Original Article

Role of L-carnitine in male infertility

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Abstract

Objective: To test the hypothesis that the free L-carnitine helps in maintaining normal fertility.

Methods: The present descriptive study was designed to show comparison of seminal free L-carnitine and sperm quality. Case controlled convenient sampling was used to assess infertile male subjects from fertile. A total of 61 adult males were selected by consent, and were categorized as fertile and infertile on the basis of history and semen analysis. Subjects were selected from Infertility Clinic, Reproductive Health Services Centre of Jinnah Postgraduate Medical Centre, Karachi. Subject's with history of pelvic surgery, or suffering from diabetes mellitus, thyroid diseases or using steroids, antihypertensives and antipsychotics drugs were excluded from the study. Groups were compared using student's t-test and $p < 0.05$ was considered as statistically significant.

Results: The mean values of sperm count, total motility and normal morphology of asthenospermic and oligoasthenoteratospermic were found significantly ($p<0.05$) lower when compared with fertile (control). When levels of seminal free L-carnitine were compared among groups, the result showed that infertile subjects had significantly lower ($P<0.001$) when compared to fertile subjects with lowest concentration in azoospermic group.

Conclusion: The results of this study suggested that L-carnitine level in seminal plasma plays an essential role in maintaining male fertility. However larger studies on Pakistani population with this approach are warranted.

Keywords: Male, Infertility, L-carnitine (JPMA 61:732; 2011).

Introduction

Infertility is a significant problem in humans. According to WHO it is defined as the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year.[1] Infertility affects fifteen percent of couples worldwide. Male and female factors coexist in about one third of cases, while one third of cases are secondary to male factors only.[2]

Spermatozoa are non-motile and cannot fertilize an ovum after formation in the seminiferous tubules. Sperm develops the capability of motility and fertilization (post-testicular maturation) only when they pass through epididymis.[3] The epididymis is a highly coiled tube measuring 5-6 meters if unwound fully. It connects the tubules of the testes to the vas deferens and plays an important role in maintaining a physiological milieu in the epididymal canal suitable for sperm maturation.

Semen analysis is routinely used to evaluate the male partner in infertile couples and provides useful information for diagnosing male infertility. Each of the sperm measurements helps to distinguish between fertile and infertile men however none is a powerful discriminator.[4] In male infertile patiens semen analysis reveals a decreased number of spermatozoa (oligozoospermia), decreased motility (asthenozoospermia) and many abnormal forms on morphological examination (teratozoospermia). These abnormalities usually occur together and are described as the oligoastheno-teratozoospermia.[5]

Free L-carnitine (beta-hydroxy-gamma-N-trimethylaminobutyric acid) is biologically active amino acid that was first isolated from beef muscle in 1905.[3] Meat and milk are the most significant dietary sources of exogenous carnitine for humans.[6] Approximately 75% of the body stores of L-carnitine are derived from the diet, where as only 25%

are synthesized de novo from lysine and methionine.[7]

L-carnitine is concentrated in high energy demanding tissues such as skeletal and cardiac muscles and in a specialized reproductive tract organ, the epididymis. It plays an important role in transferring long-chain fatty acids into the mitochondria for -oxidation, producing energy. In addition, modulation of acyl-CoA / CoA ratio, storage of energy as acetylcarnitine, and the modulation of toxic effects of poorly metabolized acyl groups by excreting them as carnitine esters are the functions of L-carnitine.[8]

In 1973, Casillas[9] demonstrated that spermatozoa accumulate carnitine in mammalian epididymis, which is closely related with the development of fertilizing capacity by spermatozoa. The concentration of L-carnitine in epididymal plasma and spermatozoa varies from 2 to 100 mmole, which is nearly 2000 fold greater than circulating levels (10-50 mole). In epididymis, free L-carnitine is taken up from the blood plasma and is transported into the epididymal fluid. It is then passively diffused into the spermatozoa, where it accumulates as both free and acetylated L-carnitine. The initiation of sperm motility occurs in parallel with the increase in concentration of free L-carnitine in the epididymal lumen.[6]

Five mammalian transporters OCTN1, OCTN2, OCTN3, CT2 and ATBº+ are identified to transport carnitine.[10] Primarily high affinity sodium dependent carnitine transporter OCTN2 mediates carnitine supply to epididymal epithelial and sertoli cells from the systemic circulation[11] and then secreted into the lumen by an active transport mechanism.[12] Toshimori et al[13] demonstrated that defect of carnitine transporter gene octn2 causes obstructive dysfunction of the epididymis and progressive spermatogenesis arrest leading to infertility in a juvenile visceral steatosis mouse — an animal model of primary carnitine deficiency. This strongly suggests that OCTN2 transporter is functional and essential for carnitine transport in epididymis and sertoli cells. CT2 is another human carnitine transporter which transports carnitine with high affinity in a sodium independent manner. It plays an important role in the maturation of human spermatozoa by transporting carnitine selectively in the luminal membrane of epididymal epithelium and within the sertoli cells of testis.[12]

Another potential use of seminal free L-carnitine is in the diagnosis of the etiology of azoospermia. Men with obstructive azoospermia whose level of obstruction is post epididymal, such as those with agenesis of vas deference, have extremely low concentrations of carnitine. On the other hand, men with pre-epididymal obstruction like at the level of rete testis have normal concentrations of carnitine in the seminal fluid. In 1986, Tomamichel and Bandhaur[14] demonstrated that free L-carnitine concentration in human semen correlates with the level of epididymal obstruction. Lower the carnitine levels, the more distal the occlusion is likely to be located and better the prognosis is after surgery. Evaluation of seminal free L-carnitine will not only diagnose the level of obstruction but also helps in postoperative prognosis regarding fertility.

