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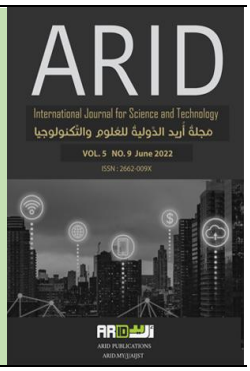

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Estimation of the concentrations of manganese, nickel, and aluminum in the semen of infertile men as oxidative stress factors

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تقدير تراكيز المنغنيز والنيكل والالمنيوم في مني الرجال غير الخصبين كعوامل مؤكسدة للإجهاد

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ABSTRACT

Semen samples were collected from healthy people (control group for comparison), asthenospermia infertility patients, and unexplained infertility patients from male donors who attended the Fertility Center at Al-Sadr Teaching Hospital in Al-Najaf Al-Ashraf / Iraq for the period from 1/12/2020 to 25/3 /2021. The present study aims to estimate the concentration of manganese, nickel, and aluminum in semen as one of the factors that cause oxidative stress in male infertility. The atomic absorption spectrometry was used to estimate the concentrations of the elements and the statistical analysis by the Tukey One Way method in the Graph Pad Prism program to determine the relationships between the study elements and the parameters of semen in men. The results of the study showed a significant increase ($P < 0.05$) in the level of nickel and aluminum concentrations (490 ± 27.57 and 450.3 ± 17.92 µg/L, respectively) and a significant decrease ($P < 0.05$) in the concentration of manganese (202 ± 8.718 ppm).) in the semen group of men with asthenospermia in comparison with the healthy fertile group (as a control group) (161.7 ± 5.963, 307.5 ± 10.58 µg/L and 309.2 ± 1.470 ppm), respectively. The results also revealed the existence of many negative relationships between the concentration of elements and semen parameters and between the same concentrations. The study concluded that the concentrations of these elements are a factor of stress oxidants that appear in cases of asthenozoospermia or may not change in other types of infertility and remain unexplained infertility.

Key World: manganese, nickel and aluminum elements, Asthenozoospermia, infertility

الملخص

تم جمع عينات السائل المنوي من الأشخاص الأصحاء (مجموعة سيطرة للمقارنة)، ومرضى العقم لوهن النطف ومرضى العقم غير المفسر من الرجال المتبرعين الذين حضروا إلى مركز الخصوبة في مستشفى الصدر التعليمي في النجف الأشرف / العراق للفترة من 2020/12/1 إلى 2021/3/25. هدفت الدراسة الحالية إلى تقدير تركيز عناصر المنغنيز والنيكل والألمنيوم في السائل المنوي كأحد العوامل التي تسبب الإجهاد التأكسدي للعقم عند الرجال. تم استخدام مطياف الامتصاص الذري لتقدير تراكيز العناصر و التحليل الإحصائي بطريقة توكي المنهج الواحد من برنامج كراف باد بريسم والعلاقات بين عناصر الدراسة ومعلمات السائل المنوي عند الرجال. أظهرت نتائج الدراسة زيادة معنوية (تحت مستوى احتمالية للخطأ 0.05) في مستوى تركيزات النيكل والألمنيوم (490 ± 27.57 و 450.3± 17.92ميكروغرام / لتر على التوالي) وانخفاض معنوي (تحت مستوى احتمالية للخطاء 0.05) في تركيز المنغنيز(202 ± 8.718 جزء في المليون) في مجموعة السائل المنوي للرجال المصابين بوهن النطف بالمقارنة مع المجموعة الخصبة الصحية (كمجموعة سيطرة) (161.7 ± 5.963 ، 307.5± 10.58ميكروغرام / لتر و 309.2 ± 1.470 جزء في المليون)، على التوالي. كما أظهرت النتائج وجود العديد من العلاقات السلبية بين تركيز العناصر ومعايير السائل المنوي وبين نفس التركيزات. وخلصت الدراسة إلى أن تراكيز هذه العناصر تعد عاملاً من عوامل مؤكسدات الإجهاد التي تظهر حالات ضعف او وهن النطف أو قد لا تغير في أنواع أخرى من العقم ويبقى عقم غير مفسر.

