ORIGINAL ARTICLE

Relationship of seminal free L-Carnitine with functional spermatozoal characteristics: Results from an observational study conducted in a tertiary care hospital of Karachi, Pakistan

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Abstract

Objective: To investigate the relationship of seminal free L-carnitine with functional spermatozoal characteristics.

Methods: This observational study was conducted at the Jinnah Postgraduate Medical Centre, Karachi, from August 2009 to June 2013, and comprised fertile and infertile subjects. Semen analysis was performed and reported for its volume, sperm count, motility and morphology according to the World Health Organisation's guidelines. Seminal free L-carnitine was estimated by high-performance liquid chromatography. Mean values of demographic characteristics, semen analysis and seminal free L-carnitine were compared, and possible relation of seminal free L-carnitine with functional spermatozoal characteristics was explored.

Results: Of the 61 participants, 19(31.1%) were fertile controls, while 42(68.9%) were infertile men. The mean age of controls was 32.7±1.44 years and that of infertile patients was 33.4±0.75 years (p=0.655). The mean seminal free L-carnitine was 447.6±23.86µmol/L among controls and 154.6±12.99 among patients (p=0.001). There was a strong positive correlation of seminal free L-carnitine with sperm count, total motility and normal morphology (p<0.01 each).

Conclusion: The role of seminal free L-carnitine in the maintenance of normal functional spermatozoal characteristics was validated.

Keywords: Carnitine, Male, Infertility, Spermatozoa, Semen analysis. (JPMA 67: 280; 2017)

Introduction

Male infertility is becoming a significant problem. There is a substantial body of evidence suggesting that human semen is getting poorer with the passage of time. A number of studies suggest that sperm concentration is decreasing and the number of defective sperm as well as other male pathologies is increasing.[1,2] Causes of male infertility can be broadly grouped among urogenital, infections, endocrine, genetic and immunological factors. However, 30-45% of cases are of idiopathic male infertility in which no causal factor is found producing pathological levels of semen parameters.[3]

Semen analysis provides basic information regarding sperm count, motility and morphology, but the ability of a male to fertilise cannot be completely expressed.[4] However, conventionally semen analysis is used to distinguish male infertile from fertile in clinical practice while estimation of serum testosterone, follicle-stimulating hormone and serum luteinising hormone is used to evaluate the hormonal status.[5,6] Spermatozoa are formed in seminiferous tubules but are still non-motile and cannot fertilise an ovum. Later, sperm develops the capability of motility and fertilisation only when it passes through epididymis. During this post-testicular maturation, biochemical modifications in metabolic pathways occur in spermatozoa.[7]

Epididymis provides an ideal milieu for sperm maturation by developing motility and fertilising capability. Epididymal epithelium produces hyperosmotic epididymal fluid by concentrating with organic constituents through secretion and absorption. It has been shown in rodents that organic osmolytes such as L-carnitine, glycerophosphocholine, D-glutamate and myo-inositol are present in millimolar concentrations, which are 1,000 times higher than the blood.[8] Ultimately, the hyperosmotic epididymal fluid is intended to load spermatozoa with these osmolytes and can act as reserves with higher intracellular concentrations in mature spermatozoa.[9]

L-carnitine is an essential metabolite that plays an important role in transferring long-chain fatty acids across the mitochondrial membranes, facilitating beta (β)-oxidation within mitochondria producing energy.[10] L-

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carnitine is concentrated in high energy-demanding tissues such as skeletal and cardiac muscles, and in a specialised reproductive tract organ, the epididymis.[7] Casillas[11] in 1973 demonstrated the accumulation of L-carnitine by spermatozoa during its passage through epididymis in mammals and it was found to have strict correlation with the development of fertilising capacity. Subsequently, a number of molecular studies revealed the presence of carnitine transporters in sperm, sertoli cells and epididymal epithelium and their importance in maintenance of male fertility.[12-15] During the epididymal transit spermatozoa takes up free L-carnitine by passive diffusion and accumulates free as well as acetylated form.[7] Spermatozoa in epididymis utilises different substrates as energy sources, but fatty-acid oxidation, involving the carnitine-dependent system seems to be the major energy supplying process.[16] The acetylated L-carnitine regulates the reserves of acetyl coenzyme A (acetyl-CoA), which is essential for tricarboxylic acid(TCA) cycle function in addition to the role of energy storage for motility when required.[7]

Moreover, L-carnitine has also shown antioxidant properties that protect sperm membranes against toxic reactive oxygen species probably via interfering arachidonic acid incorporation into phospholipids and protein kinase C mediated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system, removing toxic levels of intracellular acetyl-CoA and by replacing fatty acids in membrane phospholipids.[17,18] Genetic predisposition and environmental factors like oxidative stress may be the cause of previously unexplained idiopathic male infertility[3] and may represent unified molecular mechanism of sperm deoxyribonucleic acid (DNA) damage. Patients who are at risk of seminal oxidative stress, sperm DNA damage and apoptosis are of idiopathic infertility, genital tract infections, varicocele, teratozoospermia, asthenozoospermia, azoospermia and smoking.[19]

The growing incidence of male infertility opens up many arguments on its possible reasons, investigations and its treatment modalities.

