Influence of Letrozole and Co Q10 on Sex Hormones and Spermiogram in Infertile Men; sample of Iraqi patients

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Abstract :

Background: The definition of World Health Organization (WHO) to the infertile couple is the failure of female get pregnancy in spite of having regular sexual activity for at least 1 year without using any contraceptive methods, worldwide it is estimated that 15 % of reproductive-age couples are struggling with infertility.
In many cases, infertility cannot be treated, new treatment options with promising value were involved in the recent clinical trials.
Aim: This study was designed to evaluate the effects of letrozole plus coenzyme Q10 combination on spermiogram and sex hormones in men with idiopathic oligoasthenoteratozoospermia (iOAT) syndrome.
Patient and methods: fifty-five patients are enrolled in this study, but only 40 patients complete the study, they are treated with a combination of Letrozole 2.5 mg tablet orally twice a week plus Co-enzyme Q10 400mg per day for three months. Seminal fluid sample, follicle-stimulating hormone, estradiol, and testosterone were analyzed before starting the treatment and at the end of month 1, 2 and 3.
Results: sperm concentration, sperm morphology, total sperm count and motility, serum testosterone and follicle stimulation hormone levels, in addition to testosterone/estradiol ratio were significantly improved, while estradiol levels significantly decreased after 3 months of treatment. However, seminal fluid volume showed no significant change. Finally, as a notable outcome, one spontaneous conception occurred after treatment as well as three azoospermia cases responded well after completing the course of treatment.
Conclusions: a combination of Letrozole and CO Q10 can effectively improve sperm parameters in Iraqi men with iOAT.

Key words: Male infertility, spermiogram, Letrozole, CO enzyme Q 10.

تأثير عقار ليتروزول والانزيم المساعد Q10 على الهرمونات الجنسية ومخطط الحيوانات المنوية في الرجال المصابين بالعقم؛ عينة من المرضى العراقيين

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الخلاصة:

خلفية البحث: تعريف العقم حسب منظمة الصحة العالمية هو عدم قدرة الزوجين على الانجاب على الرغم من ممارسة العملية الجنسية بشكل منتظم و لمدة عام كامل على الأقل من دون استخدام أي وسيلة من وسائل منع الحمل، في جميع أنحاء العالم يقدر عدد الازواج المصابين بالعقم حوال ١٥٪, في كثير من الحالات لا يمكن علاج العقم ، قبل بضع سنوات تم اكتشاف خيارات علاجية جديدة ذات قيمة واعدة وحاليا تستخدم في التجارب السريرية .

اهداف البحث: هذه الدراسة هي لتقييم فعالية عقار ليتروزول بالإضافة إلى الإنزيم المساعد Q10 على مخطط الحيوانات المنوية والهرمونات الجنسية لدى الرجال المصابين بمتلازمة إمساخ ووهن وقلة النطاف.

في هذه الدراسة تمت مشاركة ٥٥ مريضاً، ولكن ٤٠ مريضًا فقط هم من اكملوا الى نهاية الدراسة ، المرضى المشاركين بالبحث تم علاجهم بمزيج من عقار ليتروزول اقراص بجرعة ٢,٥ ملغ عن طريق الفم تعطى مرتين في الأسبوع بالإضافة إلى الانزيم المساعد Q10 بجرعة ٤٠٠ ملغ يوميًا و لمدة ثلاثة أشهر. تم تحليل عينة السائل المنوي والهرمون المنشط للحوصلة وهرمون الإستراديول وهرمون التستوستيرون قبل بدء العلاج وكذلك في نهاية الشهر الأول والثاني والثالث.