كلمات مفتاحية: عناصر المنغنيز والنيكل والالمنيوم، وهن النطف، عدم الخصوبة.

1. Introduction

In view of the importance of the male reproductive system in maintaining the species of the organism, studies related to reproductive aspects proceeded in order to identify the foundations of preserving the species, the integrity and completeness of the genetic material, and the continuity of survival[1][2][3]. The wife did not have any clear reason for not getting pregnant, so the man would be considered sterile[4]. Infertility is a pathological condition that affects about 15% of the total couples who want to have children, and it is a widespread medical problem and has significant psychological effects. Male infertility factors constitute about 50% of this case, and about 25% of them are caused by idiopathic diagnostic [5]. Impaired sperm motility affects approximately 40% of all cases and is asthenozoospermia because of many factors and can result from abnormal semen liquefaction, bacterial infection, genetic defects, or be the result of as yet unknown changes at the level molecular, including proteomic changes [6].

Asthenozoospermia in infertile patients was defined as the condition in which the percentage of sperms with forwarding progression is less than 32% according to [7].

Unexplained infertility is a term that has been applied to up to 15% of infertile couples[8] and usually refers to a diagnosis made in couples for whom all standard examinations are good, such as ovulation tests, and semen analysis is normal but pregnancy has not occurred[9]. Therefore, the deficiency of these trace elements could be an important factor for poor spermatogenesis, poor sperm quality and male fertility [10].

The molecular mechanism of the genotoxic activity of nickel compounds and the underlying mechanisms affecting male fertility are not yet fully understood. One plausible mechanism is the generation of reactive oxygen species that initiate lipid peroxidation and whose

products can create bonds with DNA affecting gene expression and lead to the degradation of acid peroxides. Unsaturated fatty acids to produce malondialdehyde[11]. Aluminium causes toxic effects on sperm shape, number, vitality, and motility. Oxidative stress is suggested as one of the mechanisms by which aluminium negatively affects sperm parameters. Biological membranes contain unsaturated fatty acids. An imbalance between the level of oxidative stress and antioxidant capacity may damage the structure of the sperm. Membranes, which in turn leads to disruption of membrane-dependent functions [12] .

Manganese is able to quench peroxyl radicals and acts as a chain-breaking antioxidant. Some studies have also indicated that manganese - as a potent inhibitor of oxidative stress - can reduce oxidative stress and improve sperm motility or semen quality in laboratory conditions[13][14][15].

The current study aimed to find out the effect of the level of trace elements (nickel, aluminium and manganese) on the fertility parameters of men by measuring the fertility parameters of men suffering from asthenozoospermia (or unexplained) infertility and comparing it with the control group.

2. Material and methods:

This study was conducted in the laboratories of the Fertility Medical Center in Al-Sadr City in Al-Najaf Governorate / Ministry of Health / Iraq. The study spanned from 12/1/2020 to 25/3/2021. Semen samples were collected from donors with asthenozoospermia (n=35) and unexplained infertility (n=35) of men and fertile (n=20) as a control group (The norm criteria -as unexplained sperm - are similar to those of fertile sperm-normospermia) [16] with a lifespan ranging from 30 to 38 years.

The liquefied seminal plasma was diluted 1:10 by adding deionized water to the samples at room temperature, and. they were run in 1, 2, 3, 4, and 5 ppm to obtain a stock standard for trace elements (Ni, Al, Mu). and assayed with an atomic absorption spectrophotometer (AAS) – Varian model spectra AA 300/400, Germany) All the trace element stock standards (of concentration 1000 ppm) were obtained from Fluka Chemical Switzerland , and the results were calculated as mg / ml .[17] the statistical analysis was used by the Tukey One Way method in the Graph Pad Prism program ($p<0.05$) , as well as the Excel program to determine the relationships between the study elements and the parameters of semen in men.

Results

Table (1) showed a significant increase ($p \leq 0.05$) in semen volume, liquefaction time, and percentage of abnormal sperm shape (2.934, 34.89, 45.23, respectively) in the group of asthenozoospermia patients compared to the control and patients groups. Unexplained infertility, while there was a significant decrease ($p \leq 0.05$) in the percentage of progressive motility (15.37) in asthenozoospermia group compared with groups of unexplained infertility and control.

