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A CLINICAL STUDY TO EVALUATE SOME ANTIOXIDANTS AND TRACE ELEMENTS ON A HUMAN SEMINAL FLUID OF INFERTILITY PATIENTS IN MOSUL CITY

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Abstract

The significance of antioxidants and trace elements in the genesis and therapy of male infertility has garnered substantial interest. Male infertility is a common illness that affects couples worldwide. The purpose of this systematic review is to examine the available data on the effects of catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPX), Malondialdehyde (MDA), Calcium (Ca) and Magnesium (Mg) on male infertility. The study included the collection of 180 samples participating in the study and was distributed into three groups, the first group included men who have infertility with reason, and the second group included men who have infertility without reason, numbering (65 men). Comparison with a control group of (50 men), with ages ranging from 20 to 50 years. The levels of biochemical variables were estimated for them: CAT, GSH, GPX, MDA, Ca, and Mg. In addition, the study investigated the effect of age, smoking, BMI, and Period Infertility on these variables in male infertility patients. The study was conducted in the laboratories Scientific Center for Chemical Analysis (Baghdad - AL-Hartheyah - Kindy Street). The results were as follows: The result found significant differences ($P \leq 0.05$) in tests of CAT and GSH at (5%) probability level of men infertile with reason and men infertile without reason compared with a control group. and showed the resultant found that GSH, GPX, and MDA have not revealed significant differences ($P \geq 0.05$ n. s) among the infertile groups and the control group. Also, the show found decreased significant differences ($P \leq 0.01$) in tests of Ca, and Mg at (1%) probability level of men infertile with reason and men infertile without reason compared with a control group. Also,

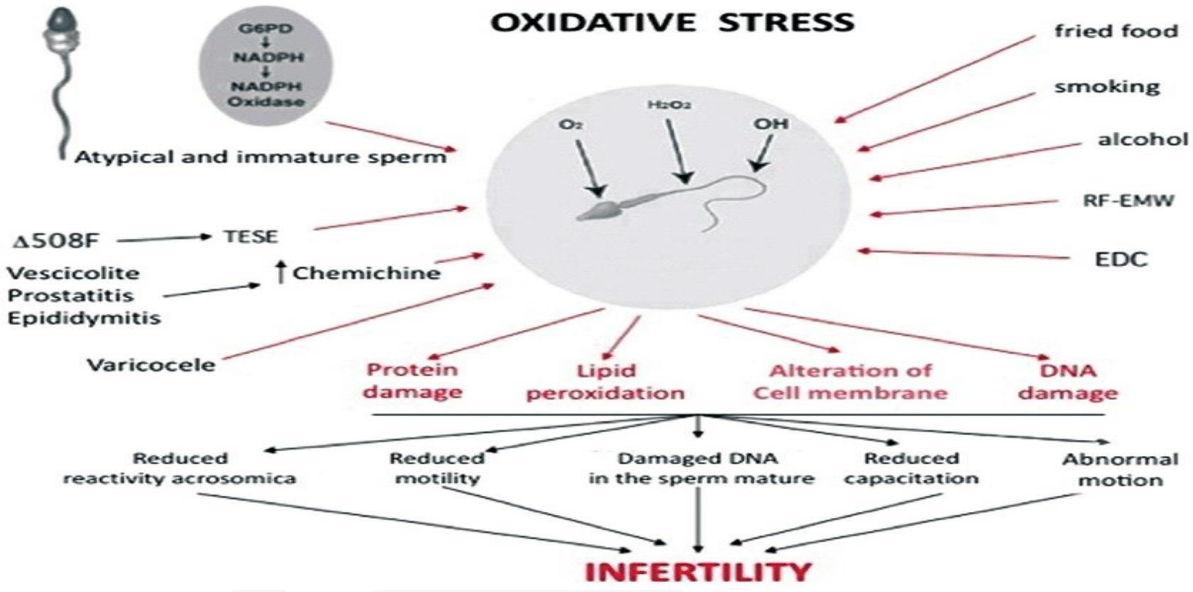
show mean values of GSH, GPX, and MDA were significantly affected by BMI significant differences. But CAT, Ca, and Mg had no significant differences in GSH, GPX, and MDA had not revealed significant differences (P ≥ 0.05 n.s) among the infertile groups and the control group. additionally, no significant differences in catalase CAT, GSH, GPX, MDA, Ca, and Mg according to a period of infertility among patient groups.

Keywords: Infertility, Antioxidant, Trace element, catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPX), Malondialdehyde (MDA), Calcium (Ca) and Magnesium (Mg).

1.Introduction:

