MMJ

Malta Medical Journal, 2025; 37(4)
http://mmsjournals.org/index.php/mmj/46

ORIGINAL ARTICLE

The role of serum total antioxidant capacity as a diagnostic tool of male infertility and its correlation to semen parameters

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Background

Male infertility has been linked to the role of reactive oxygen species (ROS) and antioxidants. ROS are harmful molecules that can cause damage to cells, while antioxidants are substances that can prevent or counteract the formation and actions of ROS. Sperm cells are especially susceptible to damage from ROS because they lack adequate protection from intracellular antioxidant enzymes. Therefore, maintaining a robust total body antioxidant capacity is crucial to safeguarding sperm health.

Aim of the study

To evaluate the impact of T-AOC on male infertility.

Materials and methods

Serum T-AOC was measured by using Elabscience ELISA kit. A case-control study included fertile group as control(n= 40) and infertile group (n=50). All subjects were included in this study between October 2022 to June 2023. Diagnosis of participants were executed by medical senior on the basis of clinical characteristics, history of patients, and biochemical tests. Age, family history, and BMI were measured. Blood samples were provided by each participant for the biochemical estimation of serum T-AOC, while semen samples were collected from each participant for the seminal fluid analysis.

Results

The results of this study showed a decreased in the level of T-AOC in infertile men as compared with fertile men. Moreover, there was a significant difference and correlation between T-AOC and some semen parameters. There was a significant difference in BMI (p-value =0.0087) between fertile and infertile groups.

Conclusions

Serum T-AOC could be used as a diagnostic tool for male infertility.

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The World Health Organization (WHO) defines infertility as the failure to achieve pregnancy after one year of regular unprotected coitus. There are many potential causes of male infertility, including: anatomical abnormalities, sexual dysfunction, varicocele, obesity, oligospermia, smoking and heavy metal toxicity.[1] Infertility is the problem of 15% of young couples in different countries and male factor infertility accounts for 50% of cases. Thus, any support for this issue would develop the health status of the human being.[2] Male infertility has been linked to genetic and pathological factors such as abnormal hormonal levels, varicocele, cystic fibrosis gene mutations, Y chromosome abnormalities, testicular cancer, epigenetic errors, pituitary tumors, and even idiopathic factors.[3] Moreover, it has believed that environmental, occupational, and lifestyle factors can affect male infertility, compromising sperm quality. Indeed, studies have demonstrated that elements like alcohol consumption, cigarette smoking, obesity, radiation, genital heat stress, dietary practices, and illicit drug use contribute to a decline in human sperm quality.[4]

Treatment for male infertility depends on the underlying cause. Options include medication to improve sperm production, surgery to correct obstructions or other problems in the reproductive tract, and assisted reproductive technologies(ART), such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). It's important for men experiencing fertility issues to seek medical evaluation and treatment.[5]

Oxidative stress is a condition that occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the ability of the body to detoxify these harmful molecules. ROS are highly reactive molecules that are produced as a natural byproduct of cellular metabolism.[6] They play a crucial role in several physiological processes, including cell signaling and immune response. However, excessive ROS production can lead to oxidative stress, which can damage cellular biomolecules such as proteins, DNA, and lipids.[7] Oxidative stress has been linked to several chronic diseases, including cancer, neurodegenerative disorders, cardiovascular disease, and diabetes.[8] It is also a major factor in the aging process. The body has several mechanisms to counteract oxidative stress, including the production of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase.[9] These enzymes work together to neutralize ROS and prevent damage to cellular biomolecules.[10] However, exposure to environmental toxins, such as cigarette smoke, air pollution, and radiation, can overwhelm the body's antioxidant defenses, leading to oxidative stress. Additionally, lifestyle factors such as poor diet, lack of exercise, and chronic stress can also contribute to oxidative stress. To combat oxidative stress, it is important to maintain a healthy lifestyle that includes a balanced diet rich in antioxidants, regular exercise, and stress-reducing activities such as meditation and yoga. Antioxidants such as vitamins C and E, beta-carotene, and flavonoids can neutralize ROS and protect against oxidative stress. Additionally, several natural compounds, such as resveratrol found in grapes, quercetin found in apples, and curcumin found in turmeric, have been shown to have potent antioxidant properties.[11,12]

