



Seminal L-Carnitine In Infertile Oligoasthenoteratozoospermic Men with Varicocele

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Abstract

Background: Few studies have investigated the relationship of seminal L-Carnitine (LC) with male infertility associated with varicocele. The purpose of this prospective cross-sectional study was to assess seminal plasma LC levels in infertile oligoasthenoteratozoospermic (OAT) men with varicocele.

Methods: Overall, 86 men were investigated. They were divided into infertile OAT men with varicocele (n=45), infertile OAT men without varicocele (n=21), and fertile men (n=20) as a control group. According to WHO guidelines, these men were subjected to history taking, clinical examination, and semen analysis. Seminal LC levels were evaluated by the colorimetric method. Statistical comparisons were done using Kruskal-Wallis and Mann-Whitney U tests and correlations were verified by the Pearson test. P-value<0.05 was set to be statistically significant.

Results: The mean seminal plasma LC levels were significantly lower in infertile OAT men with varicocele (216.3±57.1 ng/ml) compared to infertile OAT men without varicocele (252.9±62.9 ng/ml, p=0.01), or fertile men (382.8±63.6 ng/ml, p=0.001). Besides, the mean seminal plasma LC level exhibited statistically significant decreases in infertile OAT men of varicocele grade III compared to varicocele grade II cases, and in infertile OAT men with bilateral varicocele compared with unilateral varicocele cases. Collectively, there was a statistically significant positive correlation between seminal LC levels with sperm concentration, motility, and normal morphology.

Conclusion: Seminal LC levels are expressively reduced in infertile OAT men with varicocele and are influenced by an increase in varicocele grade and laterality.

Keywords: Oligoasthenoteratozoospermic, Infertility, L-Carnitine, Men, Semen, Sperm, Varicocele.

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Introduction

Approximately, in 50% of infertile couples, nonstandard semen parameters point to male factor infertility (1). Various reasons have been offered for male factor infertility, the most important of which are hormonal imbalance, congenital anomalies, inflammatory problems, envi-

ronment/lifestyle, as well as varicocele (2-5).

Varicocele has been reported in about 20% of adults and adolescents and 19-41% of males are suffering from infertility problems (6). Varicocele is habitually linked to decreased sperm count, total sperm motility, and normal morphology per-

centages (7-9). Many concepts were raised to elucidate how varicocele has a negative influence on male fertility. Those concepts comprise scrotal hyperthermia, Leydig cell dysfunction, metabolites retrograde flow, hypoxia, oxidative stress (OS), and apoptosis (10, 11).

L-Carnitine (LC) is an endogenous branched-chain non-essential amino acid derivative that is manufactured in multiple organs such as liver, kidneys, and testes, originating from L-lysine and L-methionine (12). Intracellular LC takes part in the lipid metabolism by transporting fatty acids from the cytoplasm to the mitochondria for β -oxidation (13, 14).

Higher concentrations of LC were reported in the human epididymis than in the peripheral blood (15, 16). Seminal LC was shown to play its role by improving the environment in the epididymal lumen and affecting β -oxidation of fatty acids in the mitochondria. Besides, seminal LC was demonstrated to imitate the effects of glucocorticoid that suppresses the macrophages protecting sperm membrane and chromatin from the damage of free oxygen radicals (17).

Numerous studies showed the valuable effects of LC intake on sperm motility, and sperm DNA fragmentation concluding that combining metabolic and micro-nutritive aspects is helpful for male factor infertility treatment (18-22). Tsampoukas et al. (23) pointed out that although the evidence regarding the role of LC as a primary or adjuvant treatment of varicocele is sparse, the pathophysiological significance of LC implicates a potential role of the molecule in the management of varicocele.

In this context, scarce studies investigated the relationship of seminal LC with male factor infertility associated with varicocele (24-26). Therefore, there is a gap in the literature and clinical practice regarding association of LC deficiency with both the grade as well as severity of varicocele. Therefore, this work was designed to evaluate seminal plasma LC levels in infertile OAT men with varicocele.

