

Coenzyme Q10 and oxidative stress markers in seminal plasma of Iraqi patients with male infertility

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ABSTRACT

الأهداف: تقييم مستويات سائل الإنزيم المنوي CoQ10 و الإجهاد التأكسدي في المرضى المصابين بأنواع مختلفة من عقم الرجال.

الطريقة: أجريت دراسة حالة في قسم الكيمياء والكيمياء الحيوية - كلية الطب - جامعة النهرين - بغداد - العراق خلال الفترة من نوفمبر 2004م حتى يوليو 2006م. اشتملت الدراسة على 60 مريض مصاب بعقم الرجال راجعوا مستشفى الكاظمية التعليمي - بغداد - العراق. تم تقسيم المرضى طبقاً لمؤشرات السائل المنوي، وسجلت قلة النطاف في 32 مريض، وانعدام النطاف في 22 مريض، والحركة الناقصة للحيوانات المنوية في 6 مريض. حصلنا على جميع النتائج من مجموعات الذكور المصابين بالعقم ومقارنتها مع الأفراد السليمين المطابقين في العمر والتي تحتوي على 39 فرد. تم تحليل عينات أنزيم السائل المنوي CoQ10 بطريقة استشراب السائل العالي الأداء (HPLC)، و المندوليهيد (MDA) كمؤشر للإجهاد التأكسدي.

النتائج: كان مستوى السائل المنوي $1.10 \pm 0.169 \text{ mg/L}$ في المجموعة المصابة بندرة النطاف، و $0.567 \pm 0.098 \text{ mg/L}$ في المجموعة المصابة بانعدام النطاف، و $1.652 \pm 0.139 \text{ mg/L}$ في المجموعة المصابة بالحركة الناقصة للحيوانات المنوية، و $1.652 \pm 0.139 \text{ mg/L}$ في مجموعة التحكم. أظهرت مستويات أنزيم السائل المنوي CoQ10 في المجموعة المصابة بالعقم اختلاف إحصائي مهم من مجموعة التحكم $p \leq 0.0001$. لوحظ ارتفاع ملحوظ في مستويات المندوليهيد (MDA) بمعدل إحصائي بلغ $p \leq 0.001$ في السائل المنوي للمجموعة المصابة بندرة النطاف $11.37 \pm 1.64 \mu\text{mole/L}$ ، كما بلغ $13.87 \pm 1.62 \mu\text{mole/L}$ في المجموعة المصابة بانعدام النطاف، و $9.508 \pm 0.533 \mu\text{mole/L}$ في المجموعة المصابة بقلة الحيوانات المنوية، وبلغ $8.517 \pm 0.622 \mu\text{mole/L}$ في مجموعة التحكم. ارتبط السائل المنوي بشكل عكسي، وإحصائي مع المندوليهيد (MDA) ($r = -0.760$; $p = 0.000$).

خاتمة: ارتبط ارتفاع أنزيم السائل المنوي CoQ10 بعلاقة مباشرة مع مؤشرات السائل المنوي الجيدة وعكسية مع الإجهاد التأكسدي.

Objectives: To evaluate seminal plasma coenzyme Q10 (CoQ10) levels and oxidative stress in patients with different types of male infertility.

Methods: A case-control study was carried out in the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq from the period of November 2004 to July 2006. Sixty patients with male infertility were recruited from Al-Kadhimia Teaching Hospital, Baghdad and included in this study. The male patients were categorized according to their seminal fluid parameters to oligozoospermia ($n=32$), azoospermia ($n=22$), and asthenozoospermia ($n=6$). All obtained results from infertile men groups were compared with age-matched healthy volunteers as control group consisting of 39 subjects. Seminal plasma samples were analyzed for CoQ10 by an improved high performance liquid chromatography (HPLC) method and for malondialdehyde (MDA) as an index of oxidative stress.

Results: The mean seminal plasma CoQ10 was $1.10 \pm 0.169 \text{ mg/L}$ in oligozoospermia, $0.567 \pm 0.098 \text{ mg/L}$ in azoospermia, $0.740 \pm 0.06 \text{ mg/L}$ in patients with asthenozoospermia, and $1.652 \pm 0.139 \text{ mg/L}$ in control group. The seminal plasma CoQ10 levels in all infertility groups showed a significant difference from the control group ($p \leq 0.0001$). High significant increase ($p \leq 0.001$) in the MDA levels was noted in the seminal plasma of oligozoospermia $11.37 \pm 1.64 \mu\text{mole/L}$, azoospermia $13.87 \pm 1.62 \mu\text{mole/L}$, and asthenozoospermia group $9.508 \pm 0.533 \mu\text{mole/L}$ whereas the level in the control group was $8.517 \pm 0.622 \mu\text{mole/L}$. Seminal plasma CoQ10 was inversely and significantly correlated with MDA ($r = -0.760$; $p = 0.000$).

