ORIGINAL ARTICLE

The impact of vitamin E supplementation on semen parameters and pregnancy rates after varicocelectomy: a randomised controlled study

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Summary

In this study, we aimed to investigate the impact of vitamin E supplementation on semen parameters and pregnancy after varicocelectomy. Forty-five infertile male patients who were diagnosed with varicocele and underwent subinguinal varicocelectomy were included in the study. After performing subinguinal varicocelectomy, the patients were randomised into two groups: 22 receiving vitamin E for 12 months, and 23 as the control group without receiving any supplementation. The pre-operative parameters of semen analyses and pregnancy rates of both groups were compared with those of post-operative parameters. There were no statistically significant differences between the groups in terms of sperm count and motile sperm percentage, in pre-operative, post-operative 3rd month, post-operative 6th month and post-operative 12th month periods. Repeated-measures ANOVA was performed, and sperm count, percentage of change in sperm count, motile sperm count and percentage of change in motile sperm count of the groups were compared. The administration of vitamin E increased all of these parameters; however, they were not found to be statistically significant. In conclusion, vitamin E supplementation might improve the sperm parameters after varicocelectomy; however, further studies including larger number of samples are needed to make a proper decision on vitamin E supplementation after varicocelectomy.

Introduction

A varicocele is an abnormal dilatation of the internal spermatic veins, and is known as the most surgically correctable cause of infertility in males (Ding *et al.*, 2012). The rate of varicoceles in the general population is 15%, whereas it is 35% in males with primary infertility and 75–81% in males with secondary infertility (Greenberg, 1977). The exact association between diminished male fertility and varicocele is still unclear; thus, varicoceles continue to be a debated issue. Although a deterioration in semen analysis is considered to be the first indication for surgery in adults, the treatment of adolescent varicocele is indicated with testicular growth retardation and testicular pain, considering that these patients may suffer from impaired fertility in the future (Zampieri & Cervellione, 2008).

Vitamin E is the primary antioxidant component of the spermatozoa and a major cell membrane protector against the reactive oxygen species (Jaarin *et al.*, 2002; Lee *et al.*, 2008). Previously, it has been shown that vitamin E deficiency might cause impaired fertility in humans (Rengaraj & Hong, 2015). Besides, infertile men are at a greater risk of developing pathogenic levels of reactive oxygen species (Keshtgar *et al.*, 2012).

In this prospective study, we aimed at investigating the impact of vitamin E supplementation on semen parameters and pregnancy rates after varicocelectomy.

Material and methods

This study was approved by the institutional review board, and each patient's consent for the use of their information was taken in writing. Forty-five infertile male patients who were diagnosed with a left-sided clinical varicocele in the urology polyclinic, and for whom subinguinal varicocelectomy was planned, were included in this prospective study. The physical examination was performed in a warm room by applying the Valsalva manoeuvre, while the patient was in a standing position (Hudson, 1988). The diameter of the largest vein in the pampiniform plexus was measured, and the retrograde flow, both during the Valsalva manoeuvre and in a relaxed state, was detected by applying a colour Doppler ultrasound (CDU) to confirm the diagnosis. The diagnostic criteria for varicocele were the presence of two or more varicose veins in the relaxed state and retrograde flow for a duration of more than two-seconds during the Valsalva manoeuvre. The use of alcohol, tobacco or any drugs including vitamins were used as exclusion criteria. After performing subinguinal varicocelectomy, the patients were randomised into two groups; 22 of them received daily oral vitamin E capsules (2 × 300 mg) for 12 months, while the remaining 23 patients served as the control group and did not receive any supplementation. Ephynal soft gelatin capsule 300 mg (dl-alpha-tocopheryl acetate) (Roche®, Basel, Switzerland) was used daily as a supplemental treatment.

