The Effects of Combined Oral Antioxidants on Male Infertility

Ali Hadi Sabhan *
*Lecturer (F.I.B.M.S Urologist), College of Medicine, Al-Qadisiya University, e-mail:
Email: Ali.sabhan@qu.edu.iq
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خلاصة البحث:

مقدمه:

يعتبر ارتفاع نسب أصناف الأكسدة التفاعلية في السائل المنوي للرجال من احد أسباب العقم لديهم ولذلك فان استعمال العلاجات المضادة للاكسده تعمل على خفض مستوياتها من خلال تحقيق توازن بين عملية إنتاجها وعملية التخلص منها والذي يؤدي إلى تحسين نوعية النطف ورفع نسبة الإنجاب.

طريقة البحث:

أجريت هذه الدراسة على 66 مريض من الذكور بمعدل عمر 29 عام ومعدل فترة عقم 1.5 سنه حيث تم اختيارهم عشوائيا لهذه الدراسة التي امتدت من شهر كانون الثاني 2013 وحتى شهر تشرين الأول 2014 للمرضى المراجعين لعيادة العقم في مستشفى النسائية والأطفال في الديوانية.تم اخذ التاريخ المرضي لجميع المرضى واجري لهم الفحص السريري العام والتناسلي وفحصت عينتان من السائل المنوي وإجراء التحاليل الهرمونية اللازمة وفحص سونارالدوبلر الملون للخصيتين.أعطي جميع المرضى مجموعة من علاجات مضادات الأكسدة لمدة ثلاثة اشهر وتم بعدها إعادة فحص السائل المنوي لمتابعة التغيرات في كمية السائل،تركيز النطف،مظهر النطف وحركتها مع توثيق حدوث إي حمل.

النتائج:

تحقق الحمل لثلاثة عشر مريض (19%) خلال فترة الدراسة في حين حصلت تغيرات ايجابية في السائل المنوي لدى المرضى الآخرين الذين لم يتحقق لهم الحمل حيث إن 30 مريض (45%) ازدادت لديهم كمية السائل المنوي و 28 مريض (42%) ازداد لديهم تركيز النطف في السائل.

22 مريض (33%) حققوا زيادة في حركة النطف و 34 مريض (51%) تحسن مظهر النطف لديهم كما وكانت هناك نتائج مختلفة بين المرضى في تحسن عدد عناصر السائل المنوي (عنصر واحد أو أكثر) وبنسب مختلفة.

الاستنتاج:

أظهرت هذه الدراسة دورا مشجعا لدمج علاجات مضادات الأكسدة في تحسين عناصر السائل المنوي وتحقيق الحمل لدى بعض الرجال الذين يعانون من العقم وكما أظهرت أفضلية دمج تلك المضادات على استعمالها بشكل منفرد.

Abstract

Background

High reactive oxygen species (ROS) levels was considered as an important factor of male infertility that can result in DNA damage, decrease motility and damage membrane integrity. The role of antioxidants is to maintain the balance between ROS production and their clearance which improve sperm parameters and enhance male fertility.

Patients and methods

Sixty six male patients with median age of 29 years with a mean period of infertility of 1.5 year that attend the infertility clinic in Al-Diwaniya Maternity and Pediatric Hospital were selected randomly in this study which extend from January 2013 to October 2014 (22 months).The patients were selected to have idiopathic oligo-astheno-teratozoospermia, they underwent a detailed history, general and genital physical examination, Two semen analyses, baseline hormone profile and scrotal Doppler ultrasound examination . All

patients were given a combination of oral antioxidants including vitamin E (400 IU. once daily), coenzyme Q10 (75 mg. tablet twice a day), zinc sulphate (15 mg. tablet three time a day) and L-carnitine (1000 mg. twice a day) for three months duration of treatment. Semen analysis was repeated after three months from initiation of therapy and was evaluated for changes in volume, concentration, motility and morphology in comparison with baseline semen analyses and any pregnancy was also documented.

