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Investigation of oxidative stress parameters and hormone levels in men with suspected infertility

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ABSTRACT

Aim: It was suggested that oxidative stress (OS) is one of the major causes for male infertility. Therefore, the purpose of this study was to evaluate the OS parameters and some hormone levels in men with suspected infertility.

Methods: A total of 100 males was included in the study. Study groups were designed as 5 groups of normozoospermia (n=20), azoospermia (n: 20), oligozoospermia (n=20), oligo-asthenozoospermia (n=20), idiopatic infertility (n:20) according to sperm parameters and etiology. Sperm parameters and OS markers including plasma total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), 8 Hydroxy 2 deoxy Guanosine (8-OHdG) were evaluated in all groups. In addition, FSH, LH, total testosterone, TSH, prolactin, estradiol, folic acid, Vitamin B12 and Vitamin D were analyzed.

Results: TAC was the lowest in azoospermia group compared to other groups while the highest TAC was in normozoospermia group (*p=0.002*). Total FSH and LH levels were higher in azoospermia group compared to other groups.

Conclusions: As far as we know, this is the first study evaluating OS markers and hormones in men with suspected infertility. Our results show that the OS is increased in men with azoospermia compared to other groups.

Keywords: Male infertility, oxidative stress, sperm parameters, hormones.

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Introduction

Infertility is defined as the absence of spontaneous pregnancy despite at least 12 months or more of regular, unprotected sexual intercourse. It is a multifactorial condition and affects 10-15% of couples [1]. Approximately 40% of all cases are male-caused [2]. Because male infertility due to semen deficiencies is common, semen analysis is important in assessing male fertility potential. Semen analysis is an informative analysis of spermatogenesis, secretory activity of the gonads, and patency of the male genital tract [3]. Semen deficiencies are usually seen as oligozoospermia, aspermia, hypospermia, azoospermia, teratospermia, asthenozoospermia, or combinations thereof [4]. Etiological causes include genetic problems, immunological disorders, obstructive lesions, varicocele, cryptorchidism, etc. However, in most cases, the exact cause is

not well known, which complicates the treatment [4]. About 25% of couples have one or a combination of this condition called idiopathic infertility [4]. Etiological causes include genetic problems, immunological disorders, obstructive lesions, varicocele, cryptorchidism, etc. However, in most cases, the exact cause is not well known, which complicates the treatment [4]. About 25% of couples have this condition, called idiopathic infertility.

Oxidative stress (OS), caused by an imbalance between reactive oxygen species (ROS) production and antioxidant defense systems, has been suggested as a factor in unexplained male infertility [5]. Although human body cells have effective mechanisms to deal with factors that disrupt normal cell homeostasis, the OS can occur due to an imbalance between oxidant production and antioxidant mechanisms and cause cell damage. For this reason, it has been extensively researched in recent years to explain male infertility. ROS can originate from endogenous and exogenous sources. Intracellular ROS concentrations are determined by the balance between ROS production rates and clearance rates of various antioxidant defense factors. Oxidative phosphorylation, drug metabolism catalyzed by cytochrome P450, peroxisomes, and inflammatory cell activation are endogenous sources of ROS [6]. The main ROS found in seminal plasma are superoxide anion, hydroxyl radicals, and hydrogen peroxide. In physiological amounts, ROS is required for signal transduction within the male reproductive system, regulation of junction within the blood-testis barrier, and mediation of cytotoxic and other important physiological changes in spermatozoa [7]. However, excessive amounts of ROS impair sperm motility and the ability of sperm to fuse with oocytes, damaging the sperm plasma membrane. ROS can also alter sperm DNA, leading to implantation failure [8]. In a meta-analysis of 65 studies involving 11 OS markers, including 3819 infertile men and 2012 controls, it has been suggested that decreased seminal plasma OS due to decreased antioxidant defense is associated with male infertility [4].

Therefore, the purpose of this study was to evaluate the OS parameters in men with suspected infertility. We also evaluated some of the hormones that are important for reproductive function in men.