Methods

This prospective cross-sectional study comprised 66 infertile OAT men who were recruited from the University Hospital, in addition to 20 healthy fertile men as controls, after obtaining the ethics committee approval in addition to informed consents. Sample size calculation was based on a single proportion formula (27). The cases were di-

vided into 3 groups of fertile males without varicocele (n=20), infertile OAT men without varicocele (n=21), and infertile OAT men with varicocele (n=45). Fertile controls fulfilled the criteria for normozoospermia (sperm concentration >15 million/ml, total sperm motility $>40\%$, and sperm normal forms $>4\%$) and having offspring in the previous two years. Inclusion criteria of the patients' group were OAT (sperm concentration <15 million/ml, total sperm motility $<40\%$, and sperm normal forms $<4\%$), lack of initiating pregnancy within a year of unguarded sexual relation, and normal female factor. Exclusion criteria were azoospermia, secondary varicocele, congenital anomalies, smoking, and leukocytospermia.

All participants were subjected to history taking, genital examination, and semen analysis. The ejaculates were collected after 4-5 days of sexual abstinence and were inspected according to WHO guidelines (28). Clinical examination was carried out in the standing position with/without the Valsalva maneuver. Color Doppler was carried out to diagnose varicocele (29). Varicocele was classified into grade I (only palpable during Valsalva maneuver), grade II (palpable distension on standing upright), and grade III (visible through scrotal skin) (30). LC levels in the seminal plasma were estimated utilizing a colorimetric assay kit (Bio-Vision, USA). The range for L-Carnitine is ~ 1 -200 μM with a detection sensitivity of 10 μM (colorimetric).

Statistical analysis: SPSS software version 23 (IBM, USA) was used for the statistical analysis. Kolmogorov-Smirnov test demonstrated that the data were not normally distributed. Comparisons amongst the investigated groups were carried out using Kruskal-Wallis and Mann-Whitney U tests for non-parametric data. Correlations between the variables were verified by the Pearson test. The p-value <0.05 was set to be statistically significant.

Results

The participants demonstrated matched age and no statistically significant difference was found between all investigated groups ($p=0.173$). Clinically, the group of infertile OAT men with varicocele consisted of 20 cases that had grade II varicocele and 25 cases that had grade III varicocele. Additionally, 10 cases had unilateral varicocele and 35 cases had bilateral varicocele. The mean seminal plasma LC levels were lower in infertile OAT men with varicocele compared to infertile OAT men with no varicocele as well as fertile

Table 1. Baseline characteristics of the investigated groups [mean±SD (median)]

	Fertile men (n=20)	Infertile OAT men without varicocele (n=21)	Infertile OAT men with varicocele (n=45)	p-value
Age (years)	29.5±5.5 (30)	29.4±5.4 (28)	30.8±3.8 (32)	0.173
Semen volume (ml)	2.5±1.0 (2.25)	2.6±1.7 (2.3)	2.3±0.97 (2.2)	0.813
Sperm concentration (10 ⁶ /ml)	49.9±20.2 (53.3)	6.7±4.1 (6.3)	5.7±2.7 (6.0)	0.001 *
Sperm total motility (%)	57.5±8.0 (60)	26.2±8.8 (30)	16.9±7.7 (15.0)	0.001 *
Sperm normal morphology (%)	5.5±0.9 (5.0)	2.4±0.9 (2.0)	2.1±0.8 (2.0)	0.001 *
Seminal LC (ng/ml)	382.8±63.6 (386.9)	252.9±62.9 (262.2)	216.3±57.1 (221.7)	0.001 *

* Statistical analysis using Kruskal-Wallis test

LC: L-Carnitine, OAT: Oligoasthenoteratozoospermia

Table 2. Baseline characteristics according to varicocele grade [mean ± SD (median)]