To evaluate the fertility status of the patients, background investigations and semen analyses were performed. The semen for the analyses was obtained from the men via masturbation after 3 or 4 days of sexual abstinence. Sperm concentration, motility and morphology were assessed using the World Health Organization guidelines (World Health Organization, 2010) at four points in time : before the varicocelectomy, and 3, 6 and 12 months after the varicocelectomy. Subinguinal varicocelectomy was performed under general anaesthesia using optical magnification (HEINE® Cx2.3 binocular loop; Dusseldorf, Germany) in all patients. The patient was placed in the supine position, and an approximately 3–4 cm subinguinal incision was made. The external oblique fascia is not opened in this technique. After exposing the subcutaneous fat, the spermatic cord was grasped and elevated with a Babcock clamp and placed on a Penrose drain. The varicose veins were separated and ligated while preserving the arterial and lymphatic vessels. In the post-operative follow-up period, semen analysis were performed again. The pre-operative parameters of the semen analyses of both groups were compared with those of the post-operative parameters. Additionally, the number of pregnancies of both groups were compared at the post-operative 12th month follow-up.

Statistical analysis

In descriptive statistics, mean ± standard deviation was used for numeric variables. The distribution of the data was analysed using the Kolmogorov–Smirnov test, kurtosis–skewness and box plot graphics. Paired comparisons were made by the Student's *t*-test. The impact of the groups and the varicocelectomy on the sperm count and the percentage of motile sperm was analysed by a repeated-measures ANOVA test. As the sperm count and the percentage of motile spermatozoon (motile sperm count) were different between the groups in t_0, these variables were accepted as 100% and therefore, the 'percentile change' in t_3, t_6 and t_{12} was calculated. Statistical significance was pre-defined as $P < 0.05$ for all tests. During the study set-up, the type 1 error was calculated as 0.05 and the power as 0.90, for two groups and four measurements (using the repeated-measures ANOVA test). The sample size was calculated as 56.

Results

A total of 45 patients were included in the study. The mean age of the patients was 25.8 ± 4.6 years, and the mean duration of unsuccessful attempts to obtain pregnancy was 2.8 ± 1.1 years. The patients were randomised into two groups; 22 of them received daily oral vitamin E capsules (2 × 300 mg) after the varicocelectomy for 12 months (Group1 – G1), while the remaining 23 patients comprised the control group and did not receive any medications after the varicocelectomy (Group2 – G2). There were no statistically significant differences between the groups in terms of age and duration of infertility ($M_{\text{age}}G_1 = 26.5 \pm 5$ years, $M_{\text{age}}G_2 = 25.2 \pm 4.3$ years, $t(1) = 0.981$ $P = 0.332$; $M_{\text{duration of infertility}}G_1 = 2.9 \pm 1$ years, $M_{\text{duration of infertility}}G_2 = 2.7 \pm 1.2$ years, $t(1) = 0.762$ $P = 0.450$).

The sperm count (mil ml^{-1}) and the percentage of motile spermatozoon (%) of the groups were compared in pre-operative (t_0), post-operative 3rd month (t_3), post-operative 6th month (t_6) and post-operative 12th month (t_{12}) periods. Also, the number of pregnancies of the groups at post-operative 12th month was compared. There were no statistically significant differences between the groups in terms of sperm count (mil ml^{-1}) and motile sperm percentage (%) in the pre-operative (t_0), post-operative 3rd month (t_3), post-operative 6th month (t_6) and post-operative 12th month (t_{12}) periods (see Table 1). Furthermore, no statistically significant difference was found in the number of pregnancies between the groups at the post-operative 12th month (number of pregnancies for $G_1 = 5$, $G_2 = 5$; χ^2 (1) = 0.006, $P = 0.936$).

The variables were analysed with the repeated-measures ANOVA.