Materials and methods

A total of 100 males aged between 20 and 48 years were included in the study. This study, carried out from April 2021 to July 2021 at the Gazi Yasargil Training and Research Hospital Andrology Laboratory, Diyarbakır, Turkey. Study groups were designed as 5 groups: normozoospermia (n = 20), azoospermia (n = 20), oligozoospermia (n = 20), oligo-asthenozoospermia (n = 20), and idiopatic infertility (n = 20) according to sperm parameters and etiology. Men with at least one child were classified as having normospermia. All subjects were of Turkish origin, and written informed consent was obtained. Total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), and 8-hydroxy-2-deoxyguanosine (8-OHdG) were evaluated as OS biomarkers in all men. In addition, *follicle-stimulating hormone (*FSH), *luteinizing hormone (*LH), total testosterone, *thyroid stimulating hormone* (TSH), prolactin, estradiol, folic acid, vitamin B-12, and vitamin D were examined. The principles outlined in the Declaration of Helsinki were followed. The study was approved by the same hospital's local ethics committee for human research (No. 2021/910).

Semen sample collection and analysis

All semen samples were evaluated by two experienced embryologists in the andrology laboratory. After 2–5 days of abstinence, participants were trained to take samples through masturbation into a sterile plastic container. The sample was transferred to the laboratory within 30 minutes, without exposure to cold, hot, or sunny environments. The samples were evaluated for volume, liquefaction time, and pH value. After semen liquefaction, the count and motility were determined with a Makler camera (counting chamber, Sefi Medical Instruments) using a light microscope (Olympus CX31), in conformity with WHO criteria. Trained embryologists evaluated semen analyses, including semen volume, sperm concentration, total sperm count, motility, and total progressively motile sperm count (TPMSC) according to World Health Organization (WHO) guidelines.

Sperm morphology assessment

Spermatozoon morphology was evaluated by Kruger criteria; staining was evaluated by the Spermac method. The staining kit (Ref. no.: SPS050, FertiPro NV Industriepark Noord 32 8730 Beernem, Belgium) includes a stain fixer and three stains (A, B, and C). 10 µl of semen was placed on the slide and left for 20 minutes. The slides were washed 8–10 times with distilled water 10-15 minutes after the fixative solution was added. Afterwards, the slides were incubated with A, B, and C stains for 1 minute, and washed 8–10 times with distilled water after each cycle. Slides were dried for at least 20 minutes and evaluated under a light microscope (Olympus CX31) at x100 magnification. The percentage of sperm with normal morphology was determined from 100 sperm counted on each slide. The census was carried out in at least five different areas.

Measurement of OS markers and hormones

OS biomarker and hormone measurements were made from venous blood taken between 9:00 and 11:00 in the morning on the day of semen sample collection. The blood taken from all subjects was centrifuged at 4000 rpm for 15 minutes, and hormone levels were evaluated using Cobas 6000 (Roche Diagnostic; Germany). The TAC and TOS tests were evaluated with the spectrophotometric method, and the 8-OHDG test was evaluated with the ELISA method. OSI: The ratio of TOS to TAS was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAS was converted to µmol/L, and the OSI value was calculated according to the following Formula: OSI (arbitrary unit) = TOS (µmol H2O2 equivalent/L) / TAC (µmol Trolox equivalent/L) × 100.

Statistical analysis

SPSS 21.0 (IBM SPSS Inc., Armonk, NY, USA) was used for statistical analysis. The conformity of the data to the normal distribution was analyzed by the Shapiro-Wilk test, and the homogeneity was analyzed by the Levene test. A one-way comparison of normally distributed parametric data and an analysis of variance (ANOVA) post-hoc Tukey HSD test were used. The study collected data in the form of a number, a percentage, and an arithmetic mean standard. The statistical significance level of $p < 0.05$ was considered significant.

Results

A total of 100 males were included in the study. Sperm parameters in the groups are shown in Table 1.