	Grade II varicocele (n=25)	Grade III varicocele (n=20)	p-value
Age (years)	30.6±3.4 (32.0)	31.2±4.3 (31.0)	0.810
Semen volume (ml)	2.0±0.8 (2.0)	2.4±0.81.1 (2.7)	0.067
Sperm concentration (10 ⁶ /ml)	6.1±2.9 (6.5)	5.1±2.1 (5.4)	0.150
Sperm total motility (%)	18.6±7.7 (20.0)	14.8±7.3 (15.0)	0.114
Sperm normal forms (%)	2.3±0.8 (2.0)	1.8±0.6 (2.0)	0.026 *
Seminal LC (ng/ml)	237.3±29.3 (234.7)	174.0±62.3 (182.5)	0.001 *

* Statistical analysis using Mann-Whitney U test

LC: L-Carnitine, OAT: Oligoasthenoteratozoospermia

Table 3. Baseline characteristics in OAT men with unilateral versus bilateral varicocele [mean±SD (median)]

	Unilateral varicocele (n=10)	Bilateral varicocele (n=35)	p-value
Age (years)	31.7±2.8 (32.0)	30.6±4.1 (31.0)	0.308
Semen volume (ml)	2.3±0.7 (2.4)	2.2±1.00.8 (2.0)	0.653
Sperm concentration (10 ⁶ /ml)	4.6±2.6 (4.8)	6.0±2.6 (6.0)	0.158
Sperm total motility (%)	14.0±5.2 (15.0)	17.7±8.17.7 (20.0)	0.215
Sperm normal forms (%)	2.3±0.7 (2.0)	2.0±0.8 (2.0)	0.363
Seminal LC (ng/ml)	242.5±37.0 (255.0)	199.3±57.8 (211.5)	0.036 *

* Statistical analysis using Mann-Whitney U test

LC: L-Carnitine, OAT: Oligoasthenoteratozoospermia

controls and the differences were statistically significant ($p=0.001$) (Table 1). In infertile OAT men with varicocele, the mean seminal LC levels were lower in infertile OAT men with grade III varicocele ($n=21$) compared to infertile OAT men with grade II ($n=25$) ($p=0.001$) (Table 2), and in infertile OAT men with bilateral varicocele ($n=35$) compared with infertile OAT men with unilateral varicocele ($n=10$); the obtained differences were statistically significant ($p=0.036$) (Table 3). Collectively, seminal LC levels exhibited statistically

significant positive correlations with sperm concentration ($r=0.638$, $p=0.001$), total motility percentages ($r=0.705$, $p=0.001$), and normal forms percentages ($r=0.690$, $p=0.001$) (Figure 1).

Discussion

Varicocele is one of the common correctable causes of male infertility. It has been demonstrated that varicocelectomy in men with abnormal semen parameters was associated with better fertility outcome. The adjuvant drug therapy, especially

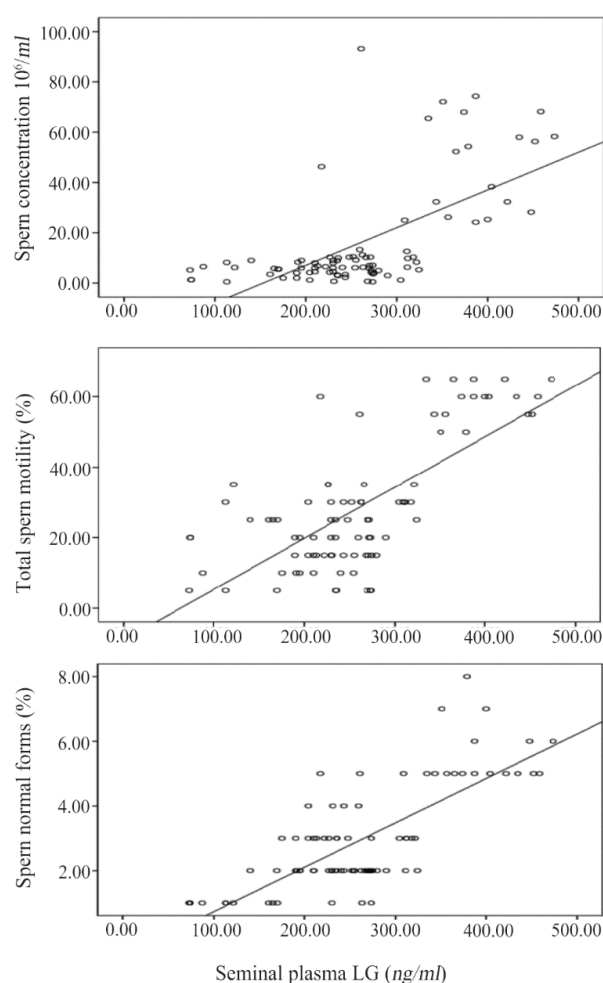


Figure 1. Significant positive correlations between seminal plasma LC (ng/ml) levels with sperm concentration, total sperm motility percentages, and sperm normal forms percentages