النتائج: عند اكمال الدراسة لوحظ ان هناك تحسن في تركيز الحيوانات المنوية، الشكل الطبيعي، العدد الكلي للحيوانات المنوية وحركتها، مستويات هرمون التستوستيرون و الهرمون المنشط للحوصلة في الدم، بالإضافة تحسن في نسبة التيستوستيرون/ الاسترادايول ، بينما انخفضت مستويات الاستراديول بشكل ملحوظ بعد ٣ شهور من العلاج. ومع ذلك، لوحظ ان كمية السائل المنوي لم تغيير كثيرا، وأخيرًا وكنتيجة ملحوظة، حدث حمل تلقائي واحد بعد العلاج بالإضافة إلى ثلاث حالات كانت تعاني من فقد نطاف قد استجابت بشكل جيد بعد الانتهاء من العلاج.

الاستنتاجات: أن مزيج من عقار اللترزول والانزيم المساعد Q10 يمكن أن يحسن بشكل فعال معايير الحيوانات المنوية لدى الرجال العراقيين المصابين بمتلازمة إمساخ ووهن وقلة النطاف.

الكلمات المفتاحية: عقم الذكور، مخطط النطاف، ليتروزول، إنزيم المساعد Q 10.

Introduction:

The definition of World Health Organization (WHO) to the infertile couple is the failure of female to achieve pregnancy after engaging in regular sexual activity for at least one year without using any kind of contraception[1]. Globally, about 15% of partners who are in reproductive age deal with infertility[2], male factors are implicated in around half of this problems [3]. A variety of factors contributing to male infertility includes environmental factor[4], infection[5], modification in post-testis organ function, such as the prostate [6], hereditary factor [4], and disturbance of sex hormones [7]. The evaluation of the infertile patient should include thorough history, physical examination and seminal fluid analysis (SFA) with stick to WHO standard values [8], in addition, every infertile partner should always have a complete andrological examination even if semen analysis reveals no abnormalities or normal sperm parameters when compared to reference values, the hormone analysis includes FSH, LH, testosterone and prolactin [9]

In response to gonadotropin-releasing hormone, the anterior pituitary releases luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Accordingly, the FSH acts on Sertoli cells to establish spermatogenesis and to produce inhibin B, which suppresses FSH secretion in a negative feedback loop, whereas LH acts on Leydig cells to enhance the release of testosterone that improves the production of sperm and virilization, as well as having a negative feedback to prevent the pituitary from releasing LH and FSH. As a result, neither FSH nor LH alone can generate a good quality and quantity of sperm [10],[11].

The spermatogenesis is further boosted when endogenous testosterone levels rise in conjunction with reduced estrogen suppression on the hypothalamic-pituitary-gonadal (HPG) axis [11]. However, the aromatase enzyme is belong to cytochrome p-450 enzymes and mainly present in liver, testis, female reproductive organ and adipose tissue, it's have a function of converting testosterone (T) to estradiol (E_2). Therefore, by preventing the conversion of T to E2, aromatase inhibitors

result in enhance testosterone levels while decreasing estrogen levels. Accordingly, When plasma E_2 levels are changed in men within a range of normal physiological values, this has a favorable influence on FSH, LH, and testosterone levels through a pituitary gland effect [12]. Consequently, aromatase inhibitors increase the production of testosterone in men with low levels of the hormone [13].

Letrozole is the most common used aromatase enzyme inhibitor, it belongs to third generation aromatase inhibitor and exert it effects by inhibit estrogen biosynthesis reversibly [14].

The reactive oxygen species (ROS) and oxidative stress (OS), are considered injurious to spermatozoa causing 30% to 80% of male infertility cases [15]. ROS in semen are triggered by both internal and external factors. However, Round cells, epithelial cells, and leukocytes are examples of endogenous sources, while drinking alcohol, smoking, and environmental factors (radiation and toxins) are considered sources of exogenous ROS [16]. Increased ROS generation results in oxidative stress and lowers the antioxidant capacity of spermatozoa [17]. The plasma membrane of spermatozoa is composed of lipids and polyunsaturated fatty acids; when there are too many (ROS) this membrane undergo to lipid peroxidation and damage[18]. Lipid peroxidation causes the decreased motility and fluidity of the membrane in sperm which is related to the sperm's diminished capacity to fertilize[19].