The current study was planned to find out the variation of seminal free L-carnitine between diagnosed male infertile cases and healthy controls. Furthermore, relationship of seminal free L-carnitine with functional spermatozoal characteristics like sperm count, motility and morphology was also examined.

Subjects and Methods

This observational case-control study was conducted at the Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, from August 2009 to June 2013, and comprised fertile and infertile men. Ethical approval for the study was obtained from the BMSI and the National Research Institute for Fertility Care.

Subjects receiving medication, history of pelvic surgery or with comorbidities were excluded. After 3-5 days of sexual abstinence, semen samples were obtained via masturbation. Semen analysis was done immediately after liquefaction and reported for its volume, sperm count, motility and morphology according to the World Health Organisation's (WHO) reference values.[4] Supernatant of the remaining portion of semen was acquired by refrigerated centrifugation and was kept at 80°C. Later, seminal free L-carnitine was estimated by high-performance liquid chromatography (HPLC) described by Li and Huang.[20] Pre-column derivatisation of free L-carnitine was done by p-bromophenacyl bromide to form its ultraviolet absorbing ester. Lichrosphersilicon dioxide (SiO2) was used to separate the sample solution and detected by ultraviolet absorbance at 260 nm.

Convenient sampling was used to collect cases and controls. Prior sample size was not calculated and was limited over a period of 2 years. Due to our modest sample size, we performed power analysis using G*Power programme.[21] Post-hoc calculations showed that the sample size of our study was adequate to detect significant correlations having power of 0.99 and an alpha of 0.05. Due to non-normality and the small sample size, we used non-parametric tests like Mann-Whitney U test to compare variables between fertile and infertile groups, and Spearman's correlation to explore the possible relation among the variables.

Results

Of the 61 participants, 19(31.1%) were fertile controls, while 42(68.9%) were infertile men. The mean age of controls was 32.7±1.44 years and that of infertile patients was 33.4±0.75 years (p=0.655).Mean ejaculate volume, sperm count and total motility among controls were 3.1±0.12ml, 78.3±15.56 million/ml and 70.2±1.92%, respectively, while the respective values among infertile participants were 3.3±0.17ml, 24.4±5.33 million/ml and 22.6±3.01% (p<0.001each). The mean seminal free L-carnitine was 447.6±23.86μmol/L among controls and 154.6±12.99 among patients (p<0.001) (Table).

A strong positive correlation of seminal free L-carnitine with levels of sperm count, motility and morphology having r value (Spearman's correlation) of 0.751, 0.790 and 0.709, respectively, was observed (p<0.01) (Figure).