Results

Thirteen patients (19%) get pregnancy after a period of 2 to 3 months, other patients show variable changes in their seminal analysis after 3 months. Thirty patients (45%) show increasing in seminal volume of 0.5 to 2 ml. , while the sperm concentration was increased in 28 patients(42%) . Twenty two patients (33%) show increasing in motility , morphology was increased in 34 patients (51%) .From all 66 patients; 6 patients (9 %) get improvement in all four parameters, while 9 patients (14 %) get improvement in only 3 parameters, 20 patients (30 %) get improvement in only 2 parameters, 23 patients (35 %) get improvement in only one parameter and 8 patients (12 %) had no improvement in any parameter.

Conclusion

This study demonstrates a possible role of combined oral antioxidants in the improvement of semen parameters and pregnancy rate for selected infertile men with superiority for the use of combination of antioxidants rather than single agent treatment.

Introduction

Infertility can be defined as a failure to achieve pregnancy after one year or more with unprotected regular sexual intercourse. Nearly 15%- 20% of couples at reproductive age found to be infertile [(1)].

Several causes were suggested for male infertility. idiopathic oligoasthenoteratozoospermia (OAT) is the most common cause in which sperm concentration, morphology and the motility of sperm are less than the reference values of World Health Organization [(2)].

Approximately 25% of infertile men have no identifiable cause .These patients can be treated with different medications to improve their semen parameters and fertility potential [(3)] .

Leukocytes are the main source of reactive oxygen species(ROS) production in semen that results from their activation in response to inflammation and infection[(4)]. An abnormally high leukocyte concentrations as in leukocytospermia ($>1\times10^6$ per milliliter of semen) which defined by WHO can result in sperm damage from leukocyte source of ROS .Sperm damage can also happens even with leukocyte concentration lower than the cut-off value of WHO for leukocytospermia[(2,5)].

Recent research on the effects of ROS and its effects in the pathogenesis of male factor infertility has received a major interest from the medical practitioners and scientists [(6)].

The spermatozoa produce a small quantity of ROS which are important for some processes such as hyperactivation, capacitation, , acrosome reaction and sperm-oocyte fusion [(7)]. Seminal antioxidants are the most important body mechanism for the reduction of ROS damage and providing a protection of sperm against their insults [(8)].Furthermore , excessive production of ROS with reduction

of its clearance can results in oxidative stress within the sperm leading to series of sperm damage and malfunctioning. Antioxidants can restore the balance between the production of ROS and clearance and thus help to improve sperm parameters [9, 10] .

ROS can stimulate apoptosis process resulting in spermatozoa death and decreased sperm count that reduced the chance of getting a pregnancy [11] .

Studies found that a high levels of ROS correlate with reducing motility of spermatozoa because of sperm ATP depletion [12] .

The damaging effects of ROS on the membrane of sperm lead to impairment of the sperm-oocyte fusion and its ability to start the necessary reactions of acrosome or zona pellucida binding and penetration of oocyte [13].

The use of oral antioxidants to reduce oxidative stress and improvement of fertility was made its basis from these described findings.

Vitamin E is a major antioxidant in the semen that can decrease lipid peroxidation, minimize free radical and improved spermatozoa fusion with oocyte and zona pellucida binding .it is available in many formulations, as oral supplements and had a significant role in increasing motility and pregnancy rates [14].

It is well known that coenzyme Q10 (ubiquinone) is a fundamental component participating in the generation of energy in the form of ATP . Coenzyme Q10 is ubiquitous which ideally help to scavenge free radicals and prevent lipid peroxidation[15] . In addition to direct anti-oxidant activity, coenzyme Q10 helps restore other anti-oxidants, such as Vitamin E and C, to their reduced state and can significantly improve sperm motility and pregnancy rate in males with idiopathic infertility [16].