We then evaluated OS markers and hormone levels. There was a significant difference between groups in terms of TAC, FSH, and LH levels. TAC was the lowest in the azoospermia

Table 1. Comparison of sperm parameters of the groups.

Sperm Parameters (Mean ± SD)	Normozoospermia n: 20	Azoospermia n:20	Oligo-zoospermia n: 20	Oligo-astenozoospermia n: 20	Idiopathic infertility n: 20
Age (year)	31.5 ± 8.58	32 ± 5.85	29.45 ± 4.03	29.85 ± 6.08	30.75 ± 4.21
Ejaculate volume (ml)	2.91 ± 1.41	2.62 ± 2.03	2.8 ± 1.14	3.05 ± 1.16	2.94 ± 1.32
Sperm concentration (million/ml)	55.7 ± 49.31	0	6.45 ± 3.86	4.32 ± 4.06	61.9 ± 27.43
Total sperm count (million)	132.74 ± 70.42	0	16.38 ± 9.5	9.4 ± 13.76	177.05 ± 118.04
Motility (%)					
Progressive	56.25 ± 18.13	0	58.95 ± 16.8	8.3 ± 10.18	60.10 ± 16.06
Nonprogressive	9.85 ± 6.42	0	7.35 ± 4.92	10.9 ± 15.39	7.75 ± 4.01
Total Motility	66.10 ± 16.26	0	66.3 ± 14.26	19.2 ± 16.31	67.4 ± 13.96
Immotility	33.9 ± 16.26	0	33.7 ± 14.26	78.55 ± 17.99	32.05 ± 14.44
TPMSC (million)	71.22 ± 38.2	0	10.01 ± 6.67	0.57 ± 0.75	109.9 ± 88.57
Normal morphology	7.2 ± 2.98	0	3.68 ± 2.72	3.95 ± 3.42	6.80 ± 3.77
Head anomaly	64.85 ± 7.54	0	64.05 ± 10.9	60.4 ± 11.4	61.30 ± 11.10
Neck anomaly	12.45 ± 4.95	0	12.21 ± 6.21	14.85 ± 8.42	14.65 ± 8.59
Tail anomaly	4.65 ± 3.06	0	6.68 ± 4.43	8.15 ± 4.54	6.20 ± 5.64
Mixt	10.9 ± 3.47	0	13.68 ± 7.18	14.10 ± 7.73	11.10 ± 5.70

Total Progressive Motile Sperm Count; SD: Standard Deviation.

group compared to other groups, while the highest TAC was in the normozoospermia group (p=0.002). Total FSH and LH levels were higher in the azoospermia group compared to other groups. FSH level was the lowest in the normozoospermia group, while LH level was the lowest in the idiopatic infertility group (p=0.000). There was no significant difference between the groups in terms of TOS, OSI, 8-OHdG, total testosterone, TSH, prolactin, estradiol, folic acid, vitamin B12, and vitamin D (p>0.05) (Table 2).

Discussion

Male infertility is often due to a lack of semen, and semen quality is an indicator of male fertility. Semen analysis is the single most useful and fundamental study in men with male infertility, with a sensitivity of 89.6% (9). Men whose sperm parameters are below the normal values determined by the WHO are considered to have male factor infertility [9]. Human spermatozoa are polarized cells that have the ability to evade recognition by the female immune system for a sufficient amount of time to induce fertilization of the egg. Damaged or defective spermatozoa not only affect the outcome of pregnancy but also have a major impact on the health trajectory of the offspring. In addition to miscarriages, it can cause neuropsychiatric disorders such as autism, schizophrenia, and childhood cancers in offspring [10]. Therefore, investigating the factors that help reduce and maintain sperm function is crucial to understanding infertility and the risks of genetic disorders in offspring.

Spermatozoa are largely vulnerable to ROS as they have cell membranes rich in

Table 2. The oxidative stress markers and hormone levels in the groups.