The World Health Organization defines infertility as a disease of the reproductive system, which is the inability of a couple to conceive after one year of regular intercourse and affects (10-15) % of couples both males and females[1]. The World Health Organization (WHO) estimates that (50–80) million people worldwide have infertility. Large-scale research has shown that (50%) of reasons are female and (20-30%) are male, while (20-30%) are prevalent in both genders and (11%) have unknown causes. A diagnosis of infertility is primarily determined by the study of the semen, which takes into account the sperm's concentration, appearance, and motility [2]. There are several causes of male infertility brought on by a variety of factors, such as hormone imbalances, physical conditions, smoking, obesity, poor sperm quality or quantity or quality-specific problems in the gametes, as well as the impact of aging, gene disorders, and other factors [3]. Oxidative stress is a result of too many oxidants, also known as reactive oxygen species (ROS), attacking cells' inadequate antioxidant defense system. Excessive generation of reactive oxygen species (ROS) is expected to play a crucial role in the pathogenesis of male infertility and has been seen in 30% to 80% of infertile male sperm [4]. One of the main causes of poor sperm function is DNA damage, which is mostly caused by oxidative stress [5], [6]. A common underlying cause of male infertility, recurrent miscarriages, complex neuropsychiatric disorders, and childhood cancers in children whose fathers had defective sperm cells may be high levels of oxidative stress, which harms sperm DNA, RNA transcripts, and telomeres [7]. Increased oxidative stress in sperm results from excessive levels of ROS production or insufficient antioxidant defense capacities of the male reproductive tract or seminal plasma. Studies have indicated that human spermatozoa may create ROS, and it has also been shown that seminal leukocytes, particularly neutrophils, which infect the majority of human sperm suspensions, can contribute significantly

to the ROS production seen [8],[9]. Both antioxidant regimens and lifestyle changes may be viable treatment options for easing the burden of oxidative stress-related male factor infertility[10],[11]. In general, antioxidants are chemicals that either dispose of, scavenge, or limit the formation of ROS [12]. Spermatozoa, like all other cell types, may defend themselves against oxidative damage. Although the existence and relevance of catalase in human spermatozoa are controversial, they are known to contain two alternative defensive mechanisms against the dioxygen species O2- and H2O2, namely SOD and the glutathione peroxidase/reductase pair (GPX/GRD) [13],[14]. Oxidative stress has been related to poor sperm quality and male infertility While low levels of reactive oxygen species (ROS) are required for male fertility[15].



Association of increased reactive oxygen (ROS) production with infertility[16]

Trace elements (or trace minerals) are minerals that individuals consume in amounts ranging from 1 to 100 mg/day or less than 0.01% of their entire body weight. Ultra-rare minerals are those that are required in amounts smaller than one microgram per day [17]. Minerals account for barely 5% of the average human diet, but they are critical for good health and function[18]. Macro minerals, Human seminal plasma includes a number of macro and trace elements, including magnesium (Mg), calcium (Ca), copper (Cu), zinc (Zn) and iron (Fe), all of which play important roles in the appropriate functioning and quality of sperm[19]. Mg is required as the primary cofactor for kinase enzymes and functions as an intracellular calcium (Ca) antagonist. Increased Mg levels in comparison to Ca increase erection and ejaculation processes. The purpose of this study was to determine the seminal plasma levels of Mg, and Ca in Infertility with Reason and Infertility without Reason, and Control group their

levels to evaluate the effects of each element on activate of Acrosin and sperm motility [20], [21].

2.Materials and Methods:

2.1. Seminal Fluid (Semen):

Semen samples were collected in the laboratory by masturbation method after a while of abstinence for a period of not less than three days and not more than five days and the samples were placed in plastic containers clean and sterile made of polypropylene material that is not harmful to sperm. After that, the sample is marked according to the code of the questionnaire form with the recording of the collection time and date, after which the sample is transferred to the Incubator at a temperature of (37 ˚C). Follow-up is carried out to calculate the viscosity and the complete liquefaction time of the sample after the completion of the liquefaction process. Semen SFA test and evaluation of semen parameters (number, motility, morphology) [22].

2.2. Seminal Plasma:

The seminal plasma was obtained by centrifuging the semen samples the fluidized at a speed of (3000 r/min) for (10Min), after which the filtrate was transferred and distributed on Eppendorf tubes and kept at (-20 ˚C) for the purpose of conducting biochemical tests[23].

2.3. Determination of Antioxidant Parameters in Seminal Plasma:

Catalase(CAT), Glutathione(GSH), Glutathione peroxidase(GPx), and Malondialdehyde (MDA) levels were determined in the Seminal Plasma of the men of participants who have Infertility with reason, Infertility without reason, and men fertile, by a kit manufactured by the Scientific Center for Chemical Analysis (Baghdad - AL-Hartheyah - kindy Street). with specifications with very high accuracy and efficiency in analysis. by using U.V Spectrophotometer manufactured by Apple Company (Japan).

2.3.1. Estimation of Catalase (CAT):

Seminal plasma Catalase was determined by using the Sinha Method (Colorimetric Method).

A. Principle:

Catalase first reacts with H_2O_2 to produce water and oxygen

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O2$$

The dichromate / acetic acid reagent can be thought of as a "stop bath" for catalase activity. The hydrogen peroxide which hasn't been split by the catalase will react with the dichromate to give a blue precipitate of per chromic acid. This unstable precipitate is then decomposed by heating to give the green solution which measures at 530 nm[24].

B. Reagents:

Reagents (1): (pH=7): Preparation of pH: {(0.25gm) of KH_2PO_4 + (0.55gm) of K_2HPO_4}, Dissolve it in (45mL) of distilled water.

Reagents (2): H_2O_2 (30%): Preparation of H_2O_2 (30%): (2mL) of H_2O_2 and Completed to (100 mL) of distilled water.

Reagents (3): Potassium Dichromate: Preparation of Potassium dichromate: (1gm) of Potassium Dichromate Dissolve it in (20mL) of distilled water.

Preparation of solution: (POTASSIUM DICHROMATE + ACIDIC ACID) (10:30), ((10mL) of Potassium dichromate + (30mL) Acidic acid).

