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Original Research Article

Effects of levo-carnitine in infertile men with asthenozoospermia: a randomized placebo-controlled trial

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ABSTRACT

Background: Infertility is defined as the inability of a sexually active couple to conceive within one year of regular unprotected coitus. Worldwide, it is estimated that 15% of couples are infertile; among them, the male factor is responsible for 50% of cases. This may be the sole underlying cause or a contributory factor to infertility. We aimed to assess the effects of a complementary treatment with a strong antioxidant (levo-carnitine) on sperm function and fertility of infertile men.

Methods: This was a randomized controlled trial study and was conducted in the department of reproductive endocrinology and infertility, Bangabandhu Sheikh Mujib medical university (BSMMU), Dhaka, Bangladesh. during the period from July 2022 to June 2023. In our study, we included 72 infertile men presenting with asthenozoospermia. There were two groups-group A (Participants who received tab levo-carnitine 330 mg twice daily orally for three months) and group B (Tab placebo twice daily orally for three months)

Result: The majority of patients in both groups, 51% in group A and 49% in group B, were aged 30-40 years, with no significant difference in mean age (35.36±5.50 vs 34.50±5.50, $p>0.05$). Overall, 62.5% of patients reported primary sub-fertility. Levo-carnitine administration leads to significant improvements in sperm motility (15±2.68 vs. 36.58±5.16, $p<0.05$). In the case of placebo treatment, there were no significant improvements in sperm motility (13.91±5.53 vs. 16.36±1.19, $p>0.05$). We found that the comparison of TMC of both groups reflected statistically significant differences ($p<0.05$) before treatment and after treatment with levo-carnitine and placebo (6.40±2.87 vs 22.91±14.88) 5.64±3.96 vs 7.71±4.91).

Conclusions: Levo-carnitine treatment can lead to significant improvements in semen parameters, particularly in motility.

Keywords: Effectiveness, Levo-carnitine, Infertile men, Asthenozoospermia

INTRODUCTION

Infertility is defined as the inability of a sexually active couple to conceive within one year of regular unprotected coitus.[1,2] Worldwide, it is estimated that 15% of couples are infertile; among them, the male factor is responsible for 50% of cases. This may be the sole underlying cause or a contributory factor to infertility.[3]

Semen analysis is used for the diagnosis of male infertility. Abnormal semen parameters such as oligozoospermia, asthenozoospermia, teratozoospermia, or a combination of these may be shown. There may be a complete absence of sperm in the ejaculate (azoospermia), which is identified in 10-15% of infertile men.[2,4,5]

Male infertility may result from varicocele, hypogonadism, cryptorchidism, infections of the genital tract or testes, autoimmune disease, systemic diseases, testicular cancer and genetic abnormalities. In around 30%-40% of cases, no known cause is identified, and this condition has been termed idiopathic oligo-astheno-teratozoospermia (OAT) in men who do not have any disease.[6] Idiopathic OAT includes a combination of low sperm concentration (<16 million/ml), reduced motility (progressive motility <30% and total motility <42%), and abnormally shaped spermatozoa (<14% normal morphology by the 2020 world health organization criteria or <4% by the Kruger strict criteria). Sperm motility is defined as a hydrodynamic impulse pushing the spermatozoa toward the oocyte through the female genital tract. This impulse is produced as transverse waves propagate in a proximal-distal direction along the flagellum.[7] The flagellum forms the large and important part of the sperm tail structure of the mammalian spermatozoon which is responsible for moving spermatozoa.[8] Sperm channel also plays a crucial role in sperm motility which is located in the sperm tail membrane and is responsible for controlling cell motility, mediating the transduction of ciliary signals, and sensing environmental cues which are important for successful fertilization.[9] For these reasons, spermatozoa must be controlled by energy (ATP production) and flagella ion homeostasis.[9]

ATP is used as an energy source by spermatozoa in a large number of cellular processes including capacitation, motility, hyper-activation, acrosome reaction, and maintaining the intracellular milieu.[10] Glycolysis and oxidative phosphorylation are two metabolic pathways responsible for ATP production in sperm cells.[11] Lifestyle changes, cigarette smoking, exposure to chemical pesticides, and air pollution can disrupt sperm motility.[12,13] Free radicals or reactive oxygen species (ROS) play an important role in cell signalling and homeostasis and are produced by sperm cells in small numbers. They provide multitudes of beneficial effects but are not limited to initiation of sperm capacitating, regulation of sperm maturation, and enhancement of cellular signalling pathways.[14]