OS markers and hormones (Mean ± SD)	**Normozoospermia (control group) (n: 20)**	**Azoospermia n: 20**	**Oligozoospermia n: 20**	**Oligo-astenozoospermia n: 20**	**Idiopathic infertility n: 20**	***P Value***
TAC (mmol/L)	1.83 ± 0.34	1.55 ± 0.17	1.67 ± 0.15	1.62 ± 0.20	1.79 ± 0.30	***0.002****
TOS (μmol/L)	6.39 ± 2.77	7.29 ± 3.11	7.88 ± 3.55	7.06 ± 2.74	8.14 ± 2.74	*0.38*
OSI	0.38 ± 0.17	0.45 ± 0.20	0.47 ± 0.21	0.43 ± 0.18	0.46 ± 0.17	*0.58*
8-OHdG	14.99 ± 7.24	18.30 ± 4.35	16.90 ± 5.88	18.09 ± 5.61	16.08 ± 6.13	*0.36*
FSH (IU/L)	3.54 ± 1,24	17.5 ± 15.94	7.30 ± 5.29	6.47 ± 5.71	4.71 ± 2.35	***0.000****
LH (IU/L)	5.97 ± 1.47	11.68 ± 8.89	7.04 ± 2.68	6.61 ± 2.72	5.58 ± 2.28	***0.000****
Total Testosterone (ng/mL)	4.94 ± 1.46	4.45 ±2.42	4.71 ± 1.95	4.92 ± 2.63	5.49 ± 2.57	*0.67*
TSH (mU/L)	1.58 ± 0.99	1.70 ± 0.87	1.75 ± 0.84	1.75 ± 0.95	1.84 ± 1.19	*0.94*
Prolactin (μg/L)	14.06 ± 5.44	17.62 ± 8.38	19.47 ± 7.93	16.13 ± 5.97	16.02 ± 7.07	*0.17*
Estradiol (pg/mL)	23.97 ± 6.77	31.55 ± 27.25	25.51 ± 9.30	23.12 ± 11.02	22.18 ± 8.41	*0.27*
Folic Acid (ng/mL)	7.41 ± 4.83	7.67 ± 4.33	7.35 ± 2.99	7.38 ± 3.88	7.60 ± 2.04	*0.99*
Vitamin B12 (ng/L)	314.10 ± 103.65	280.34 ±104.08	239.92 ± 90.49	278.35 ± 94.56	315. 69 ± 102.75	*0.10*
Vitamin D (ng/mL)	25.07 ± 5.97	22.69 ± 7.61	24.13 ± 5.89	24.37 ± 5.41	25.39 ± 6.25	*0.69*

TAC: Total antioxidant capacity; TOS: Total Oxidative Status; OSI: Oxidative stress index; 8-OHdG: 8 Hydroxy 2 deoxy Guanosine; TSH: T*hyroid stimulating hormone*; *FSH: Follicle-stimulating hormone*; *LH: Luteinizing hormone. SD: Standard Deviation.*
** p < 0.05 for ANOVA, p< 0.05 vs. Group 1 and Group 5.*
*** p < 0.01 for ANOVA, p< 0.01 vs. Group1, Group 2 and Group 5.*

polyunsaturated fatty acids (PUFAs). Lipid peroxidation (LPO) leads to the depletion of intracellular ATP, resulting in axonemal damage, low sperm viability, and increased defects in the midpiece of sperm [11]. Spermatozoa lack essential repair enzymes to overcome the damage caused by ROS. It has also been shown that OS disrupts the hormonal balance that regulates male reproductive functions. High levels of ROS may increase the likelihood of infertility, not only by directly inducing OS but also indirectly via the hypothalamic axes of hormone release [12, 13]. This not only affects the communication between the testis and the hypothalamic-pituitary unit but also disrupts the mutual communication between the hypothalamus-pituitary-gonadal axis and other hypothalamic hormonal axes [14].