C. Procedure:

Control	Sample
Add(250µL) of pH in the tube.	Add (250µL) of pH in the tube.
Then add (125µL) of H_2O_2.	Add(25µL) Sample.
Wait 1 Min.	Then add (125µL) of H_2O_2.
Then add (500µL) of solution 1:3.	Wait (1Min.).
Read the Absorbance by set the device on (530nm).	Then add (500µL) of solution 1:3
	Read the Absorbance by set the device on (530nm).

D. Calculation:

Y = Abs. & X = Conc.

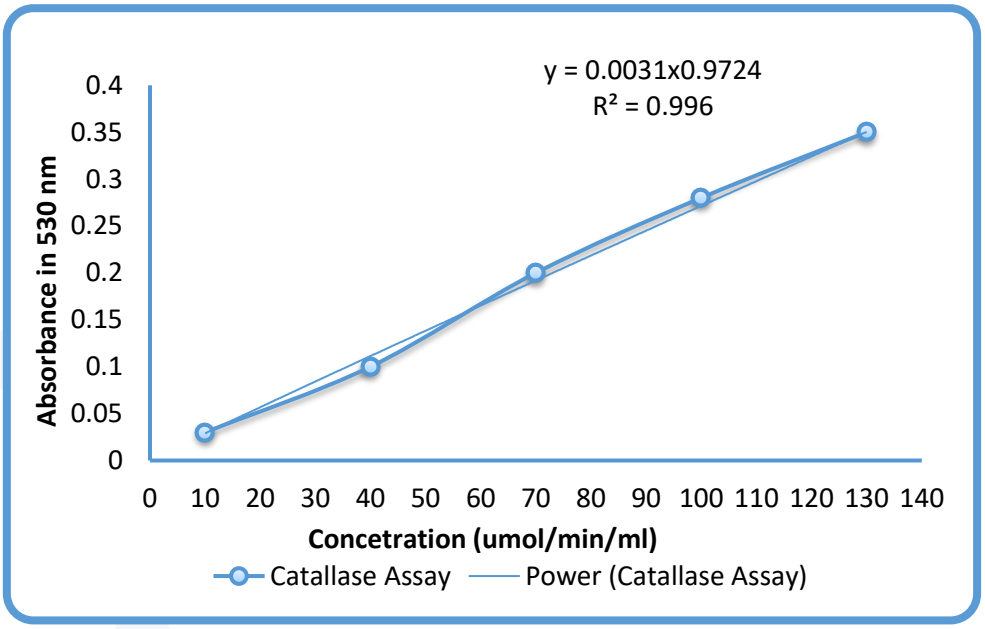
Y = 0.0031X ^0.9724

Y = 0.0031 X^1/0.9724 = 1.0283

X Conc. = (y Abs. / 0.0031) ^1.0283

Total equation is …

X Conc. = (Abs. / 0.0031) ^1.0283



Standard Curve of Catalase (CAT)

2.3.2. Estimation of Reduced Glutathione (GSH):

Seminal plasma Reduced Glutathione (GSH) was determined using Ellman method.

A. Principle:

DINB (Ellman's reagent) and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid and GSSG. Since 2-nitro-5-thiobenzoic acid is a yellow-colored product, GSH concentration can be determined by measuring absorbance at 412 nm [25].

B. Reagent Preparation:

Reagent	Amount	Solvent
(0.3M) phosphate buffer (pH 7.4)	Chapter 1	
0.1% EDTA	(0.1gm)	(100mL) HO with 6MNaOH (EDTA dissolve in alkaline water)
20% TCA	(30gm)	(150mL) H_2O

Procedure:

C.a. Control:

Take (450 µL) of DTNB and read the absorption at (412 nm).

C.b. Sample:

1.Add(25µL) of sample in the tube then add(225µL) EDTA
2. Add (375µL) of TCA then stop (5Min.) and start centrifuging for (10Min.) at (3000gm) rpm.
3. Take (50µL) of supernatant to the new tube then add (450mL) DTNB.
4. After (2 - 3Min.). Read the absorption at (412 nm).

D. Calculation:

GSH Concentration = (Abs. × 250) × 1000 / 13600 × 0.025
0.025 is (25µL of sample / 1000).

2.3.3. Estimation of Glutathione peroxidase (GPX):

Seminal plasma glutathione peroxidase (GPX) was determined by using the rotruck method (Ellman method) [25].

A. Principle:

A known amount of enzyme was allowed to react with H_2O_2 in the presence of GSH for a specified time period. Then the remaining GSH was measured by the method of Ellman.

$$2GSH + H_2O_2 \xrightarrow{GPX} GSSG + 2H_2O$$

B. Reagents:

Reagents (1): (pH=7): Preparation of pH: ((0.25 gm) of K_2HPO_4 + (0.55 gm) of KH_2PO_4), Dissolve it in 45 mL of distilled water.

Reagents (2): Sodium Azide (10mM): Preparation of Sodium azide: (0.0065gm) of Sodium Azide in Dissolve it in (10mL) of D.W.

Reagents (3): EDTA (0.4 mM): Preparation of EDTA (0.1%): (0.0021gm) of EDTA Dissolve it in (20mL) of Alkaline water

Reagents (4): TCA (10%): Preparation of TCA: (10gm) of TCA Dissolve it in (100 mL) of distill water.

Reagents (5): GSH (2mM %): Preparation of GSH: (0.021gm) of GSH in (20mL) of distill water.