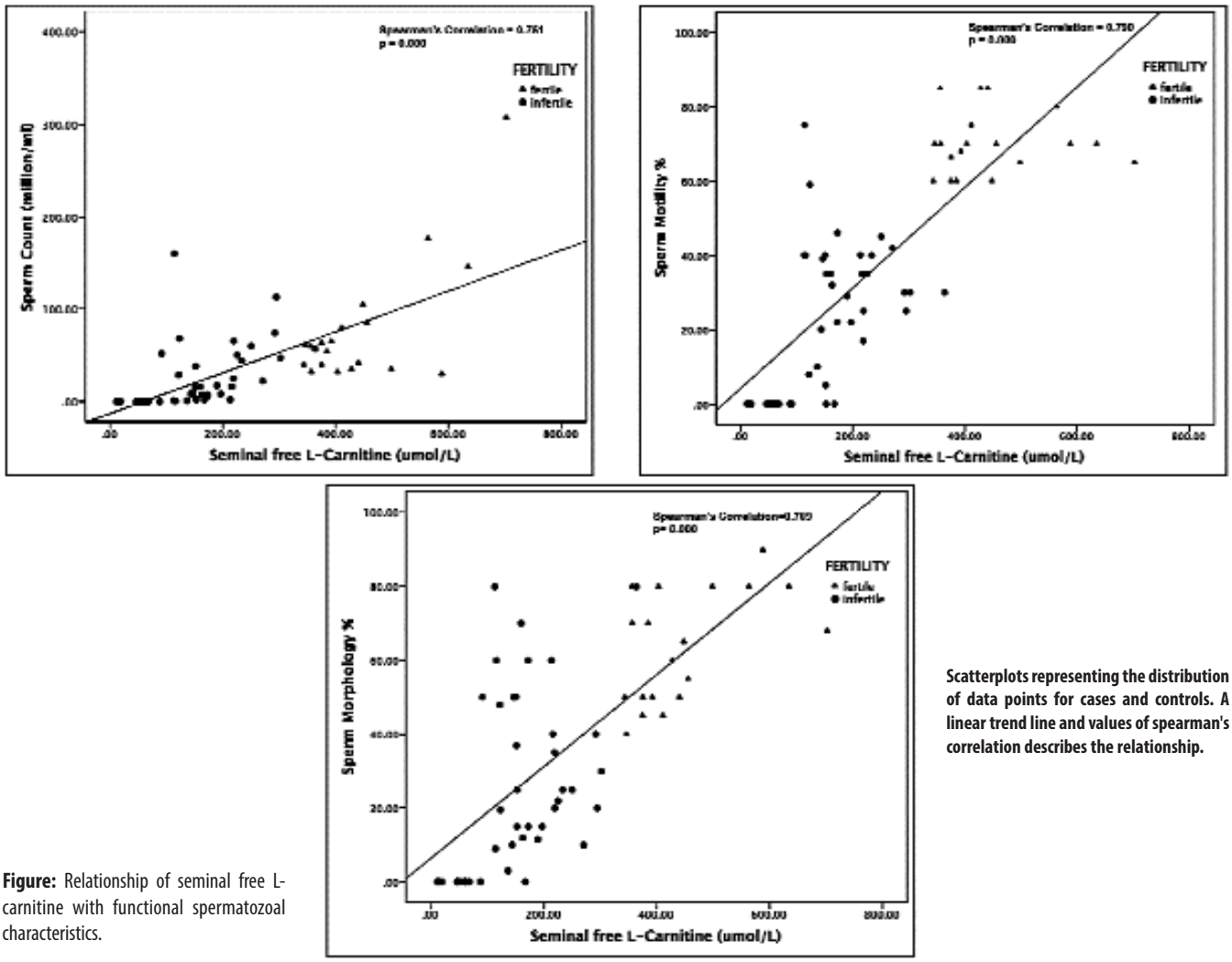

Figure: Relationship of seminal free L-carnitine with functional spermatozoal characteristics.

Scatterplots representing the distribution of data points for cases and controls. A linear trend line and values of spearman's correlation describes the relationship.

Table: Demographic characteristics, Semen Analysis and Seminal Free L-Carnitine levels.

Parameters	Fertile (Control) n=19	Infertile n=42	p-value
Age (years)	32.7±1.44	33.4±0.75	0.655
Body Mass Index (Kg/m^2)	25.1±1.05	24.3±0.58	0.703
Ejaculate Volume (ml)	3.1±0.12	3.3±0.17	0.450
Sperm Count (million/ml)	78.3±15.56	24.4±5.33*	0.000
Total Motility (%)	70.2±1.92	22.6±3.01*	0.000
Sperm Morphology (%)	63.6±3.51	24.9±3.71*	0.000
WBC (per HPF)	2.4±0.57	2.8±0.67	0.399
Seminal Free L-Carnitine (μmol/L)	447.6±23.86	154.6±12.99*	0.000

HPF: High power field

WBC: White blood cell

p-value determined by Mann-Whitney U test.

*P<0.001, significantly decreased when the infertile group was compared with the fertile group.

Discussion

This piece of research discovers the relationship of seminal free L-carnitine with the three functional spermatozoal characteristics, i.e. sperm count, motility and normal morphology. In this study, each of the functional spermatozoal characteristics showed strong positive correlation with seminal free L-carnitine levels attaining statistical significance. Similar studies[22-24] showed a significant positive correlation, while another study showed weak correlations.[25] Furthermore, other studies[20,26,27] have shown lower carnitine levels in infertile subjects than fertile, suggesting that there is a potential relationship between L-carnitine and semen quality.

Free L-carnitine is a semen biochemical parameter of epididymal function.[20,27] Epididymal epithelium secretes L-carnitine into the lumen by an active transport mechanism primarily mediated by organic carnitine

transporter 2 (OCTN2) from the systemic circulation,[12] whereas carnitine transporter 2 (CT2) is also involved,[14] both playing an important role in the maturation of human spermatozoa. Decreased levels of seminal plasma free L-carnitine among infertile subjects is due to defect in the L-carnitine uptake mechanisms in epididymis that could be caused by defective carnitine transport,[15] epithelial inflammation, obstruction or low levels of testosterone.[28]

The demonstration of strong positive relationship of free L-carnitine with functional spermatozoal characteristics in this study have further validated the fact that intrasperm L-carnitine is involved in mitochondrial energetics, keeping in mind the various studies which demonstrated carnitine uptake and expression of OCTN2 and OCTN3 carnitine transporters in mouse sperm,[13] correlation of intrasperm L-carnitine with motility and survival in cervical mucus of bovine,[29] and acetylcarnitine contributing acetyl groups for energy production in Boar spermatozoa.[30]

The study design and small sample size were among the limitations of the study and, therefore, warrant careful interpretation of its results. Further multi-centric studies on a larger population are needed to establish its external validity.

Conclusion

Significantly lower concentrations of seminal free L-carnitine among infertile participants and strong positive correlations of seminal free L-carnitine levels with sperm count, motility and morphology were found. This validated the role of seminal free L-carnitine in maintenance of normal functional spermatozoal characteristics. Thus, estimation of seminal free L-carnitine provides additional information for evaluation of male infertility. In addition, our findings also suggest its therapeutic implication in the treatment of male infertility and artificial reproductive techniques.

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