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Research Article

Correlation between MDA Level and Chitotriosidase-1 Activity in Seminal Fluid of Iraqi Infertile Males

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Abstract

Background: Male infertility is a multifactorial condition influenced by various physiological and biochemical factors. Seminal fluid composition plays a crucial role in sperm function and fertilization potential. Chitotriosidase is a chitinase enzyme released by activated macrophages and is highly conserved and controlled. The notable chitinase in humans plays a significant role in the body's immunological response and is linked to inflammation, infection, tissue damage, and remodeling processes. On the other hand, malondialdehyde is a marker of lipid peroxidation, reflecting oxidative stress levels.

Objective: This study aimed to explore the correlation between malondialdehyde levels and Chitotriosidase-1in seminal fluid in Iraqi infertile males.

Methods: Ninety males aged between twenty and forty-five were included in this cross-sectional study, all diagnosed with infertility by specialists at the infertility unit of Al-Batool Teaching Hospital between February 2022 and February 2023. The participants were categorized into three groups: the Normozoospermic Group (G1), the Asthenospermia Group (G2), and the Oligozoospermic Group (G3). Seminal malondialdehyde and Chitotriosidase-1 levels were measured by competitive Enzyme-linked immunosorbent assay.

Results: The study findings showed significantly higher levels of seminal fluid Chitotriosidase-1 found in the G2 group compared to the G3 and G1 groups. The seminal fluid malondialdehyde level for G1 was significantly lower than those for G2 and G3, which revealed a significant positive correlation between seminal fluid Chitotriosidase-1 activity and malondialdehyde levels ($r = 0.37$, $P < 0.05$) in the Asthenospermia Group.

Conclusion: There is a correlation between seminal fluid Chitotriosidase-1 activity and malondialdehyde level in the Asthenospermia Group. Novel diagnostic and therapeutic approaches for the treatment of male infertility may result from our growing understanding of the roles played by Chitotriosidase-1 and malondialdehyde in male reproductive health.

Keywords: Chitotriosidase-1; Male infertility; Malondialdehyde; Seminal plasma; Sperm quality.

Introduction

Fertility is the ability of the individual to reproduce through normal sexual acts. Normal fertility requires the production of enough healthy sperm, a problem with this step causes infertility (1). Several studies were carried out on the association of infection and inflammation with male infertility, which revealed great variations in the prevalence of genital infection in different parts of the world (2). Involvement of chitotriosidase-1 (CHIT1) in macrophage activation and differentiation has consequences for other immune cell types. Although this may point to a role for cHit1 in triggering an inflammatory response, data on the enzyme's involvement in the inflammation that contributes to male infertility is still lacking (3). Chitotriosidase-1 is an enzyme found in various tissues and bodily fluids, including blood and seminal fluid. In humans, CHIT1 is primarily produced and secreted by macrophages, where it serves as part of the innate immune response against chitin-containing pathogens. Elevated levels of CHIT1 activity have been associated with various conditions, including lysosomal storage disorders and certain inflammatory diseases (4). Malondialdehyde (MDA) is the most well-studied by-product of polyunsaturated fatty acid peroxidation caused by oxidative damage (5). Seminal levels of reactive oxygen species and MDA also increase in tandem with these findings, during infection and tissue

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damage, semen contains high amounts of the same cytokines that play an important role in immune regulation for the male gonad (6). This study aimed to explore the correlation between malondialdehyde levels and Chitotriosidase-1in seminal fluid in Iraqi infertile males.

Patients and Methods:

Study Population

Ninety males aged between twenty and forty-five were included in this cross-sectional study all were diagnosed with infertility by specialists at the infertility unit of Al-Batool Teaching Hospital / diyala governorate between February 2022 and February 2023. Subjects were divided according to seminal fluid analysis into three groups: the Normozoospermia Group (G1), the Asthenospermia Group (G2), and the Oligozoospermia Group (G3).

Inclusion criteria (according to Seminal Fluid Analysis (SFA) (WHO,1999)

❖ Normozoospermic Group (G1): must have a normal sperm count, Sperm morphology Particiy (shape and structure) should meet standard criteria for normal sperm, Sperm motility (ability to move) should meet standard criteria for normal sperm.

