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Research Article

Effect of L-carnitine on sperm parameters in patients with Thalassemia major: A pilot study

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A B S T R A C T

Background: More than one-half of men with transfusion dependent thalassemia major (TDTM) are dealing with decreased fertility potentials. As antioxidants such as L-carnitine were reported to improve fertility in healthy males, we aimed to assay the effect of L-carnitine on sperm parameters in adult males with TDTM.
Materials and Methods: Twenty six male TDTM patients older than 18 years were included in a pilot study, all had semen analysis; 3 were found to be azoospermic and were excluded. So further analysis for sperms and DNA defects was conducted on the remaining 10 participants. Participants were then given L-carnitine for 3 months. All sperm parameters were reassessed after therapy.
Results: In patients with low volume ejaculation, the mean volume of semen increased significantly (p=0.034). In oligospermic patients with a mean sperm concentration of 4.62 million/mL, the mean sperm concentration raised to 22.50 million/mL after therapy (p=0.008). In patients who had lower than normal sperm motility percentage and sperm progressive motility percentage, both variables raised after therapy.

Conclusion: Thalassemia major patients showed significant increase in sperm concentration and sperm motility with the use of L-carnitine.

Key words: Thalassemia major, Male Infertility, Semen Quality, Sperm Parameter

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INTRODUCTION

Infertility is a note worthy issue, which affects almost 7.5% of the male population. Sperm quality as a keystone of reproductive potential is impaired by several abnormal conditions. In transfusion dependent thalassemia major (TDTM) men, decreased reproductive capacity is estimated to be much higher than the general population. Recent studies show that more than one-half of men with TDTM are affected by oligospermia and asthenospermia and have abnormal sperm quality.[i]Oxidative stress is considered as a major influential factor on male fertility potential causing damage to the sperm membrane, nucleus, and proteins, thereby impairing sperm quality.[ii]In β-thalassemia, oxidants originate from various sources including iron overload and the excess unpaired α-hemoglobin chains.[iii]Normal seminal plasma contains glutathione, carnitine, folate, vitamins E, C, and A, zinc, and seleniumas defensive molecules against oxidants.[iv] In thalassemia patients, excess non-transferrin bound iron and high seminal plasma iron, acting as pro-oxidants, is coupled with low level of antioxidants.[v]

Carnitine, a vitalantioxidant molecule, is highly concentrated in the epididymis, and serves as an intra-mitochondrial vehicle in the process of energy production for sperm respiration and motility. This critical role of carnitine has led to its use as treatment modality toincrease sperm motility.[vi]On the other hand, the anti-apoptotic effects of carnitine are manifested in many tissues, suggesting that carnitine may also exert inhibitory effects on apoptosis of germ cells within the testis. Whether abnormalities involving these biochemical pathways are the basis for abnormal spermatogenesis and subfertility in thalassemia men with a high iron burden is not well studied. Thus, we investigated the effect of L-carnitine on sperm parameters in TDTM patients.

Patients

We studied 26TDTMmale patients (age range 19-35 years) who had been regularly transfused since early childhood and underwent different chelation therapies using subcutaneous desferrioxamine and/or oral deferasirox and/or deferriperone. No participant was on medications known to cause hyperprolactinemia or steroids. We excluded patients with renal insufficiency; hematopoietic stem cell transplanted patients; HIV or HCV positive patients and patients with heart failure. Sperm parameters were compared before and after administration of L-carnitine.

Semen processing

Semen samples were obtained by masturbation after 5 days of sexual abstinence. After liquefaction at 37°C, seminal volume and pH, sperm concentration and motility were evaluated according to World Health Organization guidelines.[vii]**Treatment**

Patients were orally and daily supplemented with L-carnitine (1 g) for 3 months. Seminal analyses were performed at the start of the study (T0), and after 3 months of carnitine therapy (T3).

Ethical approval

All patients were informed about the aim and the methods of the study. All patients signed an informed consent prior to recruitment, and the study protocol was approved by the Iran university of medical sciences ethics committee.

Statistical analysis

Data were analysed by the Statistical Package of SPSS version 16.0. The data were presented as number and percentages, mean±standard deviations and ranges for the quantitative data with parametric distribution. The comparison between two independent groups with quantitative data and parametric distribution were done by using Independent t-test while comparison between two groups with non-parametric data was done by using Mann-Whitney test. The paired groups with quantitative data and parametric distribution were compared by using Paired t-test; while the non-parametric distribution was done by using Wilcoxon-rank test. Spearman correlation coefficients were used to assess the relation between two quantitative parameters in the same group. The confidence interval was set to 95% and the margin of error accepted was set to 5%.