Table (1): Semen analysis parameters in asthenozoospermia patients and unexplained infertility patients in comparison with the control group.

Parameters	Asthenozoospermic n=35 Mean S.E	Unexplained n=35 Mean S.E	Control (Normospermic)n=20 Mean S.E
Semen volume (ml)	2.934 ± 0.187 b	2.519 ±0.176 b	0.139 ±3.615 a
Liquefaction time (min)	0.370± 34.89 b	0.355 ±32.86 a	33 ± 0.465 a
Sperm concentration (million/ml)	33.14 ± 1.469	33.26 ± 1.875	33 ± 0.465
Progressive motile **%**	15.37 ± 1.566 c	60.26 ± 1.279 b	69.55 ± 1.268 a
Abnormal sperm morphology %	45.23 ± 1.455 c	34.37 ± 0.754 b	25.50 ± 1.085 a

Different letters mean a significant difference between groups

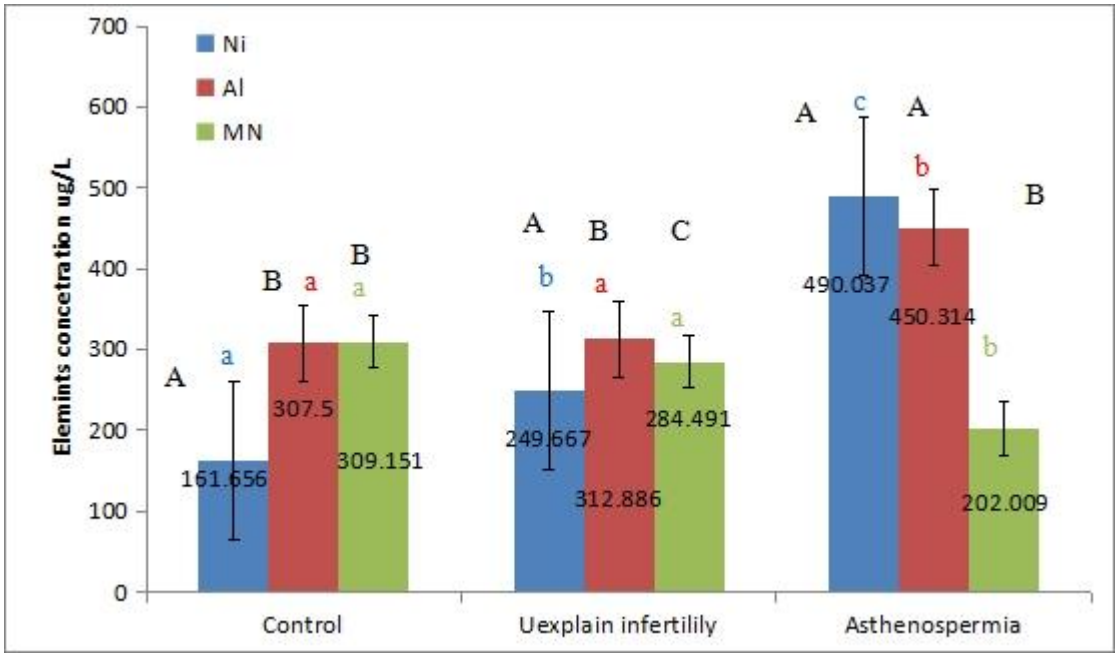

Figure (1): Evaluation of the concentration of elements (nickel, aluminium, manganese) in the seminal fluid of asthenozoospermia patients in comparison with the control and unexplained infertility groups.

Different capital letters mean a significant difference between the groups of the same element .Different small letters and the same colour element means a significant difference between the groups.

In addition, Figure 1 showed a significant increase in the elements nickel (490.037 & 249.667) and aluminium (450.314 & 312.886) and a significant decrease in manganese (202.009 & 284.491) in the semen of men with asthenozoospermia and unexplained infertility when compared with the control groups.

