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The effect of adjuvant vitamin C after varicocele surgery on sperm quality and quantity in infertile men: a double blind placebo controlled clinical trial

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ABSTRACT

Varicocele is one of the most common causes of male infertility and spontaneous pregnancy rate after varicocelectomy is only about 30%. The most important seminal antioxidant is vitamin C but recent studies about the effects of vitamin C on spermatogenesis are controversial; therefore, we decided to evaluate its role after varicocelectomy. In a double blind randomized controlled clinical trial, 115 men with infertility and clinical varicocele with abnormal semen analyses were recruited. After surgery, the intervention group received vitamin C (250 mg bid) and the control group received placebo for three months. Mean sperm count, motility, and morphology index of two semen analyses (before and after surgery) were compared between the two groups. Univariate general linear model and stepwise linear regression were used in analysis. The mean age (±SD) of participants was 27.6±5.3 years. Vitamin C group had statistically significant better normal motility (20.8 vs. 12.6, P=0.041) and morphology (23.2 vs. 10.5, P<0.001) than placebo group. Considering the values prior to surgery as covariate, vitamin C was not effective on sperm count (P=0.091); but it improved sperm motility (P=0.016) and morphology (P<0.001) even after excluding the confounding effect of age (P=0.044 and P=0.001, respectively). Vitamin C was also an independent factor in predicting motility and normal morphology after surgery. Ascorbic acid can play a role as adjuvant treatment after varicocelectomy in infertile men.

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INTRODUCTION

The overall prevalence of infertility is up to 15% of couples and in half of the cases male factor is involved. Infertility has had an increasing trend during recent decades in Iran (1). In addition, the quality of semen in men has declined during the past few decades (2) and impaired sperm function has been considered as the most common cause of infertility (3).

Infertility is an important issue for couples with sometimes severe consequences. The treatment of infertility requires a multi-faceted therapeutic approach, consisting of improvements in environmental and occupational risk factors, nutritional imbalances, antioxidants, medical therapies, surgeries, and assisted reproductive technology (ART).

Selvakumar E et al. established the effect of lipoic acid (an antioxidant like substance) in improvement of semen quality and reducing the oxidative stress and DNA damage induced by cyclophosphamide in rats (4). Other antioxidants like vitamin C (ascorbic acid) and vitamin E have been proved to ameliorate the oxidative stress and sperm toxicity induced by endosulfan in rats (5). One before-after study evaluated the effects of vitamins C and E (each one 500 mg/day) and demonstrated no efficacy of these antioxidants on DNA peroxidation in fertile and subfertile dogs; but, this controversial result could have been due to the very low sample size (6). The protective effects of ascorbic acid on p-dimethylaminoazobenzene induced genotoxicity and cytotoxicity have also been confirmed in mice (7).

Another study with a low sample size (eight healthy volunteers with azoospermia) illustrated that administration of testosterone propionate over a four-week period did not change the level of ascorbic acid in human semen even after 12 injections (8).

A recent review showed that vitamin C has controversial effects on sperm parameters and pregnancy rate in subfertile males with idiopathic oligoasthenoteratozoospermia (9).

Because the effect of vitamin C in spermatogenesis is still controversial and the proof for its clinical use challenging, we decided to evaluate its role as a supplement (as an antioxidant nutrient) after varicocele surgery (known as improving a definite cause of infertility). Previous population-based study on Iranian couples showed varicocele as a main risk factor for infertility (1).

Varicocele is one of the most common causes of male infertility and although its surgical repair results in improved spermatogenesis in 70% of patients, spontaneous pregnancy rate is only about 30% after varicocele repair without other treatments (10). Regarding the high prevalence of varicocele and suboptimal results of its surgical repair in improvement of pregnancy rates, additional treatment modalities are necessary. Recently, oxidative stress has been proposed to be an important factor in the pathophysiology of varicocele-induced infertility. The most important seminal antioxidant is ascorbic acid (vitamin C) which comprises 65% (11, 12) of antioxidant capacity of semen and is currently used in-vitro to improve sperm quality in infertility clinics. Therefore, we decided to evaluate the effects of supplemental vitamin C medical therapy after varicocelectomy for its probable improvement of infertility.

In this double-blind placebo controlled clinical trial, we assessed the effects of adjuvant vitamin C after varicocele surgery on sperm quality and quantity in infertile Iranian men.

