average BMI ($p=0.212$). Table 1 displays the mean values of the studied sperm parameters in the healthy and infertile groups. The healthy group had significantly higher levels of sperm count, normal motility, and the proportion of normal morphology than the infertile male group ($p<0.001$). However, only proportion of abnormal motility and abnormal morphology in infertile male group had significantly higher levels than healthy group ($p<0.001$).

Table 1: Parameters of semen quality in infertile males and the healthy group

Variables	Infertile male group Mean (SD) n=47	Healthy group Mean (SD) n=25	P value
Age (years)	34.36 (4.1)	32.28 (2.1)	<0.05*
BMI (kg/m^2)	22.32 (3.6)	21.26 (2.9)	0.212 NS
Sperm counts (million/ml)	34.61 (10.9)	77.20 (12.6)	<0.001**
Sperm motility (%)			
Normal	39.34 (10.2)	73.84 (10.7)	<0.001**
Abnormal	60.66 (10.2)	26.16 (10.7)	<0.001**
morphology (%)			
Normal	48.20 (16.0)	81.32 (6.1)	<0.001**
Abnormal	51.80 (16.0)	18.68 (6.1)	<0.001**

NS: Non-significant, ** (P<0.01), * (P<0.05)

According to the data in the table 2, serum non-enzymatic antioxidant levels were significantly lower in the infertile male group than in the healthy group. The results showed that serum glutathione levels in infertile male group were lower than in the healthy group (P<0.001). In addition, serum vitamin E levels were lower in the infertile male group than in the healthy group (P<0.001). Furthermore, both serum zinc and Selenium levels were lower in the infertile male group than the healthy group (P<0.001).

Table 2: Comparison of levels of glutathione, vitamin E, zinc and selenium between infertile males and the healthy group

Variables	Infertile male group Mean (SD) n=47	Healthy group Mean (SD) n=25	P value
Glutathione µmol/L	7.94 (1.1)	10.39 (0.9)	<0.001**
Vitamin E mg/L	4.77 (0.7)	5.88 (1.2)	<0.001**
Zinc µmol/L	9.10 (1.6)	11.42 (1.2)	<0.001**
Selenium µg/dL	4.30 (0.8)	4.84 (0.7)	<0.001**

** (P<0.01)

Table 3 indicates that there was no significant correlation between serum glutathione and the parameters of semen quality in infertile male group. Moreover, there was no significant correlation between serum vitamin E and the parameters of semen quality in infertile male group. Additionally, no significant correlation between serum selenium and the parameters of semen quality in infertile male group. However, a significant correlation was found between serum zinc level and morphology of sperm. There was no correlation between serum zinc level and residual parameters of semen quality in infertile male group.

Table 3: correlation coefficient (r) values between serum glutathione, vitamin E, zinc and selenium levels with semen parameters for Infertile male group

Variables	Infertile male group n=47			
	Glutathione (r)	Vitamin E (r)	Zinc (r)	Selenium (r)
Sperm counts	0.086	-0.111	-0.243	-0.167
Normal Sperm motility (%)	-0.012	0.105	-0.015	0.076
Normal morphology (%)	0.127	-0.113	-0.369*	0.130

* (P<0.05)

Table 4 indicates a significant decrease in serum glutathione levels, azoospermia, oligozoospermia and asthenozoospermia in infertile group compared to healthy group (P<0.05). There was a significant decrease in the serum levels of vitamin E of oligozoospermia, asthenozoospermia and Azoospermia groups compared to healthy group (P<0.05). Additionally, there were also a significant decrease in serum zinc levels in oligozoospermia, asthenozoospermia and Azoospermia groups compared to healthy group (P<0.05). Furthermore, the result showed a significant decrease in serum selenium levels in oligozoospermia, asthenozoospermia, and azoospermia groups compared to healthy group (P<0.05).

Table 4: Comparison of levels of glutathione, vitamin E, zinc and selenium between abnormal infertile groups and the healthy group

groups	Variables			
	Glutathione µmol/L Mean (SD)	Vitamin E mg/L Mean (SD)	Zinc µmol/L Mean (SD)	Selenium µg/dL Mean (SD)
Healthy	10.39 (0.9) *	5.88 (1.2) *	11.42 (1.2) *	4.84 (0.7) *
Asthenozoospermia	8.08 (0.9)	4.75 (0.7)	8.94 (1.5)	4.26 (1.0)
Oligozoospermia	7.83 (1.2)	4.90 (0.6)	9.50 (1.8)	4.51 (0.5)
Azoospermia	7.71 (1.3)	4.57 (0.8)	8.80 (1.0)	4.01 (0.9)

* (P<0.05)