Antioxidants are compounds that either neutralize or scavenge ROS. Because spermatozoa are especially sensitive to ROS due to inherent deficiencies in intracellular antioxidant enzyme protection; total body antioxidant capacity must increase to protect sperm.[13] Seminal fluid antioxidants are divided into two types: enzymatic antioxidants and non-enzymatic antioxidants. Low antioxidant levels in seminal plasma have been linked to abnormal sperm morphology, motility, and concentration.[14] Total antioxidant capacity has been found to be highly correlated with sperm quality. In its natural state, seminal plasma possesses inherent antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which can effectively neutralize (ROS) and safeguard the spermatozoa from harm. Conversely, studies have demonstrated that exogenous antioxidants like vitamin E, vitamin C, CoQ10, and glutathione reductase can provide protection against oxidative stress in men experiencing infertility.[15]

MATERIALS AND METHODS

Study design: Case-control comparative study was used to find out the effect of serum level of total antioxidant capacity on infertile men as compare with fertile ones, in the private medical clinics in Babylon Governorate; Iraq.

Setting of the study: This study was conducted on patients with infertility, taking all the details of the patient's condition such as (family history, age, weight, height). All the tests were conducted in the laboratories of the Department of Medicinal Chemistry, College of Medicine, University of Al-Qadisiyah.

Patients: The number of patients (infertile) was 50 with the average age 20-49 years old. While, 40 healthy subjects(fertile) as control with the same age, having at least one child. All subjects were included in this study between October 2022 to June 2023, they signed a written informed consent form by themselves. All study methods were approved by the Ethical Committee of University of Al- Qadisiyah Al-Diwaniyah, College of Medicine, Iraq. Diagnosis of participants were executed by medical senior on the basis of clinical characteristics, history of patients, and biochemical tests. Age, family history, and BMI were measured.

Inclusion criteria: All males diagnosed with primary infertility by specialist doctors.

Exclusion criteria: All males with diabetes mellitus, autoimmune diseases, and chronic conditions were excluded from the study. Males with secondary infertility, azospermia were also excluded. In addition to several other males who have not abstained from sexual intercourse for at least 2 days.

Collection of blood samples: In this study, each participant provided a blood sample of 5 ml through vein puncture. The collected blood was transferred into sterile gel tubes and left to clot for 30 minutes. Afterward, the serum was separated by centrifugation at 4000 rpm for 15 to 20 minutes at room temperature (24-25°C). The resulting serum was then preserved for biochemical analysis using Eppendorf tubes, which were kept at a temperature of -40°C.

Collection of semen samples: The male participants were asked to masturbate to obtain semen samples, which were then immediately collected in a sterile, wide-mouth container. To improve the quality and quantity of the seminal fluid, both the patients and normal individuals were advised to abstain from sexual intercourse for three days prior to the sample collection.

Seminal fluid analysis: In the field of reproductive health, semen analysis is a crucial diagnostic tool to assess male fertility. To obtain accurate results, semen samples undergo a 30-minute liquefaction process before undergoing basic semen analyses. The liquefaction process is necessary to break down semen coagulation and facilitate the examination of the semen sample. After the liquefaction process, the World Health Organization (WHO) 2010 guidelines are utilized to assess the quality of the semen sample. The guidelines assess the presence of oligozoospermia, asthenozoospermia, and teratozoospermia based on the percentage of sperm motility, sperm count ($1x10^6$ cells/ml), and abnormal head or tail forms of sperm. These parameters are observed under a light microscope at a magnification of x100 using a haemocytometer. Furthermore, deformity rate (%) is determined by using an auto-analyzer microscope, which measures the percentage of abnormal sperm morphology. The results of the semen analysis provide an insight into the male reproductive system's health and can guide the appropriate treatment options for infertility. Therefore, the accurate assessment of semen parameters is essential for the diagnosis and management of male infertility.[16-18]

Total Antioxidant Capacity: serum level was measured using E-lab science ELISA kits.[19,20] For the standard tube, 10 µL of standard solution with varying concentrations were pipetted into each of the 96 wells in the micro-plate. Similarly, for the sample tube, 10 µL of the sample were pipetted into each well of the micro-plate. 20 µL of reagent 4 application solution were dispensed into each well of the micro-plate. 170 µL of ABTS working solution was added to each well of the micro-plate. The contents of each well were thoroughly mixed and the plate was allowed to stand at room temperature for 6 minutes. After the incubation period, the optical density (OD) values of each well were measured at 414 nm using a microplate reader.