Coenzyme Q10 (CoQ10) is a fat-soluble vitamin that is naturally present in human cell membranes as well as in food and can generated endogenously [20]. Co Q10 has a powerful antioxidant properties [21], and it is among the most widely used antioxidants in the treatment of males with idiopathic infertility because of its potent antioxidant properties and role in the production of mitochondrial energy, which is crucial for maintaining spermatozoa's power source and protecting their membranes from damage due to lipid peroxidation [22].

In previous studies, letrozole as well as Co Q10 were used separately. While in the current study a combination of letrozole and Co Q10 are used to evaluate their effects on sperm parameter and sex hormones profile in infertile males.

Methods:

The total number of patients enrolled in this study is 55 patients, however, only 40 patients finish the study, while the remaining 15 patients were lost during a follow-up. Subjects who had idiopathic oligoasthenoteratozoospermia (iOAT) were chosen while visiting the "High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Nahrain University". Demographic data have been collected and includes patient age, smoking status, body mass index (BMI), duration of infertility and co-existing disease. Furthermore, each patient gave their signed informed permission before participating in this study. The time frame for this study was from December 2021 to October 2022. Participants in this study aged from 18 to 60 years old. However, patients that excluded from this study were:

- Individuals who have other infertility issues, such as varicocele or ejaculatory duct obstruction.
- Patients have undergone surgery to treat male factor infertility
- Patients who have sexual transmitted diseases (STDs) or other infectious diseases.
- Patients with liver or renal disease.

Patients participate in this study received Letrozole 2.5mg twice a week plus Co Q10 400 mg per day for 3 months. However, to monitor any potential changes in the studied parameters, the seminal fluid analysis (SFA) and hormone profile

(testosterone, E_2, and FSH) are assessed before taking the medication (baseline value) and at the end of 1, 2, and 3 months after treatment.

Statistical Analysis:

The data were analyzed using the Microsoft excel software, Minitab v17, and IBM SPSS V28. The results reported in this study were expressed as mean ± SD for numeric data and number, one-way analysis of variance (ANOVA) were used to examine the degree of significance between three or more group for various observations, Probability values <0.05 were considered significantly different and values <0.01 were considered highly significant differences.

Results:

The male reproductive capacity is affected by many factors including age, obesity, smoking and the presence of chronic disease. However, most of patients were obese and overweight, in addition, more than half of them were smokers. While, just two patients have coexisting disease, and about half of them have duration of infertility from one year to five years. Accordingly, table (1) explains the demographic information for participants.

Table (1): Demographic data and disease characteristics.

Parameters		No %
Age (years)		35.1 ± 8.3
BMI (kg/m^2)	**≤ 18.4**	0 (0)
	18.5 – 24.9	0 (0)
	25 – 29.9	15 (19.5)
	≥ 30	25 (32.5)
Smoking	**Smoker**	24 (31.2)
	Non-Smoker	16 (20.8)
Co-existing disease	**H.T**	1 (1.3)
	D.M	0 (0)
	D.M+HT	1 (1.3)
Duration of infertility (years)	**1-5**	23 (29.9)
	> 5	17 (22.1)

Data presented as mean ± SD for Age, Data presented as N (%) for other parameters, N: Number of patients, % Percentage, Diabetic (DM), Hypertension (HT).

Table (2) represent the results of treatment by letrozole plus CO Q10 on sperm production after the end of month 1, 2 and 3. The results showed a statistically significant increase in the mean value of sperm concentration and total sperm count after each month in respect to the baseline ($p < 0.01$). However, seminal fluid volume show no significant difference ($P>0.05$).

Table (2): Effect of letrozole plus CO Q10 on sperm concentration.

