Does L-carnitine Therapy Add any Extra Benefit to Standard Inguinal Varicocelectomy in Terms of Deoxyribonucleic Acid Damage or Sperm Quality Factor Indices: A Randomized Study

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OBJECTIVE To evaluate if addition of L-carnitine therapy to standard varicocelectomy adds any extra benefit in terms of improvement in semen parameters or deoxyribonucleic acid (DNA) damage.

MATERIALS AND METHODS One hundred patients enrolled in this study and were randomly divided into 2 groups (50 patients in each group). In group 1, standard inguinal varicocelectomy and, in group 2, standard inguinal varicocelectomy plus oral antioxidant therapy (oral L-carnitine, 250 mg 3 times a day) were performed for 6 months. For all patients, routine semen analysis and DNA damage test of spermatozoa (by 2 methods of terminal deoxynucleotidyl transferase dUTP nick end labeling and protamine damage assay) were performed at baseline and at 3 and 6 months postoperatively.

RESULTS In both groups, the improvement in semen analysis parameters and DNA damage was observed, but there was not any statistically significant difference between the 2 groups in these parameters, although the slope of improvement in DNA damage was slightly better in group 2 (that was not statistically significant).

CONCLUSION We observed that addition of 750 mg of L-carnitine orally daily to standard inguinal varicocelectomy does not add any extra benefit in terms of improvement in semen analysis parameters or DNA damage. UROLOGY 84: 821–825, 2014. © 2014 Elsevier Inc.

Varicocele is the leading cause of male factor infertility, responsible for up to 40% and 80% of primary and secondary infertilities, respectively.[1-3] Varicocele might have an impact on spermatogenesis by decreasing the sperm count, motility, and morphologically normal sperm cells as well as testicular volume.[4,5]

The exact pathologic mechanism of varicocele impact on spermatogenesis is controversial. Multiple pathogeneses such as venous stasis, heat stress, hypoxia, and accumulation of toxic metabolites in the testes are defined. These toxic metabolites will lead to increased reactive oxygen species production and apoptosis during specific stages of spermatogenesis.[6,7]

In addition, the semen of these patients can present high levels of oxidative stress, as evidenced by increased levels of reactive oxygen species and reduced total antioxidant capacity, which lead to sperm deoxyribonucleic acid (DNA) damage.[8,9]

DNA damage is one of the possible varicocele mechanisms causing infertility. A higher frequency of spermatozoa with damaged DNA has been reported in the ejaculate of patients with varicocele in comparison with fertile men.[10] It has been noted that increased sperm nuclear DNA damage is strongly and negatively associated with natural and assisted fertility outcomes.[11]

Recent studies on infertile men with high levels of sperm DNA damage declared that antioxidant therapy is effective in improving sperm DNA integrity or pregnancy rates.[12] On the other hand, it is clearly defined in multiple randomized studies that DNA damage can be improved by varicocelectomy.[13]

The role of antioxidants in male factor infertility treatment has been studied previously. Different types of antioxidants (such as vitamins E and C, glutathione,

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pentoxifylline, L-carnitine, and so forth) with different dosages and different treatment plans (single-agent therapy or combination therapy) are used in male factor infertility treatment.[14,15]

L-carnitine supplements are used to increase L-carnitine levels in people whose natural level of L-carnitine is too low. In addition to infertility, L-carnitine is also used for conditions of the heart and blood vessels including heart-related chest pain, congestive heart failure, heart complications of a disease called diphtheria, heart attack, leg pain caused by circulation problems (intermittent claudication), and high cholesterol.

L-carnitine is likely safe for most people when taken orally. It can cause side effects such as nausea, vomiting, stomach upset, heartburn, diarrhea, and seizures. It can also cause the urine, breath, and sweat to have a "fishy" odor.[16]

It is proved that L-carnitine has a positive effect on sperm quality at least in patients with idiopathic asthenozoospermia in different doses (2-3 g/d for 2-4 months) individually or in combination with other agents such as cinnoxicam and acetyl-L-carnitine.[15,17,18]

In our country, Iran, L-carnitine is available in a 250-mg pearl form, and to reach these doses, it is needed to take 8-12 pearls a day, which decreases the patient compliance. On the other hand, it is not investigated if lower doses of L-carnitine would have a positive effect on semen quality especially when combined with other interventions such as surgical varicocelectomy.