Carnitines (L-acetyl carnitine and L-carnitine) play an important role in generating cellular energy . The increase in L-carnitine in the epididymal lumen corresponds with the initiation of sperm motility [17].The mechanism of the anti-oxidant abilities of carnitines has not fully been elucidated, but carnitines have been shown to scavenge ROS and inhibit lipid peroxidation and found that carnitines can improves sperm straight progressive velocity, total oxyradical scavenging capacity, sperm motility, sperm concentration, and semen volume [18].

Zinc acts as a cofactor for approximately 100 metalloenzymes . Zinc has direct anti-oxidant effects and synergistically helps other anti-oxidants . Zinc is also thought to have membrane stabilizing effects and anti-apoptotic properties . Low levels of zinc in seminal plasma are associated with male infertility. Higher levels of zinc in seminal plasma correlate significantly with increased sperm concentration and normal sperm morphology [19]. Zinc was found to improve sperm parameters, reduce oxidative stress, decrease sperm apoptosis, and decrease sperm DNA fragmentation, alone or in combination with vitamin E and C [20].

Patients and methods

From January 2013 to October 2014 (22 months period); sixty six male patients with median age of 29 years (range from 20 to 43 years) with a mean period of infertility of 1.5 year (range from 1 to 5 years) that attend the infertility clinic in Al-Diwaniya Maternity and Pediatric Hospital (Al-

Diwaniya, Iraq) were selected randomly in this study.

The patients were selected to have idiopathic oligo-astheno-teratozoospermia. The exclusion criteria were cases with varicocele, azoospermia, small testicular size, an abnormal hormonal analysis (including serum FSH, LH, prolactin and testosterone), presence of female factor of infertility, patients with poor compliance to treatment and those with known or developed hypersensitivity to any of medications used.

The study was approved by ethical committee of College of Medicine, Al-Qadisiya University. Informed consent was taken from each patient. All men underwent a detailed history, general and genital physical examination, Two semen analyses, baseline hormone analysis (serum FSH, LH, prolactin and testosterone) and scrotal Doppler ultrasound examination to exclude correctable cause for infertility. Abnormal semen parameters were defined based on the values suggested by World Health Organization criteria[(2)].

All patients were administered a combination of oral antioxidants including vitamin E 400 IU. once daily in form of gelatinous tablet (vitamin E-400®, Vitane's Nature company, USA.), coenzyme Q10 75 mg. tablet twice a day (Covitan 75® ,Vitane's Nature company, USA.), zinc sulphate 15 mg. tablet three time a day (All-zinc®, DietRoute company, UK.) and L-carnitine 1000 mg. tablet twice a day (L-carnitine® , Ultimate Nutrition company , USA.) for three months duration of treatment.

Patients were followed during the treatment period to insure their compliance to treatment and for detection of any side effects or complications of treatment, Semen analysis was repeated after three months from initiation of therapy and was evaluated for changes in volume, concentration, motility and morphology in comparison with baseline semen analyses that were done before initiation of therapy.

Any reported pregnancy during the period of follow up was documented. The statistical analysis was performed using SPSS software, t- test was applied and the results are expressed as percentage and median values ± SD.

Results

All the sixty six patients that included in our study complete the treatment trial of three months with no adverse effects. Thirteen patients (19%) get pregnancy after a period of 2 to 3 months (mean of 2.6 ± 0.4 months), the other patients show variable changes in their seminal analysis after 3 months in comparison with their baseline seminal analysis that was done before starting the treatment trial.

Thirty patients (45%) show increasing in seminal volume of 0.5 to 2 ml. with a mean of 1.2 ± 0.4 ml., while the sperm concentration was increased in 28 patients(42%) with a range of increment of 1.2 to 12 million and mean of 7.9 ± 3.5 million.

