Total antioxidant biomarkers in sperm and its association on Male reproductive capacity

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Abstract:

Indicators of total antioxidants are widely used in the assessment and detection of biochemical causes of male infertility. The main purpose of this study was to identify biomarkers that aid in the diagnosis and monitoring of male reproductive capacity. We prefer to measure total antioxidants (TRAP) in seminal plasma, for people with reproductive problems. Trap as a potential biomarker of male sterility in semen plasma has the highest sensitivity and complete specificity, Furthermore, we provide evidence that the male reproductive capacity is It is closely related to the basic parameters of sperm, the integrity of the sperm cell membrane, its morphology, Lifestyle, eating habits, occupation and mental health. Our results provide evidence on the importance of oxidative stress and defense against free radicals in diagnosing and monitoring men with infertility that are consistent with previously conducted research. We provide an alternative approach on the possibility of interpreting the combination of the biomarkers that can bring benefits to a multi-threaded approach to the diagnosis and treatment of male infertility.

Objective: - To study the relationship between the levels of total antioxidants in semen plasma of infertile and fertile men and the effect of an increase and decrease on fertility

Subjects and Methods: The study was conducted on 119 semen samples, 31 men with Asthenozoospermea, 28 men with Oligoasthenozoospermia, while another 60 healthy controls were diagnosed, Semen samples were collected from Iraqi patients who attended infertility clinics for infertility diagnosis and assisted reproductive technologies in Karbala for the period between November 2021 and May 2022. and total antioxidant levels were analyzed using ELISA technology from Sun Long Biotech Co/China.

Result**: -** The mean total antioxidants in the control group were significantly higher than in the patient group ($P<0.001$). In addition, in comparison to Asthenozoospermea groups with Oligoasthenozoospermia Found a significant difference in the mean level of Total anti-oxidant between Asthenozoospermea groups and Oligoasthenozoospermia groups. Total anti-oxidant levels showed a positive significant correlation with the Concentration, Progressive cell and Non- progressive cell, and negative significant correlation with the sample volume, viscosity of semen, p value<0.05.
Conclusion: - The total level of antioxidants in the semen of men is lower in infertile men (Asthenozoospermea and Oligoasthenozoospermia), while we find higher levels in the semen of fertile men. A positive relationship was also found between the level of total antioxidants with semen concentrations and sperm motility and an inverse relationship with the volume of the semen sample and its viscosity.

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1. Introduction

The World Health Organization's (WHO) defines infertility as the inability to conceive despite regular unprotected intercourse during 12 months or longer (1). A couple's fertility is diagnosed based on various reasons, including a man's age being more than 35 years old and a woman's age above 30 years old, with diagnosis of conditions, such as oligo menorrhea or amenorrhea, and with pathological changes in

the reproductive organs, as well as men exhibiting infertility attributed to female factors (approximately 1/3 of infertility) (2). Some of the factors that determine the occurrence of a pregnancy are the genomic quality of the oocyte and sperm, implantation capability of the embryo and the endometrial receptivity, most biological systems depend on preservation of equilibrium. Within the male reproductive system, oxidative harmony is crucial to fertility, Reactive oxygen species (ROS) make functional contributions at appropriate concentrations, but quickly become destructive if left unchecked (3). The share of the male factor in infertility is close to that of women (ca. 50%), while about 20% of infertility cases involve overlapping male and female factors. Idiopathic infertility remains to be a major problem in all cases of not-recognized male infertility (4), (5). Oxidative stress, which arises from an unbalance between reactive oxygen species and protective antioxidants, influences the entire reproductive lifespan of men and women (6). Reactive oxygen species may act as key signaling molecules in physiological processes, but in excess, uncontrolled levels may also mediate pathological processes involving the reproductive tract/reproduction. biological antioxidant, which has scavenger effects on ROS of cells against O2·- produced in the external cellular milieu. It protection against cellular damage, preventing extracellular (7). WHO recommends a seminogram in male fertility evaluation, which is a general examination of semen; however, this does not always enable the determination of the cause of infertility and spontaneous abortions and pregnancy losses after in vitro procedures, inter alia, after intracytoplasmic sperm injection (ICSI). The quality of sperm and oocytes plays a decisive role in the ICSI fertilization, in which OxS may participate, It has been demonstrated that a 25% increase in ROS production in seminal fluid activates cellular response, OxS induction, and apoptosis in the p53-dependent pathway associated with inhibition of natural antioxidants, such as GSH, superoxide dismutase (SOD), catalase (CAT), or glutathione peroxidase (GPx). Increased OxS, co-accompanying other overlapping factors, reduces the ability of sperm to reproduce (8), (9), (10), (11).