In this study, we aimed to investigate whether lower doses of L-carnitine (750 mg/d for 6 months) have any extra benefit in terms of improvement in semen parameters or DNA damage to standard varicocelectomy.

MATERIALS AND METHODS

Design

The study was a randomized clinical trial and an add-on intervention.

Population and Setting

All patients attending our clinic (Infertility Clinic, Sina Hospital, Tehran University of Medical Sciences) for male factor infertility underwent complete medical history, general and genital examinations, and color Doppler ultrasonography in the standing position. Varicocele diagnosis was confirmed by clinical and radiologic findings. Physical examination was done in the standing position by Valsalva maneuver for detecting varicocele and for clinical grading if palpable varicocele was present. Color Doppler ultrasonography was performed to confirm the clinical diagnosis and diagnose subclinical varicocele (no palpable varicocele on physical examination but dilated veins on color doppler ultrasonography).

Inclusion and Exclusion Criteria

The indication for varicocelectomy (inclusion criteria) in our study was made by the presence of left-sided clinical or subclinical varicocele plus one of these factors: primary infertility, secondary infertility, or impaired semen analysis. All patients with prior medical (opium or drug abuse, any prior medical treatment for infertility, recurrent urinary tract infection, sexually transmitted disease, prostatitis, mumps in childhood, epididymo-orchitis, and so forth) or surgical (cryptorchidism, orchiopexy, prior varicocelectomy repair, inguinal hernia repair, other inguinal surgeries, and so forth) history of male factor infertility were excluded from the study. All patients with right-sided isolated varicocele, bilateral varicocele, and each side varicocele that did not decompress in lying position were also excluded from the study (because of the need for further evaluation). One hundred patients with left-sided clinical or subclinical varicocele who were admitted to our ward (Urology 2, Sina Hospital, Tehran University of Medical Sciences) for left-sided varicocelectomy were included in our study.

Randomization

Block randomization was performed for controlling less probable variation in varicocelectomy technique or surgeon within the time of study.

Intervention

Fifty patients underwent varicocelectomy surgery alone (group 1) and 50 patients underwent varicocelectomy plus 250-mg oral L-carnitine 3 times a day for 6 months (group 2). All patients underwent standard left-sided inguinal varicocelectomy with Loupe magnification (×3 magnification power) in 1 center, by 1 surgeon.

Data Gathering

Routine semen analysis and DNA damage were assessed in all patients using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and protamine damage assay (PDA) at baseline and at 3 and 6 months after the intervention.

Semen sample was obtained 2 hours before the surgery and 3 and 6 months later. History of smoking and alcohol consumption was obtained from all participants, preoperatively.

Genital examination was performed on all patients to evaluate major surgical complications (hydrocele formation, clinical recurrence, and testis atrophy) at 3 and 6 months after the surgery. In these visits, a brief history of the major adverse effects (gastrointestinal distress, headache, dizziness, and fever) plus blood pressure measurement were obtained.

DNA damage was evaluated by 2 methods, PDA and TUNEL.

Ethical Consideration

The process and purpose of the study were explained for all the participants, and an informed consent was obtained. The present research has been approved by both Ethics Committee and Scientific Committee of the Tehran University of Medical Sciences and registered under no. 11982 in the institutional review board.

Statistical Analysis

Measures were described in terms of mean, standard deviation, maximum, and minimum. Measure comparisons between intervention groups have been done using repeated-measure analysis of variance. All descriptive statistics and data analysis were performed by the SPSS, version 17 software (SPSS, Inc, Chicago, IL).

RESULTS

During the study period, of 145 patients admitted to our clinic for male factor infertility, 38 patients were excluded

Table 1. Baseline comparison between 2 groups

	Standard Group (Mean ± SD)	Standard + L-carnitine (Mean ± SD)	*P* Value
Age, y	27.52 ± 5.23	26.73 ± 6.25	.5
Sperm counts	$108.62 \times 10^6 \pm 87.6 \times 10^6$	$102.57 \times 10^6 \pm 86.15 \times 10^6$	.74
Sperm motility (a + b), %	32.54 ± 17.94	33.20 ± 18.23	.86
Normal morphology, %	21.75 ± 15.32	21.6 ± 9.38	.95
DNA damage (PDA), %	41.91 ± 15.94	44.98 ± 11.81	.295
DNA damage (TUNEL), %	14.08 ± 10.57	13.97 ± 5.33	.747