MATERIALS AND METHODS

In this double blind randomized controlled clinical trial, we recruited infertile males who had palpable varicocele (grades 2 and 3) from February 2010 to May 2011. In the presence of a palpable varicocele in physical examination and accompanying abnormalities in count, motility, or morphology of sperm in two separate semen analyses, they became candidates for surgery.

Other inclusion criteria were: age range between 18 and 50, weight between 50 and 100 kilograms, and being married; the negative inclusion criteria were: absence of azoospermia, diabetes mellitus, hormonal disorders (according to medical history and clinical examination), tobacco smoking, opium or recreational drugs addiction, regular usage of vitamins or nutritional supplements, active or chronic genitourinary infection (based on medical history, physical examination, semen and urine analysis), history of peptic ulcer, and previous reaction to or intolerance to vitamin C. Exclusion criteria were missed follow-up, incorrect usage of the capsules, demonstrating side effects due to vitamin C, and commencement of smoking or opium addiction during the follow-up period, and delayed complications of varicocelectomy such as: hydrocele, recurrence of varicocele, and testicular atrophy.

In this study, infertility was defined as the inability to achieve a pregnancy after one year of unprotected regular intercourse. Normal semen sample was considered as defined by WHO (1999) (13): volume more than or equal to 2.0 mL, sperm count greater than or equal to 20 million per mL, motility (movement of the sperm) value greater than or equal to 50% with forward progression (grades “a+b”), or greater than or equal to 25% with rapid progression (grade “a”) within 60 minutes of ejaculation, morphology greater than or equal to 30% with normal forms, and white blood cell count less than 1 million per mL. All semen samples were obtained by masturbation in the lab and were analyzed in a reference laboratory (Sina Laboratory of Arak) by an experienced specialist in pathology and clinical laboratory medicine. Sperm counts were measured by the hemocytometer method using a Neubauer sperm counting chamber after immobilization of spermatozoa with neutral formalin. Sperm motility was calculated by scanning several fields under high-dry objective, until a total of at least 200 spermatozoa were registered. Morphology was assessed by differential counts of morphologically normal and abnormal spermatozoa types on Pap-stained slides.

The surgical approach was open inguinal method (Ivanissevich) which does not need special equipment and is still the most prevalent approach in Iran. All patients in both groups had varicocelectomy without image magnification. Our secondary complications were rare and they were excluded from the study and only those with clinically cured varicocele were selected for the final analysis. If there was any other unaccounted factor from Ivanissevich method that could affect the results, since both groups had the same type of operation, it would be balanced in the two groups.

Randomization

After including 115 eligible patients that had surgery, we randomly allocated these patients into vitamin C (intervention) or placebo group according to simple randomization method using Excel 2010 software (Microsoft Corporation, Washington, USA) by "RANDBETWEEN(0;1000000)" function. The allocation sequence was produced by our statistician and was delivered to our pharmacist. Participants were enrolled by the two executive urologists who were unaware of the results of the allocation table. Then based on the numbers in the sequence being odd or even each new patient after varicocele surgery was assigned to intervention or placebo group by our pharmacist who supplied the drugs. The ratio of placebo to intervention group was 1.5. Therefore, 46 cases were allocated to vitamin C and 69 cases to placebo group. Lost to follow-up patients were substituted with matching new cases. The intervention group received capsules containing 250 mg of vitamin C (OSVAH Pharmaceutical Co. Tehran, Iran) in two daily doses, one in the morning and one in the evening. Controls received starch-filled capsules (Osveh Co., Tehran, Iran) as placebo. Both groups received capsules for three months after surgery.

Then, two separate semen analyses were obtained, each after 3 days of abstinence. Samples were collected inside the laboratory by masturbation into sterile containers and allowed to liquefy at 37°C for 30 min. Mean sperm count, motility, and morphology index of each of the two semen analyses (before and after surgery) were compared between two groups as our primary outcomes.Moreover, complications of surgery, varicocele grade, age and weight were determined.

Motility was defined as mean percent of good motility (type A plus type B) divided by all motility types (Type A+Type B+Type C+Type D). These types are defined as: Fast progressive (Type A), Slow progressive (Type B), Non-progressive (Type C), Immotile (Type D).

Statistical analysis

Descriptive indices like percent, mean, standard deviation (SD) were employed in this study. We also used chi-square for evaluating association between categorical variables, repeated measure ANOVA for evaluating the simultaneous effect of both treatments (vitamin C or placebo) and time (before and after surgery). Student-t test was used for comparing quantitative variables in different groups. We used univariate general linear model for comparing the status of quantitative indices of semen analysis after varicocelectomy considering baseline values as covariates (omitting their confounding effect on post-surgery values) and stepwise linear regression for predicting post-surgery semen indices. P-value less than 0.05 was considered statistically significant. All statistical analyses were executed by SPSS 20 (SPSS Inc., Chicago, Illinois, USA).