❖ Asthenospermia Group (G2): must have reduced sperm motility, other parameters such as sperm count and morphology may still fall within the normal range.

❖ Oligozoospermic Group (G3): must have a low sperm count, Sperm morphology and motility may be normal.

Exclusion criteria

Systemic disease such as DM.

1- Auto immune disease such as SLE, RA, Hashimoto's thyroiditis.
2- Severe oligospermia 5 million / ejaculation.
3- Azoospermia
4- Patients with varicocele.
5- Patients with disorders in his wife reproductive system.
6- Patients with undiscerning testis and with testicular torsion.
7- Hypogonadism
8- Patients previously taken Antimicrobials, corticosteroid, and Antioxidants.

Seminal fluid samples

All patients' semen samples were taken in sterile, clean cups, and placed in an incubator for 15-20 minutes to cause the semen to liquefy, throughout the course of three to four days of abstinence. The samples were then examined under a light microscope. Seminal plasma was obtained by centrifugation at 4000 rpm for 15 minutes were divided into two portions and kept until assay.

Measurements of MDA and Chitinase1 (CHIT1) by enzyme-linked immunosorbent assay (ELISA)

Seminal MDA and CHIT1 levels were measured by competitive Enzyme-linked immunosorbent assay (ELISA) according to the manufacturer (Cloud-Clone Corp/USA/Cat No. CEA597Ge, SEJ374Hu, SEA181Hu).

Statistical Analysis:

The statistical analysis was conducted using Microsoft Excel for data input and preparation, which included organizing and cleaning the data for analysis, One-way ANOVA followed by multiple comparisons test was performed using GraphPad Prism version 19.5.1 for Windows, GraphPad Software, San Diego, California USA, and MedCalc® Statistical Software version 20.215was used to calculate and examine the strength and direction of relationships between variables, particularly between seminal plasma Malondialdehyde (SF-MDA) and CHIT1 levels. The statistical significance level was set at 0.05 for most tests, indicating that results with a [P-value less than 0.05 were considered statistically significant.

Results:

Age appeared to be uniformly distributed across the three groups with mean ages of 29. 67±1.043, 29.86±0.896, and 29.96±0.752 years, for G1, G2, and G3, respectively. The *P*= 0.972, strongly suggests that the differences between these groups were not statistically significant, demonstrated more discernible differences among the groups as shown in figure (1).

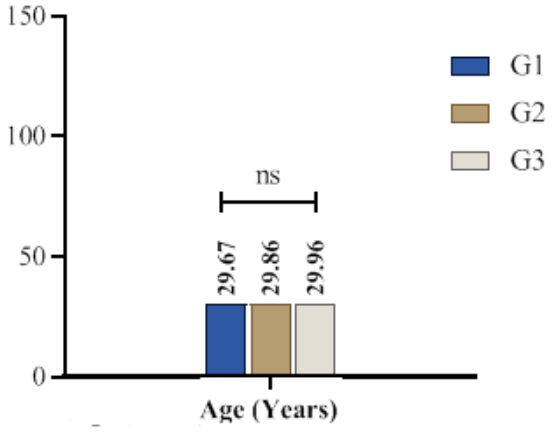

Figure 1: Age of males in the study groups.

In table 1 and figure (2), the results showed, that the Seminal Fluid Volume (SF Vol) revealed mean values of 2.550±0.1701, 2.083±0.1337, and 2.033±0.1809 ml for study groups, respectively. Although differences were observed, the effect size indicated by the R^2 value of 0.07 and a borderline *P*= 0.05 suggested that these differences are not substantial.

For Sperm Count, however, a striking disparity among the groups was evident. The means were 71.833±1.8489, 39.333±2.3700, and 11.000±0.7350 for

study groups, respectively. The R^2 value of 0.87 and a *P*= 0.001 indicated not only a statistically significant difference but also a large effect size, denoting a considerable variation in sperm count among these groups.