RESULTS

The mean age of the patients was 28.05±3.73. The mean ferritin level was 1540.8±718.91.

Three patients were excluded due to azoospermia and 3 patients did not continue the drug use, one of them had experienced gastrointestinal disturbance, the other one had insomnia, and the third one did not continue the drug usage due to no obvious reason.

Semen analysis data of the zoospermic participants before and after treatment and their statistical comparison are listed in table 1. It is evident that sperm parameters show significant difference after treatment. Among all the patients, seven cases had low volume ejaculation, in whom the mean volume of semen increased significantly after l-carnitine administration in the second semen specimen (p=0.034). Among the included patients,nine had oligospermia with a mean sperm concentration of 4.62 million/mL. In the second sperm specimen evaluation, the mean sperm concentration in the oligospermic patients raised to 22.50 million/mL after therapy (p=0.008). Considering sperm motility, 10 patients had lower than normal sperm motility percentage (<40%) with a mean sperm motility of 16.40, which raised to 32.44 after therapy (p=0.022). Thirteen patients had lower than normal progressive motile sperm percentage (<32%), in whom this factor also raised significantly after carnitine therapy (p=0.05).Considering normal sperm morphology percentage, nine patients had low percentage of normal sperm morphology, which this variable also raised after carnitine therapy (p=0.015).

Discussion

Remarkable improved medical care and better preventive strategies for complications has led to successful survival of TDTM patients into adult life. Decreased reproductive capacity in TDTM men is common but inadequately addressed and ineffectively managed.

Hypogonadotropic hypogonadism which is mostlydue to iron deposition and generation of reactive oxygen species (ROS), is reported in 80-90% of TDTM patients worldwide.[viii] The harmful effects of ROS on sperm structural components are recognized in different studies. Sperms are particularly vulnerable to oxidant pleaded injuriesowing to their high content of unsaturated fatty acids in their lipid membrane, leading to loss of functionality and sperm death.[ix]Susceptibility of sperm to ROS, has led to emergence of antioxidants as possible treatments for infertile men. L- carnitine, a well-recognized antioxidant drug,is demonstrated to have beneficial effects on sperm parameters in a number of clinical studies and could be capable of improving sperm forward motility and viability.[x]This could be due to carnitine's crucial role in energy metabolism and in the maturation of spermatozoa, suggested by its high concentration in the male reproductive tract, especially in the epididymis.[xi]On the other hand, it has been proven that L-carnitine and its ester, acetyl-L-carnitine, has antiapoptotic action on human germ cells.[xii]It has been shown that the overall average effect of carnitines on pregnancy rate could be as high as 4.10 with a large statistical significance compared with placebo.[xiii] Different doses and durations of administering L-carnitine has been used in different trials. Costaet al. administered 3 g/day of oral L-carnitine for 4 months to patients with idiopathic asthenozoospermia, and reported an improvement in progressive and total sperm motility and an increase in sperm concentration in their treated subjects.[xiv] Vicari and colleagues evaluated the antioxidant properties of L-carnitine in the treatment of asymptomatic infertile patients with evidence of prostato-vesiculo-epididymitis andshowed an increase in the motility and viability of spermatozoa after 3 months of 2g/day L-carnitine therapy.[xv]Lenziet al. showed that combined treatment with L-carnitine (500 mg every 12 hours for 2 months) and acetyl-L-carnitine was effective in increasing sperm motility, especially in groups with lower baseline levels.[xvi]In a study by Balercia and collaborators[xvii], 3 g/day of L-carnitine for 6 months and in another study by De Rosa et al.[xviii], 1 g/day of L-carnitine for 6 months led to an improvement in sperm motility.

In thalassemia subjects, for the first time, Elsedfy and coworkers[xix], administered 2 g/day L-carnitine and 600 mg/day N-acetyl cysteine for 6 months to 10 fully pubertal TDTM patients and reported that sperm deformity index and teratozospermia indexincreased significantly in their post treatment semen samples.However, the increase in mentioned indexesnegatively correlates with the fertilization rate in conventional in vitro fertilization techniques and predicts fertilization failure, so the results of their study advocates against improvement of fertility by the use of antioxidants in men with TDTM.[xx]In our study, administring a lower dose and duration of L-carnitine compared to other studies, to zoospermic adult TDTM males with abnormalities in their basic semen specimens, led to significant improvement in their post therapysemen and sperm parameters.