Reagents (6): H_2O_2 (0.2mM): Preparation of H_2O_2: (0.02 mL) of H_2O_2 and Completed to (99.98 mL) of distill water.

Reagents (7): DTNB (0.1mM): Preparation of DTNB: (0.071gm) of DTNB Dissolve it in (180mL) of Phosphate Buffer pH 7.4.

C. Procedure:

Blank	Sample
(50µL) of pH added to the tube.	(50µL) of pH added to the tube.
(50µL) of EDTA.	(50mL) of EDTA.
(25µL) of Sodium Azide.	(25µL) of Sodium Azide.
(50µL) of GSH.	(125µL) of Seminal Plasma.
(25µL) of H_2O_2.	(50µL) of GSH.
Incubation at (37°C) for (10Min).	(25µL) of H_2O_2.
Adding (125µL) of TCA.	Incubation at (37 °C) for (10Min.).
Centrifugation for (5Min.).	Adding (125µL) of TCA.
Take (50µL) of Supernatant to the new tube.	Centrifugation for (5Min.).
Add (450µL) of DTNB.	Take(50µL) of Supernatant to the new tube.
After (2-3Min.). record the Abs. at (412 nm).	Add(450µL) of DTNB.
	After (2-3Min.) record the Abs. at (412 nm).

D. Calculation:

GPX Concentration = (Blank - Sample) × 18.38 / 0.125

2.3.4. Estimation of Malondialdehyde(MDA):

The Malondialdehyde levels in the semen were estimated by using the Ohkawa method.

A. Principle:

In the TBA test reaction, one molecule of MDA reacts with two molecules of TBA with the production of a pink pigment having an absorption maximum at 532-535 nm[25].

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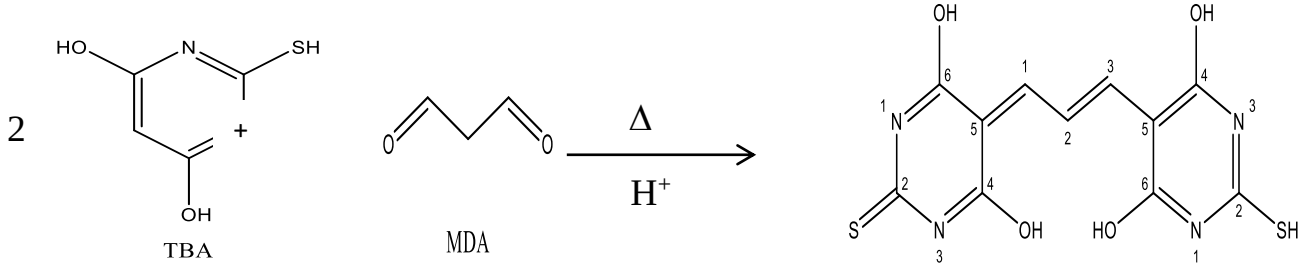

The reaction should be performed at pH 2-3 at 90-100°C.

B. Reagent Preparation:

Reagent	Amount	Solvent
10% TCA	10 gm	(100mL) H_2O
1% TCA	0.5 gm	(50mL) hot H_2O

C. Procedure:

1.Add (150µL) of Seminal Plasma in the tube.
2.Then add (1mL) of TCA (A), then add (1mL) of TBA and Incubate the mixture (15Min.).
3.Cooling the mixture, then add 1 mL of TCA (B) and Incubate (20Min.) at (37 °C).
4.Centrifuging (5Min.) then Read the absorption at (532 nm) and Calculate the concentration

D. Calculation:

Conc. MDA (mMole/L) = (Absorbance of Sample) / (E_o × CM × L)
L: Light path (1cm), E_o: Extinction coefficient = 21, CM = 1

2.4. Determination of Trace Elements Parameters in Seminal Plasma:

Calcium (Ca) and Magnesium (Mg) levels were determined in the Seminal Plasma of the male participants who had Infertility with reason, Infertility without reason, and men fertile, by a kit Giesse diagnostics from Guidonia Montecelio (RM) - Italia that had specifications with very high accuracy and efficiency in the analysis. by using a Chemistry Analyzer Smart-120 manufactured by Geno Tek Inc., No. device S/N: AES2KIKH01212, Issued V4.8.3.1.

2.4.1. Estimation of Calcium (Ca):

A. Principle:

Calcium and Arsenazo III combine to generate a stable blue-violet complex in a pH-neutral solution. The quantity of calcium contained in the sample is directly correlated with the color's intensity [26].

B. Procedure:

Pipette	Blank	Sample	Standard
Reagent	1000 µL	1000 µL	1000 µL
Water	10 µL		
Sample		10 µL	
Standard			10 µL

Mix, incubate at (37°C) for (2Min.) and read the absorbance of the sample and the standard against blank reagent

C. Calculation:

$$\text{Concentration Calcium mg/dl} = \frac{\text{Absorbance of the Sample}}{\text{Absorbance of the Standard}} * 10(\text{Standard Valve})$$

2.4.2. Estimation of Magnesium (Mg):

A. Principle:

Magnesium creates a blue-violet complex with xylidyl-blue dye, the strength of which is correlated with the sample's magnesium content [27].

B. Procedure:

Pipette	Blank	Sample	Standard
Reagent	1500 µL	15000 µL	15000 µL
Water	10 µL	-	-
Sample	-	10 µL	
Standard	-	-	10 µL

Mix, and incubate at 25, 30, and 37°C for (3Min.). Read the absorbance of the standard and the sample against a blank reagent.