RESULTS

Demographic characteristics of infertile and fertile men outlined in **Figure 1**. The mean age of fertile was 30.75±5.665 years and infertile 29.62 ± 6.546 years, there was no significant difference in mean age among study groups (p-value = 0.3904). The body mass index (BMI) (kg/m^2) of fertile (24.72 ± 2.614) (kg/

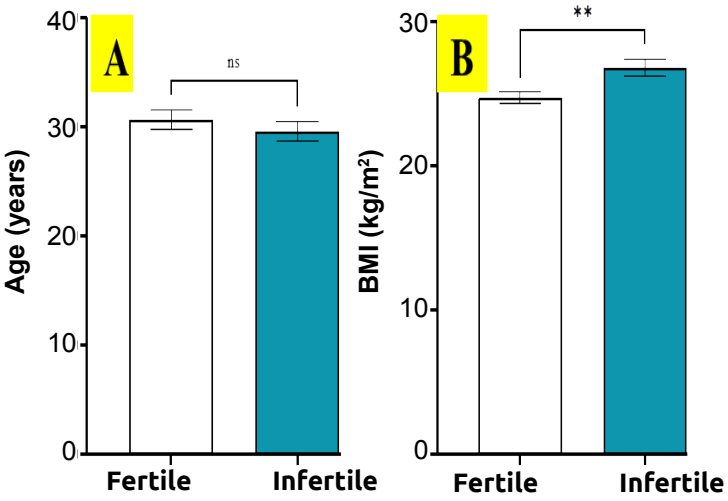

Figure 1 *(A) Age and (B) BMI of the studied groups. Data are expressed as means ± SD. **indicates significant differences compared to the control, P≤0.05*

Table 1 *Semen analysis Characteristics of infertile and fertile groups*

Characteristic	Fertile	Infertile	P value
Volume			
Range	2-6	1-9	0.25
Mean ± SD	3.9±1.1	3.5±1.9	
Total count			
Range	100-276	10 -90	*<0.0001*
Mean ± SD	169.5±46.3	43.5±21	
Total motility			
Range	0.45-0.7	0 - 65	*<0.0001*
Mean ± SD	56.33±6	32.4±18.9	
Immotile			
Range	0.3-0.55	35-100	*<0.0001*
Mean ± SD	0.44 ± 0.06	65.2±18.6	
Deformity rate			
Range	0.03-0.13	13-45	*<0.0001*
Mean ± SD	0.06±0.03	20.9±6.6	

m²), while that of infertile was (26.81 ± 4.32) (kg/m²) and there was a significant difference in BMI (p-value =0.0087).

Seminal fluid analysis is a vital diagnostic test of male fertility that demonstrates whether a human male has a normal semen parameters or not. Results showed that both fertile and infertile groups have a significant difference in total count, total motility, immotile sperm and deformity rate. Semen volume was lower in infertile group but there was no significant difference between study groups (Table1).

The measurement of Total Antioxidant Capacity: The meaurement of concentration of total antioxidant capacity (T-AOC) (mmol/L) in serum was found to be significantly lower in the infertile group as compared with the fertile group (0.6347 ± 0.1173),(0.8685 ± 0.0743) (mmol/L) respectively; the significant difference (p-value <0.0001) (Figure 2).

Correlation between serum T-AOC and semen parameters among infertile: The study showed that there was a significant weak positive correlation between T-AOC and sperm total motility (r=0.3744,