Sperm production		Mean ± SD
Volume/ ml (ml)	(baseline)	3.1 ± 0.9 [a]
	(1st month)	3 ± 0.8 [a]
	(2nd month)	3.1 ± 1.1 [a]
	(3rd month)	3.1 ± 1 [a]
P. Value [B]		0.769 [NS]
Sperm concentration/ml (Mil./ml)	(baseline)	7.3 ± 5.2 [a]
	(1st month)	11.9 ± 9.2 [b]
	(2nd month)	20 ± 14.1 [c]
	(3rd month)	25.2 ± 16.5 [d]
P. Value [B]		< 0.001**
Total sperm count / ml (Million)	(baseline)	24.8 ± 20.6 [a]
	(1st month)	36.5 ± 32 [b]
	(2nd month)	66 ± 60.4 [c]
	(3rd month)	80.1 ± 62.6 [cd]
P. Value [B]		< 0.001**

Data presented as mean ± SD, [B] one way repeated measures ANOVA used for comparison between (Time-wise values) within same group. Different small case letters indicate significant difference. NS: No significant changes ($p \geq 0.05$), * significant changes ($p < 0.05$), ** highly significant changes ($p < 0.01$).

The results in Table (3) clarifies the effects of letrozole plus CO Q10 combination on sperm motility and morphology upon the completion of months 1, 2, and 3. Statistically, after receiving medication for three months, there has been a significant increase in sperm motility and morphology.

Table (3): Effect of letrozole plus CO Q10 on sperm motility and morphology

Parameters		Mean ±SD
Progressive motility (%)	(baseline)	12.9 ± 11.2 [a]
	(1st month)	20.0 ± 14.7 [b]
	(2nd month)	27.7 ± 15.7 [c]
	(3rd month)	31.7 ± 15.5 [dc]
P. Value [B]		< 0.001**
Non-progressive motility (%)	(baseline)	12.9 ± 10.6 [a]
	(1st month)	14.9 ± 11.0 [ab]
	(2nd month)	14.8 ± 9.7 [abc]
	(3rd month)	16 ± 8.7 [bd]
P. Value [B]		0.013*
Immotile (%)	(baseline)	73.4 ± 19.7 [a]
	(1st month)	66.4 ± 18.3 [b]
	(2nd month)	58.5 ± 18.8 [c]

	(3rd month)	51.3 ± 18.1 d
P. Value B		< 0.001**
Normal morphology (%)	(baseline)	2.3 ± 1.6 a
	(1st month)	2.7 ± 2.1 ab
	(2nd month)	4.6 ± 3.7 c
	(3rd month)	5.8 ± 4.0 d
P. Value B		< 0.001**

Data presented as mean ± SD, B one way repeated measures ANOVA used for comparison between (Time-wise values) within same group. Different small case letters indicate significant difference. NS: No significant changes (p≥0.05), * significant changes (p<0.05), ** highly significant changes (p<0.01).

Results of the effect of letrozole plus CO Q10 on sex hormones after 1, 2 and 3 months are illustrated in table (4). Both testosterone and FSH levels demonstrated a high significant (p<0.01) increase in the mean value. Conversely, E_2 levels decreased significantly (p<0.01). Regarding to the ratio of T/E_2, the growth was about 312% from the baseline.

Table (4): Effect of letrozole plus CO Q10 on sex hormones

		Mean(±SD)
Testosterone ng/ml	(baseline)	3.3 ± 1.3 a
	(1st month)	4.8 ± 1.2 b
	(2nd Month)	6.2 ± 1.6 c
	(3rd Month)	7 ± 2 d
P. Value B		< 0.001**
Estradiol Pg/ml	(baseline)	50.4 ± 11.1 a
	(1st month)	40.8 ± 8 b
	(2nd Month)	33 ± 7.7 c
	(3rd Month)	26.1 ± 6.6 d
P. Value B		< 0.001**
FSH mIU/ml	(baseline)	6.4 ± 2.6 a
	(1st month)	8.2 ± 2.9 b
	(2nd Month)	10 ± 3.6 c
	(3rd Month)	11.2 ± 3.5 d
P. Value B		< 0.001**
Ratio T/E_2	(baseline)	7 ± 3.2 a
	(1st month)	12.3 ± 3.7 b
	(2nd Month)	20 ± 7.6 c
	(3rd Month)	28.9 ± 11.3 d
P. Value B		< 0.001**

Data presented as mean ± SD, B one way repeated measures ANOVA used for comparison between (Time-wise values) within each group. Different small case letters indicate significant difference. NS: No significant changes (p≥0.05), * significant changes (p<0.05), ** highly significant changes (p<0.01).

