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Alpha-lipoic acid improves sperm motility in infertile men after varicocelectomy: a triple-blind randomized controlled trial

BIOGRAPHY

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KEY MESSAGE

An 80-day course of alpha-lipoic acid (ALA) medication after microsurgical repair improves sperm motility and progressive motility in men with a varicocele. Thus, ALA supplementation could be considered as an adjunct therapy to varicocelectomy.

ABSTRACT

Research question: Does supplementation with alpha-lipoic acid (ALA) enhance sperm parameters and/or the status of sperm lipid peroxidation and DNA fragmentation in men who have undergone microsurgical repair of a varicocele?

Design: Individuals with a varicocele who had undergone varicocelectomy were divided into two groups receiving either 600 mg of ALA or an identical placebo for 80 days. Semen samples obtained from the participants before surgery and after completion of the course of medication were analysed and compared. Participants, clinicians and data analysts were blinded to the randomization sequence.

Results: In the ALA group, total motility ($P = 0.01$) and progressive motility ($P = 0.002$) of the spermatozoa were significantly higher compared with the placebo group after surgery. Sperm lipid peroxidation and DNA damage (assessed by sperm chromatin structure assay) showed significant decreases in both the ALA and placebo groups ($P \leq 0.02$) after treatment.

Conclusions: An 80-day course of ALA medication after surgical repair improves total motility and progressive motility of the spermatozoa in individuals with a varicocele.

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KEY WORDS

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DNA fragmentation
Male infertility
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TUNEL
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INTRODUCTION

The term 'infertility' depicts the failure to conceive after a minimum of 12 months of regular unprotected intercourse, and affects 10% of the couples of reproductive age. A male aetiology contributes to as many as half of all cases of infertility (*Winters and Walsh, 2014*): classically, an impairment on sperm analysis implies male infertility (*WHO, 2010*). While the environment, physiological alterations and genetics have long been acknowledged to affect male fertility status, the exact underlying molecular mechanisms are not well recognized (*Coutton et al.,2016*). However, the evidence indicates that oxidative stress plays a substantial role in approximately 50% of cases. Oxidative stress arises as a result of an imbalance between oxidizing agents and reductant molecules due to the overproduction of oxidants, namely reactive oxygen species (ROS) (*Agarwal et al., 2018*; *Scott et al.*). ROS, naturally produced through normal cellular metabolism, serve as signalling molecules and promote sperm penetration into the oocyte at physiological levels; some studies have shown that incubation of spermatozoa with particular types of ROS upholds capacitation, acrosome reaction, hyperactivation and fusion to the zona pellucida (*de Lamirande et al., 1997*).

Morphologically abnormal spermatozoa and seminal leukocytes are the principal suppliers of excessive ROS in human semen, the main aetiologies being considered to be mitochondrial dysfunction and a provoked myeloperoxidase system, respectively (*Lobascio et al., 2015*). In the seminal plasma, antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase work hand in hand with antioxidant compounds, namely ascorbic acid (vitamin C), tocopherol (vitamin E) and glutathione, in order to maintain optimal oxidative homeostasis (*Agarwal et al., 2005*).

Varicocele – defined as aberrant dilation/elongation of the pampiniform venous plexus of the spermatic cord – is the most common treatable cause of male infertility. Its prevalence ranges between 15% and 20% in the normal adult male population. It is estimated that 30% and 70–85% of men with primary and secondary male factor infertility, respectively, manifest variable degrees of varicocele (*Alsaikhan et al., 2016*). The physiopathology of varicocele is not well-understood. However, the spermatic veins tend to dilate, supposedly as a consequence of structural defects, leading to incompetency followed by a backflow of hazardous components of venous blood to the testicles, as well as the production of higher mean temperatures (*McClure and Hricak, 1986*; *Miyaoka and Esteves, 2012*).

