



Assessment of Seminal L-Carnitine in Infertile Men with Varicocele

Mohamed adel abdallah^a, Taymour Mostafa Ibrahim^b, Khadiga Mohamed Abugabal^c,
Ahmed Fathy Mohamed AboSief^a

^aAndrology, Sexology & STIs Faculty of Medicine, Beni-Suef University

^bAndrology, Sexology & STIs Faculty of Medicine, Cairo University

^cClinical and Chemical Pathology Faculty of Medicine, Beni-Suef University

Abstract:

Introduction: Most studies that linked varicocele (Vx) with male infertility focused mainly on the testis affection, yet it was suggested that Vx can disturb also the epididymis that plays a central role in sperm maturation. Higher concentrations of L-Carnitine (LC) were reported in the human epididymis but few studies have investigated the relationship of seminal LC with male infertility associated with Vx. This study aimed to assess seminal plasma L-Carnitine (LC) levels in infertile oligoasthenozoospermic (OAT) patients with varicocele (Vx). **Methods:** Overall, 86 men were investigated. They were divided into; infertile OAT patients with Vx (n=45), infertile OAT patients without Vx (n=21), and fertile men (n=20) as controls. These cases were subjected to history taking, clinical examination, and semen analysis. Seminal LC levels were estimated by the colorimetric method. **Results:** The mean seminal plasma LC levels were significantly lower in infertile OAT patients with Vx (216.3 ± 57.1 ng/ml) compared to infertile OAT patients without Vx (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 significant decreases in infertile OAT patients of Vx grade III compared to Vx grade II cases, and in infertile OAT patients of bilateral Vx compared with unilateral Vx cases. Collectively, there was a significant positive correlation between seminal LC levels with sperm concentration, motility, and normal forms. **Conclusion:** Seminal LC levels are expressively reduced in infertile OAT patients with Vx influenced by an escalation of its grade and bilaterality.

Keywords: male infertility, Vx, carnitine

1. Introduction:

Approximately, in 50% of infertile couples, nonstandard semen parameters point to male factor infertility [1]. Various reasons have been raised for male factor infertility, the most important of which are: hormonal imbalance, congenital causes, inflammatory problems, environment/lifestyle, as well as varicocele (Vx) [2-5].

Vx has been reported in about 20% of adults and adolescents and 19-41% of males looking for fertility issues [6]. Vx is habitually linked to decreased sperm count, total sperm motility, and normal morphology percentages [7-9].

Many concepts were raised to elucidate how Vx 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 non-essential amino acid derivative that is manufactured in multiple organs as; liver, kidneys, and testes, originating from L-lysine and L-methionine [12]. Intracellular, LC takes a

part in the lipid metabolism by transporting fatty acids from the cytoplasm to the mitochondria aimed at β -oxidation [13,14].

In this context, 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 with a role in β -oxidation of fatty acids in the mitochondria. Besides, seminal LC was demonstrated to imitate the effects of glucocorticoid that suppresses the macrophages protecting the DNA and cell membranes from the free oxygen radicals' damage [17].

Numerous studies showed the valuable effects of LC intake on sperm motility, and sperm DNA fragmentation concluding that merging metabolic and micro-nutritive aspects is helpful for male factor infertility [18-22]. However, few studies investigated the relationship of seminal LC with male factor infertility associated with Vx. This work was designed to evaluate seminal plasma LC levels in infertile OAT patients with Vx.

2. Methods:

This study comprised 66 infertile OAT patients that were recruited from the University Hospital, in addition to 20 healthy fertile men as controls, after IRB approval as well as informed consent. These cases have been divided into 3 groups; fertile males (n=20), infertile OAT patients with no Vx (n=21), and infertile OAT patients with Vx (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 at 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 Vx, 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 [23]. Clinical

examination was carried out in the standing position with/without the Valsalva maneuver. Color Doppler US was carried out to diagnose Vx [24]. Vx was classified into; grade I: Only palpable during Valsalva maneuver, grade II: Palpable distension on standing upright, and grade III: Visible through scrotal skin [25]. LC levels in the seminal plasma were estimated utilizing a colorimetric assay kit (BioVision, Milpitas, CA, USA).

Statistical analysis:

The Statistical Package for the Social Sciences program (SPSS© Inc., Chicago, IL, USA) version 23 was used for the statistical analysis. Kolmogorov-Smirnova 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 tests for non-parametric data. Correlations between the variables were verified by the Pearson test. P-value <0.05 was set to be statistically significant.