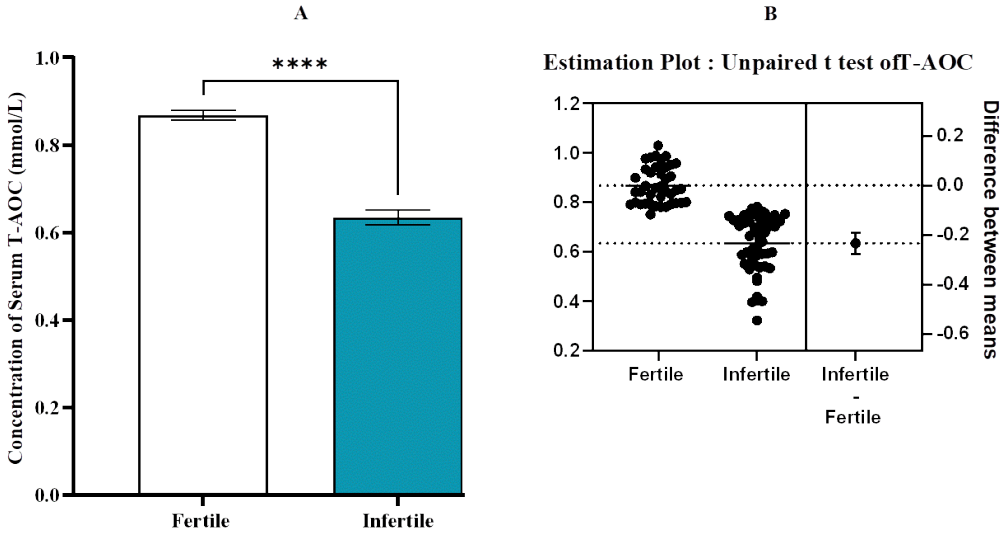

Figure 2 *Estimating serum total antioxidant capacity (T-AOC) (mmol/L). (A) a comparison between the fertile and infertile groups, (B) an estimation plot that illustrates the presence of a significant decrease in the level of T-AOC in the infertile group as compared to the infertile, the significant difference (p-value <0.0001). Data are expressed as means ± SD. indicates *significant differences compared to the control, P≤0.05.*

Table 1 *Correlation between serum T-AOC and semen parameters among infertile*

Characteristic	T-AOC vs. volume	T-AOC vs. Total count	T-AOC vs. Total motility	T-AOC vs. Immotile	T-AOC vs. Deformity rate
Pearson r	-0.3	-0.08949	0.3744	-0.3959	-0.4421
95% confidence interval	-0.5127 to 0.005379	-0.3589 to 0.1937	-0.5911 to -0.1072	0.1321 to 0.6073	0.1868 to 0.6415
R squared	0.07474	0.008009	0.1402	0.1567	0.1955
p value	0.0547	0.5365	*0.0074*	*0.0044*	*0.0013*

p value=0.0074). In terms of semen parameters, the results showed that there was a significant weak negative correlation between T-AOC and number of immotile sperm and deformity rate (r=-0.3959, p value=0.0044 and r=-0.4421, p value=0.0013) respectively. No significant differences were observed between T-AOC, semen volume and sperm total count (Table 2).

Diagnostic performance of total antioxidant capacity (T-AOC): To evaluate the diagnostic performance of total antioxidant capacity (T-AOC) (mmol/L) in infertile, receiver operator characteristic (ROC) curve analysis was performed (Figure 3). Regarding T-AOC , the cutoff value was ≤0.775 mmol/L with 98.00% sensitivity, 97.50% specificity, and 0.996 area (Table 3).

DISCUSSION

Our study showed that the concentration of total antioxidant capacity (T-AOC) (mmol/L) in serum was found to be significantly lower in the infertile group as compared with the fertile group, the significant difference (p-value <0.0001). The low level of antioxidants in infertile group is due to several environmental causes such as: smoking, alcohol intake, exposure to radiations, psychological issues and stress. All these factors could lead to ROS accumulation and ultimately OS. Many factors are participated in the pathophysiology of male infertility such as OS, the male infertility is strongly associated with (ROS).[21] The evaluation of oxidative stress in the male infertility workup can be done through Total Antioxidant Capacity (TAC), which is a diagnostic test that measures the level of total antioxidants present in the serum.[22] The results obtained by the study are consistent with other studies[23,24], who have showed that serum levels of TAC are lower in infertile group as compared with fertile group. While other studies like Eroglu et al showed that Serum TAC levels did not differ significantly among fertile and infertile.[25]

Based on the results of semen analysis, it seems that abnormal semen parameters may negatively impact men fertility. To further understand the underlying causes behind abnormal semen parameters, we studied the correlation between T-AOC and semen parameters. As previously mentioned, that there was

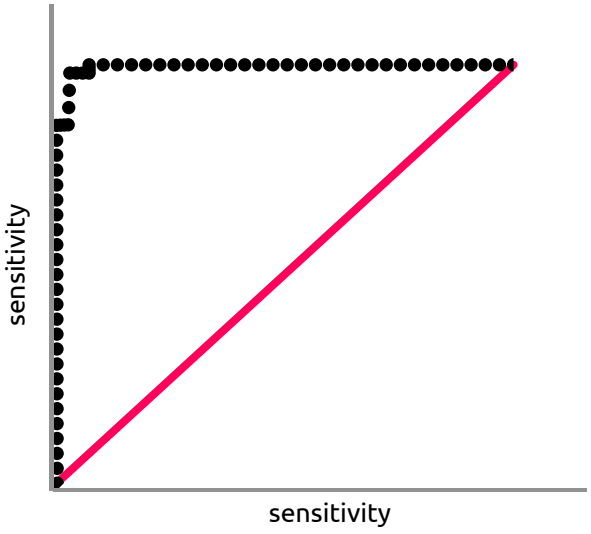

Figure 3 *Receiver operator characteristic (ROC) curve analysis to find the best serum total antioxidant capacity (T-AOC) (mmol/L)cutoff value that can predict a diagnosis of infertile*