Ethics

We explained the aim and method of the study for the patients. All cases were aware of and accepted that they will receive vitamin C or placebo; however, they did not know which drug (intervention or placebo) they would receive. All cases signed an informed written consent form before entering the study and they were informed that they could terminate their cooperation with use whenever they wanted without any consequences.

The trial is registered in Iranian Registry of Clinical Trials (IRCT) site (www.irct.ir), with the code number "IRCT201103042134N2".

RESULTS

Mean age (±SD) of the participants was 27.6±5.3 years. Five patients from the intervention group and eight patients from controls did not show-up for the follow-up visits and were substituted with matched new cases. We performed the intention to treat analysis in all parts of the study. The grades of varicocele were 2 and 3 in 9 (19.6%) and 37 (80.4%) of cases in vitamin C group and 10 (14.5%) and 59 (85.5%) cases in placebo group, respectively. The grade of varicocele was not statistically different between vitamin C and placebo groups (P=0.473). Totally, in both groups, in 35 (30.4%) cases the sperm count worsened after the intervention. Also, in 22 (19.1%) and 14 (12.2%) cases the motility or normal morphology declined, respectively.

Basic characteristics of the two groups are presented in Table-1. As it shows, the intervention group was younger; but semen analysis indices were not significantly different between the two groups.

Evaluating the simultaneous effect of treatment and time by repeated ANOVA showed that vitamin C group had statistically significantly better status than the placebo group except for the sperm count which although was higher in the intervention group, the difference was not statistically significant (Table-2). Similarly, we compared the mean difference of values of different indices of semen analysis (for example: mean of differences of sperm count before and after surgery in each intervention or placebo group) between the two groups using student t--test (Table-3). Again, motility and morphology, but not sperm count, were significantly better in vitamin C as compared to the placebo group.

Using univariate general linear model, we considered the values of before surgery as covariate and evaluated the effects of vitamin C in comparison with placebo on sperm indices. According to this analysis, vitamin C was not effective on sperm count (P=0.091), even after excluding the confounding effect of age (P=0.151). But, vitamin C was effective on sperm motility (P=0.016) and morphology (P<0.001) even after excluding the confounding effect of age (P=0.044 and P=0.001, respectively).

Table 1 - Basic characteristics of the intervention and placebo groups before treatment*

	Group		Sig.
	Vitamin C	Placebo	
Age, year	26.3±4.9	28.5±5.5	0.025
Count, million/mL	42.5±28.4	37.5±28.1	0.362
Motility, %	33.8±15.5	32.4±14.3	0.618
Morphology, %	52.2±16.1	57±18.2	0.147
Oligozoospermia, %	30.4	26.1	0.610
Asthenozoospermia, %	89.1	91.3	0.698
Teratozoospermia, %	4.3	7.2	0.524

* = All variables refer to the condition of patients before surgery, values are presented as mean±SD

Table 2 - Comparison of the results of the surgery between the intervention and placebo group*

	Group		Sig.
	Vitamin C	Placebo	
Mean count, million/mL	58.4±24.3	48.7±27.8	0.328
Mean normal motility**, %	54.5±18.3	44.9±21.4	**0.041**
Mean normal morphology, %	75.3±13.1	67.5±16.4	**<0.001**
Normal count, %	95.7%	81.2%	**0.025**
Normal motility, %	67.4%	44.9%	0.066
Normal morphology, %	100.0%	98.6%	0.033

* = Values are presented as mean±SD; ** = Sum of (a+b) motility type

Table 3 - Comparing the mean differences of indices before and after surgery status between the intervention and placebo groups*

	Group		Sig.
	Vitamin C	Placebo	
Count[a], million/mL	15.9±25.3	11.1±26.2	0.328
Motility[b], %	20.8±21.3	12.6±20.5	0.041
Morphology[c], %	23.2±18.7	10.5±15.8	<0.001

* = all values are presented as mean±SD

a = (mean count after surgery - mean count before surgery); b = (mean motility after surgery - mean motility before surgery); c = (mean normal morphology after surgery - mean normal morphology before surgery)

Stepwise linear regression showed that sperm count before intervention could significantly predict post-intervention sperm count (Table-4). The regression formula for count after surgery without vitamin C was:

Sperm count after surgery = (0.53 × sperm count before intervention) + 31.6

It shows that varicocelectomy by itself can increase the sperm count from 10 to 36.9 million/mL irrespective of administration of vitamin C supplementation.