As Mauchly's test indicated that the assumption of sphericity had been violated, F values were calculated with a Huynh–Feldt correction. The compared variables

Table 1 The comparison of sperm count (mil ml^{-1}), and percentage of motile sperm (%) between G1 and G2 in t_0, t_3, t_6 and t_{12}

	Groups	*n*	*M*	SD	*t*	*P*
Age (year)	G_1	22	26.5	5.0	0.981	0.332
	G_2	23	25.2	4.3		
Sperm count (mil ml^{-1})						
t_0	G_1	22	36.5	29.8	0.997	0.324
	G_2	23	28.2	25.7		
t_3	G_1	22	49.5	27.9	2.475	0.017
	G_2	23	30.6	23.0		
t_6	G_1	22	53.9	22.0	0.684	0.494
	G_2	23	48.0	34.2		
t_{12}	G_1	22	58.6	20.2	1.583	0.121
	G_2	23	47.2	27.2		
PMS (%)						
t_0	G_1	22	54.1	20.4	1.319	0.194
	G_2	23	45.2	24.6		
t_3	G_1	22	61.4	18.3	2.635	0.012
	G_2	23	42.5	28.7		
t_6	G_1	22	60.1	16.1	0.779	0.441
	G_2	23	55.0	26.9		
t_{12}	G_1	22	59.3	16.2	0.390	0.698
	G_2	23	57.1	20.2		

G1, Group 1 (E vitamine supplement); G2, Group 2 (comparison); PMS, Percentage of motile sperm.

of the groups were sperm count (mil ml^{-1}) (see Fig. 1), percentage of change in sperm count (%) (see Fig. 2), motile sperm count (mil ml^{-1}) (see Fig. 3) and percentage of change in motile sperm count (%) (see Fig. 4). When the groups were compared, it was seen that the administration of vitamin E increased all of these variables; however, they were not found to be statistically significant. When the sperm count (mil ml^{-1}) of t_0 is compared with the sperm count (mil ml^{-1}) of t_6 and t_{12}, a significant relationship was found between varicocelectomy and an increase in sperm counts. Comparing the change of percentage (%) in sperm counts of t_0 with t_3, t_6 and t_{12}, statistically significant differences were found. Also, the change of percentage (%) in sperm counts of t_3 was compared with those of t_{12}, and a significant difference was detected. No significant difference was found in repeated comparisons of motile sperm counts.

The change of percentage in motile sperm count (%) was analysed, and a statistically significant difference was found in t_0 when compared with t_3, t_6 and t_{12}. The details of the above-mentioned results are given in Table 2. The pregnancy rate in t_{12}, regardless of vitamin E supplementation, was found to be 22.2% (n = 10).

Discussion

Many theories have been put forward to explain developing varicoceles, including the incompetence of the venous valves, hydrostatic pressure difference between the testicular veins, the nutcracker effect and increased arterial blood flow to the testis at puberty that exceeds the venous capacity, resulting in venous dilatation (Goldstein & Eid, 1989; Sweeney *et al.*, 1991; Ener *et al.*, 2015). It appears that, when a varicocele develops, the venous stasis in the testes is disrupted, and there is an increase in venous pressure and a decrease in arterial blood flow, resulting in hypoxia that leads to oxidative stress.

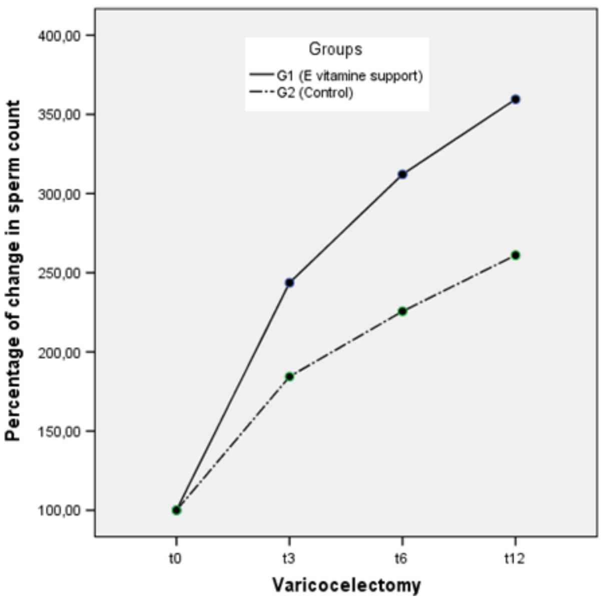

Fig. 1 Percentage of change in sperm count in G1 and G2 after varicocelectomy.