Studies have shown that men with a varicocele show higher concentrations of oxidative stress markers. The abundance of polyunsaturated fatty acids subjects the human sperm membrane to lipid peroxidation, which is a major consequence of seminal oxidative stress, leading to defects in the morphology and functionality of the sperm membrane (*Alaa Hamada, 2016*). Additionally, elevated concentrations of ROS decrease intracellular pH, which unbalances the relationship between total antioxidant capacity and ROS production, as ROS-scavenging enzymes work optimally in mildly alkaline environments (*Alaa Hamada, 2016*). Besides damaging multiple cell organelles, the resultant thermal/oxidative stress may harm sperm chromatin; however, the situation is shown to be reversible following surgical repair (*Telli et al., 2015*; *Zaazaa et al., 2018*).

Favourable outcomes have repeatedly been reported for supplementation with antioxidant compounds in humans and animals with low sperm quality (*Showell et al., 2014*). Oral antioxidants (e.g. vitamins E and A, zinc, folate and selenium) may reduce the oxidative stress of the ejaculate, which may eventually improve sperm parameters (*Zini and Al-Hathal, 2011*). Various studies have investigated the impact of peri-varicocelectomy antioxidant administration (*Azadi et al., 2011*; *Barekat et al., 2016*; *Paradiso Galatioto et al., 2008*). Although a few reports have confirmed beneficial effects on sperm parameters, the evidence is not yet conclusive. Moreover, as a result of poor outcomes in comparison with varicocelectomy, applying antioxidant supplementation as an alternate therapy has not gained much attention.

Alpha-lipoic acid (ALA) is a natural short-chain fatty acid containing sulfhydryl groups generated from octanoic acid and cysteine in the mitochondria, and is found in both the aqueous and lipid phases. ALA delivers a vast number of antioxidant features: it (i) suppresses free oxygen radicals; (ii) chelates metals; (ii) rejuvenates oxidized antioxidants, namely glutathione and vitamins C and E; and (in) promotes the functionality of enzymes with antioxidant features (e.g. glutathione peroxidase, catalase and superoxide dismutase). ALA can also be reduced to dihydrolipoic acid, which exhibits more robust antioxidant capabilities.

While endogenous ALA is protein bound and acts as a co-enzyme for mitochondrial enzymes (*Rochette et al., 2013*; *Solmonson and DeBerardinis, 2018*; *Suzuki et al., 1993*), supplementation increases the amount of the free form, which can function as an antioxidant. Several studies have shown that ALA supplementation is able to deliver benefit in conditions in which oxidative stress plays a major role, such as diabetic neuropathy and heavy metal toxicity (*Haghighian et al., 2015*; *Rochette et al., 2015*; *Smith et al., 2004*). Numerous animal studies have investigated the efficacy of ALA supplementation: it has been shown that administration of ALA can lower ROS generation and contribute to improved reproductive potential by ameliorating damaged testicular architecture, impaired steroidogenesis, poor sperm parameters and damaged DNA (*Jana et al., 2014*; *Pınar et al., 2018*; *Prathima et al., 2017*; *Prathima et al., 2018*; *Ren et al., 2018*).

ALA medication for individuals with varicocele has rarely been considered in the literature. In a previous study on rats with induced varicocele, the current authors showed that ALA supplementation was able to lessen the hazardous effects of elevated ROS concentrations on sperm parameters/chromatin (*Shaygannia et al., 2018*). Applying a triple-blinded controlled clinical trial methodology, the present study aims to assess the consequences of ALA supplementation on sperm lipid peroxidation, sperm parameters and DNA fragmentation in men with varicocele after microsurgical repair.

MATERIALS AND METHODS

Semen collection

The present study was approved by the Royan Institute's Ethics Committee for

Research Involving Human Subjects and conducted at the Royan Institute (IR.ACECR.ROYAN.REC.1398.120 and IRCT20110804007223N10, approved 16 July 2019) in collaboration with Isfahan Fertility and Infertility Center, between 2018 and 2019.

Each of the participants was informed about the aim and methodology of the study; all their questions were answered, and a formal consent form was filled and signed by each of the participants. A total of 60 men aged 19 to 45 years, with uni/bilateral grade II–III varicocele (confirmed by Doppler duplex ultrasonography if ambiguous on palpation) met the inclusion criteria and were enrolled in the study. After undergoing microsurgical repair of the varicocele, the participants were randomly allocated to treatment (n = 30) and control (n = 30) groups, receiving daily doses of 600 mg of ALA or an identical placebo (Raha, Iran), respectively, for the following 80 days.