Damage to mitochondrial DNA impairs the maintenance of the fertilized ovum and permanently disturbs the genetic material passed on to the progeny. This is important in maintaining fertilized embryos and reducing the still high number of abortions after in vitro procedures (12), (13) The main objective of this study was to evaluate the usefulness of an indicator of total antioxidants

Defense in seminal plasma as a biomarker of semen quality in male sterility. their own evaluation Correlation with semen parameters such as mobility, concentration, morphology and vitality, as well as Questionnaire data, may contribute to improving knowledge about the etiology of male infertility and the effectiveness of its treatment. This could allow for better identification of men at risk of infertility Without any obvious clinical signs, prediction of semen damage caused by ROS, and its prevention, which leads to pregnancy and the birth of a healthy child in infertile patients, Although the parameters of oxidative stress tested so far have not been supported by sufficient scientific evidence as biomarkers of male infertility, they are still among its pathogenesis factors available in the recommendations of international urological, gynecological, or reproductive medical associations (14) , (15).

2. Experimental section

Subjects enrolled in this study are review participants and were recruited from Infertility treatment center in Karbala. Written informed consent Obtained by gynecologists and andrologists with relevant clinical experience from all studies Participants and their partners. All of them were informed of the purpose of this study. the study It was approved by the Bioethics Committee of Karbala University. The clinical examination was performed in accordance with the principles of the Declaration of Helsinki from 2008. Infertile men were recruited into the study group who reported to an infertility clinic because of Repeated failed attempts to have a baby for at least 12 months or due to repeated failure Intrauterine insemination without any apparent reason. Introduce male's t infertility clinic They were examined and enrolled in the study if they had repeated unsuccessful attempts to become pregnant the child for at least 12 months or due to repeated unsuccessful IUIs without any obvious reason. The exclusion criteria include the factors clearly affecting the oxidative-antioxidant status, i.e., genetic diseases, microbial infections, stimulants

(alcohol, drugs, marijuana); age < 20 or > 40; use of the following drugs in the last three months: antibiotics, nonsteroidal anti-inflammatory drugs, corticosteroids, vitamins, dietary supplements; inflammation of the genitals or abdominal cavity; diseases and inflammations of the genitourinary tract in the last 6 months; surgery; metabolic syndrome features (insulin resistance, hypertension, atherosclerosis, or hyperlipidemia); or withdrawal of a patient from the study without providing a clear reason . Men whose clinical history showed an increased susceptibility to infertility due to the use of hormonal pharmacotherapy or exposed to occupational factors generating mental or oxidative stress were also excluded from the study. The control group comprised men who had offspring (one or two children) a year or two before the examination and reported to the Infertility Clinic to perform control tests at the same time. They were followed up in connection with an earlier history of infertility and were included in the study due to the current lack of negative changes in sperm parameters and success in conceiving children. Data were prospectively collected from participants during the study, and questionnaire data was collected which included questions about age, sexual history, lifestyle, diet, psychosocial conditions, past diseases including autoimmune diseases, metabolic syndrome, and genetic diseases. 28 men with oligoasthenozoospermia, and 31 with asthenozoospermia were recruited to the study group. The qualification was based on the international guidelines of the World Health Organization on semen analysis (2),which define Oligoasthenozoospermia as the total number of spermatozoa < 39 million & progressive motility < 32% per ejaculate; and Asthenozoospermia as progressive motility < 32% (regards spermatozoa that do not swim quickly with a progressive movement, which reduces the likelihood of distant fertilization of a female ovum in the genital tract). In total, 119 individuals were recruited and divided into two groups: patients with infertility and controls with offspring. Demographic data, i.e., the average age of fertile and infertile men, their partners' age, and, body mass index (BMI) were assessed in the groups (Table 1).