DNA, deoxyribonucleic acid; PDA, protamine damage assay; SD, standard deviation; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

Table 2. Baseline distribution of varicocele grade, alcohol intake, and smoking between 2 groups

	Standard Group, Number (%)	Standard + L-carnitine, Number (%)	Pearson Chi-square (*P* Value)
Varicocele grade (left side)	50	50	
Subclinical	5 (10)	6 (12)	.844
Grade 1	6 (12)	4 (8)	
Grade 2	13 (26)	11 (22)	
Grade 3	26 (52)	29 (58)	
Smoking	6 (12)	7 (14)	.766
Alcohol intake	18 (36)	20 (40)	.680

because of not meeting the inclusion criteria and 7 patients declined to participate. The remaining 100 patients with left-sided clinical or subclinical varicocele and infertility (primary or secondary) or semen analysis disturbance were randomly allocated to 2 groups of 50 patients each. Group 1 underwent varicocelectomy, and group 2 underwent varicocelectomy plus 750-mg L-carnitine orally daily for 6 months (Supplementary Fig. 1). The mean age of group 1 and group 2 patients were 27.52 ± 5.23 and 26.73 ± 6.25 years, respectively ($P = .50$).

There was no statistical difference between the 2 groups in terms of semen analysis parameters (sperm count, motility a + b, and morphology), percentage of PDA and TUNEL assays, and distribution of the grade of varicocele at baseline (Tables 1 and 2).

Smoking and alcohol intake frequencies were not different between the 2 groups at the beginning of the study (Table 2).

Table 3 lists the result of comparing the semen analysis parameters (sperm count, motility a + b, and morphology). The evolving process in group 1 (standard group) was better than group 2 (standard + L-carnitine group), and this difference was statistically significant ($P < .001$). The evolving process of sperm morphology and motility improvement was noted in both the groups, although this process was not statistically significant in group 1. The evolving process was much better in group 2, but the difference was not statistically significant ($P = .53$ for motility index, and $P = .464$ for morphologically normal sperms).

Sperm DNA damage analysis by PDA showed an increase in both groups, although the improvement was significant in group 2, and the slope of improvement was much better in this group; however, this difference was not statistically significant ($P = .235$).

Although DNA damage analysis by TUNEL demonstrated a more improving slope in group 2, like PDA, the difference between the 2 groups was not statistically significant ($P = .255$; Table 4).

In follow-up visits, no evidence of major surgical complications (including hydrocele formation, clinical recurrence, or testis atrophy) was observed in the study groups.

In the L-carnitine plus surgery group, only mild gastrointestinal disturbance was observed in 5 patients, but all of them could tolerate continuing the medication. On the other hand, no new onset of hypertension was observed.

COMMENT

In this study, the patients were assigned into 2 groups. In group 1, standard inguinal varicocelectomy was performed, and in group 2, oral antioxidant therapy (L-carnitine of 250 mg 3 times a day) was added to the standard treatment for 6 months. In both groups, an improvement was observed in the semen analysis parameters. Sperm count was improved from 108,620,000 preoperatively to 177,120,000 and 207,770,000 at 3 and 6 months, respectively, postoperatively in group 1 and from 102,570,000 at baseline to 136,390,000 and 140,390,000 at 3 and 6 months, respectively, postsurgically in group 2. Sperm motility index (class a + b from the World Health Organization classification) was improved from 32.54% at baseline to 38.88% and 38.27% at 3 and 6 months, respectively, postprocedurally in group 1 and from 33.2% to 44.11% and 46.33% in group 2. Over time, this slope of improvement was statistically significant for sperm count in group 1 and for motility and morphology indexes in group 2. There was no statistical difference between the 2 groups, although the slope of improvement for morphology and motility indexes was better in group 2. It was previously documented that surgical varicocelectomy significantly improves semen parameters in infertile men with palpable varicocele; abnormal semen parameters and varicocelectomy in selected patients do indeed have beneficial effects on fertility status.[19,20] In our study, the improvement effect of varicocelectomy was observed in semen parameters, and although this improvement was more significant in group 2, our study failed to show added beneficial effects of

Table 3. Comparison of semen analysis parameters between 2 groups

	Standard Group			Within-group *P* Value	Standard + L-carnitine			Within-group *P* Value
	Before Surgery	3 mo	6 mo		Before Surgery	3 mo	6 mo	
Sperm counts	108.62×10^6	177.12×10^6	207.7×10^6	.000	102.57×10^6	136.39×10^6	140.39×10^6	.166
Sperm motility, %	32.54	38.88	38.27	.101	33.20	44.11	46.33	.000
Normal morphology, %	21.75	22.65	24.4	.666	21.60	24.25	28.56	.006

L-carnitine therapy to standard inguinal varicocelectomy to improvement in semen analysis parameters.