The permutation block randomization method was used, applying nine blocks containing eight units (individuals) for the sample size, and a random sequence was built using all the possible permutations. Drug and placebo packaging was identical, and medications were given to the participants according to the randomization sequence, to which the clinician, healthcare providers, individuals in charge of data collection and analysis, and statistician were all blinded. The codes were revealed only after the final analysis of the data.

Individuals with azoospermia, occupational exposure to heat, radiation, and pesticides, a history of mumps, cryptorchidism, solitary testis, urogenital malignancies/infections, endocrinopathies, Sertoli cell-only syndrome, leukocytospermia, scrotal trauma, high fever prior to sampling, recurrent varicocele, severe alcoholism and heavy smoking were not included in this study.

A semen sample was obtained from each of the participants before and 80 days after surgery. All samples were provided by masturbation after 3–4 days of abstinence, subsequently liquefied at room temperature, fixed and analysed based on the World Health Organization (WHO) criteria by an instructed operator who was blinded to the type of the treatment given to each donor (*WHO 2010*). To evaluate the sperm concentration, a duplicate or replicate count was carried out using a sperm counting chamber with a 10 µm depth (sperm meter, sperm processor; Garkheda, India) and a Labomed CxL optical microscope (magnification 20 ×). Volumes of 10 µl of liquefied semen were loaded into the sperm meter chamber and the number of spermatozoa was counted. For semen samples with a high number of spermatozoa, the semen was diluted (1:10) in 1% formalin in a sodium bicarbonate solution. The number of spermatozoa was then counted and expressed as million per microlitre. If the difference between duplicates fell outside the ranges provided by WHO tables, a second duplicate sample was used.

For each sample, the morphology and motility of the spermatozoa were evaluated using a computer-assisted sperm analysis CASA system (VideoTesT-Sperm 2.1; Russia). To assess sperm morphology and protamine deficiency, the samples were stained with Diff-Quik and chromomycin A3 (CMA3), respectively. DNA fragmentation was then evaluated by TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick-end labelling (TUNEL) and sperm chromatin structure assay (SCSA), and sperm lipid peroxidation was assessed by BODIPY staining.

Assessment of sperm protamine deficiency applying CMA3 staining

CMA3 staining was performed as described by Iranpour and colleagues (Iranpour et al.,): semen samples were washed with phosphate-buffered saline (PBS) and subsequently fixed in Carnoy's solution (methanol:glacial acetic acid 3:1; Merck, Germany). Next, two smears were obtained from each sample. Smears were stained with CMA3 solution (0.25 mg/ml in McIlvaine buffer [citric acid 0.1 mol/l, $Na_2HPO_4.7H_2O$, 0.2 mol/l, pH 7.0, containing 10 mmol/l $MgCl_2$]) for 20 min followed by washing and mounting. Using an Olympus fluorescence microscope (BX51; Olympus, Japan) and the appropriate filters (460–470 nm), the percentage of protamine-deficient spermatozoa (determined by spermatozoa with bright yellow staining) was assessed for each sample by counting at least 500 sperm cells per smear.

Assessment of sperm DNA fragmentation applying TUNEL assay

DNA fragmentation was assessed using the TUNEL assay. After the semen samples had been washed with PBS, they were fixed in 4% methanol-free formaldehyde for 30 min. Fixed samples were then washed and permeabilized using 0.2% Triton X-100 (Merck, Germany) in PBS for 5 min. DNA fragmentation was detected using the Apoptosis Detection System Fluorescein kit (Promega, Germany), and samples were evaluated using a FACSCalibur fluorescence-activated cell sorter (Becton Dickinson, USA).

Assessment of DNA fragmentation by SCSA

Volumes containing 1–2 million spermatozoa were isolated from seminal fluid, and the volume was boosted to 1 ml TNE buffer (Tris HCl [Merck, Germany]/NaCl [Merck]/EDTA [Merck]). A volume of 1200 µl of acridine orange (Sigma, USA) staining solution was added to 200 µl of a diluted semen sample in the control tube; for the main tube, 200 µl of diluted semen sample was mixed with 400 µl acid-detergent solution for 30 s before adding the staining solution as mentioned above. Finally, a FACSCalibur fluorescence-activated cell sorter (Becton Dickinson) was used to evaluate the percentage of DNA fragmentation). Approximately 10,000 spermatozoa were analysed for each sample.