Motility after surgery was also predictable by motility and morphology before intervention and vitamin C or placebo (Table-4). The regression formula was:

Sperm motility after surgery = (0.425 × motility before intervention) + (10.154 × D#) + (0.238 × morphology before intervention) + 17.6

D="1" if the intervention was vitamin C and "zero" if it was placebo

The value of normal morphology after surgery was only dependent on morphology before intervention (Table-4). Its regression model was:

Sperm morphology after surgery = (0.404 × morphology before intervention) + (9.758 × D#) + 44.5

D="1" if the intervention was vitamin C and "zero" if it was placebo

Adjusted R-squares of these models were 0.307, 0.170 and 0.248 for count, motility and morphology, respectively. It shows that the models for predicting count and morphology had higher accuracy, respectively, irrespective of the number of independent variables included in the model. In each model, higher standardized beta

Table 4 – Stepwise linear regression model of post-surgical indices of semen analysis.

Dependent variable	Independent variable	Unstandardized Coefficients		Standardized Beta	Sig.	Model	
		Beta	SE of Beta			R-square	Sig.
Count	Sperm count before intervention	0.53	0.074	0.56	<0.001	0.31	<0.001
Motility	Motility before intervention	0.425	0.120	0.303	0.001	0.192	<0.001
	Giving vitamin C	10.154	3.630	0.241	0.006		
	Normal morphology before intervention	0.238	0.102	0.201	0.022		
Morphology	Normal morphology before intervention	0.404	0.073	0.452	<0.001	0.261	<0.001
	Giving vitamin C	9.758	2.604	0.307	<0.001		

showed the more important predictors of the value of dependent variables.

DISCUSSION

Totally, in 30.4%, 19.1% and 12.2% of cases the sperm count, motility and normal morphology worsened after the intervention, respectively. Vitamin C was not effective on sperm count; but effective on sperm motility and morphology even after excluding the confounding effect of the age. Despite the statistically different age between vitamin C and placebo groups, the age difference of 2.2 years does not seem clinically important and based on the mean semen parameters before the surgery the two groups were comparable.

All our analyses in this study showed that vitamin C supplementation had significant positive effects on sperm motility and morphology; but not in sperm count in infertile young adult males with low quality sperm. It means that ascorbic acid could positively affect qualitative and not quantitative characteristics of sperm analysis.

It is obvious that better sperm quantity or quality before surgery resulted in better situation after surgery for most patients (totally and not individually because the model predicts the mean indices for all patients and not for each individual). However, the aim of our study was to answer whether vitamin C had an additive role for improvement of semen parameters after varicocelectomy, since our regression models showed vitamin C could increase post-operative motility and normal morphology but not sperm count. These findings confirmed the results of our univariate analyses.

One Egyptian study has shown that smoking significantly decreases seminal plasma ascorbic acid and it is also associated with reduced semen parameters which can worsen male fertilization potential (14). Subsequently, we excluded smokers and drug addicts from our study to restrict the confounding effect of smoking and related habits.

Colagar AH and Marzony ET showed that fertile smoker men have lower concentrations of seminal ascorbic acid than fertile non-smokers. This study also illustrated that infertile smokers have lower vitamin C level than fertile smoker men (12).

One observational study on healthy non--smoking American volunteers established that higher intake of antioxidants consisting of vitamin C by normal diet or supplement usage is associated with higher sperm count and motility (15). Our study was a randomized controlled clinical trial (RCT) and included cases after varicocele surgery, thus these differences make these two studies non-comparable. However, both results support the same issue which is effectiveness of vitamin C on fertility indices of the semen.

The evaluation of the effects of different dosages of vitamin C on infertility needs more sophisticated studies; however, one study has shown that an increase in vitamin C dosage from 200 to 1000 mg per day significantly improved sperm quality (16).

Before surgery, most of our cases had poor sperm motility but over two-thirds had normal sperm counts. Thus, the improvement in semen indices with vitamin C cannot be generalize to all patients and more studies in different populations with different pre-surgery patterns of sperm analysis especially in those with more severe oligozoospermia, are needed.

Varicocelectomy is not a definite treatment for infertility in cases who have varicocele (17) and in some of our cases sperm analysis even worsened after surgery. Since none of our patients had testis atrophy, vascular damage during surgery could not be a culprit and progressive degeneration of spermatogenesis as the natural course of varicocele should have been the cause.