A limitation of our study was the unavailability of the data about the level of L-carnitine in serum and/or semen and lack of a placebo controlled group. By measuring of L-carnitine level in serum/semen, the effect of the drug is better sensed.

CONCLUSION

Thalassemia major patients showed significant increase in sperm concentration and sperm motility with the use of L-carnitine. Further studies should be conducted to examine the effect of L-carnitine on fertility outcomes.

Table: 1. Sperm parameters of patients at the before and after drug administration.

		Before treatment (Mean±SD)	After treatment (Mean±SD)	P-value
Semen Volume (ml)	Low (<1.5)	0.87±0.27	1.67±0.92	0.34
Sperm Concentration (million/mL)	Low(<15)	4.62±5.72	22.50±22.10	0.008
Sperm Motility (%)	Low(<40)	16.40±13.75	13.44±19.27	0.022
Sperm progressive motility (%)	Low(<32)	10.66±10.81	23.91±23.39	0.05
Sperm morphology (%)	Low(<20)	3.22±3.63	25.33±19.78	0.015

REFERENCE:

1. Perera D, Pizzey A, Campbell A, et al. Sperm DNA damage in potentially fertile homozygous beta-thalassaemia patients with iron overload. Hum Reprod. 2002; 17:1820–1825.
2. Safarinejad MR. Evaluation of semen quality, endocrine profile and hypothalamus-pituitary-testis axis in male patients with homozygous beta-thalassemia major. J Urol. 2008;179:2327–2332.
3. Agarwal A, Virk G, Ong C, et al. Effect of oxidative stress on male reproduction. World J Mens Health. 2014; 32:1–17.
4. Walter PB, Fung EB, Killilea DW, Jiang Q, Hudes M, Madden J, Porter J, Evans P, Vichinsky E, Harmatz P. Oxidative stress and inflammation in iron-overloaded patients with β-thalassaemia or sickle cell disease. British journal of haematology. 2006 ;135(2):254-63.
5. Scott, M.D., Van Den Berg, J.J., Repka, T., Rouyer-Fessard, P., Hebbel, R.P., Beuzard, Y. & Lubin, B.H. (1993) Effect of excess alpha-hemoglobin chains on cellular and membrane oxidation in model beta-thalassemic erythrocytes. Journal of Clinical Investigation, 91, 1706–1712.
6. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility.
7. Ebisch IM, Thomas CM, Peters WH, Braat DD, Steegers-Theunissen RP. Hum Reprod Update. 2007 Mar-Apr; 13(2):163-74.
8. Livrea MA, Tesoriere L, Pintaudi AM, et al. Oxidative stress and antioxidant status in beta-thalassemia major: Iron overload