Conclusion: Elevated seminal plasma CoQ10 levels are associated directly with good semen parameters and inversely with the oxidative stress.

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Infertility is defined as the failure of a couple to achieve a pregnancy after at least one year of frequent unprotected intercourse.¹ Studies in the United States and Europe showed a one-year prevalence of infertility in 15% of couples. As shown in multicenter studies, 30-35% of sub-fertility can be attributed to predominantly female factors, 25-30% to male factors, and 25-30% to problems in both partners; in the remaining cases, no cause can be identified.^{1,2} Excessive production of free radicals or reactive oxygen species (ROS) can damage sperm, and ROS have been extensively studied as one of the mechanisms of infertility.³ Superoxide anion, hydroxyl radical, and hydrogen peroxide are some of the major ROS present in seminal plasma. Cells living under aerobic conditions constantly face the oxygen (O₂) paradox: O₂ is required to support life, but its metabolites such as ROS can modify cell functions, endanger cell survival or both.^{3,4} Hence, any excess ROS must be continuously inactivated by antioxidants present in the seminal plasma in order to maintain normal cell function. Thus, when there is an excessive production of ROS or the presence of any impairment in the antioxidant defense mechanisms, oxidative stress (OS) occurs, which is so harmful to spermatozoa.³ Coenzyme Q10 (CoQ10), an integral redox and proton translocating component of the mitochondrial respiratory chain,^{5,6} plays a key role in energy metabolism and has potent antioxidant properties for cellular membrane integrity. Bio-synthetic machinery for CoQ10 is present at remarkably high levels in testis where ubiquinone could cover important functions for its metabolic and antioxidant properties. In fact, a large amount of mitochondria are present in spermatozoa, in which the motile activity requires a high energy expenditure; in addition, the sperm for its high contents of unsaturated fatty acids in its membrane, is particularly exposed to peroxidative stress due to the action of reactive oxygen species (ROS).⁷⁻⁹ These reactive molecules caused a loss of sperm motility and decreased the capacity of sperm-oocyte fusion; they also affect the sperm axoneme because of ATP depletion and inhibit mitochondrial functions and synthesis of DNA, RNA, and proteins.⁹ Recently, studies started to define the CoQ10 activity in male fertility, suggesting a possible role for this molecule as a possible fertility marker.⁹ Coenzyme Q10 function in biological fluids and cells has been widely investigated in the recent years, highlighting its importance in the mechanism of electron transfer in the mitochondrial respiratory chain and in the neutralization of O₂ reactive species.⁹ In the reproductive field, several studies have attempted to establish a link between CoQ10 and sperm quality and function, but the results appeared to be conflicting.⁹ The objective of this study was to explore the CoQ10

and malondialdehyde (MDA) levels as oxidative stress markers in seminal plasma of infertile men.

Methods. A case-control study was conducted in the Chemistry and Biochemistry Department, College of Medicine, Al-Nahrain University, Baghdad, Iraq. After obtaining the approval of the Research and Ethics Committee of Al-Nahrain Medical College and written consent from the patients, 60 infertile patients were enrolled through this study from November 2004 to July 2006.

All patients were diagnosed according to a protocol described by the World Health Organization (1999).¹⁰ Men without any treatment and had regular unprotected intercourse for at least 12 months without conception with their partners were included in the infertile men group. Wives of the infertile subjects with no obvious causes of infertility such as hormonal disorders, tubal blockage or ovulation disorders were included. Patients who had infertility secondary to infection, were taking medication, or had a congenital defect and more than 106 leukocyte/mL in semen analysis were excluded from this study. Also, individuals with diabetes or thyroid diseases, patients who were on antipsychotic or antihypertensive drugs, or drinking alcohol, smoking, taking vitamins, minerals, and antioxidants supplementations within the past 3 months were also excluded from the study. Thirty-nine healthy donors with proven fertility who was initiated a successful pregnancy within the last year and had a normal spermogram at the time of study as controls were selected. Human semen samples were collected after 3-5 days of abstinence of sexual activity from healthy and infertile individuals. Semen samples were obtained by masturbation and samples were collected in a disposable sterile container. The freshly ejaculated samples were allowed to liquefy for 30 minutes at room temperature. Sperm parameters were evaluated according to the World Health Organization (WHO) criteria (1999).¹⁰ All individuals signed a consent form to allow the use of 4 mL of their seminal fluid in this study.