DNA damage was an important factor in infertility and a factor related to varicocele and the oxidants in the semen fluid. DNA damage was assessed by 2 methods, PDA and TUNEL. In PDA, we observed an improvement in the DNA damage from 41.9% at baseline to 36.45% at 6 months postsurgically in group 1; however, this improvement was not statistically significant ($P = .133$). Yet, the DNA damage in group 2 improved from 44.98% at baseline to 33.75% at 3 months after the surgery.

In group 2, the improving slope in PDA analysis was more considerable than that in group 1, and it was statistically significant comparing the baseline value with that at 3 and 6 months postsurgically plus L-carnitine therapy (44.98% vs 38.34% and 33.75%, respectively; $P = .001$).

The slope of improvement was more evident in group 2, but the difference between the 2 groups was not significant ($P = .235$).

By analyzing the DNA damage by TUNEL, statistically significant improvement was observed in both groups (from 14.08% to 10.43% and 9.54% in group 1 and from 13.97% to 9.25% and 8.49% in group 2, respectively, at baseline and at 3 and 6 months postoperatively; $P = .02$ vs .000, respectively). A more improved slope was observed in group 2 (5.48% reduction in group 2 vs 4.54% in group 1), but the observed difference was not statistically different ($P = .255$).

It was previously documented that there was increased sperm DNA damage in patients with varicocele and varicocelectomy may be a possible treatment.[13]

On the other hand, the role of oxidative stress on semen parameters and DNA damage was reviewed in several studies.[11,12] In these studies, the etiology of suboptimal semen quality due to oxidative stress was elucidated.

Findings of the present study show that over time, the slope of the sperm DNA damage improves more by adding L-carnitine to standard varicocelectomy; yet, the difference was not statistically significant in neither of the methods (PDA/TUNEL). Although we observed more reduction in sperm damage by adding L-carnitine, we failed to show that this difference is significant, and so we could not show that L-carnitine therapy adds extra benefits to varicocelectomy in terms of improvement in the semen parameter indices or DNA damage. Albeit, this insignificant slope of improvement may be significant in longer follow-ups (9 and 12 months or longer) or by using a greater sample size.

However, in this study, just 1 regime of fixed-dose antioxidant therapy was administered, and all the patients underwent surgery. It seems that the role of antioxidant therapy with other agents (other antioxidants, different doses, or combination therapy) and a larger number of patients could be promising.

A well-designed, randomized, controlled trial will be required to assess the potential of different combinations of antioxidant regimens, their dosage, and exact course of therapy.[12,14]

Table 4. Comparison of DNA damage between 2 groups using PDA and TUNEL

	Standard Group			Within-group *P* Value	Standard + L-carnitine			Within-group *P* Value
	Before Surgery	3 mo	6 mo		Before Surgery	3 mo	6 mo	
PDA, %	41.91	43.77	36.45	.133	44.98	38.43	33.75	.001
TUNEL, %	14.08	10.43	9.54	.022	13.97	9.25	8.49	.000

Abbreviations as in Table 1.

CONCLUSION

In this study, we failed to show that L-carnitine therapy can add any extra benefits to varicocelectomy in terms of improvement in the semen parameter indices or DNA damage. Although it is documented that varicocelectomy and L-carnitine therapy individually will improve DNA damage, it seems that addition of 250-mg L-carnitine 3 times a day to varicocelectomy in comparison with varicocelectomy alone will not add any extra laboratory benefit in terms of improvement in DNA damage or semen analysis parameters.

In this present study, we used lower doses of L-carnitine (750 mg daily) in comparison with previous studies (2-3 g daily). Therefore, further studies are needed to conclude whether higher doses of L-carnitine add extra benefit to surgery in terms of semen analysis parameters or DNA damage. On the other hand, all grades of varicocele (including subclinical varicocele) were included in this study. It may be possible that the beneficial effects of L-carnitine supplementation would be realized if only high-grade varicoceles were included.

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APPENDIX

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.urology.2014.07.006.