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Andrology

The effects of L-carnitine on sperm parameters in smoker and non-smoker patients with idiopathic sperm abnormalities

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***Purpose*:** To determine the effects of L-carnitine on sperm parameters in patients with idiopathic sperm abnormalities.
***Methods*:** In an academic reproductive care center, 170 patients including 48 smokers and 122 non-smokers participated in a before–after study. Men were given 1 g of L-carnitine orally 3 × daily for 3 months. Sperm assessment was done before and after the treatment. Main outcome measures were sperm concentration, motility, and morphology before and after the treatment.
***Results*:** L-Carnitine was effective in improvement of percentile of motile sperms, grade A sperms, and normal-shaped sperms. L-Carnitine significantly improved percentile of motile and grade A sperms in non-smokers. Only the change in the median percentile of normal forms was within the significant range in smokers.
***Conclusions*:** The results of this study indicate that smoker patients should not be excluded from the treatment with L-carnitine based on smoking alone.

KEY WORDS: L-Carnitine; non-smokers; smokers; sperm; sperm morphology; sperm motility.

INTRODUCTION

One couple in 10 seeks medical help because of infertility. In a review article, Whitman-Elia *et al.* showed that in 40% of cases of infertility, the problem is predominantly male (1). Therefore, offering some type of treatment may help approximately half of the couples with infertility. In nearly 40% of male factor infertility, no specific etiological factor could be found (2). Few empirical pharmacological therapies have been developed for the treatment of idiopathic male infertility.

The beneficial effects of L-carnitine on the quality of sperm and/or spontaneous pregnancy outcome in patients with idiopathic asthenozoospermia have been demonstrated in a number of clinical studies (3–5). High concentrations of carnitine, a 3-hydroxy-4-trimethylaminobutyric acid, are present in both seminal plasma and spermatozoa. Carnitine plays a major role in the transport of fatty acids through mitochondrial membranes and in the intracellular storage of acetate moieties derived from acetyl-co (6). L-Carnitine and acetylcarnitine are important for sperm metabolism and providing energy for use by spermatozoa (7,8). However, it has been shown that the seminal plasma of oligoasthenozoospermic men contain lower levels of L-carnitine compared with fertile men (9). Consequently, these observations create a rationale for treatment with L-carnitine

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and/or acetylcarnitine. The first article was introduced in 1989 (10). Prescribing 2–4 g/day L-carnitine for 2–4 months resulted in increased sperm concentration and sperm motility (3,5).

Personal habits such as smoking may have adverse effects on sperm quality (11,12). Smoking is a prevalent habit. According to the latest statistics (13), the median prevalence of current cigarette smoking among men in the United States has been reported to be about 24.8%. As a result, verifying the effects of smoking in relation to treatment is an important issue. In light of these observations, estimation of treatment effect in smokers and non-smokers can be of value.

The purpose of this study is evaluation of L-carnitine effect on sperm parameters in patients with idiopathic sperm abnormalities and evaluation of L-carnitine effect in smokers and non-smokers.

MATERIALS AND METHODS

A prospective study was performed among infertile men who were treated with L-carnitine. The study was approved by Ethics Committee of Tehran University of Medical Sciences. Written informed consent was obtained from all subjects.

The subjects were selected from infertile couples who visited the out patient infertility clinic, Shariati Hospital, Tehran University of Medical Sciences in Tehran, Iran. Only men with idiopathic oligozoospermia or/and asthenozoospermia or/and teratozoospermia were enrolled in the study.

The diagnosis was made after the medical assessment of these men, which included a comprehensive history and physical exam with emphasis on the evaluation of the male reproductive system (i.e., varicocele, testicular volume evaluation, etc.) and sonography of genitalia. Semen samples from these men were evaluated for basic parameters (semen analysis) and presence of antisperm antibodies (SperMAR test, Fertipro NV, Beernem, Belgium). These men were also evaluated for FSH, LH, testosterone, estradiol, and prolactin levels using commercial RIA kits. Only subjects with normal hormonal profiles were included. The subjects with corrected undescended testes, varicocele, atrophy of testes, alcohol or/and opium addiction, occupational chemical exposure, systemic diseases, and abnormal semen volume, pH, agglutination or viscosity were excluded (11,14–16).

All men were asked to take L-carnitine (Sigma tau, Italy) orally at a dose of 1 g every 8 h for 3 months (3). The patients were evaluated for drug-related adverse events and compliance every month.

Three semen analyses covering a period of 3 months were performed before any treatment was given and the mean value was used for statistical analysis. Semen analyses were performed following 2–7 days of abstinence and according to the WHO criteria. Patterns of sperm motion were assessed according to the WHO classification described as A (rapid progressive motile sperms), B (slow or sluggish progressive motile sperms), C (non-progressive motile sperms) and D (immotile sperms) (16). Sperm morphology was assessed using Kruger Strict Criteria (17). At the end of treatment another two semen analyses covering a period of 2 weeks were performed.