The liquefied semen samples were centrifuged at 600 x g for 20 minutes at 4°C. The supernatant seminal plasma samples were stored at -20°C until assay. Coenzyme Q10 concentrations were determined according to our previous method.¹¹ Malondialdehyde levels were analyzed according to the method described by Rao et al.^{12,13} Thiobarbituric acid reactive substances (TBARS) levels were estimated from the absorbance measurement at 534 nm using a molar absorption coefficient of 1.56 x 10⁵ cm⁻¹M⁻¹ for MDA-TBA complex.^{14,15}

Statistical analyses were performed using the Statistical Package for the Social Sciences computer program (SPSS for windows Version 10.0, SPSS Inc., Chicago, IL, USA). All values were given as mean with corresponding standard deviation. Independent student's t test and one-way analysis of variance were used for data analysis. Correlations between individual variables were examined by linear-regression analyses (Pearson correlation coefficient). Statistical tests were considered to be significant at the $p < 0.05$ level. All p values reported in this study were corresponded to 2-sided test for differences.

Results. The characteristics of subjects participated in this study with their seminal fluid parameters were listed in Table 1. It was found that there were no significant differences ($p \leq 0.05$) between the age values of different patient groups and control group. Analysis of variance revealed the presence of high significant differences in the sperm counts and progressive motility percents between infertile groups and the control group.

The levels of CoQ10 were measured in seminal plasma of different types of male infertility patients, and these results were compared with those obtained in age matched healthy controls. These results were listed in Table 2. Table 2 shows that there was a significant decrease ($p \leq 0.005$) in the seminal plasma CoQ10 concentration of the azoospermic patients compared with the other infertility groups and with the controls. Analysis of variance revealed that there were significant differences between infertility groups and the control group in seminal plasma CoQ10 levels. The results may also be expressed in relation to fertile and infertile groups as shown in Table 3 and Figure 1. Seminal plasma MDA levels were measured, and the results were compared with those of the healthy controls; values are listed in Table 4. Student t test showed significant differences ($p \leq 0.005$) in the seminal plasma MDA levels between infertility groups and the control group. It was found that the control group had the lowest MDA levels in seminal plasma samples. In this study, seminal plasma CoQ10

postulated a negative correlation coefficient and very high significant difference with seminal plasma MDA levels ($r = -0.760$; $p = 0.000$) as shown in Figure 2 and a positive correlation coefficient with high significant difference with progressive motility ($r = 0.424$; $p = 0.000$) as shown in Figure 3.

Table 2 - Seminal plasma coenzyme Q10 (CoQ10), levels (mean±SD) in infertile patients, and healthy controls.

Group	Seminal plasma (mg/L)	P-value
Oligozoospermia (n=32)	1.1015 ± 0.1690	0.000*
Azoospermia (n=22)	0.5677 ± 0.0982	0.000*
Asthenozoospermia (n=6)	0.7400 ± 0.0606	0.000*
Control (n=39)	1.6523 ± 0.1397	
Analysis of variance		0.000

All values are expressed as mean ± SD
*indicates significant difference from the control group at $p \leq 0.005$

Table 3 - Coenzyme Q10 levels in fertile and infertile groups.

Group	Seminal plasma (mg/L)
Infertile (n=38)	1.015 ± 0.256*
Fertile (n=39)	1.652 ± 0.139
P value	0.000

*indicates significant difference from the control group at $p \leq 0.005$

Table 4 - Seminal plasma malondialdehyde levels in fertile and infertile subjects.

Group	Seminal plasma (µmole/L)	P-value
Oligozoospermia (n=32)	11.373 ± 1.640*	0.000*
Azoospermia (n=22)	13.877 ± 1.620*	0.000*
Asthenozoospermia (n=6)	9.508 ± 0.533*	0.000*
Control (n=39)	8.517 ± 0.622	
ANOVA		0.000

*indicates significant difference from the control group at $p \leq 0.005$

Table 1 - Characteristics of patients and controls with their seminal fluid.

Variables	Azoospermia (n=22)	Oligozoospermia (n=32)	Asthenozoospermia (n=6)	Control (n=39)	ANOVA
Age (years)	32.09 ± 4.51	30.90 ± 3.52	35.00 ± 3.84	31.87 ± 3.76	0.122
Seminal fluid volume (mL)	3.75 ± 1.05	3.04 ± 1.23	3.66 ± 1.40	3.46 ± 1.72	0.308
Sperm count (million/mL)	-	8.44 ± 5.13†	53.04 ± 11.10†	87.34 ± 38.72	0.000
Progressive motility (%)	-	60.89 ± 9.40	38.22 ± 14.64*	64.06 ± 8.90	0.000
Morphology (%)	-	75.34 ± 8.25	75.38 ± 7.48	78.29 ± 7.84	0.279

Data are expressed as mean ± SD. ANOVA - analysis of variance.