The results of the relationships between semen parameters and the studied elements in asthenozoospermia patients

The results of the current study showed a positive relationship between nickel concentration and semen analysis parameters (semen volume, percentage of non-motile sperm and percentage of abnormal sperm shape) and the results showed a negative relationship with the following parameters for semen analysis (liquefaction time, percentage of progressive motility, and the percentage of non-progressive motility of the sperm). The results of the current study showed a positive relationship between aluminum concentration and semen analysis parameters (sperm volume, percentage of progressive motility, percentage of non-progressive motility of sperm) and the results showed a negative relationship with the following parameters for semen analysis (liquefaction time, percentage of non-motile sperm and percentage of abnormal sperm shape). The results of the current study showed a positive relationship between manganese concentration and semen analysis parameters (sperm volume, liquefaction time, percentage of progressive motility, and percentage of non-progressive motility of sperm) and the results showed a negative relationship with the following parameters for semen analysis (percentage of non-motile sperm and the percentage of abnormal sperm shape) as shown in Table (2).

Table (2): Correlation coefficient of nickel, aluminium and manganese elements in the semen of asthenozoospermia patients compared to the unexplained infertility and control groups.

Elements	Parameters of semen	R value	R^2 value	Straight line equation values
Nickel	Semen volume (ml)	y=0.0002x+2.8379	0.0008	0.028
	Liquefaction time (min)	y=-0.0021x+35.936	0.0254	0.159
	Progressive motile %	y= -0.0034x+17.058	0.0037	0.061
	Abnormal sperm morphology %	Y=0.0041x+52.261	0.006	0.077
Aluminium	Semen volume (ml)	y=0.0014x+2.3234	0.017	0.130
	Liquefaction time (min)	y=- 0.0021x+35.817	0.01	0.1
	Progressive motile %	y=0.0128x+9.6198	0.0214	0.146
	Abnormal sperm morphology %	y= -0.0283x+66.984	0.121	0.348
Manganese	Semen volume (ml)	y=0.0022x + 2.4915	0.0105	0.102
	Liquefaction time (min)	y=0.0124x + 32.381	0.085	0.292
	Progressive motile %	y=0.0501x + 5.2414	0.0779	0.279
	Abnormal sperm morphology %	y= - 0.00489x + 64.34	0.0858	0.293

The relationship between the levels of concentrations of the studied elements

The results of the current study showed a negative relationship between the concentrations of nickel, aluminum and manganese, as shown in Figures (4, 5 and 6).

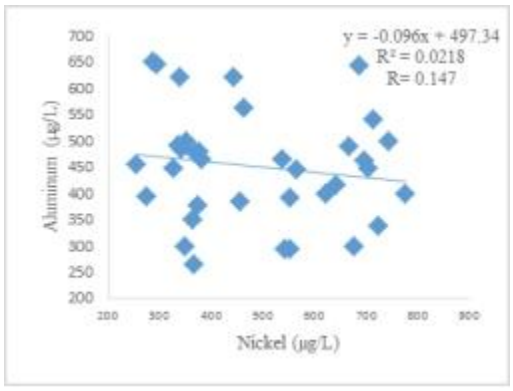

Figure (4) the relationship between the concentrations of nickel and aluminum in patients with asthenozoospermia

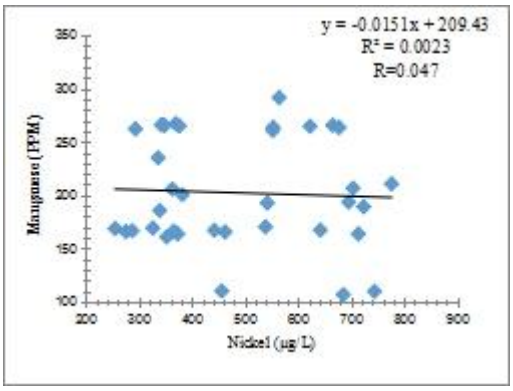

Figure (5) the relationship between the concentrations of nickel and manganese in patients with asthenozoospermia

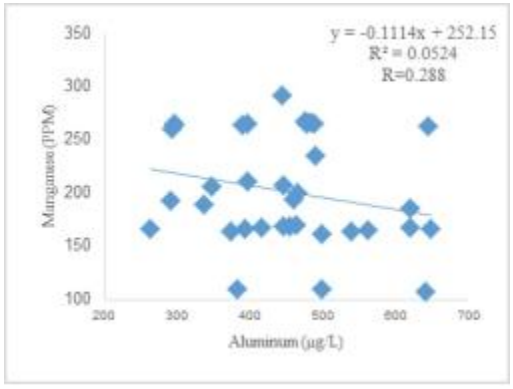

Figure (6) the relationship between the concentrations of aluminum and manganese in patients with asthenozoospermia