Table (1): The results of demographic analysis of factors, such as age, and body mass index (BMI) in Control, and Patient groups. p < 0.05 indicates significant differences between the groups

Parameter	Control (N=60)	Patient(N=59)	P value
	Mean±SD	Mean±SD	
Age	32.2±5.76	31.07±5.59	0.27[NS]
BMI	27.52±5.10	29.25±5.75	0.139[NS]

Sperm samples were obtained by masturbation 2–5 days after the last ejaculation, after a night's rest. Each semen sample was collected according to the 2010 WHO guidelines on sperm testing (2). In addition to manual assessment of the seminogram, the material was analyzed using computer sperm analysis (CASA). Seminogram and cell membrane integrity were evaluated in fresh ejaculate, while oxidative and ant oxidative parameters in the seminal plasma were evaluated after obtaining statistically insignificant differences between these parameters between the seminal plasma and the entire ejaculate.

A sperm cell membrane integrity analysis was performed on a flow cytometer using the LIVE/DEAD Sperm Viability Kit (Thermo Fisher, Munich, Germany) according to the manufacturer's instructions within 0.5 h of material collection. The liquefied semen was centrifuged at 300× g for 10 min at a room temperature. The remaining sperm in a volume of approximately 1 mL was washed twice in 3 mL of sperm washing medium (HEPES—buffered human tubal fluid (HTF) medium supplemented with human serum albumin, Irvine Scientific, Santa Ana, CA, USA). Afterward, 0.5 mL of medium was added to the suspension, and the falcon tilted at a 45◦ angle was incubated at 37 ◦C for at least 45 min. The falcon was then placed in a vertical position and used for multipara meter analysis on a flow cytometer.

2.1 Total Redox Antioxidant Potential (TRAP)

TRAP was determined in the semen plasma using the This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in the kit has been pre-coated with an antibody specific to **(**Total Oxidative Status Test)

2.2 Statistical Analysis

Information from the questionnaire from all participants were entered a data sheet and were assigned a serial identifier number. Multiple entry was used to avoid errors. The data analysis for this work was generated using The Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA). Descriptive statistics was performed on the participants' data of each group. Values were illustrated by Mean ± 2SD for continuous variable. The distribution of the data was checked using Shapiro test. The association between the analyzed factors was estimated using odds ratios (ORs) and 95% Confidence Interval Range which calculated by a no conditional logistic regression.

Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant.

3. Results

3.1. Demographic

Analysis The average age of people in fertile and infertile groups was32.2±5.76 and 31.07±5.59 years, respectively (Table 1). Due to the fact that there were no statistically significant differences between men with Oligoasthenozoospermia, and Asthenozoospermia, further comparisons were carried out in the groups of infertile men and fertile men as controls. (Table 1). Also The differences were unimportant in body mass index no statistically significant (Table 1).

3.2. Questionnaire Data

Analyzing the questionnaire data, i.e., work in variable temperature conditions, TRAP levels were significantly higher in men working at lower temperatures compared to TRAP levels in men working at higher temperatures. The presence of any harmful factor had the same impact on the TRAP parameter level. The type of performed work (sitting or standing) had no significant effect on the measured parameters. We also considered the type of work and its impact on the results. It was noted that the work that was characterized by excessive movement or soldiers, where the effects of the level of TRAP appeared to be less than in the office and comfortable work.

3.3. Analysis of Basic Semen Parameters

The analysis of standard parameters of sperm, e.g., concentration, total sperm count, motility, morphology, and cell membrane integrity and viability of both groups showed that significantly lower values of the following parameters were observed in the infertile group concentration, Progressive cell, Non-progressive cell, and Non-moving sperm was higher than in the fertile group (Table 2).