The most recent Cochrane review shows that for couples attending fertility clinics, pregnancy rates, and live births may be improved with antioxidant supplementation in infertile males. As one of many antioxidants, levo-carnitine is are naturally occurring compound in mammals.[15,16] Primary sources of carnitine are dietary intake, de novo biosynthesis, and renal tubular reabsorption.[16] Foods rich in carnitines include red meats, fish, poultry, and dairy products.[17] Aside from dietary consumption, approximately 25% of total body carnitine is synthesized by the body from the essential amino acids lysine and methionine.[18,19] L-carnitine facilitates the β-oxidation of long-chain fatty acids, and in its active form of L-acetylcarnitine, it is a vital antioxidant that protects the sperm mitochondria from oxidative.[20,21] Carnitines act as free radicle scavengers, thereby increasing anti-oxidative capabilities in spermatozoa resulting in the reduction of OS.[22-24] *In vitro*, the addition of carnitine to culture media increases sperm motility and vitality.[25]

The objective of this study will be to assess the effects of a complementary treatment with a strong antioxidant (levo-carnitine) on sperm function and fertility of infertile men.

METHODS

This was a randomized controlled trial study and was conducted in the Placebo Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib medical university (BSMMU), Dhaka, Bangladesh. during the period from July 2022 to June 2022. In our study, we included 72 infertile men presenting with asthenozoospermia. There were two groups of men, group A and B, group A (Participants who received tab levo-carnitine 330 mg twice daily orally for three months) and group B (Tab placebo twice daily orally for three months).

These are the following criteria to be eligible for the enrolment as our study participants: a) Patients aged between 20-45 years; b) Patients with asthenozoospermia; c) Patients who were willing to participate were included in the study and a) Patients with severe asthenozoospermia (sperm motility <5%); b) Patients who took antioxidants supplementation in the last three months; c) Patients with endocrinopathies (FSH >7 mIU/mL, total testosterone <300 ng/dl/10.40 nmol/L, LH >7 mIU/mL); d) Patients with OAT due to hypogonadotropic hypogonadism/ testicular failure; e) Patients with genital surgery, history of chemotherapy or radiotherapy and genetic causes of infertility; f) Patients with any history acute illness (e.g., uncontrolled DM, renal or pancreatic diseases, ischaemic heart disease etc.) were excluded from our study.

Statistical analysis

All data were recorded systematically in preformed data collection form. Quantitative data was expressed as mean and standard deviation and qualitative data was expressed as frequency distribution and percentage. Pairwise comparison of outcome variables was done with the treatment arm of levo-carnitine with control arm of placebo. Numerical variables were described as mean ± standard deviation or categorical variables as frequency (%). P values were calculated using student's t test and Chi-square test. Chi-square test or Fisher's exact test will be used for categorical variable and independent samples t-test (unpaired t test) or paired sample t-test for continuous

variable. Statistical analysis was performed by using SPSS 23 (Statistical package for social sciences) for Windows version 10. Probability value<0.05 was considered as level of significance. The study was approved by the ethical review committee of Bangabandhu Sheikh Mujib medical university (BSMMU), Dhaka, Bangladesh.

RESULTS

Table 1 shows that most of the patients of both groups (26, 51% vs 25, 49%) were aged between 30-40 years old. The mean difference of both groups (35.36±5.50 vs 34.50±5.50) was statistically not significant. Most of the participants 28 (56%) in group levo-carnitine vs 22 (44%) in the placebo were non-smokers and the statistical difference was not significant (p>0.05).

Table 2 shows that the mean difference of the duration of infertility of both groups (3.22±0.76 vs 2.88±0.82) was not statistically significant (p>0.05). The baseline semen motility (15±2.68 vs 13.91±5.53) of both groups was not statistically significant (p>0.05). Semen concentration (19.02±8.42 vs 17.50±11.81), and semen volume (2.48±1.00 vs 2.39±0.53) of both groups were not statistically significant (p>0.05).

Table 3 shows that before and after treatment with levo-carnitine showed statistically significant differences in semen motility, semen volume, while placebo showed no statistically significant differences (p>0.05). The comparison of semen analysis of both groups reflected statistically significant differences (p<0.05) in semen motility (36.58±5.16 vs 14.36±1.19), but reflected no statistically significant differences (p<0.05) in sperm concentration (24.58±17.69 vs 20.08±10.34) and semen volume (2.54±0.84 vs 2.60±0.46). TMC of both groups reflected statistically significant differences (p<0.05) in case before and after treatment levo-carnitine group (6.40±2.87 vs 22.91±14.88) and in placebo group (5.64±3.96 vs 7.71±4.91).