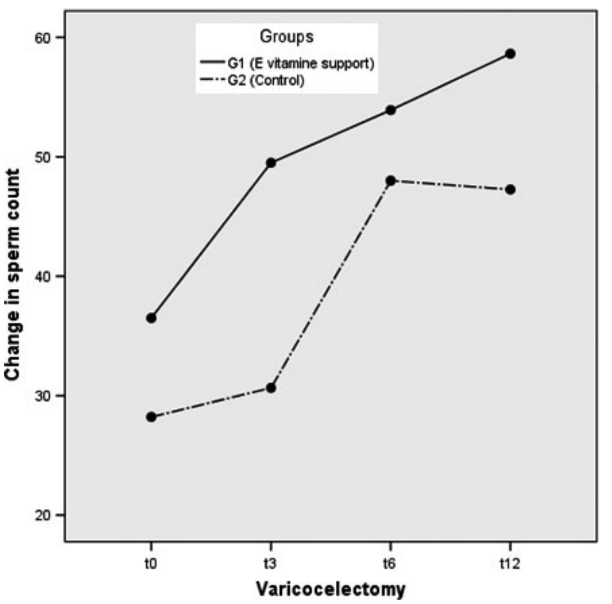

Fig. 2 Change in sperm count in G1 and G2 after varicocelectomy.

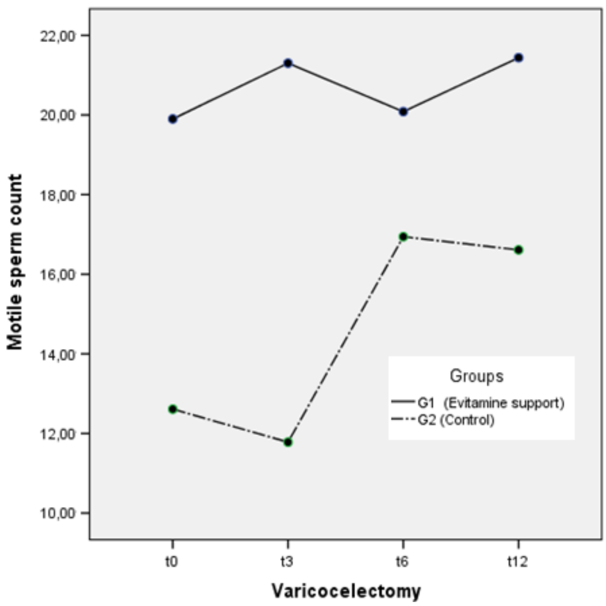

Fig. 3 Change in motile sperm count in G1 and G2 after varicocelectomy.

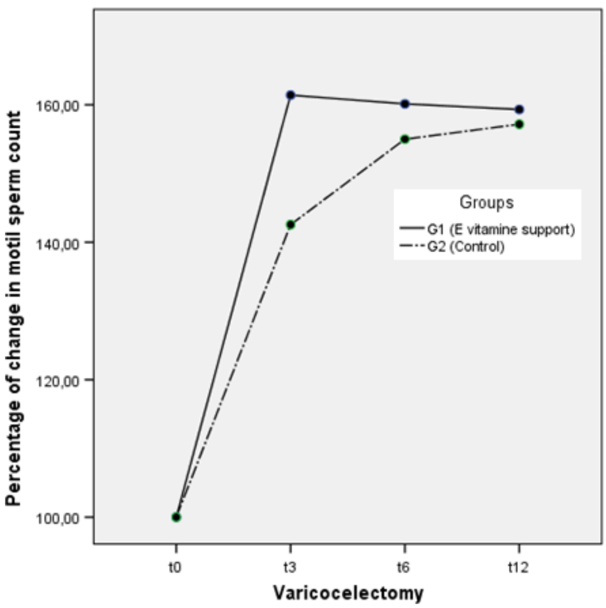

Fig. 4 Percentage of change in motile sperm count in G1 and G2 after varicocelectomy.