Assessment of sperm lipid peroxidation applying the BODIPY probe

After separating approximately 2 million spermatozoa, BODIPY C11 loading (BODIPY 581/591 C11 D3861, Molecular Probes) was added at a final concentration of 5 mmol/l. The tubes were then incubated (30 min, 37°C) and samples were washed twice in PBS buffer. Lipid peroxidation of the spermatozoa (percentage of BODIPY-positive spermatozoa) was evaluated using a FACSCalibur fluorescence-activated cell sorter (Becton Dickinson) (Aitken et al., 2007).

Statistical analysis

All the data in the present study were analysed using the IBM SPSS Statistics for Windows version 26 (IBM, USA). As the variables were normally distributed, an independent samples t-test was used to compare the variables in the ALA and placebo groups before or after treatment. A paired samples t-test was used for intra-group comparisons between the pre- and post-treatment values. For descriptive results, data were

expressed as mean ± standard error of the mean except for male age (presented as mean ± standard deviation of the mean). A *P*-value <0.05 was considered significant.

RESULTS

A total of 60 individuals met the inclusion criteria and were enrolled in the study. Of these, 41 – 22 men who had received placebo and 19 who had received ALA – attended the post-medication sampling. There was no statistically significant difference in the mean values for male age, body weight and height, or body mass index between the ALA and placebo groups (TABLE 1).

As shown in TABLE 2, semen parameters and results of sperm function tests did not differ significantly between the two groups before varicocelectomy except for abnormal morphology ($P = 0.04$). Similarly, no statistically significant difference was observed between the ALA and placebo groups for these parameters after varicocelectomy (TABLE 3).

The analysis of sperm parameters (FIGURE 1) demonstrated that the mean percentage of spermatozoa with normal morphology significantly increased after medication in both the ALA- and placebo-treated subjects ($P \leq 0.001$) while the mean percentages related to sperm motility ($P = 0.01$) and progressive motility ($P = 0.002$) showed statistically significant improvements after treatment only in the ALA group (FIGURE 1A, B). In addition, there were significant improvements in mean values for semen volume (ALA, $P = 0.01$; placebo, $P = 0.003$) and sperm concentration (ALA, $P = 0.02$; placebo, $P = 0.4$) after varicocelectomy in both the ALA- and placebo-treated participants (FIGURE 1C, D).

Sperm lipid peroxidation (FIGURE 2) as an oxidative stress marker showed a highly significant decrease in both groups after treatment (ALA, $P = 0.001$; placebo, $P = 0.009$). Regarding susceptibility to damage of the chromatin structure, a comparison of the SCSA test results showed a significant decrease in both the ALA ($P = 0.003$) and placebo ($P = 0.02$) groups after medication. No statistically significant difference was observed between the before- and after-medication TUNEL results in either of the study groups.

TABLE 1 ANTHROPOMETRIC CHARACTERISTICS OF THE PARTICIPANTS

Variable	Mean (95% CI)		*P*-value
	ALA (*n* = 19)	Placebo (*n* = 22)	
Age (years)	31.14 ± 5.54	31.89 ± 5.06	0.3
Weight (kg)	81.25 (74.63–87.86)	74.85 (71.65–78.04)	0.08
Height (cm)	179.21 (176.22–182.20)	176.48 (174.60–178.35)	0.11
Body mass index (kg/m^2)	25.39 (23.11–27.67)	24.02 (23.08–24.96)	0.26

ALA, alpha-lipoic acid; CI, confidence interval.