Table 3 *The area under the ROC curve (AUC)*

The area under the ROC curve (AUC)	0.996
Standard Error	0.004
95% Confidence interval	0.951 to 1.000
P-value	<0.0001
Cutoff value	≤0.775
Sensitivity	98.00%
Specificity	97.50%

a significant difference and correlation between T-AOC and sperm parameters such as: total motility, deformity rate and immotile sperm. Low levels of serum antioxidant in infertile men indicate that there are large amount of ROS and that leads to OS. Several studies have validated that oxidative stress (OS) has a harmful effect on semen parameters and fertility potential. OS disrupts sperm viability, motility, and fertilization potential in reproductive tissues, as demonstrated by noticeably elevated levels of reactive oxygen species (ROS) in infertile men, in contrast to fertile controls.[26] Our results are on the line with other studies who reported that serum T-AOC affect sperm parameters.[24,27] However, the work done by Eroglu et al showed that serum levels of TAC had no effect on these parameters.[25]

Male infertility is a condition that affects a significant number of men worldwide. Various diagnostic tools have been developed to identify the underlying causes of male infertility, including seminal plasma T-AOC, which has been approved by several studies. However, a recent study has shown that serum T-AOC may also be a useful diagnostic tool for male infertility. Although seminal plasma T-AOC was not measured in the current study due to insufficient semen samples and a difficulty in finding a specific diagnostic kit for it, the results of the study indicated that serum T-AOC may be a more accessible and reliable diagnostic tool for male infertility. The study's findings suggest that serum T-AOC may be a valuable addition to the existing diagnostic tools for male infertility and may help improve the accuracy and efficiency of diagnosis and treatment. Further research is needed to validate these findings and to determine the optimal use of serum T-AOC in the diagnosis and management of male infertility. Nonetheless, this study highlights the importance of exploring new diagnostic tools and approaches to improve the detection and management of male infertility, which can have a significant impact on the lives of affected individuals and their families. The results of ROC curve analysis are shown in **Table 3**. The cutoff value of ≤0.775 indicates that if the T-AOC level in the serum is less than or equal to this value, it is highly likely that the individual is infertile. On the other hand, if the T-AOC level is greater than the cutoff value, it is highly likely that the individual is fertile. This result is consistent with work done by Palani, who showed that serum T-AOC is a valuable tool can be used in andrology laboratories for diagnosis of male infertility.[29] Sperm quality evaluation has become a crucial aspect of fertility research in recent years. With increasing cases of infertility, researchers are constantly striving to find new and improved ways to measure and evaluate sperm quality. In this regard, a recent study has indicated the possibility of using not only seminal antioxidants but also blood antioxidants as biochemical markers to support sperm quality evaluation. The study suggests that antioxidants in the blood may be used as a more reliable and accurate indicator of sperm quality than seminal antioxidants alone. This is because seminal antioxidants may not always reflect the overall health status of an individual, whereas blood antioxidants are a more holistic measure of the antioxidant status of the body. The study highlights the importance of considering multiple markers when evaluating sperm quality and suggests that the use of blood antioxidants may improve the accuracy and reliability of such evaluations. This research has significant implications for the field of fertility research, as it may lead to the development of new and improved methods for evaluating sperm quality and ultimately improving fertility outcomes. By incorporating multiple markers in sperm quality evaluation, researchers can gain a more comprehensive understanding of the underlying factors that affect fertility, and develop more targeted and effective interventions to address these factors.[30]

CONCLUSIONS

In conclusion, serum TAC can be a useful diagnostic tool for male infertility. It is a non-invasive and cost-effective way to identify men with low sperm quality due to oxidative stress. Measuring serum TAC levels can guide treatment decisions and improve outcomes by identifying men who would benefit from antioxidant therapy. Further research is needed to establish the clinical utility of serum TAC in diagnosing and treating male infertility.

Acknowledgments: The University of Al-Qadisiyah/ College of Medicine's provided facilities, which aided in enhancing the quality of this work, are greatly appreciated by the authors.

Adherence to Ethical Standards: The study was approved by the ethical committee at the University of Al-Qadisiyah (registration code CMUQ3544 on 15.12.2020).

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