Figure 1 shows that most of the participants of both groups 25 (62.5%) vs 15 (37.5%) had primary sub-fertility.

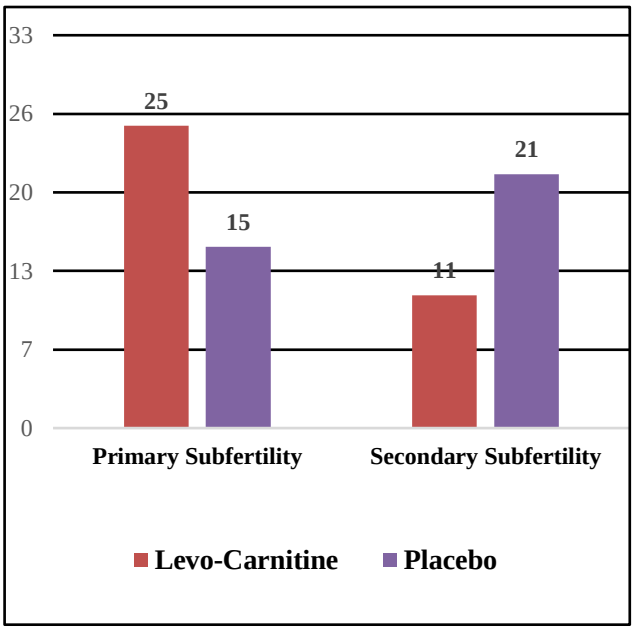

Figure 1: Types of infertility among patients.

Table 1: Sociodemographic characteristics of the study participants, (n=72).

Sociodemographic characteristics	Levo-carnitine, (n=36) N (%)	Placebo, (n=36) N (%)	P value
Age (in years)			
20-<30	6 (46.2)	7 (53.8)	>[a]0.99
30-<40	26 (51)	25 (49)	
40-45	4 (50)	4 (50)	
Mean ± SD	35.36±5.50	34.50±5.50	[b]0.509
Occupation			
Service	4 (44.4)	5 (55.6)	>[a]0.99
Business	31 (50.8)	30 (49.2)	
Student	1 (50)	1 (50)	
Socioeconomic status (BDT)			
<20,000	1 (25)	3 (75)	[a]0.851
20,000-30,000	33 (51.6)	31 (48.4)	
>30,000	2 (50)	2 (50)	
BMI (kg/m^2)			
Normal	32 (50)	32 (50)	>[a]0.99
Overweight	4 (50)	4 (50)	
Smoking habit			
Smoker	8 (36.4)	14 (63.6)	0.125
Non-smoker	28 (56)	22 (44)	

P value reached through chi-square test and [a]Fisher's exact test for categorical values, and [b]unpaired t test for normally distributed continuous variables.

Table 2: Baseline findings of the study participants.

Baseline findings	Levo-carnitine, (n=36)	Placebo, (n=36)	P value
Duration of infertility	3.22±0.76	2.88±0.82	[c]0.082
Semen concentration	19.02±8.42	17.50±11.81	[c]0.120
Semen motility	15±2.68	13.91±5.53	[c]0.487
Semen volume	2.39±0.53	2.48±1.00	[c]0.551

P value reached through [c]Mann Whitney U-test for non-normally distributed continuous variables.

Table 3: Compare the semen analysis before and after treatment with placebo and levo-carnitine of the study participants.

Semen analysis	Levo-carnitine	Placebo	P value
Sperm motility			
Before treatment	15±2.68	13.91±5.53	[b]0.001
After treatment	36.58±5.16	14.36±1.19	
	[d]0.001	[d]0.611	
Sperm concentration			
Before treatment	19.02±8.42	17.50±11.81	[c]0.087
After treatment	24.58±17.69	20.08±10.34	
	[d]0.071	[d]0.169	
Semen volume			
Before treatment	2.39±0.53	2.48±1.00	[c]0.797
After treatment	2.60±0.46	2.64±0.84	
	[d]0.026	[d]0.242	
TMC analysis			
Before treatment	6.40±2.87	5.64±3.96	
After treatment	22.91±14.88	7.71±4.91	
	[c]0.001[s]	[c]0.019[s]	

P value reached through [b]unpaired t-test and [c]Mann Whitney U-test for non-normally distributed continuous variables and [d]paired t test for continuous variables.