Therefore the present study was done to test the hypothesis (H1) that free L-carnitine helps in maintaining normal fertility.

Material and Methods

The study was carried out in the Department of Biochemistry, Basic Medical Sciences Institute (BMSI) at Jinnah Postgraduate Medical Centre (JPMC), Karachi, All subjects were selected from Infertility Clinic, Reproductive Health Services Centre of JPMC.

The study was designed as a descriptive study showing comparison of seminal free L-carnitine and sperm characteristics. Case controlled convenient sampling was used to assess infertile male subjects from fertile.

Of the sixty-one male subjects selected, forty two were primary infertile without treatment with age ranging between 20 to 40 years. Nineteen fertile subjects who came for vasectomy voluntarily were also included in the study as controls and their samples were taken before the procedure. Subjects who underwent pelvic surgery or suffering from diabetes mellitus, thyroid diseases or using steroids, antihypertensive and antipsychotic drugs were excluded from the study.

Semen samples were obtained by masturbation after 3 to 5 days of sexual abstinence. After 30 min of liquefaction at room temperature, the sample was immediately divided into two portions, the first for semen analysis and the second to be centrifuged for 10 min and the supernatant was kept at -80°C for the assay of free L-carnitine.

Seminal free L-carnitine was analyzed on HPLC (high performance liquid chromatography, Shimadzu 20A) using method developed by Ke Li et al.[15] Free L-carnitine was derivatized by p-bromophenacyl bromide to form its UV-absorbing ester after proteins precipitation with a mixture of acetonitrile and methanol. HPLC separation of the sample solution was performed on a Lichrosphere SiO2 column and detected by ultraviolet absorbance at 260 nm.

Results

The total of sixty one subjects were selected and were distributed into two main groups, fertile (control) and infertile. Fertility was established on the basis of history of proven fertility and semen analysis according to WHO guide lines.[16] Nineteen subjects were placed in fertile group and 42

Table-1: Demographic features of fertile (control), Azoospermic, Asthenospermic and Oligoasthenoteratospermic subjects.

Parameters	Group A Fertile (Control) n=19	Group B Azoospermic n=10	Group C Asthenospermic n=13	Group D OligoAstheno Teratospermic n=19
Age (years)	32.7±1.44	37.6±1.10	31.5±1.34	32.5±0.99
Duration of Infertility	5.6±1.03	12.0±2.14*	5.0±0.92	6.1±0.81
BMI	25.1±1.05	25.7±1.40	22.3±0.72	24.9±0.84

All values are expressed as mean ± SEM.
*P<0.05.when Group B compared to Group A.

Table-2: Semen microscopical features of fertile (control), Azoospermic, Asthenospermic and Ligoasthenoteratospermic subjects.

Parameters	Group A Fertile (Control) n=19	Group B Azoospermic n=10	Group C Asthenospermic n=13	Group D OligoAstheno Teratospermic n=19
Volume (ml)	3.1±0.12	2.7±0.39	3.3±0.27	3.7±0.23
Sperm Count (million/ml)	78.3±15.62	0	52.2±6.65 ^	18.3±8.60 †
Total Motility (%)	70.2±1.92	-	25.5±3.97 ^	32.6±4.18 †
Normal Morphology (%)	63.6±3.51	-	34.0±5.00 ^	31.8±5.82 †
WBC (per HPF)	2.4±0.57	2.5±0.96	1.3±0.13	3.9±1.37

All values are expressed as mean ± SEM.
^ P<0.05.when Group C compared to Group A. † P<0.05.when Group D compared to Group A.

Table-3: Comparison of seminal free l-carnitine among fertile (control) Azoospermic, Asthenospermic and Oligoasthenoteratospermic subjects.

Parameters	Group A Fertile n=19	Group B Azoospermic n=10	Group C Asthenospermic n=13	Group D OligoAstheno Teratospermic n=19
Seminal Free L-Carnitine mole/L	447.6±23.86	46.5±7.93*ϕ§	233.3±21.25^£	157.6±7.09†

The values are expressed as mean ± SEM.
*P<0.05.significantly decreased when Group B compared to Group A. ^ P<0.05 significantly decreased when Group C compared to Group A.
†P<0.05 significantly decreased when Group D compared to Group A. ϕ p<0.05 significantly decreased when group B compared to group C.
§p<0.05 significantly decreased when group B compared to group D. £ p<0.05 significantly increased when group C compared to group D.

subjects in infertile group. The infertile group was further divided and the number of subjects included were, ten in azoospermic, thirteen in asthenospermic and nineteen in oligoasthenoteratospermic group.