One study on 30 subfertile men showed that varicocelectomy decreased oxidative damage in sperm DNA and improved the quality of sperm (18). Another study on 13 infertile patients demonstrated similar results to our study; but, due to small sample size their findings were not statistically significant (19).

Pesticides produce sperm toxicity and one animal study on mice showed that vitamin C can prevent these effects. This study proved that higher doses of vitamin C resulted in more significant amelioration of sperm count and morphology after administration of pesticides (20).

Audet I et al. demonstrated that water soluble vitamins improved semen production and motile sperm counts in boars (21).

Another study on rainbow trouts showed that sperm concentration and motility (fertility) decreases in fishes with ascorbic acid deficiency and vitamin C is important for male fish reproduction (22).

On the other hand, Donoghue AM and Donoghue DJ demonstrated that vitamin C had no effect on sperm viability, membrane integrity, or sperm motility index in turkeys at any concentration or time period tested (23). Moreover, in one RCT on 31 infertile men with asthenozoospermia, administration of high-dose oral vitamin C (1000 mg) and E (800 mg) for 56 days did not show any effect on semen parameters or pregnancies during the treatment period (24). It should be noted that these patients' infertility was due to causes other than varicocele and the only treatment they received were vitamin supplements; therefore, this study could not be compared to ours.

A recent study has shown that vitamin C partially reduced semen oxidative stress level and number of abnormal sperms; but, did not improve sperm motility in hyperglycemic rats (25).

One study in Spain illustrated that 1 g vitamin C and 1 g vitamin E daily for two months in 38 cases with elevated percentage of DNA-fragmented spermatozoa decreased fragmentation in 76% of cases. This study showed no effect on fertilization and cleavage rates or on embryo morphology after ICSI; however, clinical pregnancy and implantation rates improved (26).

At present, a large proportion of the Iranian population ingests insufficient amounts of antioxidants like vitamin C. According to a recent study, vitamin C intake was lower than the recommended daily allowance (RDA) in 28% of urban men and 55% of men living in rural areas of Iran. In 6% of urban men and 13% of rural men, the daily intake of vitamin C was lower than the lowest threshold intake (LTI) (27).

In 2010, the mean intake of fruit and vegetable servings per day was 1.74 ± 1.16 in 102 elderly Iranian men (28) which is far from the minimum recommended five servings of fruits and vegetables per day in 2002 (Centers for Disease Control and Prevention (CDC)) (29). Our findings suggest that a healthy diet with sufficient supplements might be an inexpensive and safe way to improve semen quality and fertility.

There have not been many studies concerning the role of vitamin C in the treatment of male infertility and still controversies exist due to different basic characteristics of the study samples, cause of infertility, small sample sizes, comorbidities, hormonal status, and other co-variables that potentially influence the results in such clinical studies.

Since vitamin C is a safe and cheap supplement with possible therapeutic effects in some cases of male infertility, further studies are necessary.

Limitations

We did not assess the levels of ascorbic acid in serum or semen of the patients and demonstration of the relation of seminal vitamin C levels with improvement in sperm indices could have supported our findings.

We used conventional semen analysis which is not an accurate predictor of fertility as Purvis K. and Christiansen E have shown that many cases with below normal values of sperm are able to impregnate their partners (30). The best outcome for evaluation of improvement in infertility is pregnancy rate which needs long term follow-ups.

Strengths

Because of variations between multiple samples from the same person, we obtained two separate samples from each case and mean values of all indices were used in all analyzes. Our inclusion and exclusion criteria have limited the reproducibility of our results to nonsmoker young infertile males with clinical varicocele, while it increased the accuracy of the study by removing many confounders which might have distorted the results.

Thirty six percent of couples with low sperm counts are able to achieve natural conception without any therapy. Thus, studies about the effectiveness of different therapies in male infertility without control groups have doubtful results (17). As a result, we included placebo as control group in our study.

Most of the mentioned studies concerning the effects of vitamin C on male infertility in human subjects had small sample sizes. However, our larger sample size helped in obtaining statistically significant results.

CONCLUSIONS

Despite the controversies about the effects of ascorbic acid on spermatogenesis, most studies suggest a positive effect. The present study showed that vitamin C can play a role as adjuvant treatment after varicocelectomy in infertile men specifically on quality and not quantity of sperm.

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CONFLICT OF INTEREST

None declared.

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