Table (2): Results of the analysis of basic semen parameters, such as concentration, Progressive cell, Non-progressive cell, and Non-moving sperm, in Control and Patient groups. P values lower than 0.05 indicate significant differences between the groups.

Variable	Control(N=60)	Patient(N=59)	P value
	Mean± SD	Mean ±SD	
Concentration (mln/mL)	94.70±72.29	32.68±37.05	**<0.001[S]**
Progressive cell (%)	47.67±12.03	10.4051±8.47	**<0.001[S]**
Non-progressive cell (%)	27.26±6.54	20.84±14.76	**<0.001[S]**
Non-moving sperm (%)	22.89±10.77	68.5593±18.72	**<0.001[S]**

Out of 59 patients, the number of those with Oligoasthenozoospermia, and Asthenozoospermia was equal to 28, and 31, respectively. statistically significant differences were found between men with Oligoasthenozoospermia, and Asthenozoospermia (confidence interval α = 0.05); hence, further analyses were performed in

the groups of infertile (patients) and fertile (controls) men.

3.4. Analysis of Selected Total antioxidant

Before the selection of plasma for the study of oxidative stress parameters, pilot studies were performed on a group of 59 infertile and 60 healthy individuals, comparing these parameters in the seminal plasma and the spermatozoa alone. This is due to the absence of statistically significant differences They are found in antioxidant coefficients that have been studied between seminal plasma and Sperm itself, seminal plasma was used for further analysis. The total antioxidant in the seminal plasma of infertile patients was significantly higher in the patients compared with control group (Table 3)

Table (3): The results of selected oxidative stress (OxS) indices of seminal plasma, such as total redox antioxidant potential (TRAP). in fertile and infertile groups. p < 0.05 indicates significant differences between the groups

Parameter	Control(N=60)	Patient(N=59)	P value		
	Mean±SD	Mean±SD			
Total anti-oxidant	3.53±0.64	1.98±0.20		**<0.001[S]**	

Results are presented as mean ± SD, or n= number of subjects and percentage, p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant

Table (4) showed the marker examined among the groups of male infertile patients (Asthenozoospermea and Oligoasthenozoospermia) compared to the control groups, the average total level of antioxidants was significantly decreased in the Oligoasthenozoospermia compared to the other groups. The mean level was 1.88 compared to 2.07 in the Asthenozoospermea group and 3.53 in the control group.

Table (4): The results of selected oxidative stress (OxS) indices of seminal plasma, such as total redox antioxidant potential (TRAP). in fertile and infertile groups (Asthenozoospermea, and Oligoasthenozoospermia groups). p < 0.05 indicates significant differences between the groups.

parameters				P value
	Control (>39M>32%)	Asthenozoospermea (>39M-<32%)	Oligoasthenozoospermia (<39M-<32%)	
Total antioxidant	3.53±0.64	2.07±0.20	1.88±0.15	**<0.001[S]**
Results are presented as mean ± SD, p<0.05 considered significantly different, [S]= Significant,[NS]= Non significant				

Individually, the difference in Table (5) between laboratory results was also compared in terms of Oligoasthenozoospermia and the control group. The results showed a significant difference in the level of total antioxidant. The mean total level of antioxidants was 1.98 in the Oligoasthenozoospermia groups while it was 3.53 in the control group, and p-value < 0.001.

Table (5); The results of term semen Viscosity characteristic, and biochemical parameters finding mean in Oligoasthenozoospermia, and Asthenozoospermea groups. p < 0.05 indicates significant differences between the groups

Biochemical parameters	Viscosity (N=119)			P value
	Low N=79	Moderate N=22	High N=18	
Total antioxidant	3.14±0.89*	2.02±0.24*	1.99±0.19*	**<0.001[S]**