Table-1 shows mean values (± SEM) of demographic features like age, duration of infertility and body mass index (BMI). When azoospermic compared with fertile (control), only the mean value of duration of infertility was found significantly higher (p<0.05).

Table-2 shows comparison of mean values (± SEM) of semen analysis parameters like ejaculated volume, sperm count, total motility, normal morphology and white blood cell count among group A fertile (control), group B azoospermic, group C asthenospermic and group D oligoasthenoteratospermic groups. The mean values of sperm count, total motility and normal morphology of asthenospermic and oligoasthenoteratospermic were found significantly (p<0.05) lower when compared with fertile (control). All other values were found non-significant.

Table-3 explains the comparison of mean values (± SEM) of seminal free L-carnitine assay levels among group A fertile (control), group B azoospermic, group C asthenospermic and group D oligoasthenoteratospermic groups. In all azoospermic, asthenospermic, and oligoasthenoteratospermic the seminal free L-carnitine was found significantly low when compared independently with fertile (control) group. The mean value of azoospermic was also found significantly decreased (p<0.05) when compared with asthenospermic and oligoasthenoteratospermic, whereas the value of asthenospermic was significantly increased (p<0.05) when compared with oligoasthenoteratospermic. The lowest value of seminal free L-carnitine was found in azoospermic group.

Discussion

It is only in the past few decades that male factor has been recognized as a significant cause of infertility. Male infertility is not an entity but it reflects a variety of different pathogenic mechanisms. As biochemical parameters are seldom done in daily routine investigations in the seminal plasma, this study has provided a new biochemical marker for

evaluating the pathogenecity of male infertility.

Epididymal spermatozoa make use of different substrates as energy sources, but fatty-acid oxidation involving the carnitine dependent system seems to be the major energy supplying process.[17] Rat epididymal plasma is reported to contain approximately 1 mmol fatty acid and high levels of carnitine from which sperms generate a large reservoir of acetylcarnitine, which is thought to provide a ready source of energy during their post-ejaculation activity.[18] Mazzilli et al[19] established strict correlation between intrasperm L-carnitine content and sperm motility survival in bovine cervical mucus. This is possibly due to the fact that lipids are an important energy source for sperm in cervical mucus and to metabolize these lipids intrasperm L-carnitine is essential. L-carnitine not only helps in lipid metabolism but also it modulates the reserves of free CoA, essential for tricarboxylic acid cycle regulation. Therefore, L-carnitine content can be considered as an indicator of sperm motility life span in cervical mucus.[19]

L-carnitine also has antioxidant properties that protect sperm membranes against toxic reactive oxygen species. Pignatelli et al[20] demonstrated that carnitine reduces oxidative stress via interference with arachidonic acid incorporation into phospholipids and protein kinase C mediated NADPH oxidase system. It is also proposed that carnitine exerts antioxidant properties as a result of repairing mechanism by which elevated intracellular toxic acetyl-CoA is removed and fatty acids in membrane phospholipids are replaced.[21]

Male fertile subjects have higher carnitine levels in semen than infertile subjects, suggesting that there is a potential relationship between carnitine and semen quality.[22] Similar relationship was also demonstrated by Ke Li et al[15] with lowest concentration in oligoasthenopermic group. In addition, results of other studies also showed that the concentration of seminal carnitine positively correlates with the number of spermatozoa as well as the motility, and morphology.[23,24]

In the present study seminal plasma free L-carnitine in infertile subjects was found significantly decreased ($p<0.001$) when compared with fertile group. When infertile group was further divided according to their semen analysis, the azoospermic group had the lowest seminal free L-carnitine concentration. In addition seminal free L-carnitine in azoospermic, asthenospermic, and oligoasthenoteratospermic was also found significantly lower ($p<0.001$) when compared individually with fertile control. These results are in accordance with the already published studies[15,22-25] that showed significantly decreased levels in infertile men when compared with fertile group.

The mean value of seminal free L-carnitine in fertile males (control) in our study was 447.6 µmol/L which is higher than the study conducted by Ke Li et al[15] which showed mean value of 392 µmol/L, whereas Tomamichel and Bandhaur[14] showed normal range of 440-990 µmol/L. This may be explained because of different dietary habits and demographic variation as approximately 75% of the body stores of L-carnitine are derived from the diet.

Various studies[26,27] have demonstrated the effectiveness of L-carnitine in treating male infertility due to idiopathic or microbial infections by increasing sperm count, motility and semen volume significantly. Therefore, the therapeutic implication of the present study is that where decrease functional spermatozoal characteristics is due to low levels of seminal plasma free L-carnitine, exogenous L-carnitine administration could promote the acquisition of normal functional spermatozoal characteristics.

Conclusion

The results of this study suggested that L-carnitine in seminal plasma plays an essential role in maintaining male fertility. However larger studies on Pakistani population with this approach are needed.

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