All semen analyses were performed blindly by the same investigator in the laboratory of the same infertility center. Two hundred spermatozoa were evaluated in each specimen (1). The parameters of semen, which were evaluated in this study, were sperm concentration, motility, and morphology. Sperm concentration was expressed as number/ml. Sperm motility was expressed as percentile of motile sperm from total. Sperm morphology was expressed as percentile of normal-shaped sperm from total.

A total of 191 men (mean age 34.6 $\pm$ 6.1 years; range 20–56 years) were included in the study.

Starting with 54 smokers and 137 non-smokers who entered the study, 6 smokers and 15 non-smokers were removed from the study. The causes included: non-compliance (four in the smoker group and six in the non-smoker group); low volume of semen ($\leq$1.5 cc) (one in the smoker group and one in the non-smoker group); low pH (pH $\leq$ 7.2) (four in the non-smoker group); high sperm agglutination ($\geq$2) (one in the smoker group and two in the non-smoker group); and high viscosity ($\geq$3) (two in the non-smoker group) after treatment (16–19).

Data were statistically evaluated with the Statistical Package of Social Sciences (SPSS 10; SPSS, Chicago, IL). The main outcome was the comparison of sperm concentration, motility, and morphology before and after treatment in total and in smoker and non-smoker groups.

Results were expressed as mean $\pm$ SD (for parametric variables) and median, 10th, and 90th percentile (for non-parametric variables). Differences of demographics and sperm parameters between the two groups (smokers and non-smokers) were tested statistically using unpaired *t*-test and Mann–Whitney

U-test, respectively. The Wilcoxon test was used for comparison of sperm parameters before and after treatment in each group. A p-value of <0.05 was considered statistically significant.

RESULTS

Sperm parameters were compared before and after administration of L-carnitine in 170 patients. All patients were teratozoospermic (<14% normal forms). In none of the subjects was the motility within normal range (percentile of grades A and B motile sperms ≥50%). Oligozoospermia (sperm concentration <20 million/ml) was detected in 58 subjects (16 smokers and 42 non-smokers, $p = 0.5$). Table I shows sperm parameters before and after L-carnitine therapy.

Demographic characteristics of smoker (48) patients were compared with non-smoker (122) subjects. The ages of smoker men ranged from 23 to 47 years (mean age ± SD: 35.4 ± 5 years), and from 20 to 56 years (mean ± SD: 34.3 ± 6.4 years) in the non-smokers ($p = 0.3$). The mean duration of infertility was 8.9 ± 5.3 years for the smokers and 8.5 ± 5.1 years for the non-smokers ($p = 0.6$). The range of duration of infertility in smokers and non-smokers groups was 8.9 ± 5.2 and 8.5 ± 5.1 years, respectively. In the smoker group, the period of cigarette smoking ranged from 2.5 to 25 years (mean 14.7 ± 5.0 years). Smokers reported the number of cigarettes they smoked during the year prior to the study, which was an important variable to study. The range of cigarettes smoked per day was reported to be one cigarette to one pack per day (mean 12.2 ± 6.5). Smokers continued to smoke during the study except two, who were analyzed in the smoker group.

Table II shows the comparison between sperm parameters of the smoker and the non-smoker groups before the treatment with L-carnitine. There were no significant differences noted between the two groups.

Table II. Sperm Characteristics Before Treatment in Smokers and Non-Smokers

Sperm parameters	Smokers ($n = 48$)	Non-smokers ($n = 122$)	p-Value
Sperm density (10^6 ml^{-1})	35 (1–86.5)	30 (1–73)	NS
Normal form (%)	3.5 (2–8.3)	4 (1–8)	NS
Motility (%)	10 (0–30)	10 (0–30)	NS
Grade A (%)	0 (0–0.5)	0 (0–0)	NS
Grade B (%)	0 (0–30)	0 (0–15)	NS
Grade C (%)	10 (0–20)	8 (0–20)	NS

Note. Values are median (10th percentile–90th percentile). NS: not significant.

Table III shows the comparison of various sperm parameters for the non-smoker group before and after treatment. In non-smokers, the median of percentile of total motile sperms and the median of percentile of sperm with linear forward progression (grade A) increased significantly after the treatment ($p = 0.02$ and $p < 0.001$, respectively).

Table IV depicts the comparison of various sperm parameters before and after the treatment in the smoker group. Only the change in the median percentile of normal forms was within the significant range.

DISCUSSION

Depending on the diagnosis for male infertility, there are three alternative approaches to treatment: surgery, assisted reproductive technology (ART), and/or pharmacological therapy.

The purpose of pharmacological therapy is to stimulate spermatogenesis, and to increase sperm quality (percentile of total motile sperms, grade A motile sperms and normal forms), maturity and the ability

Table I. Comparison of Sperm Parameters Before and After Treatment with L-Carnitine

Sperm parameters	Before treatment	After treatment	p-Value
Sperm density (10^6 ml^{-1})	30 (1–80)	35 (2–70)	NS
Normal form (%)	4 (1–8)	4.5 (2–9)	0.03
Motility (%)	10 (0–30)	10 (0–35)	0.03
Grade A (%)	0 (0–0)	0 (0–5)	<0.00
Grade B (%)	0 (0–15)	0 (0–15)	NS
Grade C (%)	10 (0–20)	10 (0–20)	NS

Note. Values are median (10th percentile–90th percentile). NS: not significant.