Motility categories, designated as A%, B%, C%, and D%, also showed substantive differences among the groups. For category A%, the mean values were 21.833±0.9123, 6.500±1.0491, and 2.000±0.9160 for study groups, respectively. Category B% displayed similar disparities with means of 33.833±0.5715, 18.000±1.0057, and 8.833±1.6380. Both A% and B% had R^2 values of 0.73 and *P*-values less than 0.001, signifying statistical significance and substantial effect sizes. Category C%, while also statistically significant with *P*= 0.01, showed a modest R^2 value of 0.10, implying a smaller effect size. Category D% exhibited an R^2 value of 0.77 and a *P*-value less than 0.001, indicating significant differences with a large effect size. Finally, the percentages of morphologically normal and abnormal sperms were 70.000±0.8970 and 30.333±0.8949 for G1, 40.833±2.5380 and 59.167±2.5380 for G2, and 23.833±3.5934 and 76.167±3.5934 for G3, respectively. These differences were statistically significant with *P*= 0.001 and R^2 values of 0.65, highlighting a sizable effect size.

Table 1 Semen profile for study groups

SFA	Study groups				
	G1	G2	G3	ANOVA	
	Mean± SE	Mean± SE	Mean± SE	R^2	P value
SF Vol ml	2.550± 0.1701	2.083± 0.1337	2.033± 0.1809	0.07	0.05
Sperm count	71.833± 1.8489	39.333± 2.3700	11.000± 0.7350	0.87	< 0.001
A%	21.833± 0.9123	6.500± 1.0491	2.000± 0.9160	0.73	< 0.001
B%	33.833± 0.5715	18.000± 1.0057	8.833± 1.6380	0.73	< 0.001
C%	10.000± 0.0000	10.833± 0.3460	9.333± 0.4632	0.10	0.01
D%	34.333± 1.0095	64.667± 1.9613	79.833± 2.5312	0.77	< 0.001
Normal%	70.000± 0.8970	40.833± 2.5380	23.833± 3.5934	0.65	< 0.001
Abnormal%	30.333± 0.8949	59.167± 2.5380	76.167± 3.5934	0.65	< 0.001

SFA=Seminal fluid analysis, SF vol=Seminal fluid volume, SE= Standard error of mean, and Motility categories=A%, B%, C%, and D%

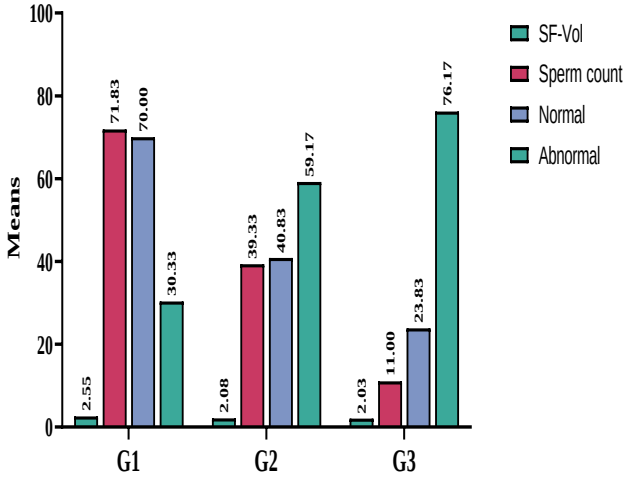

Figure 2: Means of Semen profile by the study groups.

Seminal fluid plasma Ch1t1 levels also displayed pronounced disparities among the groups with mean concentrations of 5.62±0.19, 15.96±0.29, and 12.46±0.18 (ng/ml) for study groups, respectively. As seen Table 2 and Figure 3

Table 2: The SF- Ch1t1 for study groups

Parameter	Mean ± SE			P value
	G1	G2	G3	
SF_Ch1t1 (ng/ml)	5.62± 0.19	15.96± 0.29	12.46± 0.18	< .001

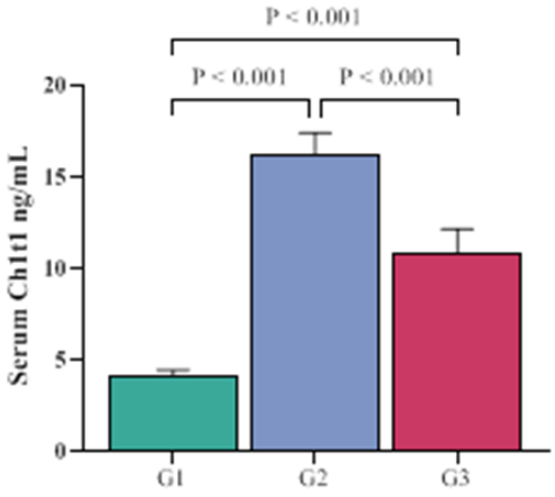

Figure3: Means of Ch1t1 by study groups.