Twenty two patients (33%) show increasing in motility in a range of 5 to 30% with a mean of 18 ± 6.2 %, morphology was increased in 34 patients (51%) in a range of 5 to 15% and a mean of 9.6± 3.6 % .Table 1 and figure 1 summarize the changes in semen parameters after treatment in the 66 patients.

From all 66 patients; 6 patients (9 %) get improvement in all four parameters, while 9

patients (14 %) get improvement in only 3 parameters, 20 patients (30 %) get improvement in only 2 parameters, 23 patients (35 %) get improvement in only one parameter and 8 patients (12 %) had no improvement in any parameter. Tables 2, 3 and 4; show the percentage of patients who got improvement in 1 or 2 or 3 parameters.

Table 1. **Semen parameters with improvement after completion of treatment trial**

Semen parameter	Number of patient show improvement	Percentage of patient show improvement	Range values of improvement	Mean ± SD for improvement values
Volume	**30**	**45 %**	**0.5 – 2 ml.**	**1.2 ±0.4 ml.**
Concentration	**28**	**42 %**	**1.2 – 12 million**	**7.9 ± 3.5 million**
Motility	**22**	**33 %**	**5 – 30 %**	**18 ± 6.2 %**
Morphology	**34**	**51 %**	**5 – 15 %**	**9.6± 3.6 %**

Table 2. **Patients who get improvement in only one parameter.**

	volume	Concentration	Motility	Morphology	total
Number of patients	**4**	**5**	**4**	**10**	**23 (35%)**

Table 3. **Patients who get improvement in only two parameters.**

	Volume - Concentration	Volume - Motility	Volume - Morphology	Concentration - Motility	Concentration - Morphology	Motility - Morphology	total
Number of patients	**5**	**2**	**6**	**2**	**3**	**2**	**20 (30%)**

Table 4. **Patients who get improvement in only three parameters.**

	Volume-Concentration-Motility	Volume-Concentration-Morphology	Volume-Motility-Morphology	Concentration-Motility-Morphology	total
Number of patients	**2**	**3**	**2**	**2**	**9 (14 %)**