Table III. Comparison of Sperm Parameters Before and After Treatment in Non-Smoker Group

Sperm parameters	Before treatment	After treatment	p-Value
Sperm density (10^6 ml^{-1})	30 (1–73)	35 (2–70)	NS
Normal form (%)	4 (1–8)	4 (1.3–8.7)	NS
Motility (%)	10 (0–30)	10 (0–35)	0.02
Grade A (%)	0 (0–0)	0 (0–5)	<0.00
Grade B (%)	0 (0–15)	0 (0–15)	NS
Grade C (%)	8 (0–20)	10 (0–20)	NS

Note. Values are median (10th percentile–90th percentile). NS: not significant.

Table IV. Comparison of Sperm Parameters Before and After Treatment in Smoker Group

Sperm parameters	Before treatment	After treatment	*p*-Value
Sperm density (10^6 ml^{-1})	35 (1–86.5)	40 (2.9–80)	NS
Normal form (%)	3.5 (2–8.3)	5 (2–9.2)	0.03
Motility (%)	10 (0–30)	10 (0–40)	NS
Grade A (%)	0 (0–0.5)	0 (0–5)	NS
Grade B (%)	0 (0–30)	0 (0–15)	NS
Grade C (%)	10 (0–20)	10 (0–15)	NS

Note. Values are median (10th percentile–90th percentile). NS: not significant.

to fertilize. L-Carnitine and acetylcarnitine have been shown to increase human sperm viability and motility (3–8,18). Based on these reports, we administered L-carnitine to a group of patients with idiopathic sperm abnormalities including smokers and non-smokers.

We found out that L-carnitine is effective in improvement of percentile of motile sperms, percentile of grade A sperms, and percentile of normal-shaped sperms.

The positive effects of L-carnitine and acetylcarnitine on sperm motility were revealed in other studies; although in almost all of these investigations smoking was an exclusion criterion (3,7,8,18).

Concerning the effects of smoking on the sperm functions, benzopyrene and cotinine, noxious metabolites of cigarette smoke, have been shown to exert a detrimental effect. This was demonstrated in terms of reduced sperm motility and/or morphology in smokers (19–21). Therefore, the effect of L-carnitine has been evaluated separately in smokers and non-smokers.

After treatment with L-carnitine, a statistically significant rise in percentile of motile and grade A sperm occurred in the non-smoker group, while there was no significant change in percentile of motile and grade A sperm in the smoker group.

In this study, there was no difference between demographic characteristics, percentile of oligozoospermic patients, and basic parameters of semen in smoker and non-smoker subjects at the beginning of the study (Table II). Therefore, it seems that the two groups were matched in the general characteristics at the beginning of the study. However, Zavos *et al.* (22) showed that although there were no statistically significant differences between seminal parameters between the smoker and non-smoker groups, the quality of spermatozoa obtained from non-smokers after processing their semen samples was superior to that of smokers.

Interestingly, we found an increase in normal-shaped sperms in smokers. In another study, we showed that the percentile of normal-shaped sperms rises after carnitine therapy in oligozoospermic patients (23). In other studies, there was no statistically significant increase in percentile of normal morphology. We estimated the morphology by strict criteria of Kruger, although in other studies WHO criteria were used.

Manufacturers of a new drug may select the best group who more likely respond positively to the drug (i.e., a kind of selection bias). After approval of the use for drug in practice, clinicians would face subjects who are not the best candidates for that treatment, although potentially will benefit (in our study the percentile of normal-shaped sperms significantly improved in smokers on L-carnitine).

In the present study, L-carnitine was shown to have no effect on sperm density. One of the reasons why our data show no change in sperm concentration is that L-carnitine induces a parallel increase both in sperm production and seminal fluid volume (3). However, no consensus exists about improvement of sperm density after L-carnitine therapy. Sperm density has been demonstrated by some to increase after L-carnitine treatment (7,24), and found by others to have no change (8,10).

A limitation of our study was unavailability of the data about the level of L-carnitine and cotinine (breakdown product of nicotine) in serum and/or semen. Using a nationally representative sample of the U.S population from the third National Health and Nutrition Examination Survey (NHANES III), CDC assessed the population's exposure to environmental tobacco smoke. The study included self-reported exposure and measurements of cotinine levels in serum. The study found that 87.9% of non-smokers had detectable levels of serum cotinine (25). The interaction of L-carnitine and smoking could be better evaluated through the drug and cotinine levels measurement in serum/semen.

Further studies should be conducted to examine the effect of smoking and L-carnitine on fertility outcome. In order to evaluate the effect of L-carnitine on semen parameters, one should, ideally conduct a randomized controlled trial (placebo vs. L-carnitine) in both smokers and non-smokers. Basic studies on interaction between L-carnitine and smoking byproducts will disclose the best way to manage selected subfertile men who smoke.

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