Discussion

According to the estimates, infertility problems impact about 25% of men globally. The process of spermatogenesis is impacted by a number of factors, including nutrition, genetics, physiological problems, and the environment (21). The current study indicated that ages of both groups were comparable (p<0.05) (table1), which is consistent with the findings of Alnajjar et al. (22). Most infertile patients were found to be between the ages of 25 and 50. The characteristics of sperm analysis revealed significant decrease in sperm count, sperm motility, and normal sperm morphology in the infertile male group as compared to the healthy group. Our findings concur with the results of Al-Baldawi et al. (23), who reported that sperm quality affects spermatozoa's ability to become fertilized. Furthermore, the findings of a study conducted by Al Jebory et al. (24) indicated that reduced consumption of some antioxidant agents in the group of infertile males is related to increased risk of poor sperm concentration, motility, and morphology. The current study showed a highly significant decline in glutathione levels in infertile male group compared to healthy group counterparts. It has been established that a glutathione deficiency leads to spermatozoa instability which results in defective sperm motility. This implies that deficient glutathione levels may have an impact on male fertility (25). Moreover, increased levels of lipid peroxidation and free radical formation are lowering antioxidant levels in the blood as a result of continual oxidation of these antioxidants during the neutralization of these free radicals, showing that the antioxidant defense system is impaired in infertile males (26). Our study results showed significant decrease of vitamin E levels in infertile male group compared to healthy group counterpart, and this finding is consistent with the study of Rengaraj et al. (27), which showed that vitamin E deficiency impairs fertility in both humans and laboratory animals. Generally, found in cell membranes, vitamin E is regarded as a fat-soluble organic molecule that shields sperm cell membranes from oxidative damage (28). Any variation in vitamin E levels may have a role in infertility. Superoxide anion, hydrogen peroxide, and hydroxyl radicals are all immediately neutralized by vitamin E; hence an increase in these free radicals may cause vitamin E to become depleted (29). Additionally, our findings showed a significant decrease in zinc levels in the group of infertile males compared to their counterparts in the healthy group, and this finding is consistent with the results of Al-Baldawi et al. (23), who demonstrated the importance of zinc in fertility through its direct and indirect effects on spermatogenesis. Zinc plays important roles in the early phases of germ cell development and spermatogenesis, sperm cell maturation and development, ejaculation, liquefaction, and fertilization (30). As a result, zinc deficiency may be a major risk factor for poor sperm quality and idiopathic male infertility (31). Regarding the results of the study, there was a significant decrease in selenium levels in infertile male group compared to healthy group counterparts. Selenium appears to be important for sperm cells' antioxidative defense. Spermatogenesis is significantly impacted when a breakdown in selenium homeostasis occurs (32).

The results of our study agreed with the study of Onono et al. (33) which showed that selenium deficiency in men leads to oligospermia and hence impaired spermatogenesis, decreased sperm motility and ability to fertilize, and an increase in the amount of aberrant male sex cells as a result of oxidative stress damages on integrity of their membranes. In the current study, we report a non-significant correlation between glutathione, vitamin E and selenium with semen parameters, as well as, a non-significant correlation was observed between zinc and sperm counts and motility; serum zinc levels showed significant correlation with normal morphology of sperm. Similar results have been reported in earlier researches (34,35).

There is evidence that zinc influences spermatozoa's physiological processes such as their motility and morphology, and a decline in seminal zinc content results in poor-quality semen and fewer opportunities for fertilization (36). Additionally, though the reasons for the decline in male fertility are still unknown, a number of lifestyle factors, particularly an unhealthy diet, are thought to be major causes of male reproductive health impairment. However, roughly 30% of cases of male infertility are known as idiopathic infertility since they continue to have unidentified reasons (37,38). In the present study, we report a significant decrease in serum glutathione levels in azoospermia, oligozoospermia and asthenozoospermia groups compared to healthy group. This result agree with Krzyściak et al. (39) which indicated a decrease in glutathione in the abnormal infertile groups compared to healthy group. Numerous studies have reported a decrease in glutathione concentration in the groups of oligozoospermia and asthenozoospermia, which may be related to a decrease in NDPH coenzyme. NDPH is necessary for the production of glutathione and also is a catalytic agent for the enzyme glutathione reductase, which works to convert glutathione from its GSSG (glutathione disulfide) form to glutathione (40,41). On the other hand, results of the study showed a significant decrease in serum zinc, vitamin E, and selenium levels in oligozoospermia, asthenozoospermia and azoospermia groups compared to healthy group. In summary, several studies have shown deficiency effects of zinc, selenium and vitamin E in men with infertility. A number of studies showed that insufficient zinc levels prevent spermatogenesis, contribute to sperm abnormalities, and diminish blood testosterone levels (42).

In contrast, selenium seems to have a favorable effect on the Leydig cell, and thus in turn, affects the release of testosterone (32). Vitamin E is however an essential substance for preventing oxidative damage to cell membranes by capturing and scavenging free radicals within cellular membranes (43,44). Another study indicated that zinc, selenium, and glutathione correlations with indices of sperm quality and suggested that a decrease in seminal antioxidants may be a risk factor for sperm abnormalities and idiopathic male infertility (45). According to Ritchie et al. (46), idiopathic male infertility may be caused by decreased seminal plasma antioxidant activity and increased ROS generation. Studies showed that antioxidant therapy had a significant positive impact on basic sperm parameters, advanced sperm function, outcomes of assisted reproductive therapy, and live birth rate in a comprehensive review of the impact of oral antioxidants on male infertility. The most frequently used nutrients were vitamin C, vitamin E, N-acetyl cysteine, carnitines, coenzyme Q10, selenium, zinc, lycopene, and folic acid (47).

Conclusion

In conclusion, the present study shows significant decrease in the serum levels of glutathione, vitamin E, zinc and selenium in infertile male group than in the healthy group. Thus, we believe that male infertility may be related with a low antioxidant levels. Future studies should concentrate on enzymatic and non-enzymatic antioxidants, as well as genetic susceptibility and its effects on sperm quality.

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Ethical approval

The study was approved by the Council College of Education for Pure Sciences/University of Diyala's local ethics committee (Code: CEPS.UD.REC NO.190.16/11/2023).

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