TABLE 2 SEMEN PARAMETERS OF THE STUDY PARTICIPANTS BEFORE MICROSURGICAL REPAIR OF VARICOCELE

Parameter	Mean ± SE		*P*-value
	Placebo (*n* = 22)	ALA (*n* = 19)	
Sperm concentration (10^6/ml)	47.91 ± 12.16	52.37 ± 12.55	0.8
Semen volume (ml)	2.31 ± 0.31	1.99 ± 0.28	0.45
Abnormal sperm morphology (%)	98.27 ± 0.33	97.21 ± 0.39	0.04
Sperm motility (%)	38.38 ± 5.71	36.41 ± 5.61	0.8
Sperm progressive motility (%)	24.86 ± 4.09	23.70 ± 3.44	0.83
Sperm lipid peroxidation (%)	36.22 ± 3.38	40.72 ± 3.32	0.34
Sperm lipid peroxidation (intensity)	24.13 ± 1.70	25.21 ± 3	0.72
DNA fragmentation (TUNEL) (%)	13.65 ± 2.44	11.25 ± 0.62	0.39
DNA damage index (SCSA) (%)	20.47 ± 2.31	23.22 ± 1.56	0.32
Sperm protamine deficiency (%)	41.72 ± 4.14	35.34 ± 3.29	0.24

SCSA, sperm chromatin structure assay; TUNEL, TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick-end labelling.

DISCUSSION

In spite of the extensive growth of knowledge on reproductive well-being, varicocele has tended to remain the most prevalent treatable aetiology of male primary and secondary infertility. Although the pathophysiological pathways of varicocele are well understood, the quest for the optimal treatment remains challenging (*Alaa Hamada, 2016*; *Miyaoka and Esteves, 2012*). Today, the

TABLE 3 PARTICIPANTS' POST-VARICOCELECTOMY/POST-MEDICATION SEMEN PARAMETERS

Parameter	Mean ± SE		*P*-value
	Placebo group (*n* = 22)	ALA group (*n* = 19)	
Sperm concentration (10^6/ml)	74.40 ± 12.71	81.65 ± 16.18	0.72
Semen volume (ml)	3.63 ± 0.34	3.19 ± 0.44	0.42
Abnormal sperm morphology (%)	95.45 ± 0.70	93.42 ± 0.77	0.056
Sperm motility (%)	39.76 ± 4.40	50.34 ± 5.20	0.12
Sperm progressive motility (%)	26.76 ± 3.85	35.75 ± 396	0.11
Sperm lipid peroxidation (%)	24.04 ± 1.80	22.62 ± 1.63	0.56
Sperm lipid peroxidation (intensity)	21.30 ± 1.51	21.77 ± 1.89	0.84
DNA fragmentation (TUNEL) (%)	10.38 ± 0.99	12.26 ± 1.06	0.2
DNA damage index (SCSA) (%)	16.45 ± 1.28	18.37 ± 1.41	0.32
Sperm protamine deficiency (%)	37.49 ± 3.62	33.52 ± 3.22	0.42

SCSA, sperm chromatin structure assay; TUNEL, TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick-end labelling.