C. Calculation:

$$\text{Concentration Magnesium mg/dl} = \frac{\text{Absorbance of the Sample}}{\text{Absorbance of the Standard}} * 2.5(\text{Standard Valve})$$

2.4. Statistical analysis:

In order to calculate the mean and standard deviation (SD) and evaluate if there were any significant differences between the research groups, the laboratory test data were analyzed using a one-way Analysis of Variance (ANOVA) and a T-test in the program (SPSS) (version 16 IBM Corp.). When using the $P < 0.01$it means a significant difference at the 1% probability level, P 0.01 - 0.05, it means a significant difference at a 5% probability level, $P > 0.05$, no significant differences between the probabilities.

3.Result and Dissection:

This study included the collection of 180 samples participating in the study and was distributed into three groups, the first group included men who have infertility with reason, and The second group included men who have infertility without reason, numbering (65 men). Comparison with a control group of (50 men). The samples were selected from the reviewers at Al-Jamhouri Teaching Hospital in Mosul, to be the study models according to the instructions of the supervisor. A field doctor and specialist in diseases of the kidneys, urinary tract, prostate, infertility, and sexual dysfunction in the consultation of the Republican Teaching Hospital in the city of Mosul, after obtaining the moral license from the patients and conforming to the controls of the health department in Mosul City.

3.1. Comparison of Catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPX), and Malondialdehyde (MDA) Levels between the control group and infertility patients with reason and without reason:

Catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPx), and Malondialdehyde (MDA) levels were determined in the Seminal Plasma of the men of participants who have Infertility with reason, Infertility without reason, and men fertile, by a kit manufactured by the Scientific Center for Chemical Analysis (Baghdad - AL-Hartheyah - kindy Street). with specifications with very high accuracy and efficiency in analysis. by using a U.V Spectrophotometer manufactured by Apple Company (Japan).

Table (3-1): Comparison of Antioxidant profile in normal subjects and infertile with reason, and men infertile without reason in Seminal Plasma

Biochemical Parameters	Control group		Men infertility with reason		Men infertility without reason		p-value
	Mean	± SD	Mean	± SD	Mean	± SD	
CAT (µmoll/min/mL)	4.152 a	0.670	3.279 b	0.175	3.509 b	0.860	0.015*
GSH (mMole/mL)	1.372 a	0.526	1.089 b	0.411	1.062 b	0.960	0.026*
GPX (mMole/mL)	38.083 a	1.509	36.269 a	1.183	40.793 a	2.549	0.641 n. s
MDA (mMole/mL)	1.066 a	0.481	1.869 a	0.178	1.227 a	0.799	0.868 n.s

By lowering oxidative stress and raising sperm quality, antioxidants can help with male infertility. When there is an imbalance between the body's capacity to neutralize and eliminate reactive oxygen species (ROS) and the amount of ROS produced, oxidative stress results [28]. Overexposure to reactive oxygen species (ROS) can harm sperm cells, resulting in decreased motility, morphology, and sperm count, as well as damage to DNA. It is noteworthy to acknowledge that although antioxidants have the potential to enhance male fertility, their efficacy may differ based on the specific circumstances and the fundamental reasons for infertility. It's also important to note that taking too many antioxidant supplements could not always be advantageous and might perhaps have the opposite impact. As a result, it's critical to heed expert advice and avoid self-medicating excessive antioxidant dosages without enough supervision [29]. Table (3-1) shows levels of some antioxidants in infertility patients compared with a control group. Revealed Catalase (CAT) of men infertile with reason with mean of 3.279±0.175 (µmoll/min/mL), Glutathione (GSH) with the mean of 1.089±0.411(mMole/mL), Glutathione peroxidase (GPx) with mean of 36.269 ± 1.183 (mMole/mL), and Malondialdehyde (MDA) with the mean of 1.869±0.178 (mMole/mL). Catalase(CAT) of men infertile without reason with mean of 3.509±0.860 (µmoll/min/mL), Glutathione (GSH) with the mean of 1.089±0.960 (mMole/mL), Glutathione peroxidase (GPx) with the mean of 40.793±2.549 (mMole/mL), and Malondialdehyde (MDA) with the mean of 1.227±2.0.977 (mMole/mL). Catalase (CAT) of the control group with mean of 4.152±.0.670 (µmoll/min/mL), Glutathione (GSH) with the mean of 1.372±0.526(mMole/mL), Glutathione peroxidase (GPx) with the mean of 38.083 ±1.509(mMole/mL), and Malondialdehyde (MDA) with the mean of 1.066±0.481 (mMole/mL). In men infertile with reason and men infertile without reason a significant decrease in Catalase (CAT) and Glutathione (GSH) values compared control group. also, show not any difference in levels of Glutathione peroxidase (GPx) and Malondialdehyde (MDA) in the study groups. The result found significant differences ($P \leq 0.05$) in tests of Catalase(CAT) and Glutathione(GSH) at (5%) probability level of men infertile with reason and men infertile without reason compared with a control group. This is consistent with research by Ruthaiporn Ratchamak and his group [30]. GPx and MDA have not revealed significant differences ($P \geq 0.05$ n.s) among the infertile groups and the control group.

3.2. Comparison of Calcium (Ca) and Magnesium (Mg) Levels between the control group and infertility patients with reason and without reason:

Calcium (Ca) and Magnesium (Mg) levels were determined in the Seminal Plasma of the male participants who had Infertility with reason, Infertility without reason, and men fertile.