4. Discussion

Semen assays in patients with asthenozoospermia and unexplained infertility compared to the control group

The results in this study showed a significant increase ($p<0.05$) in the parameters of semen represented by semen volume, sperm concentration, percentage of progressively moving sperm, and percentage of normal sperm in normal sperm compared to patients with asthenozoospermia and unexplained infertility, and the results with other studies [18][19],which indicated that there was a significant increase in the concentration of sperms, the percentage of sperms with progressive movement, and the percentage of normal sperms compared to fertile people, and this may explain the result of the reactive oxygen species (ROS) whose increased production leads to a negative impact on the parameters of sperm as a result of the occurrence of the state of oxidative stress, as the active oxygen species works to attack the unsaturated fatty acids, proteins, and sugars involved in the composition of the plasma membranes of the sperm, and then leads to a state of lipid oxidation in the outer plasma membranes, and cellular membranes inside the cell such as the mitochondrial membranes and the nuclear membrane, the matter which leads to a decrease in the percentage of motile sperm, and the percentage of normal sperm [20]. In this study, which agreed with [21], a significant decrease ($P < 0.05$) was observed in the percentage of motile sperm in asthenospermia compared to both normospermia and unexplained infertility, It may be caused by the cytokines that this study indicated. Where, the increase in the concentration of circulating cells in the semen beyond the normal limits, which leads to a decrease in the volume of semen[22] .

The level of trace elements in the semen of asthenozoospermia patients compared to the two control groups

The results showed a significant ($p<0.05$) increase in the level of nickel in asthenospermia and unexplained when compared to the control group, and this may explain that nickel can induce apoptosis, oxidative stress, and DNA damage, and that nickel's reproductive toxicity in males, through indicators of fertility, sperm motility, and the epididymis, it shows damage to nickel compounds in spermatogenesis[23]. This study agrees with[24] where a decrease in sperm count and testicular enzymes was recorded with severe pathological changes in the testes such as interstitial cell proliferation because of exposure to nickel. Or eat unwashed foods that are exposed to heavy metals[25].

Several studies have shown a correlation between exposure to nickel and human fertility, and laboratory experiments have shown that high exposure to nickel is associated with a low level of expression of mannose receptors in sperm, leading to a decrease in sperm quality and then disruption of fetal development. In addition, another study reported that the concentration Low nickel significantly increased the total and advanced motility of sperm by in vitro experiments [26].

The results also showed a significant increase ($p<0.05$) in the level of aluminum in the subjects with asthenozoospermia when compared with groups of unexplained infertility and the control, and this may explain that aluminum is an oxidizing, toxic, immunomodulatory, pro-inflammatory and mutagenic agent[27].

The results of the current study showed significant differences ($P\leq0.05$) in the levels of manganese, as there was a significant decrease in patients with asthenozoospermia compared to

the two groups of unexplained infertility and the control. The results of this study agree with a study that says that a deficiency in manganese intake can delay growth, impair fertility and cause birth defects[28]. While the results of this study did not agree with Gennart, *et al*., [29], as no effect of manganese on fertility. The results of the current study showed a negative relationship between the concentrations of nickel, aluminum and manganese, as noted in Figures (4, 5 and 6). Which may affect the concentration of these elements one on the other and lead to the emergence of infertility in men. Because the body and all its fluids fall within the homeostasis mechanism between oxidative stresses and antioxidants, and any imbalance between this balance leads to the emergence of cases of one of the types of infertility or remains unexplained infertility despite the completion of all the analyzes and treatments required for couples. [30].

5. Conclusion

It was found that the increase in the concentration of nickel and aluminum and the decrease in the concentration of manganese are among the possible causes of infertility in patients with asthenozoospermia. Or unexplained infertility remains in a few men who receive all the required treatments and supplements because this is subject to the genetics and environment of the same infertile man.

Abbreviations

AAS: atomic absorption spectrophotometer.;ROS: reactive oxygen species. ;DNA:Deoxyribonucleic acid

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