4. Discussion

Decrease in antioxidant levels and an increase in oxidative damage could induce OxS and causing sperm abnormalities impairing their potential for reproduction is considered one of the major sperm-damaging factors (16), (17), (18), (19). In this study, we explain the usefulness of selected oxidative-antioxidant parameters, i.e., TRAP, as well as their relationship with lifestyle, clinical data, and male infertility. Taking into account a number of published studies on the role of oxidative stress in male infertility, we tried to present a new point of view consisting of a comprehensive approach to the problem of infertility related to lifestyle, eating habits, physical activity, or mental health as factors affecting male reproductive abilities. Therefore, well-defined oxidation and antioxidant potential parameters are presented in the new perspective of the dual role of TRAP (defense against the negative effects of free radicals, regulation of cell signaling pathways, and reproductive potential), together with an interpretation of their values in seminal plasma. In addition, we present possible interpretations of existing correlations between the selected parameters and male fertility. A standard semen analysis is the first test prescribed by a clinician to assess male factor infertility in a couple. The lower reference values provided by the WHO are used to guide the diagnosis and treatment of the male partner. Diagnosis of normozoospermia may push the investigation towards the female partner as the primary cause of infertility, while a semen sample with altered sperm parameters suggests male factor as a putative cause for infertility and guide towards more advanced investigations, genetic testing and treatments (20). Furthermore, morphological abnormalities such as globozoospermia, macrocephaly, multiple tail defects and decapitated sperm syndrome are indicative of genetic disorders (21). In the present study, sperm concentrations and motility in fertile men were more than the concentrations and motility of infertile men , which agreed with reference values provided by the (22). In (Table1) this study, the BMIs of infertile men were either overweight or obese. In a previous study, the mean BMI was 26.6 ± 4.1 kg/m2 as per his study (23), while a study done by (24). reveal that the mean BMI was 26.7 ±4.6 kg/m2. Thus, the mean BMI in the current study is slightly higher than that reported by previous authors. In addition to impaired semen quality, fertility among obese men may be affected by sexual dysfunction, endocrinopathy, aromatization activity, psychological and thermal effects, sleep apnea, leptin and minor toxins According to the study (25) . When the comparison was made between men who have fertility (normozoospermia males) and men who suffer from infertility. Where Significantly higher levels of Total anti-oxidant were observed in our study in men with normozoospermia compared to men with infertility, suggesting a significant relationship between Total anti-oxidant in the seminal plasma and the integrity of the cell membrane, viability, or sperm morphology, while other studies also report a positive correlation with sperm motility , as studying (26). We conclude from previous studies and the results Table (4) of our study that the lack of antioxidants in seminal plasma is directly related to a defect in the function of sperm, its movement, Concentration, and thus a defect in the process of fertilization ability and thus results in us infertility. And this is what we found in our study through the results, it was observed that the level of antioxidants decreased in infertile men. When the comparison was made between men who have fertility (normozoospermic males) and men who suffer from (Oligoasthenozoospermia & Asthenozoospermea). Where Significantly higher

levels of Total anti-oxidant were observed in our study in men with normozoospermic males compared to men with Oligoasthenozoospermia, and this result was confirmed by studies (27), (28). on the other side Significantly higher levels of Total anti-oxidant were observed in our study in men with normozoospermic males compared to men with Asthenozoospermea, and this result was confirmed by studies (28), (29).

We conclude from previous studies and the results of our study that the lack of antioxidants in seminal plasma affects the cell membrane through free radicals and thus affects its contents such as mitochondria, which produce forms of damage to the cell through cell shape, number and movement and thus leads to pathological conditions such as Oligoasthenozoospermia & Asthenozoospermea. In This study Table (5) has demonstrated a significant relationship low antioxidants in semen with increased viscosity. Possible negative oxidative stress effect on low Sperm motility cannot be ruled out. This indicates that Antioxidant therapy may be beneficial for patients ,show abnormal semen consistency to protect sperm cells by oxidative damage and to improve their functional properties, this result was confirmed by studies (30), (31).

5. Conclusions

1. The total oxidative status (TOS) of semen of infertile men enrolled in the current study was more than that reported in fertile men in previous studies and thus it can be inferred that levels of oxidative stress that are higher than normal physiological levels are associated at least partially with male infertility.
2. The highest mean total oxidative state (TOS) was observed in Oligoasthenozoospermia and the lowest was reported in Asthenozoospermea.

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