3. Results:

The mean seminal plasma LC levels were lower in infertile OAT patients with Vx compared to infertile OAT patients with no Vx as well as fertile controls with

significant differences ($p=0.001$) . In infertile OAT patients with Vx, the mean seminal LC levels were lower at infertile OAT patients grade III ($n=21$) compared to infertile OAT patients with grade II ($n=25$) cases ($p= 0.001$), and in infertile OAT patients with bilateral Vx cases ($n=35$) compared infertile OAT patients

with unilateral Vx cases ($n=10$) with significant differences ($p=0.036$). Collectively, seminal LC levels exhibited 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 ($r=0.690$, $P=0.001$) .

Table. Comparison between the data of the investigated groups (mean \pm SD).

	Fertile normozoospermic men	Infertile OAT men without Vx	Infertile OAT men with Vx
n	20	21	45
Age (years)	28.9 \pm 5.5	29.4 \pm 5.4	30.8 \pm 3.8
Semen volume (ml)	2.5 \pm 1.0	2.6 \pm 1.7	2.3 \pm 0.97
Sperm concentration ($10^6/ml$)	49.9 \pm 20.2	6.7 \pm 4.1^a	5.7 \pm 2.7^a
Sperm total motility (%)	57.5 \pm 8.0	26.2 \pm 8.8^a	16.9 \pm 7.7^{a,b}
Sperm normal forms (%)	5.5 \pm 0.9	2.4 \pm 0.9^a	2.1 \pm 0.8^a
Seminal plasma L-carnitine (ng/ml)	377.8\pm 65.4	252.9\pm 62.9^a	212.9 \pm 58^{a,b}

4. Discussion:

In this study, the mean seminal plasma LC levels were significantly lower in infertile OAT patients associated with Vx compared to infertile

OAT patients with no Vx and fertile controls. Besides, seminal plasma LC levels exhibited significant positive correlations with sperm concentration,

motility, and normal forms percentages. Those aforementioned outcomes pointed out the importance of normal levels of seminal LC to be associated with the normozoospermic semen parameters.

In their study, Matalliotakis et al. [26] reported that seminal LC levels differ significantly between fertile men and infertile patients with significant positive correlations and sperm count, motility percentage, and normal forms percentage. Several authors reported significant positive correlations between seminal LC levels with total sperm count and sperm normal forms percentage [27-29]. Besides, other researchers validated the role of seminal free LC in the preservation of sound sperm features [30-32].

Reviewing the literature, most studies that linked Vx with male infertility focused mainly on the testis affection. Yet, it was suggested that Vx can disturb also the epididymis that plays a central role in sperm maturation, gaining of sperm motility, and is the place where the sperm nucleus undertakes changes in chromatin condensation [15,33,34].

Previously, Lenzi et al.[35] 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, in part androgen-dependent, mechanism. Moreover, epididymal sperms can concentrate LC during their passage from the caput to the cauda to give the sperms an energetic substrate. This function is of great importance since the epididymal sperms employ fatty acid oxidation for their energy metabolism; on the contrary, the ejaculated sperm employ the glycolytic process. Carnitines also possess antioxidant and anti-apoptotic properties [36,37]. Therefore, it was expected that seminal levels of LC have decreased in Vx-associated cases since Vx is recognized to be linked with the accentuated burden of the seminal OS as well as apoptotic markers [8,38,39].

These effects were perceived with the significant decreases in seminal LC levels in infertile OAT patients of grade III Vx compared to infertile OAT patients of grade II, and in bilateral Vx, patients compared with unilateral one. This relation could be explained due to the increased OS effect associated with these conditions on the antioxidant LC. In their study, Lehtihet et al.[40] concluded that left-sided grade III Vx is

proposed to cause a reversible suppression of the epididymal function where treating Vx results in improved semen quality as well as the epididymal function. Sofimajidpour et al. [41] reported significant decreases in sperm total motility in grade III Vx compared to grade II Vx. Also, Mostafa et al. [42] showed that different seminal miRNA are lowered in infertile OAT patients with Vx linked to elevated Vx grade and its bilaterality being negatively correlated with OS, apoptotic markers. Besides, Alkan et al. [43] and Ashrafzade et al. [44] linked ROS overproduction to elevated Vx grade, reduced semen concentration, and normal sperm morphology.

However, this study has still a limitation due to the relatively low number of participated subjects.

5. Conclusion:

The aforementioned results point to that seminal plasma LC levels are significantly reduced in infertile OAT patients with Vx influenced by an increase in its grade and bilaterality.

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Conflict of Interest

The authors declare no conflict of interest

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author

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