Previously, it has been suggested that infertility caused by varicocele had a relationship with oxidative stress (Sharma & Agarwal, 1996; Mostafa *et al.*, 2001; Cam *et al.*, 2004). A few chemoattractant agents were shown to enhance the production of reactive oxygen radicals in the spermatozoa of fertile and infertile men (Weese *et al.*, 1993). Although varicocele has been considered to be associated with infertility, the involved mechanism is not yet completely understood. However, the impact of varicocele on male fertility continues to be better understood with recent studies, particularly in regard to the positive impact of varicocelectomy on semen parameters and pregnancy rates. However, failing to improve fertility in all patients after varicocelectomy brings up the question of the necessity of a supplemental treatment.

It has been shown that vitamin E has positive effects on testis and sperm fertility, and the supplemental prescriptions containing vitamin E may improve the spermatozoa functions, both *in vivo* and *in vitro*, by reducing oxidative stress damage (Yousef *et al.*, 2003; Erat *et al.*, 2007). Even though oxidative damage is one of the proposed mechanisms in the etiopathogenesis of varicocele, it is still a debated issue whether or not vitamin E supplementation should be offered after varicocelectomy in patients with varicocele-associated infertility. In a prospective study, the potential *in vitro* protective effects of α-tocopherol on teratozoospermia motility, viability, acrosome reaction and DNA integrity was evaluated (Keshtgar *et al.*, 2012). The authors concluded that α-tocopherol might improve teratozoospermia motility and viability. However, its effect on DNA integrity and acrosome reaction ability as supplementation IVF culture media was unclear. Similarly, in another study, the effects of supplementation of ascorbic acid and vitamin E, and their combined effect on sperm characteristics, lipid peroxidation and seminal plasma enzymes of mature male rabbits, were evaluated (Yousef *et al.*, 2003). The results of this study indicated that the combination of ascorbic acid and vitamin E supplementation reduced the production of free radicals and could improve rabbit semen quality. Similarly, in a previous study, it was suggested that the application of antioxidants, including astaxanthin, vitamin A and vitamin E, decreased serum triglyceride and cholesterol levels, and improved the semen parameters in obese patients with subfertility or infertility (Mortazavi *et al.*, 2014). The authors proposed antioxidants in these patients as a new and efficient strategy to improve the quality of semen parameters and pregnancy rates. Contrary to the studies that show improved results of vitamin E analogues on sperm parameters, in several studies, it has been shown that vitamin E and vitamin C might act as a pro-oxidant instead of an antioxidant, particularly when used in high amounts. In a study evaluating the effects of antioxidant supplementation during Percoll preparation on human sperm deoxyribonucleic acid (DNA) integrity, Hughes *et al.* (1998) have shown that acetyl cysteine or ascorbate and alpha tocopherol together induced further DNA damage. Similarly, in another study, toxicity was reported when spermatozoa were incubated with a combination of vitamin C and

Table 2 The evaluation of the impact of varicocelectomy and vitamin E supplementation on sperm count (SC), change of sperm count (%SC), motile sperm count (MS) and change in motile sperm count (%MS) in t_0, t_3, t_6 and t_{12} by repeated-measures ANOVA