The mean of SF-MDA for G1 236.59 ± 105.30(ng/ml), was significantly lower than for G2 1904.08 ± 877.85, similarly, compared to G3 1,042.78 ± 339.03 with *P*= 0.001. As seen Table 3, and Figure 4.

Table 3: The SF-MDA for study groups

Parameter	Group	n	Mean± SD	G1	G2	G3	Pr > F(Model)	Significant
SF-MDA ng/ml	G1	30	236.59± 105.30	236.59 a	1904.08 c	1042.78 b	< 0.001	Yes
	G2	30	1904.08± 877.85					
	G3	30	1042.78± 339.03					

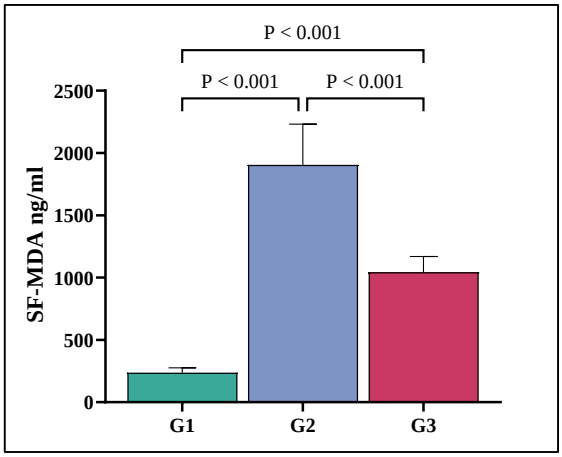

Figure 4: Comparison of SF-MDA between study groups.

The statement highlights a significant and substantial difference in sperm count among the Normozoospermic Group (G1), Asthenospermic Group (G2), and Oligozoospermic Group (G3). This indicated that the observed variation in sperm count is unlikely to have occurred by chance alone.

The correlation between SF MDA and SF Ch1t1 is modestly positive (r=0.37) and statistically significant (*P* value = 0.042) in G2 group. This suggested that there is a significant, albeit weak, positive association between SF MDA and SF Ch1t1 levels in the studied population. The modestly positive correlation between SF MDA and SF Ch1t1 may suggest a potential relationship between oxidative stress (MDA) and Ch1t1activity in the seminal fluid.

Table 4: Correlation between SF Ch1t1 and SF_MDA **in G2 group and G3**

Parameter	SF_Ch1t1 in G2		
	R	95.00% CI	*p*
SF_MDA	.37	[.01, .65]	.042

Parameter	SF_Ch1t1 in G3		
	R	95.00% CI	*p*
SF MDA	-.12	[-.46, .25]	.536

Discussion:

The observed variation is substantial and not merely a result of the sample size, a considerable difference in sperm count among the groups has important clinical implications, sperm count is a crucial factor in male fertility, and significant variations may affect the likelihood of successful conception, the study may explore potential causes or factors contributing to the observed differences in sperm count (7).

Factors such as lifestyle, environmental exposures, and genetic predispositions could be the cause of such variations among the different sperm health conditions, there are several reasons for a significant difference in sperm count among the groups included in the study (8). These factors may result from complex interactions between genetic, environmental, and health-related elements, variations in levels of hormones involved in sperm production, such as testosterone, may contribute to this difference, differences in genetic makeup could affect sperm count, genetics play a crucial role in determining reproductive characteristics(9).

For seminal fluid volume, although there are differences, the *P*-value (Table 1; $P \leq 0.05$) suggested that , there can be several reasons for differences in semen volume among different groups, including disruptions in hormone levels, particularly those involved in sperm production, can affect semen volume, imbalances in hormones like testosterone may influence semen production, variations in genes among individuals can contribute to differences in semen volume, genetic factors play a role in the physical characteristics of semen (10).

Exposure to specific environmental factors may influence natural sperm production and, consequently, semen volume. This could include exposure to harmful chemicals or elevated temperatures, as shown by (11).