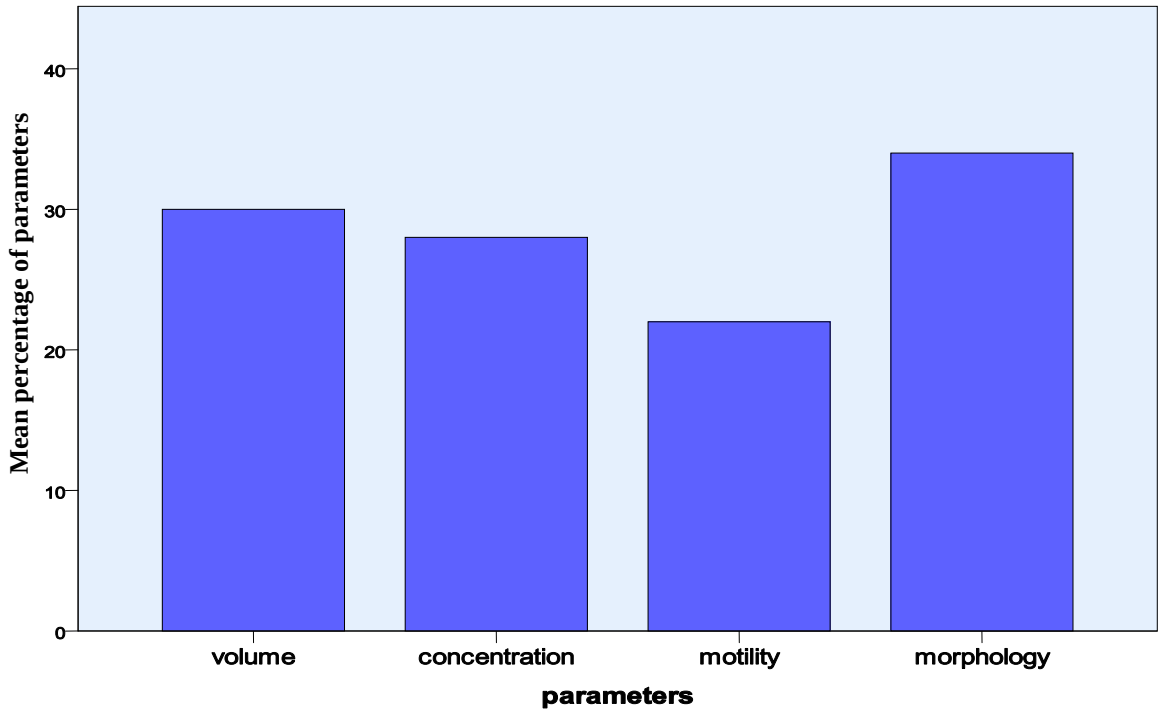

Figure 1. **Improvement of semen parameters after completion of treatment trial**

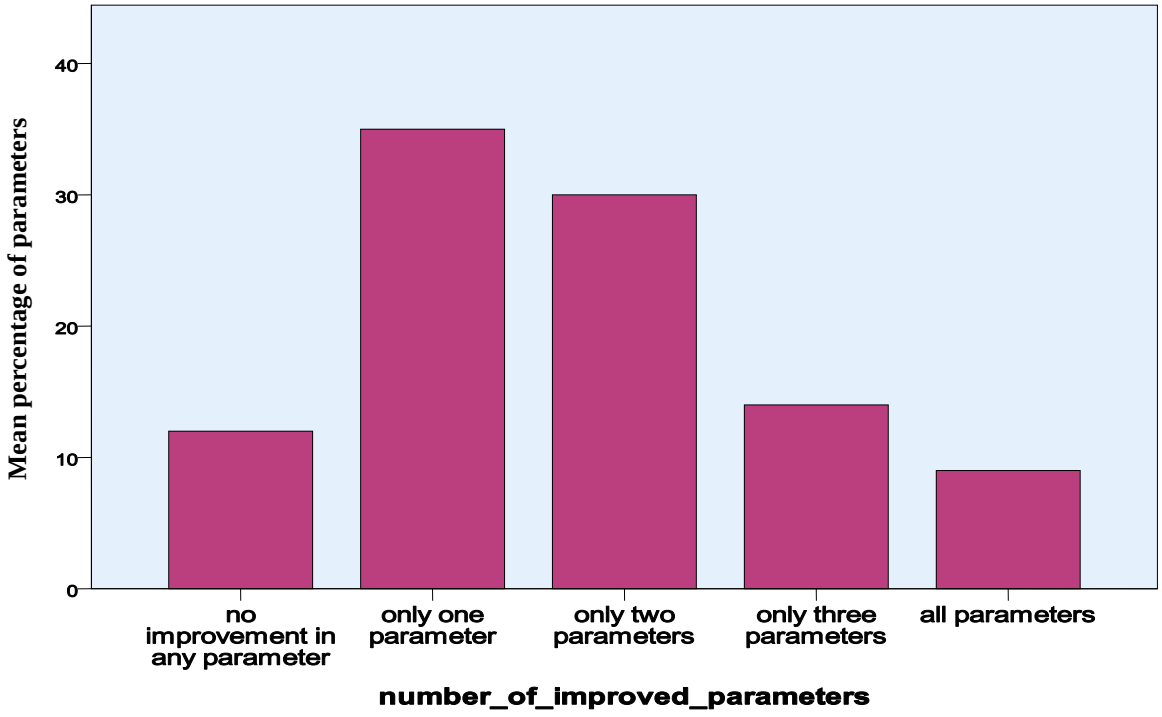

***Figure 2*. Patient's variation in number of improved parameters.**

Discussion

The cause of male infertility with abnormal semen parameters remains unknown in 25% of men . Defective sperm function with free radical induced oxidative damage has been postulated to be one factor of the underlying causes [(21)]. When ROS are present in excessive amounts, they can cause extensive sperm DNA structural damage , reduced sperm motility and defective sperm membrane integrity, all of which are important mechanisms behind sperm dysfunction [(11)].