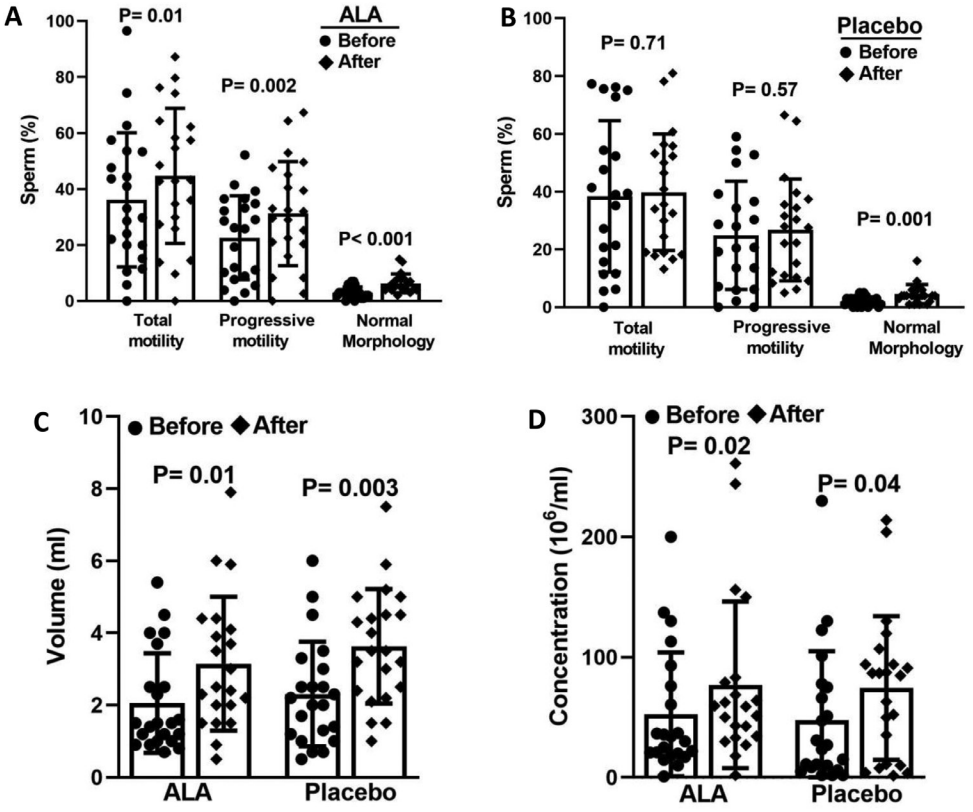

FIGURE 1 Comparison of mean values before and after medication for (A, B) total sperm motility, progressive motility and normal sperm morphology, (C) semen volume and (D) sperm concentration in the placebo and alpha-lipoic acid (ALA) groups.

understanding of fertility related issues inevitably relies on understanding the underlying interactions at a molecular level.

Physiologically, in the seminal plasma, scavenging enzymes (namely superoxide dismutase, catalase and glutathione peroxidases), together with natural antioxidants (e.g. vitamins C and E), restrict free radicals from exceeding an absolute concentration that contributes to the functionality of the spermatozoa: ROS are crucial to capacitation, hyperactivation and the acrosome reaction. When produced in excess, ROS may activate the lipid peroxidation cascade and dysregulate apoptosis factors, leading to subsequent alterations in the membrane and DNA integrity of the spermatozoa, and ultimately reducing fertility potential (*Agarwal et al., 2003*; *Moustafa et al., 2004*). In general, evidence supports the role of antioxidant supplementation in improving semen/sperm quality by alleviating oxidative stress. However, in the case of men with varicocele, such a proposition remains debatable (*Garg and Kumar, 2016*). The present study thus aimed to assess the efficacy of ALA supplementation as an adjunct therapy to surgery in these individuals. In a triple-blind clinical trial design, individuals with varicocele were treated with either 600 mg of ALA or a matching placebo for 80 days, immediately after the microsurgical repair of varicocele.

Through the study, daily supplementation with ALA improved total motility and progressive motility of the spermatozoa. The level of sperm DNA fragmentation as assessed by SCSA significantly decreased after varicocelectomy in both the ALA and placebo groups, and this reduction was more significant in the ALA group compared with the placebo group (P = 0.003 versus P = 0.02). In ALA-treated individuals, sperm motility and progressive motility increased significantly, in addition to the statistically significant decline in SCSA-assessed DNA fragmentation seen after surgery. However, the analysis revealed a statistically significant increase in semen volume and sperm concentration in both groups, while abnormal morphology and lipid peroxidation levels decreased significantly. The results clearly show that varicocelectomy surgery significantly improved all sperm parameters and functional tests in all the participants in this study in both ALA and placebo groups, except for sperm motility and progressive motility, which improved only in the ALA-treated subjects after varicocelectomy.

Unlike SCSA-assessed DNA fragmentation, mean TUNEL-assessed DNA fragmentation in the spermatozoa did not improve in either the ALA or the placebo group after varicocelectomy. This may arise from the selection of individuals for this study, in view of the fact that SCSA assesses susceptibility to DNA damage while TUNEL evaluates the actual level of DNA fragmentation, and the individuals evaluated in this study were non-smokers and had fewer than 1 million peroxidase-positive cells per millilitre. Therefore, it was not unexpected that DNA fragmentation as assessed by TUNEL would be low in these individuals; this figure would, like that for SCSA, have become significant if a higher number of individuals had been included in the study.