Table (3-2): Comparison of Calcium (Ca) and Magnesium (Mg) in normal subjects and infertile with reason, and men infertile without reason in Seminal Plasma

Biochemical Parameters	Control group		Men infertility with reason		Men infertility without reason		p-value
	Mean	± SD	Mean	± SD	Mean	± SD	
Ca (mg/dL)	31.261 a	1.094	25.430 b	1.483	20.123 a	0.931	0.000*
Mg (mg/dL)	7.897 a	0.359	5.007 a	0.495	3.731 a	0.232	0.000*

Micronutrients also referred to as trace elements, are crucial for male fertility. Although modest quantities of these vital minerals are needed, they are engaged in a number of physiological processes that support reproductive health. Many studies indicated that calcium has an important role in regulating sperm formation and fertilization processes, in addition to growth, differentiation, reproduction, and cell death. In sperm cells and seminal cells, it has an important effect on sperm movement and activity. This means that calcium is an important mineral for fertility [31]. Also, Magnesium is a trace element that acts as an activator for a variety of enzymes involved in P transfer processes[32]. The prostate gland produces seminal plasma Mg. It has an important role in spermatogenesis and sperm motility. Table (3-2) shows levels of some trace elements in infertility patients compared with a control group. Divulged Calcium (Ca) of men infertile with reason with mean of 25.430±1.483(mg/dL) and Magnesium (Mg) With the mean of 5.007 ±0.495(mg/dL). Calcium (Ca) of men infertile without reason with mean of 20.123±0.931 (mg/dL) and Magnesium (Mg) With the mean of 3.731±0.232(mg/dL). Calcium (Ca) of the control group with reason with mean of 31.261±1.094(mg/dL) and Magnesium (Mg) With the mean of 7.897±0.359 (mg/dL). In men infertile with reason and men infertile without reason a significant decrease in levels of Calcium (Ca) and Magnesium (Mg) values compared control group. The result found decreased significant differences ($P \leq 0.01$) in tests of Calcium (Ca) and Magnesium (Mg) at (1%) probability level of men infertile with reason and men infertile without reason compared with a control group This is consistent with research by Sherif Salah Azab and his group [33]. This means that calcium is an important mineral for fertility. Calcium is secreted by the prostate and

its secretion is regulated by the hormone progesterone [31]. Calcium flows from the cellular vacuoles of the prostate cells into the semen and is transported by a compound called 1,4,5-inositol triphosphate that transports calcium from the prostate cells to the semen [34]. In Semen, when there is a decrease in the secretion of calcium, the progesterone hormone stimulates the prostate cells to secrete calcium and compensate for the deficiency [35],[36]. Also, Mg levels in infertile patients' seminal plasma have been shown to be lower compared to fertile persons, and fertile patients had decreased mean Mg levels in their seminal plasma Mg was shown to be positively associated with Ca concentration. Mg may be involved in the movement of sperm. As a result of these findings, Mg appears to play a significant role in sperm motility and male fertility. Because seminal Mg is primarily produced by the prostate gland, abnormalities in the prostate gland might result in a drop in seminal Mg levels and, as a result, cause infertility[37].

3.3. Antioxidant and Trace Elements level between the control group and men infertility with reason and without reason patients according to Age

The results were analyzed for the statistical values of age and their effect on Antioxidant and Trace Elements in infertility patients. The patient group was divided into three groups according to age, as shown in Tables (3-3).

In the context of male infertility, age can affect antioxidant and trace element levels. Men's bodies naturally lose some of their antioxidant defense systems as they age. This decrease may result in more oxidative stress and a diminished ability to combat dangerous free radicals, both of which may have an adverse effect on the health of sperm. Sperm DNA damage, sperm motility issues, and sperm viability reductions can all result from oxidative stress. Age-related decreases in antioxidant levels, including glutathione, vitamin E, and GPX, may be a factor in these harmful consequences[38].

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Tables (3-3): The mean and standard deviation antioxidant and trace elements concentrations in infertility patients based on age

Groups	Age (Year)		CAT (µmol/min/mL)	GSH (mmole/mL)	GPX (mmol/mL)	MDA (mmole/mL)	Ca (mg/dL)	Mg (mg/dL)
Control	20-30	Mean	4.606 a	1.092 b	39.67 a	1.507 a	23.150 a	5.055 a
		± SD	0.300	0.010	3.737	0.220	1.030	0.817
	31-40	Mean	3.832 a	1.034 b	39.7008 b	1.836 a	26.580 a	8.382 a
		± SD	0.781	0.901	2.787	0.239	1.304	0.973
Men infertility with reason	>40	Mean	3.654 a	1.191 b	30.878 a	1.423 a	36.400 a	9.530 a
		± SD	0.359	0.559	1.122	0.217	1.994	0.933
	20-30	Mean	3.321 a	1.009 b	42.117 b	1.654 a	23.300a	6.155 a
		± SD	0.610	0.962	1.284	0.740	1.043	0.848
	31-40	Mean	3.353 a	1.426 b	29.885 a	1.445a	17.050 a	3.440 a
		± SD	0.179	1.888	1.016	0.859	0.407	0.386
	>40	Mean	3.006 a	1.283 ab	37.495 a	1.359 a	20.040 a	5.344 a
		± SD	0.296	0.235	1.226	0.190	1.664	0.883
Men infertility without reason	20-30	Mean	3.554 a	1.100 b	35.387 a	1.502 a	30.616 a	8.110 a
		± SD	0.060	0.424	3.890	0.298	3.149	0.630
	31-40	Mean	3.476 a	1.577 a	44.847 b	1.770 a	21.800 a	5.886 a
		± SD	0.110	0.286	4.463	0.344	1.321	0.319
	>40	Mean	3.676 a	1.967 a	42.647 ab	1.862 a	18.950 a	4.710 a
		± SD	0.910	0.382	2.483	0.541	0.353	0.357
p-value			0.098 n.s	0.020*	0.019*	0.840 n.s	0.079 n.s	0.332 n.s