OS measurement is increasingly used in the evaluation of infertile men as it provides clinical benefits [15]. The human body has an antioxidant defense system consisting of enzymatic and non-enzymatic antioxidants [16]. The seminal fluid contains enzymatic antioxidants such as superoxide dismutase, glutathione peroxide, and glutathione reductase that catalyze the free radical quenching reaction. Low seminal plasma antioxidants have been shown to play a role in reducing sperm concentration, motility, and morphology [17]. Various studies have confirmed the existence of significant correlations between changing semen parameters and ROS levels.

This suggests that OS has a negative effect on male fertility. In a study on this subject, it was shown that there was an association between TAC and semen quality [18]. Riaz et al. [19] Palani et al. [20] and Benedetti et al. [21] found that the serum TAC level was lower in normozoospermic infertile men compared to fertile males. It has been shown that there is a correlation between low TAC levels in the seminal plasma of asthenoteratozoospermic and oligoasthenoteratozoospermic men with low rates of sperm count, motility, and low normal morphology [22]. Riaz et al. [19] showed an increase in TOS levels in the serum and seminal plasma of infertile men compared to fertile men. In a study conducted in our country, seminal TOS and OSI were found to be higher in the oligoasthenozoospermia, teratozoospermia and azoospermia groups compared to the normozoospermia group [23]. Also, the TAC activity was higher in the normozoospermic group than the infertile group in the same study. Similarly, it was reported that the mean 8-OHdG in the seminal plasma of infertile men was significantly higher compared to fertile controls, but the mean TAC was significantly lower [24]. It was found that the TAC level was significantly lower in asthenospermic, astenoteratozoospermic, and oligoasthenoteratozoospermic groups [25]. Koca et al. showed that men with asthenoteratozoospermia, asthenospermia, and oligoasthenoteratozoospermia have lower seminal plasma TAC than fertile men [26]. They also reported a positive correlation between seminal plasma TAC and sperm motility. Low TAC levels in seminal plasma may contribute to decreased fertilization capacity and defective sperm structure.

The use of antioxidants to reduce OS for male infertility with various etiologies and risk factors has been the focus of attention. This view is supported by the availability of oral antioxidants, their safety and bioavailability profiles, and their relative cost-effectiveness. Therefore, in this study, we aimed to evaluate the blood plasma TAC, TOS OSI, 8-OHDG, and some hormone levels in men with suspected infertility living in Diyarbakir. As far as we know, this is the first study evaluating OS markers and hormones in men with suspected infertility living in Diyarbakir. For this, we examined 100 men as a result of sperm analysis in the Andrology Department of our hospital. Sperm analyses on all participants were performed. OS markers and hormones were evaluated. TAC values differed significantly between the groups. We found that the males with azoospermia had the lowest TAC level in comparison to other groups, while the males with normospermia had the highest TAC level compared to other groups (Table 2). Also, this group had the highest FSH and LH values compared to other groups (Table 2). Although not statistically significant, the lowest testosterone was found in this group. Our results are consistent with other studies.

Enzymes that metabolize the Vitamin D receptor and Vitamin D have been shown to be expressed in testes and spermatozoa. Therefore, the key role of vitamin D in the male reproductive system has been suggested. Vitamin D hypovitaminosis adversely affects semen and hormone function in humans [27]. Vitamin D levels showed no statistical difference between the groups in this study. Folate deficiency leads to OS, chaotic methylation reactions, a deficiency in protein synthesis, and spermatogenesis following homocysteine overproduction [28]. Vitamin B12 positively affects semen quality by increasing the first sperm count, sperm motility, and reducing sperm DNA damage [29]. Vitamin D, folic acid, and vitamin B12 levels

were not different between the groups in this study.

Our study has some limitations. The first is the small sample size. The other is that OS markers are not seen in seminal fluid.

The prevalence of male infertility is a worrying global health problem that has not been fully studied. Our results show that the OS is more common in men with azoospermia compared to other groups. The prevention and treatment of OS are important for improving the reproductive potential of infertile men.

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