*indicates significant difference from the control group at $p \leq 0.01$, †indicates significant difference from the control group at $p \leq 0.005$

Discussion. The data presented in this study indicate that endogenous CoQ10 is assayable in human semen. Seminal plasma CoQ10 correlated in this study positively and significantly with sperm count ($r=0.607$; $p\leq 0.0001$) and with sperm progressive motility ($r=0.424$; $p\leq 0.0001$) and this was in agreement with previous studies.^{9,16,17} Ducci et al⁹ also noticed that there

was no correlation between seminal plasma CoQ10 and sperm normal morphology percent, and this was completely in consistent with our results. Coenzyme Q10 plays an important role in energy metabolism and has potent antioxidant properties for cellular membrane integrity. Bio-synthetic machinery for CoQ10 is present at remarkably high levels in testis where ubiquinone could cover important functions for its metabolic and antioxidant properties. Actually, a large amount of mitochondria are present in spermatozoa in which the motile activity requires a high energy expenditure; in addition, the sperm for its high contents of unsaturated fatty acids in its membrane, is particularly exposed to oxidative stress due to the action of reactive oxygen species (ROS). These reactive molecules caused a loss of sperm motility and decreased the capacity for sperm-oocyte fusion. The positive correlation between the seminal plasma CoQ10 level and sperm progressive motility has a simple and logical explanation consequent to its potent antioxidant property; the molecule could counteract ROS damage on the sperm membrane so, due to CoQ10 antioxidant activity, it could be involved in the protection of sperm cell membranes from oxidative insult. On the other hand, seminal plasma CoQ10 correlated negatively and significantly

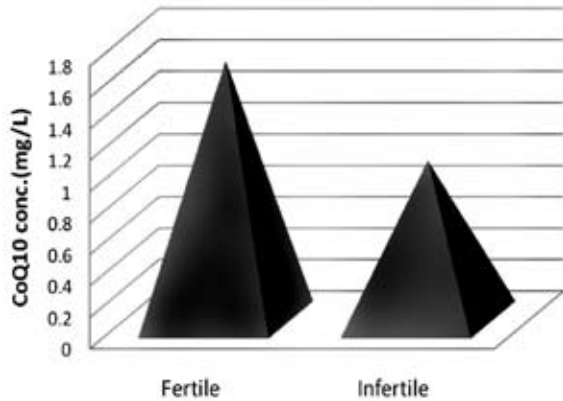
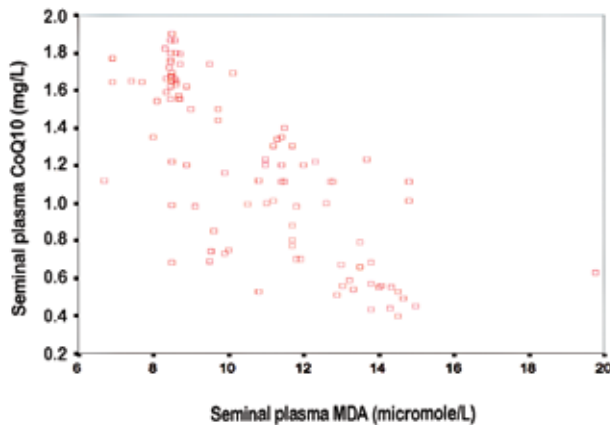
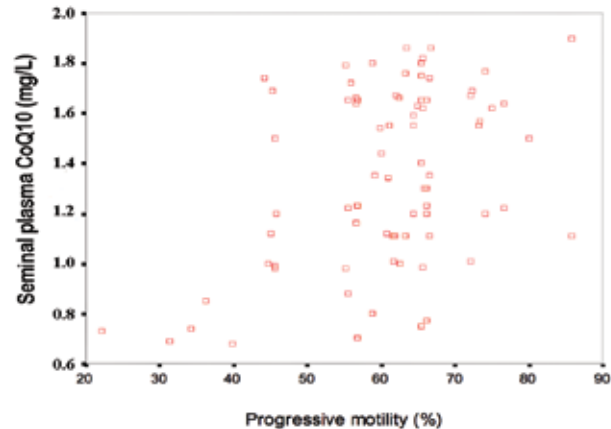


Figure 1 - Histogram of seminal plasma coenzyme Q10 (CoQ10) levels (mg/L) in fertile and infertile groups.