The results shown in Table (3-3) indicate that the mean values of most of the antioxidant and trace elements studied in male infertility patients were not significantly affected by age and did not group significant differences ($P \leq 0.05$), at (5%) probability level. Only the mean values of Glutathione (GSH) and Glutathione peroxidase (GPX) were significantly affected by Age are significant differences. but Catalase, Malondialdehyde (MDA), Calcium (Ca), and Magnesium (Mg) had no significant differences in Glutathione (GSH), Glutathione peroxidase (GPX), and Malondialdehyde (MDA), had not revealed significant differences ($P \geq 0.05$ n.s) among the infertile groups and the control group. This is consistent with research by Fatma Atig and his group[39].

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3.4. Antioxidant and Trace Elements level between the control group and men infertility with reason and without reason patients according to Body mass index (BMI)

The results were analyzed for the statistical values of age and their effect on Antioxidant and Trace Elements in infertility patients. The patient group was divided into three groups according to Body mass index (BMI), as shown in Tables (3-4).

Tables (3-4): The mean and standard deviation antioxidant and trace elements concentrations in infertility patients based on BMI

Groups	BMI		CAT (µmol/min/mL)	GSH (mmole/mL)	GPX (mmol/mL)	MDA (mmole/mL)	Ca (mg/dL)	Mg (mg/dL)
Control	18.5-25	Mean	4.017 a	1.955 ab	41.024 ab	2.826 c	23.150 a	5.055 a
		± SD	0.407	0.416	2.762	0.259	1.030	1.817
	25-30	Mean	4.326 a	1.562 ab	37.716 ab	2.469 c	26.580 a	8.382 a
		± SD	0.631	0.649	1.834	0.213	1.304	0.973
	> 30	Mean	4.139 a	1.079 ab	31.466 ab	1.859 b	36.400 a	9.530 a
		± SD	0.491	1.455	1.206	0.662	1.994	0.933
Men infertility with reason	18.5-25	Mean	3.597 a	1.970 b	49.832 ab	2.365 c	23.300 a	6.155 a
		± SD	0.289	1.351	3.847	0.081	1.043	0.848
	25-30	Mean	3.273 a	1.700 ab	46.601 ab	2.017 c	17.050 a	3.440 a
		± SD	0.539	0.950	3.789	0.991	1.407	0.386
	> 30	Mean	3.191 a	1.063 ab	27.538 b	1.949 b	20.040 a	5.344 a
		± SD	0.100	0.992	1.874	0.453	0.664	0.883
Men infertility without reason	18.5-25	Mean	2.477 a	2.000 a	52.346 a	5.262 a	30.611 a	8.110 a
		± SD	0.258	0.145	2.632	0.158	1.149	0.630
	25-30	Mean	3.437 a	1.308 ab	38.598 ab	2.282 c	21.800 a	5.886 a
		± SD	0.230	0.547	2.966	0.036	1.321	0.319
	> 30	Mean	3.804 a	1.248 ab	36.002 ab	1.690 c	18.950 a	4.710 a
		± SD	0.687	0.674	1.834	0.257	0.353	0.357
p-value			0.186 n.s	0.036*	0.006**	0.022*	0.079 n.s	0.332 n.s

Body mass index (BMI) is used to calculate their body fat percentage. It may affect the body's levels of trace elements and antioxidants. Elevated body mass index (BMI) has been linked to elevated oxidative stress and decreased antioxidant capacity inside the body, especially when obesity is present. Chronic low-grade inflammation, which can result in increased oxidative damage and free radical generation, is a hallmark of

obesity. Reduced amounts of vital antioxidants like glutathione, GPX, and Catalase can come from this unbalance between free radicals and antioxidants. The results shown in Table (3-3) indicate that the mean values of most of the antioxidant and trace elements studied in male infertility patients were not significantly affected by BMI, have group significant differences ($P \leq 0.05$), at (5%) probability level. Only the mean values of Glutathione (GSH), Glutathione peroxidase (GPX), and Malondialdehyde(MDA) were significantly affected by BMI are significant differences. This is consistent with research by Adnan J. M. Al-Fartosy and his group[40]. But Catalase, Calcium (Ca), and Magnesium (Mg) had no significant differences in Glutathione (GSH), Glutathione peroxidase (GPX) and Malondialdehyde (MDA), had not revealed significant differences ($P \leq 0.05$ n.s) among the infertile groups and the control group .This is consistent with research by Malik Adewoyin and his group [41].

3.5. Antioxidant and Trace Elements levels between the control group and men infertility with reason and without reason patients according to Smoking

The results were analyzed for the statistical values of smokers and their effect on biochemical parameters in infertility patients. The patient group was divided into three groups according to smokers or non-smokers, as shown in Tables (3-5).