This study was based on the concept that oral supplementation of antioxidants can minimized the effects of oxidative stress in patients with idiopathic infertility and a combination of oral antioxidants including vitamin E, coenzyme Q10, zinc sulphate and L-carnitine were evaluated for their effectiveness.

At the end of this study; pregnancy rate was 19% , and for those patients who fail to make pregnancy; a variable improvement in their semen was noted being highest with the morphology followed by the volume, concentration and motility with different changes in the number of improved parameters where 35% of our patients had improvement in only one parameter in their semen analysis (i.e. only single parameter was improved without improvement in other parameters) followed by decreasing percentage for those who get two, three or all parameters improvement, whereas 12% of the studied patients had no any change in their analysis at the end of this trial.

In a study conducted by Sikka SC., has been found to have a positive correlation for combined antioxidants with normal sperm morphology [(22)].

Two other meta-analyses had similar results, reporting that both sperm parameters and pregnancy rate improve with anti-oxidant therapy [(23,24)].

Comhaire and Decleer pooled data (1013 couples) that specifically looked at pregnancy rates from the two largest meta-analyses . The pregnancy rate in the anti-oxidant group was 17.2% (88/512) versus 4.4% (22/501) in the control group . The authors concluded that oral anti-oxidants quadrupled the probability of spontaneous pregnancy within 3 months and also reduced the cost per pregnancy by 60% [(25)].

Another randomized, controlled trial (n = 42) treated patients for 3 months with combined anti-oxidants. This combination significantly increased the sperm concentration, but had no impact on pregnancy rate after 12 months of follow-up [(26)].

Further randomized double-blind controlled trials that conducted by Al-Gubory *et al* and Abd-Allah *et al* involving asthenozoospermic patients in which they administered antioxidants combination result in a significant increment in sperm motility and pregnancy rate improvement [(27,28)].

One randomized, placebo-controlled study involving 60 men with idiopathic infertility assessed the use of a commercial combination of anti-oxidants as an adjuvant to IVF-ICSI treatment. The specific combination capsule

contains 6 mg lycopene, 400 IU vitamin E, 100 mg vitamin C, 25 mg zinc, 26 mg selenium, 0.5 mg folate, and 1000 mg garlic. The study found a pregnancy rate of 64% (23/36) in the treatment group compared to 38% (6/16) in the placebo group [29]. We suggest that the difference of anti-oxidant combination may explain the difference in the results.

In our study; some causes may implicated in those patients who did not get pregnancy like impaired oocyte penetration [13], this parameter may be the cause of infertility in some men and is usually not evaluated in semen analysis, this is particularly in patients with severe oligospermia as the oxidative stress may not be the cause for infertility when the sperm concentration below five million per ml. further causes may related to methodological difficulties with these works given by the short duration and inadequate sample size of this study(some patient could be able to make pregnancy if the treatment continue for a time longer than the time of the study).

No significant side-effects were noted and the most common antioxidant side-effect was gastric upset, that it was not serious.

Conclusion

Anti-oxidant therapy can be utilized as encouraging therapy for idiopathic male infertility in selected patients in an effort to reduce free radicals and in turn improve sperm function and fertility.

This study demonstrates a possible role of combined oral antioxidants in the improvement of semen parameters and pregnancy rate of these men with superiority for the use of combination of antioxidants rather than single agent treatment.

The current evidence on the effectiveness of anti-oxidants is inconclusive and that further randomized, placebo-controlled trials with pregnancy rates are recommended .

The duration and dose of antioxidants should also be determined and standardized and efforts should be directed to reach the optimum combinations of antioxidants .

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