				Varicocelectomy		Groups		Varicocelectomy* Groups	
	Groups	*M*	SE	*F**	*P**	*F*	*P*	*F**	*P**
SC (mil ml^{-1})									
$_{a}t_0$	G1	36.5	29.8	15.830	0.000	2.700	0.108	1.346	0.264
	G2	28.2	25.7						
$_{b}t_3$	G1	49.5	27.9						
	G2	30.6	23.0						
$_{a,\ b}t_6$	G1	53.9	22.0						
	G2	48.0	34.2						
$_{a,\ b}t_{12}$	G1	58.6	20.2						
	G2	47.2	27.2						
%SC									
$_{c}t_0$	G1	100	0.0	15.342	0.000	1.123	0.295	0.888	0.408
	G2	100	0.0						
$_{c,\ d}t_3$	G1	243	276.5						
	G2	184	176.7						
$_{c}t_6$	G1	312	375.5						
	G2	225	137.6						
$_{c,\ d}t_{12}$	G1	359	392.9						
	G2	261	171.3						
MS (mil ml^{-1})									
t_0	G1	19.8	19.7	1.400	0.252	1.462	0.233	1.417	0.258
	G2	12.6	13.2						
t_3	G1	21.3	22.8						
	G2	11.7	13.4						
t_6	G1	20.0	16.6						
	G2	16.9	21.7						
t_{12}	G1	21.4	18.7						
	G2	16.6	19.3						
%MS									
$_{e}t_0$	G1	100.0	0.0	162.883	0.000	2.492	0.122	3.702	0.016
	G2	100.0	0.0						
$_{e}t_3$	G1	161.4	18.3						
	G2	142.5	28.7						
$_{e}t_6$	G1	160.1	16.1						
	G2	155.0	26.9						
$_{e}t_{12}$	G1	159.3	16.2						
	G2	157.1	20.2						

a, b, c, d, e: ANOVA, Bonferroni-corrected $P < 0.05$ for multiple comparisons.
M, mean; SE, standart error; SC, sperm count; %SC, % change in sperm count; MS, motile sperm count; %MS, % change in motile sperm count.

vitamin E, resulting in the exacerbation of DNA damage (Donnelly *et al.*, 1999). In our study, it is considered that the insignificance of the increase in sperm parameters in the vitamin E supplementation group might be attributed to the high amount of vitamin used. The dosage of Ephynal is recommended as 100 or 300 mg day^{-1}, and the dose may be raised up to 1000 mg day^{-1} safely when necessary. Therefore, further investigations are needed to determine the optimal vitamin E dose in infertile men.

In the present study, we found an improvement in both groups in terms of sperm concentration and motility after varicocelectomy compared to the pre-operative parameters. However, the repeated-measures ANOVA revealed that the factor that contributed to this improvement was varicocelectomy surgery alone. As a result, two variables have been evaluated in terms of having an impact on improved sperm parameters and pregnancy rates. Although the sperm parameters improved in all the control periods, we could not achieve a statistically significant improvement with vitamin E supplementation. Several nutrients, including vitamins, minerals, enzymes and trace elements, might be beneficial in sperm cell metabolism, DNA synthesis during spermatogenesis, proliferation and antioxidative protection. However, the treatment of infertile patients who are candidates for varicocelectomy should primarily be focused on for this surgery.

Theoretically, these supplementations might be taken by subfertile subjects to ameliorate the sperm characteristics, and to improve the potential of fertility, with no adverse effects (Wirleitner *et al.*, 2012). Similar to our study, in the literature there exist a few studies that show no effect of these supplementations on sperm characteristics, despite their antioxidant effect (Raigani *et al.*, 2014).

Of note, our cohort was not without limitation. During the study set-up, the sample size was calculated as 56. However, 11 patients who could not use vitamin E regularly, or did not come to visit in control periods, were excluded from the study. Therefore, the study was completed with the remaining 45 patients. Consequently, to provide a statistically significant amelioration in semen analysis and, therefore, to obtain improved pregnancy rates with optimal vitamin E dosage, further studies with large samples must be investigated.

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