Discussion:

In numerous earlier clinical trials, men's infertility was treated with Letrozole alone at a dosage of 2.5 mg daily [23],[24]. However, in another trial, 2.5 mg of Letrozole were administered weekly to increase testosterone [25]. in the current study, the Letrozole dose used was 2.5 mg twice a week for all subjects; this was based on consultants' expertise in an effort to reduce the anticipated adverse effect which is loss of libido that was noted in prior studies [24],[26].

A research by Kooshesh *et al.* determines that use of Letrozole in treatment of men present with iOAT and T:E2 ratio ≤ 10, can successfully increase sperm quality and chromatin integrity, and consequently increase spontaneous pregnancy [27]. These findings mostly agreed with the present findings.

In current study, the administration of Letrozole lead to increase testosterone and decrease estradiol production, consequently, improving the T/E_2 ratio that producing a positive impacts on spermatogenesis and obtaining a good quality and quantity of sperm [28].

Following a three month course of therapy, Co Q10 plus Letrozole significantly improved the T/E_2 ratio which achieved growth of about (312%) from the baseline. A study by Peivandi S. *et al* (2019) attribute the enhancement of spermatogenesis to the rise in the ratio of T/E_2 [29]. Appasamy *et al*. (2019) imply that the elevated ROS levels may be capable of reversing the balance of hormones and lowering levels of the male sex hormone causing infertility [30]. accordingly, the spermiogram parameters were improved by administering Co Q10, by its potent antioxidant effects to remove ROS and reverse oxidative damage [31]. Furthermore, according to Safarinejad (2009), taking 300 mg of Co Q10 per day for six months improved sperm motility, count, and shape while lowering FSH levels [32]. However, after using Co Q10 and Letrozole together for three months, the results of this study show an improvement in sperm motility, morphology, and concentration. In addition, measurements of sex hormones show a rise in FSH and testosterone levels as well as a drop in E_2 levels.

Many personal and environmental factors have an impact on men's ability to reproduce, but age seems to be the most delicate one since aging is inversely correlated with spermatogenesis[33]. Because the majority of individuals in the current study were in the third decade of life, there may be a link between age and sperm quantity and quality. The second crucial element is smoking, according to Jin-Bo Dai *et al*. (2015), tobacco use whether it takes the form of cigarettes, water pipes, or vapes may contain a variety of harmful chemicals that disturb the antioxidant status of the testicles, lower the intratesticular testosterone levels and ultimately disrupt spermatogenesis [34], Nevertheless, many men are still fertile despite the negative effects of smoking on male fertility, while they are at danger of losing their fertility [35]. The majority of patients in the current research were smokers which may have a significant impact on the quality of sperm. Finally, most of participants in this study were overweight and obese and this may have negative effects to generate poor quality of spermatozoa. however, this finding is agreed with Belloc *et al*. (2014) that revealed a relationship between a high BMI and poor semen quality [36].

Limitation:

Low sample size is the main limitation in current study, this is because of exclusion criteria indicated above and the fact that many patients were missed during follow-up periods. Additionally, Lack of control group since it was difficult to persuade a normal man to undergo SFA and hormone analyses. Finally, Due to the lack of techniques to assess ROS in semen, ROS in seminal fluid is not measured.

Conclusion:

Letrozole and Co Q10 together had a significant positive impact on all sperm parameters, in this study, three cases of azoospermia responded well to therapy, and one spontaneous pregnancy was achieved during the course of the trial. According to the findings of this study, Letrozole with Co Q10 is recommended for the treatment of patients with iOAT syndrome who have high estradiol and low testosterone levels.

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