Correlation	Seminal plasma CoQ10	Seminal plasma MDA
<i>Seminal plasma CoQ10</i>		
Pearson correlation	1.000	-0.760*
Significant (2-tailed)	-	0.000
Number	99	99
<i>Seminal plasma MDA</i>		
Pearson correlation	-0.760*	1.000
Significant (2-tailed)	0.000	-
Number	99	99

*Correlation is significant at the 0.01 level (2-tailed)



Correlation	Seminal plasma CoQ10	Seminal progressive motility
<i>Seminal plasma CoQ10</i>		
Pearson correlation	1.000	-0.424*
Significant (2-tailed)	-	0.000
Number	77	77
<i>Seminal plasma sperm progressive motility</i>		
Pearson correlation	-0.424*	1.000
Significant (2-tailed)	0.000	-
Number	77	77

*Correlation is significant at the 0.01 level (2-tailed)

Figure 2 - Scatter plot for the correlation between seminal plasma coenzyme Q10 (CoQ10) and seminal plasma malondialdehyde (MDA).

Figure 3 - Scatter plot for the correlation between seminal plasma coenzyme Q10 (CoQ10) and sperm progressive motility.

with seminal plasma MDA ($r = -0.760$; $p \leq 0.0001$) and this may be attributed to the potent antioxidant activity of CoQ10. Also in this study, it was found that the control seminal plasma CoQ10 1.65 ± 0.14 mg/L. Reactive oxygen species (ROS) have a unique role in the human reproduction. Spermatozoa are rich in polyunsaturated fatty acids as well as susceptible to be attacked by ROS or membrane lipid peroxide ions. The equilibrium between the amounts of ROS produced and scavenged is related with the gamete cell stability and damage. Free radicals have beneficial or detrimental effects upon sperm functions, which depend on their nature and concentration.¹⁸ The production of ROS, such as superoxide anion, hydrogen peroxide, and the hydroxyl radical can result in the damage to cell membranes. Spermatozoa are highly sensitive to damage caused by high ROS concentration.¹⁸ Extreme generation of ROS in semen, mainly by neutrophils and by abnormal spermatozoa could be associated with reduced sperm fertilizing potentials.¹⁹ Spermatozoa are rendered dysfunctional by lipid peroxidation and altered membrane function, together with impaired metabolism, morphology, and motility.¹⁹ Lipid peroxidation triggers the loss of membrane integrity, causing increased cell permeability, enzyme inactivation, structural damage to DNA, DNA mutations, and cell death.²⁰ Selly et al²¹ found that the oxygen free radicals generated by spermatozoa may be involved in the production of spermicidal end products.²¹⁻²³ Concerning to male infertility Hsieh et al¹³ reported that the seminal plasma level of MDA in control group ($n=20$) was 1.52 ± 0.75 nmole/mL and in oligoasthenozoospermia group ($n=31$) was 2.25 ± 0.88 nmole/mL. On the other hand, Zarghami et al²¹ mentioned that seminal plasma MDA levels were higher in asthenozoospermic subjects than in control subjects (0.72 ± 0.06 μ M versus 0.40 ± 0.06 μ M).²² The seminal plasma MDA level measured in this study was 8.517 ± 0.622 in control group, 11.37 ± 1.64 in oligozoospermia group, 13.87 ± 1.62 in azoospermia group and 9.508 ± 0.533 μ mole/L in asthenozoospermia group. These results were different from studies obtained by Zarghami and Khosrowbeygi²² and Hsieh et al.¹³ Oligozoospermia, azoospermia, and asthenozoospermia were associated with higher seminal plasma MDA activity. Increased MDA activity could represent the pathologic lipid peroxidation of spermatozoa membrane and the following inhibition of sperm motility and viability.²⁴ A negative correlation was found in this study between the seminal plasma MDA concentration and sperm concentration ($r = -0.637$; $p \leq 0.001$) and this finding was compatible with the studies of Geva et al,²⁵ Fraczek et al,²⁶ and Kobayashi et al.²⁷ In this study, we observed that there was no significant correlation was present between seminal plasma MDA and sperm

progressive motility, and this was inconsistent with the study conducted by Suleiman et al.²⁸

The limitation of this study was the incapability to evaluate the levels of reduced and oxidized CoQ10 separately, instead the adapted method can measure the total CoQ10 content only. The present study findings support the hypothesis that seminal plasma CoQ10 levels decreased significantly in all infertility groups and correlates significantly and positively with sperm motility and negatively with seminal plasma MDA content. Seminal plasma MDA concentrations are negatively correlated with sperm concentration and motility that might provide a simple and useful tool in predicting sperm parameters.

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Related topics

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