different types of antioxidants, seems to be associated with better fertility outcome, in comparison with no medical treatment (31, 32). Therefore, assessment of some antioxidants such as LC could be beneficial for clinical purposes.

In this study, the mean seminal plasma LC levels were significantly lower in infertile OAT men with varicocele compared to infertile OAT men without varicocele and fertile controls. Besides, seminal plasma LC levels exhibited statistically significant positive correlations with sperm concentration, motility, and normal forms percentages. Those aforementioned outcomes show the association of normal levels of seminal LC with normozoospermic semen parameters.

Matalliotakis et al. (33) reported that seminal LC levels differ significantly between fertile men and infertile men and there is statistically significant

positive correlation between the levels and sperm count, motility percentage, and normal forms percentage. Moreover, several researchers reported statistically significant positive correlations between seminal LC levels with total sperm count and sperm normal morphology percentage (34-36). Furthermore, the role of seminal free LC in the preservation of normal sperm features was validated in previous research (37-39).

Reviewing the literature, most studies that linked varicocele with male infertility focused mainly on the role of testis. Yet, it was suggested that varicocele can also disturb the epididymis that plays a central role in sperm maturation, sperm motility, and is the place where the sperm nucleus alters chromatin condensation (15, 40, 41).

Previously, Lenzi et al. (42) pointed out that the effects of LC on the male genital function are connected mainly with its high concentration in the epididymis where the uptake of the LC from the blood is active. Moreover, epididymal sperm can concentrate LC during their passage from the caput to the cauda to provide patterns of energetic substrate. This function is of great importance since epididymal sperm employ fatty acid oxidation for their energy metabolism; on the contrary, the ejaculated sperm employ the glycolytic process. Besides, carnitines also possess both antioxidant as well as anti-apoptotic properties (43, 44) that could counteract the accentuated burden of the seminal OS as well as apoptotic markers in infertile men associated with varicocele (8, 45, 46).

These effects were perceived with the statistically significant decreases in seminal LC levels in infertile OAT men of grade III varicocele compared to infertile OAT men of grade II, and in men with bilateral varicocele compared with unilateral one. This relation could be explained due to the increased OS effect associated with these conditions on the antioxidant LC. Lehtihet et al. (47) concluded that left-sided grade III varicocele could induce a reversible suppression of the epididymal function where treatment of varicocele results in improved semen quality as well as epididymal function. Sofimajidpour et al. (48) reported statistically significant decreases in sperm total motility in grade III varicocele compared to grade II varicocele. Also, Mostafa et al. (49) showed that different seminal miRNA levels are lowered in infertile OAT men with varicocele which is linked to elevated varicocele grade and its bilaterality is negatively correlated with OS (apoptotic

markers). Besides, Alkan et al. (50) and Ashrafzade et al. (51) linked ROS overproduction to elevated varicocele grade, reduced semen concentration, and normal sperm morphology.

However, this study still has some limitations due to the relatively low number of participants, the need to investigate fertile men with varicocele, and the necessity to assess seminal LC after varicocele surgical repair in infertile OAT men with varicocele.

Conclusion

The aforementioned results show that seminal plasma LC levels are significantly reduced in infertile OAT men with varicocele and are influenced by an increase in its grade and laterality. This finding indicates the benefits of assessing the seminal plasma LC in clinical practice among these men. Additionally, LC supplementation should be evaluated in such cases with/without varicocele surgical repair.

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None.

Conflict of Interest

The authors declare no conflict of interest.

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