Tables (3-5): The mean and standard deviation of antioxidant and trace elements concentrations in infertility patients based on smokers or non-smokers

Biochemical Parameters	Control		Men infertility with reason		Men infertility without reason		p-value
	Smoker	non-smoker	Smoker	non-smoker	Smoker	non-smoker	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
CAT (µmol/min/mL)	3.185 a 0.685	5.132 b 0.760	2.269 c 0.620	4.299 d 0.915	3.285 a 0.886	4.809 d 1.089	0.045*
GSH (mmole/mL)	1.119 a 0.338	3.072 b 0.700	1.111 a 0.106	90.808 c 1.526	1.325 a 0.497	3.436 b 0.955	0.022*
GPX (mmol/mL)	36.575 a 1.001	41.087 b 1.597	33.568 c 1.286	36.672 a 1.066	42.627 b 1.170	34.347 a 2.699	0.032*
MDA (mmole/mL)	1.889 a 0.252	2.167 b 0.896	1.088 a 0.414	2.431 b 0.187	1.692 a 0.392	2.606 b 0.337	0.036*
Ca (mg/dL)	25.100 a 1.959	29.450 b 1.232	18.585c 0.338	21.916 d 0.184	23.700 ad 0.171	27.485 a 1.988	0.042*
Mg (mg/dL)	7.763 a 0.363	4.965 b 0.086	6.194 c 0.848	4.956 b 0.973	6.860 c 0.991	5.621 a 0.575	0.038*

It has been demonstrated that smoking negatively impacts male fertility and that one of the ways it does this is by interfering with antioxidant function. Antioxidants are chemicals that assist in shielding cells from the damaging effects of dangerous free radicals. Antioxidants are essential for preserving sperm cell health and function in the setting of male fertility. Numerous investigations have indicated a correlation between smoking and reduced antioxidant levels in male smokers' seminal fluid. For instance, studies have indicated that smoking lowers semen's concentrations of crucial antioxidants including glutathione, GPX, and MDA. The sperm's capacity to fend off oxidative stress and shield their DNA from harm may be compromised by this drop in antioxidant levels. Also, cigarette smoking has been demonstrated to have deleterious effects on trace elements. Essential minerals known as trace elements are needed in trace levels by the body for a number of physiological functions, including healthy reproduction. The results shown in Table (3-5) indicate that the mean values of most of the antioxidant and trace elements studied in male infertility patients were significantly affected by smoking. Catalase(CAT), Glutathione (GSH), Glutathione peroxidase (GPx), Malondialdehyde (MDA) and Calcium (Ca) had the highest mean values in the nonsmoker groups, while Magnesium (Mg)had the highest mean values in the smoking group of infertility patients. This is consistent with research by T.A. Kumosani and his group [42].

3.6. Antioxidant and Trace elements levels between the groups of infertility patients according to a period of infertility

The results were analyzed for the statistical values of a period of infertility and their effect on antioxidant and trace elements in infertility patients. The patient group was divided into two groups according to a period of infertility, as shown in Table (3-6).

Tables (3-6): The mean and standard deviation of antioxidant and trace elements concentrations in infertility patients based on a period of infertility

Biochemical Parameters	Men infertility with reason			Men infertility without reason			p-value
	(1-5)	(6-10)	(>10)	(1-5)	(6-10)	(>10)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
CAT (µmol/min/mL)	3.294 a 0.653	3.159 a 0.189	3.408 a 0.855	3.410 a 0.153	3.584 a 0.972	3.604 a 0.972	0.936 n.s
GSH (mmole/mL)	91.806 a 0.322	1.221 a 0.251	1.213 a 0.223	1.402 a 0.179	1.351 a 0.651	1.415 a 0.651	0.115 n.s
GPX (mmol/mL)	36.048 a 1.409	30.878 a 1.137	45.141 a 1.455	37.495 a 1.335	43.266 a 2.869	44.266 a 1.689	0.534 n.s
MDA (mmole/mL)	1.6978 a 0.659	1.581 a 0.289	1.400 a 0.131	2.138 a 0.718	1.543 a 0.303	1.494 a 0.303	0.500 n.s
Ca (mg/dL)	22.560 a 1.159	20.525 a 2.535	16.675 a 1.668	28.300 a 1.144	25.166 a 1.041	19.050 a 0.212	0.194 n.s
Mg (mg/dL)	5.694 a 0.488	5.085 a 0.202	4.072 a 0.368	7.604 a 0.440	6.980 a 0.714	3.805 a 0.077	0.173 n.s

The results shown in Tables (3-6) indicate that there are no significant differences in catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPX), Malondialdehyde (MDA), Calcium (Ca), and Magnesium (Mg) according to a period of infertility among patient groups. However, the tables do show significant differences in catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPX), Malondialdehyde (MDA), Calcium (Ca), and Magnesium (Mg) based on the period of infertility among patient groups. This indicates that the duration of infertility does not have an influence on antioxidant and trace element levels. This is consistent with research by KESKES-AMMAR and his group[43].

Conclusion:

The clinical investigation assessing trace elements and antioxidants in the seminal fluid of Mosul City infertility patients offers important new information about the possible involvement of these variables in male infertility. When compared to healthy persons, the study found that male infertility patients' seminal fluids had significantly different quantities of antioxidants and trace elements. Also, showed that age, smoking, and BMI have a significant effect on some antioxidants and trace elements but periods of infertility did not have a significant effect on many antioxidants and trace elements in male infertility patients.

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