Sustained integrity of the mitochondrial structure is the backbone of maintaining normal motility of spermatozoa,

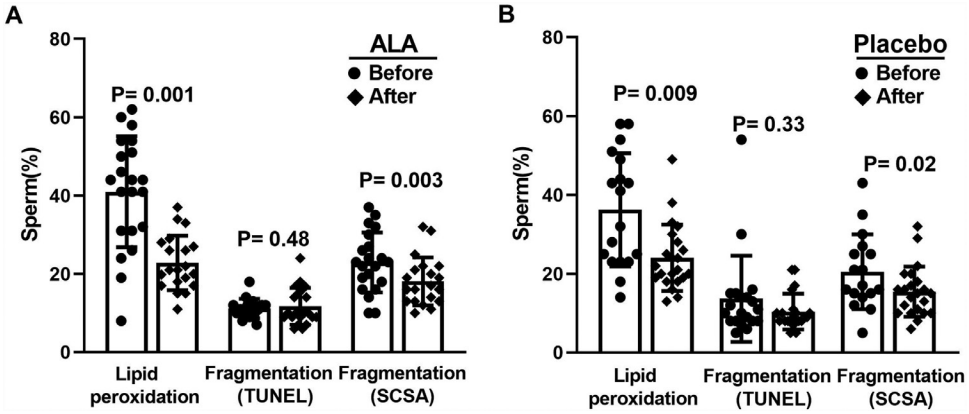

FIGURE 2 Mean levels of sperm lipid peroxidation (used as an oxidative stress marker) and DNA fragmentation (evaluated simultaneously by TUNEL and SCSA), before and after administration of (A) ALA or (B) an identical placebo. ALA, alpha-lipoic acid; SCSA, sperm chromatin structure assay; TUNEL, TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick-end labelling.

as sperm motility relies hugely on efficient ATP synthesis (*Ibrahim et al., 2008*). A normally functioning sperm mitochondrion produces notable amounts of free radicals, which may eventually result in damage to its external/internal membranes due to the abundance of polyunsaturated fatty acids (*Ibrahim et al., 2008*). ALA actively forms a shield over the sperm membrane in the midpiece through interrelation with glutathione and ascorbic acid, the initiator and regulator of sperm motility occupied by the flagellum and the principal piece. The midpiece section of the flagellum contributes to the initiation of sperm motility, while the principal piece controls hyperactivation. The shield produced by ALA over the midpiece protects the inner organelles from oxidative stress induced by free radicals, potentially originating from the varicocele (*Gawish, 2010*). In addition, ALA acts as a mitochondrial co-enzyme augmenting cytochrome C concentrations, and this enhances its membrane capacity (*Plotnikov et al., 2007*). Motility is highly dependent on the availability of ATP generated by the mitochondria, which may increase following ALA administration through the aforementioned mechanisms.

In this study, sperm DNA integrity was evaluated in individuals who had received 600 mg of ALA for 80 days: DNA fragmentation significantly decreased in both ALA- and placebo-treated individuals. Numerous studies have confirmed the increased levels of sperm DNA damage in men with varicocele, highlighting heat and hypoxia as the precipitating factors. DNA injuries have been associated with raised concentrations of ROS in individuals with varicocele (*Agarwal et al., 2016*).

To date, numerous studies have addressed the effect of medical supplementation in the form of either an alternative or an adjunctive therapy to varicocelectomy. For instance, in a controlled clinical trial including 102 individuals with varicocele, the authors' group concluded that treatment with Zaditen (ketotifen, a mast cell stabilizer) after subinguinal microembolization of varicocele improved various semen parameters as well as chromatin integrity and number of spontaneous pregnancies (*Azadi et al., 2011*). Moreover, researchers have investigated the role of either the sole or the combined use of various antioxidant compounds in this regard. Applying *N*-acetylcysteine (NAC) in combination with several other antioxidants (namely vitamins C, E and A), Paradiso Galatioto and colleagues showed a significant increase in sperm count in individuals with persistent oligozoospermia after surgical repair of varicocele (*Paradiso Galatioto et al., 2008*). The authors of the present study came to a similar conclusion when conducting an animal model study recruiting rats with surgically induced varicocele receiving a one-carbon cycle regimen (NAC, B vitamins, vitamin E, betalains and quercetin): sperm concentration, motility and abnormal morphology, DNA fragmentation and sperm lipid peroxidation had improved 2 months after varicocele induction (*Mohammadi et al., 2018*). These findings were confirmed in a clinical trial involving 35 infertile men with varicocele who received NAC for 3 months immediately after surgery. In this study, semen parameters and DNA integrity improved significantly in comparison to the control group (*Barekat et al., 2016*).