Table 4: Compare the sperm motility after treatment with levo-carnitine of the study participants, (n=36).

Sperm motility	Before treatment, (n=36)	After treatment, (n=36)
	N (%)	**N (%)**
10%-<20% (moderate asthenozoospermia)	34 (94.4)	0 (0)
20%-<42% (mild asthenozoospermia)	2 (5.6)	29 (80.6)
≥42% (normal)	0 (0)	7 (19.4)

Data presented as frequency and percentage over columns.

Table 4 shows that before treatment with levo-carnitine, 34 (94.4%) patients had moderate asthenozoospermia. After treatment with levo-carnitine, 29 (80.6%) patients' sperm motility improved and lay in mild asthenozoospermia and after treatment with levo-carnitine, a total of 7 patients' sperm motility became normal.

DISCUSSION

After analyzing the result, we found that most of the patients of both groups (51% vs 49%) were aged between 30-40 years old. The mean difference of both groups (35.36±5.50 vs 34.50±5.50) was statistically not significant. We found that most of the participants of both groups 25 (62.5%) vs 15 (37.5%) had primary sub-fertility. The mean difference in the duration of infertility of both groups (3.22±0.76 vs 2.88±0.82) was statistically not significant (p>0.05).

Our findings were slightly supported by a previous study done by Mehni and Ketabchi.[26] Mehni and Ketabchi found 6.28 years of infertility duration range, with 73% primary and 27% secondary infertility (p=0.788), and 54.28% of the patients had opium addiction without significant difference between groups (p=0.454).[26]

We observed that the baseline semen motility (15±2.68 vs 13.91±5.53) of both groups was not statistically significant (p>0.05). Semen concentration (19.02±8.42 vs 17.50±11.81), and semen volume (2.39±0.53 vs 2.48±1.00) of both groups were not statistically significant (p>0.05).

We also observed that semen analysis before and after treatment with placebo showed no statistically significant differences (p>0.05) in all parameters. But semen analysis before and after treatment with levo-carnitine showed

reflected statistically significant differences ($p<0.05$) in semen motility (15±2.68 vs 36.58±5.16), and semen volume (2.39±0.53 vs 2.60±0.46).

Manssor et al observed a significant increase in sperm count in infertile men treated with (L-carnitine, and the mean and standard deviation of increment in sperm count after four months of treatment (51±4.78), which is similar to our study.[27]

We also found that the comparison of semen analysis of both groups reflected statistically significant differences ($p<0.05$) in semen motility (36.58±5.16 vs 14.36±1.19).

Our findings were similar to Mehni and Ketabchi who found that the single use of PX and L-C only improved sperm motility rate in OAT patients, but the combined use of them improved all main sperm parameters.[26] Our findings coincided with Lenzi et al where they stated that subjects undergoing carnitine therapy showed no improvement in semen volume and sperm concentration. The differences between the therapy and placebo groups for all the semen variables analyzed were not statistically significant for either analysis of variance for repeated measures or t-test for independent samples (on differences between T+6 and T0).[28]

We found that the comparison of TMC of both groups reflected statistically significant differences ($p<0.05$) before treatment and after treatment with levo-carnitine and placebo (6.40±2.87 vs 22.91±14.88) 5.64±3.96 vs 7.71±4.91).

In studies comparing LC and LAC to placebo, Balercia et al showed a significant increase in total sperm motility in all three study arms when compared to a placebo (MD=18.75, 95% CI=14.78-22.73; n=30, $p<0.05$), which is similar to our study.[22]

Our findings also coincided with Lenzi et al who stated that subjects undergoing carnitine therapy did have an improvement in total and forward sperm motility as compared with placebo patients (from 23.17±6.50 to 31.11±13.46 and from 14.83±5.17 to 25.00±13.06).[28]

Limitations

Our study was a single-center study. We took a small sample size due to our short study period. We primarily focused on semen parameters as outcomes and did not thoroughly examine additional measures related to fertility and reproductive success. After evaluating those patients, we did not follow up with them for the long term and did not know other possible interference that may happen in the long term with these patients.

CONCLUSION

In our study, we found that levo-carnitine treatment can lead to significant improvements in semen parameters, particularly in motility. Some earlier research found similar results, whereas others did not.

So further study with a prospective and longitudinal study design including a larger sample size needs to be done to prove that levo-carnitine improves male fertility and spontaneous conception.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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