The provided data revealed significant differences in motility categories (A%, B%, C%, and D%) among the study groups, namely the Normozoospermic Group (G1), Asthenospermic Group (G2), and Oligozoospermic Group (G3).

The statistically significant differences in Category A% suggested that there is a significant variation in the percentage of sperms showing progressive motility among the different groups ($P < 0.001$). This is a crucial parameter for male fertility, as sperm with progressive motility have a higher chance of reaching and fertilizing the egg (12).

Similar to Category A%, the significant differences ($P<$ 0.001) with a substantial effect size in Category B% indicated notable disparities in the percentage of sperms showing non-progressive motility. Non-progressive motility may still allow sperm to move but with less efficiency compared to progressive motility (13).

Although statistically significant ($P<0.01$), the smaller effect size in Category C% suggested a less substantial impact compared to Categories A% and B%. This indicated that the percentage of sperms with local motility varies among the groups but to a lesser degree. Local motility may have limited functional relevance for fertilization compared to progressive motility (14).

The significant differences ($P<$ 0.001) with a large effect size in Category D% highlighted considerable variations in the percentage of immotile sperm among the study groups. Immotile sperms have reduced or no movement, which can significantly impact fertility (15).

The findings have clinical relevance, as sperm motility is a critical factor in male fertility, understanding the specific patterns of motility among different sperm health conditions can guide clinicians in diagnosing and addressing fertility issues in couples (16), as found by (17). With advancing age, there can be an effect on sperm motility, aging is often associated with a decline in overall body functions.

The provided information indicated significant differences in the percentages of morphologically normal and abnormal sperms among the three groups (G1, G2, and G3), these morphological differences may have implications for fertility and reproductive health in each group, this agreed with (18).

This finding can be important for the study's validity and interpretation, it suggested that the differences in sperm characteristics observed among the groups are more likely to be related to the specific condition (G1, G2, and G3) rather than being confounded by age or BMI differences (19).

As shown in Table (1) and Figure (4), seminal plasma MDA levels for G1 were significantly lower ($P<0.001$) than those for both G2 and G3.

Malondialdehyde (MDA) is a naturally occurring compound that serves as a marker for oxidative stress and lipid peroxidation in cells, it is a byproduct of the degradation of polyunsaturated fatty acids in cell membranes when cells are exposed to oxidative stress, such as from reactive oxygen species (ROS) (20), lipid peroxidation can occur, leading to the formation of MDA, this agrees with (21). Lower MDA levels in G1 might suggest reduced oxidative stress in the Normozoospermic Group (G1) compared to the Asthenospermic Group (G2) and Oligozoospermic Group (G3), oxidative stress is known to negatively impact sperm quality and function, high levels of ROS can lead to DNA damage, lipid peroxidation, and impaired sperm function. The observed difference in MDA levels among the groups may be associated with the differences in sperm parameters, especially considering that G2 is associated with asthenospermia (reduced sperm motility) (22).

Elevated MDA levels are associated with various pathological conditions, including inflammation, cardiovascular diseases, neurodegenerative disorders, and reproductive health issues (23).

Studies often investigate MDA levels concerning male fertility. Elevated MDA levels in seminal plasma or sperm cells may be linked to reduced sperm motility, viability, and overall sperm function, this agreed with (24, 25).

The study demonstrated significant differences in CHIT1 concentrations in seminal plasma between the normozoospermic and oligozoospermic groups, which aligned with the findings of this study. Inflammation in the male reproductive system can cause oligozoospermia (26).

This suggests a potential link between chitin metabolism and oxidative stress in the male reproductive system. Furthermore, subgroup analysis based on semen parameters revealed varying correlations between CHIT1 and MDA levels among different fertility profiles.

Malondialdehyde is a marker of oxidative stress, and elevated levels of MDA in seminal fluid have been associated with decreased sperm quality and male infertility. Asthenospermia, a condition characterized by reduced sperm motility, can be influenced by oxidative stress (20)

Ch1t1, on the other hand, is a protein involved in sperm maturation and function. Studies have shown that alterations in Ch1t1 levels in seminal fluid may be associated with male infertility and impaired sperm function (3).