Several animal studies have investigated the administration of ALA in terms of infertility. Generally, these studies have indicated that ALA treatment in fertility-compromised rodents has a restorative effect against oxidative stress-induced damage, reducing ROS production (*Jana et al., 2014*; *Prathima et al., 2018*), improving sperm motility (*Ibrahim et al., 2008*; *Othman et al., 2012*; *Ren et al., 2018*; *Yeni et al., 2012*), intactness of the membrane (*Ren et al., 2018*) and DNA integrity (*Othman et al., 2012*; *Ren et al., 2018*), improving testicular architecture and function (*Lebda et al., 2014*; *Pınar et al., 2018*; *Prathima et al., 2018*) and restoring male reproduction (*Prathima et al., 2017*; *Prathima et al., 2018*; *Shen et al., 2016*). In line with these results, the current authors observed a significant improvement in sperm parameters and DNA integrity in surgically induced varicocele in Wistar rats after ALA treatment (*Shaygannia et al., 2018*).

To the authors' knowledge, only two studies to date have surveyed the effect of administering ALA on infertility in human participants (*Canepa et al., 2018*; *Haghighian et al., 2015*), but neither of these studies recruited individuals with varicocele. Despite lacking a relatively larger sample size, the present study is the first to evaluate the effects of ALA supplementation on semen samples and sperm quality in humans with varicocele. Canepa and colleagues conducted an uncontrolled trial on 143 subfertile men, 100 of whom completed the study course (*Canepa et al., 2018*).

Participants were given two tablets a day of Sinopol – two tablets containing ALA (800 mg), myo-inositol (1000 mg), folic acid (400 mg) and relatively small doses of betaine and vitamins B2, B6 and B12. Compared with the pre-medication samples, sperm concentration, number of spermatozoa, progressive motility, total motile sperm count and sperm morphology significantly increased after 90 days of treatment. The authors concluded that co-administration of myo-inositol and ALA could enhance sperm quality. However, because of limitations of the study design, they were not able to elucidate a causality between ALA treatment and improvement of semen parameters (*Canepa et al., 2018*).

In the only clinical trial on the subject, Haghighian and colleges, using a random design, gave 600 mg of ALA or the matching placebo to 44 oligoasthenozoospermic individuals on a daily basis. After 12 weeks of medication, sperm concentration, sperm count and motility showed a significant improvement in the group administered ALA. Moreover, the authors noticed a decline and an increase, respectively, in malondialdehyde and total antioxidant capacity concentrations in the ALA group (*Haghighian et al., 2015*), indicative of an alleviation of oxidative stress after taking the medication. Likewise, in the present study, there was a significant increase in sperm motility as well as progressive motility in the ALA group. Regarding oxidative stress markers, a decline in lipid peroxidation level was observed in both groups. However, Haghighian and colleagues did not eliminate the source of excessive ROS in the participants, which may have boosted the contrast in results between the case and control groups. In the current study, the participants underwent microsurgical varicocelectomy, which consequently abolished the leading cause of ROS production. An improvement in sperm motility and progressive motility was, however, still seen, indicating the advantages of ALA administration after varicocelectomy.

CONCLUSION

An 80-day course of ALA medication after surgery improves semen quality in individuals with varicocele. In this study, mean sperm motility and progressive motility significantly improved after the participants took ALA for approximately 3 months. Additional ALA treatment after surgical repair of varicocele could thus enhance sperm quality more efficiently than treatment with surgery alone. ALA supplementation could therefore be considered as an adjunct therapy to varicocelectomy. More extensive trials are needed to further investigate this association and the mechanisms involved.

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