and depletion of lipid-soluble antioxidants. Blood. 1996; 88:3608–3614.
9. Claster S, Wood JC, Noetzli L, et al. Nutritional deficiencies in iron overloaded patients with hemoglobinopathies. Am J Hematol. 2009;84:344–348
10. Singer ST, Killilea D, Suh JH, Wang ZJ, Yuan Q, Ivani K, Evans P, Vichinsky E, Fischer R, Smith JF. Fertility in transfusion-dependent thalassemia men: Effects of iron burden on the reproductive axis. American journal of hematology. 2015 ;90(9):E190-2.
11. A. Lenzi, F. Lombardo, P. Sgro, P. Salacone, L. Caponecchia, F. Dondero, et al.Use of carnitine therapy in selected cases of male factor infertility a double blind crossover trial. Fertil Steril, 79 (2003): 292-300
12. C. Jeulin, L.M. LewinRole of free L-carnitine and acetyl-L-carnitine in post-gonadal maturation of mammalian spermatozoa. Hum Reprod Update, 1996; (2):87-102
13. Zopfgen, F. Priem, F. Sudhoff, K. Jung, S. Lenk, S.A. Loening, et al.Relationship between semen quality and the seminal plasma components carnitine, alpha-glucosidase, fructose, citrate and granulocyte elastase in infertile men compared with a normal population
14. Hum Reprod.2000; 15:840-845
15. E. Ruiz-Pesini, E. Alvarez, J.A. Enriquez, M.J. Lopez-PerezAssociation between seminal plasma carnitine and sperm mitochondrial enzymatic activities
16. Int J Androl, 2001; (24):335-340
17. Garolla A, Maiorino M, Roverato A, Roveri A, Ursini F, Foresta C. Oral carnitine supplementation increases sperm motility in asthenozoospermic men with normal sperm phospholipid hydroperoxide glutathione peroxidase levels. Fertility and sterility. 2005; 1; 83(2):355-61.
18. World Health OrganizationLaboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press, New York (1999)
19. Perera D, Pizzey A, Campbell A, Katz M, Porter J, Petrou M, Irvine DS, Chatterjee R. Sperm DNA damage in potentially fertile homozygous beta-thalassaemia patients with iron overload. Hum Reprod 2002; 17:1820-5.2.
20. Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jen-nings Z, Irvine DS. Relative impact of oxidative stress on the functional competence and genomic integrity of human sper-matozoa. Biol Reprod. 1998; 59:1037-46
21. Aitken RJ, Smith TB, Jobling MS, Baker MA, De Iuliis G. Oxidative stress and male reproductive health. Asian J Androl 2014; 16:31-8.
22. Costa M, Canale D, Filicori M, D'Iddio S, Lenzi A: L-Carnitine in idiopathic asthenozoospermia: A multicenterstudy. Italian Study Group on Carnitine and Male infertility.Andrologia 1994; 26:155–1594.
23. Lenzi A, Lombardo F, Sgro P, Salacone P, Caponecchia L,Dondero F, Gandini L: Use of carnitine therapy in selectedcases of male factor infertility: A double-blind crossover trial. Fertil Steril 2003; 79:292–3005.
24. Vicari E, Calogero AE: Effects of treatment with carnitinesin infertile patients with prostato-vesiculo-epididymitis. HumReprod 2001;16:2338–2342
25. Campaniello E., Petrarolo N., Meriggiola M.C., et al. (1989) Carnitine administration in asthenospermia (Abstract). 6 International Congress of Andrology, Firenze, May 14-18.
26. Vicari E, La Vignera S & Calogero AE. (2002) Antioxidant treatment with carnitines is effective in infertile patients with prostate vesiculo epididymitis and elevated seminal leukocyte concentrations after treatment with non-steroidal anti-inflammatory compounds. Fertil Steril78, 1203–1208.
27. [xii] Abad C, Amengual MJ, Gosalvez J, Coward K, Hannaoui N, Benet J,Garcıa-Peiro A & Prats J. (2013) Effects of oral antioxidant treatmentupon the dynamics of human sperm DNA fragmentation andsubpopulations of sperm with highly degraded DNA.Andrologia45,211–216.
28. Ng CM, Blackman MR, Wang C & Swerdloff RS. (2004) The role ofcarnitine in the male reproductive system.Ann N Y Acad Sci1033,177–188
29. Zhou X, Liu F, Zhai S. Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review. Asia Pacific journal of clinical nutrition. 2007;16:383.
30. Costa M, Canale D, Filicori M, D'lddio S & Lenzi A. (1994) L-carnitine in idiopathic asthenozoospermia: a multicenter study. Italian Study Group on Carnitine and Male Infertility.Andrologia26, 155–159.
31. Vicari E, Rubino C, De Palma A, Longo G, Lauretta M, Consoli S &Arancio A. (2001) Antioxidant therapeutic efficiency after theuse of carnitine in infertile patients with bacterial or nonbacterial prostato-vesiculo-epididymitis.Arch Ital Urol Androl73,15–25.
32. Lenzi A, Sgro P, Salacone P, Paoli D, Gilio B, Lombardo F, Santulli M, Agarwal A & Gandini L. (2004) A placebo-controlled double-blindrandomized trial of the use of combined l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia.Fertil Steril81,1578–1584.
33. Balercia G, Regoli F, Armeni T, Koverech A, Mantero F & Boscaro M.(2005) Placebo-controlled double-blind randomized trial on the use ofL-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia.Fertil Steril84, 662–671.
34. De Rosa M, Boggia B, Amalfi B, Zarrilli S, Vita A, Colao A & Lombardi G.(2005) Correlation between seminal carnitine and functionalspermatozoal characteristics in men with semen dysfunction ofvarious origins.Drugs R D6, 1–9.
35. Elsedfy H, De Sanctis V, Ahmed AY, Mohamed NR, Arafa M, Elalfy MS. A pilot study on sperm DNA damage in β-thalassemia major: is there a role for antioxidants?. Acta bio-medica: Atenei Parmensis. 2018;89(1):47.
36. Hammadeh ME, Radwan M, Al-Hasani S, Micu R, Rosen-baum P, Lorenz M, Schmidt W. Comparison of reactive oxygen species concentration in seminal plasma and semen parameters in partners of pregnant and non-pregnant patients after IVF/ICSI. Reprod Biomed Online 2006; 13: 696-706.