In the context of asthenospermia, it is possible that elevated levels of MDA and alterations in Ch1t1 levels in seminal fluid could contribute to the condition. Oxidative stress induced by high MDA levels may affect sperm motility, while changes in Ch1t1 levels could impact sperm maturation and function.

Limitations:

There were some factors and reasons behind the limitations of the study: The small number of sample size that we were able to collect in the study, sample contamination was an obstacle to collecting a larger number of samples, Community customs in collecting semen samples restricted obtaining other samples, Some patients not providing their personal information to obtain the sample prevented obtaining more samples and information and the limited geographical area affected the generalization of the study to all patients in Iraq.

Conclusion:
The observed correlation between seminal fluid CHIT1 activity and MDA levels underscores the interplay between chitin metabolism and oxidative stress in male fertility. Understanding the role of CHIT1 and MDA in male reproductive health could lead to novel diagnostic and therapeutic strategies for male infertility management.

Authors' declaration:
We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in (Department of Biochemistry) according to the code number (19634) on (20/ 5/ 2024).
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Authors' contributions:
Study conception & design: (Ali S. Abdul Aziz, Hedef Dhafir El-Yassin, HK Kadhem). Literature search: (Ali S. Abdul Aziz, Hedef Dhafir El-Yassin, HK Kadhem). Data acquisition: (Ali S. Abdul Aziz, Hedef Dhafir El-Yassin, HK Kadhem). Data analysis & interpretation: (Ali S. Abdul Aziz, Hedef Dhafir El-Yassin, HK Kadhem). Manuscript preparation: (Ali S. Abdul Aziz, Hedef Dhafir El-Yassin, HK Kadhem). Manuscript editing & review: (Ali S. Abdul Aziz, Hedef Dhafir El-Yassin, HK Kadhem).

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العلاقة بين مستوى كيتوتريوسايديز-1 والمالونديالدهيد في السائل المنوي للذكور العراقيين العقيمين

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الخلاصة

خلفية البحث: العقم عند الرجال هو حالة متعددة العوامل تتأثر بعوامل فسيولوجية وكيميائية حيوية مختلفة .يلعب تكوين السائل المنوي دورا حاسما في وظيفة الحيوانات المنوية وإمكانية الإخصاب .كيتوتريوسايديز -1 هو إنزيم يشارك في استقلاب الكيتين، في حين أن المالونديالدهيد (هو علامة على بيروكسيد الدهون، مما يعكس مستويات الإجهاد التأكسدي . تهدف هذه الدراسة إلى استكشاف العلاقة بين مستويات المالونديالديهايد و كيتوتريوسايديز (في السائل المنوي عند الذكور العراقيين المصابين بالعقم والذين يعانون من التهاب صامت .

طرق العمل : تم تضمين تسعين رجلا تتراوح أعمارهم بين عشرين وخمسة وأربعين عاما في هذه الدراسة المقطعية، وتم تشخيص جميعهم بالعقم من قبل متخصصين في وحدة العقم في مستشفى البتول التعليمي بين فبراير 2022 وفبراير 2023. وتم تصنيف المشاركين إلى ثلاث مجموعات :مجموعة طبيعية النطاف(G1) ، ومجموعة وهن النطاف(G2) ، ومجموعة قليلة النطاف.(G3)

النتائج: النتائج التي توصلنا إليها، كانت هناك مستويات أعلى بكثير من كيتوتريوسايديز في السائل المنوي الموجودة في المجموعة G2 مقارنة بمجموعتي G3و .G1كانت مستويات المالونديالديهايد للسائل المنوي لـ G1 أقل بكثير من تلك الخاصة بـ G2 و .G3كشفت عن وجود علاقة إيجابية مهمة بين نشاط السائل المنوي كيتوتريوسايديز ومستويات المالونديالديهايد($p < 0.05$، $r = 0.37$)في مجموعة. قليلة النطاف

الاستنتاجات: وجود علاقة إيجابية معنوية بين نشاط السائل المنوي بمستويات المالونديالديهايد و مستويات كيتوتريوسايديز في مجموعة وهن النطاف .

الكلمات المفتاحية :السائل المنوي البشري، كيتوتريوسايديز -1، الالتهاب الصامت، المالونديالدهايد. بيروكسيد الدهون