Impact of Spirulina Supplementation on Semen Parameters in Patients with Idiopathic Male Infertility: A Pilot Randomized Trial

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Purpose: To evaluate the efficacy of therapy with spirulina supplement on semen parameters in patients with idiopathic male infertility.

Materials and Methods: A total of 40 men with idiopathic infertility were randomly assigned into two groups. Group A received 2 g spirulina supplement as well as conventional regimen for the treatment of infertility selected by their physician (220 mg/day zinc sulfate, 500mg/day L-carnitine, and 50 mg/day clomiphene) during 12 weeks of the study, while group B received placebo plus conventional therapy during the study period. Semen parameters were analyzed at baseline and at the end of the study as a primary endpoint. The secondary endpoint was the rate of pregnancy occurring in the patients. wives.

Result: No significant differences in semen parameters were observed between the spirulina and control groups [count (16.43 vs. 46.00, $P = .164$), motility (51.00 vs. 48.7, $P = .008$), and morphology (47.50 vs. 15.00, $P =$ NA)]. Our results showed a pregnancy rate of 5% in the spirulina group versus 0% in the control group.

Conclusion: This pilot randomized trial provides initial evidence on the possible beneficial effects of spirulina mainly in patients with impaired sperm motility or morphology. Due to the limited sample size, further larger randomized trials not only at the level of semen parameters but at the scope of paternity are required to confirm these potential benefits.

Keywords: idiopathic male infertility; pregnancy; semen parameters; spirulina supplement.

INTRODUCTION

Idiopathic infertility is one of the most common reproductive disorders in men worldwide. Approximately one out of ten couple is infertile and infertility in about half of them is the result of male factors. The pathogenesis and etiology of infertility are not completely understood in most cases; therefore, it is named idiopathic infertility. This disorder results from interaction between genetic and environmental factors and can be easily manipulated.[1,2] Because of the society's shift toward industrialism during the last decades, concerns have arisen about the effect of higher exposure to chemicals and radiations in everyday life and workplace that could also lead to infertility.[3,4]

Although some progression has been reported in the treatment of infertility in the literature, there is still no standard treatment with acceptable efficacy for this problem. Vitamins and minerals as efficient anti-oxidants help to protect the body from oxidative damage. Therefore, these supplements have been studied for the management of fertility problems in both men (oligospermia) and women (anovulation).[5,6]

Spirulina is a cyanobacterium blue-green micro-alga. It has protein content of 50-70% of total dry weight and also it is full of vitamins such as A, E, D, K, B1, B2, B3, B6, B12, panthotenic acid, folate and minerals such as mg, Zn, Fe, Cu, and selenium.[7,8] It has been mentioned as a safe edible alga. Various studies on mice have shown no adverse effects on non-pregnant and pregnant mice that were given spirulina in their daily routines.[9,10]

To our best of knowledge, no clinical trial has been done to address the effect of spirulina on idiopathic male fertility. Therefore, in this pilot trial, we investigated the effect of sprirulina as a rich supplement for the management of male patients with infertility for the first time.

MATERIALS AND METHODS

Study design

This is a pilot randomized clinical trial with double-blind study design that was conducted in two infertility clinic, Shahid Motahari and Shahid Faghihi, affiliated to Shiraz University of Medical Sciences (SUMS), Shiraz, Iran. The study was approved by the ethical committee of SUMS. The identifier code of

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Table 1. Semen parameters analysis at baseline and after 12 weeks of treatment in two study groups.

Groups	Baseline				12 weeks				*P* value			
	Control (N = 20)		Spirulina (N = 20)		Control (N = 20)		Spirulina (N = 20)					
	Mean ± SD	N (%)	Mean ± SD	N (%)	Mean ± SD	N (%)	Mean ± SD	N (%)	Baseline control-baseline Spirulina	Baseline control-12 weeks control	12 weeks control-12 weeks Spirulina	Baseline Spirulina-12 weeks Spirulina
Oligospermia (Sperm count) (10^6/mL)	16.23 ± 3.69	3 (15)	5.42 ± 2.25	3 (15)	46.00 ± 29.13	3 (15)	16.43 ± 7.72	3 (15)	.0123	.153	.164	.076
Asthenosperima (Sperm motility) (% motile)	33.04 ± 9.72	10 (50)	37.3 ± 10.97	8 (40)	48.7 ± 13.82	10 (50)	51.00 ± 7.12	8 (40)	.39	.008	.675	.01
Teratospermia (Sperm morphology) (% normal)	19.0 ± 0.00	1 (5)	5.5 ± 0.5	2 (10)	15 ± 0.00	1 (5)	47.5 ± 10.5	2 (10)	NA	NA	NA	.02
Oligospermia + Asthenospermia	10.3 ± 4.59 + 21.42 ± 15.94	4 (20)	14.90 ± 3.1 + 31.34 ± 3.66	2 (10)	17.75 ± 13.2 + 38.25 ± 8.31	4 (20)	6.88 ± 1.28 + 35.50 ± 6.50	2 (10)	.28 .46	.327 .110	.334 .703	.077 .512
Asthenospermia + Teratospermia	40 ± 0.00 + 15 ± 0.00	1 (5)	30.00 ± 0.00 + 15.00 ± 0.00	1 (5)	55 ± 0.00 + 65 ± 0.00	1 (5)	40.00 ± 0.00 + 65.00 ± 0.00q	1 (5)	NA NA	NA NA	NA NA	NA NA
Oligospermia + Asthenospermia + Teratospermia	17 ± 0.00 + 29 ± 0.00 + 5 ± 0.00	1 (5)	8.03 ± 5.30 + 18.00 ± 11.8 + 13.00 ± 3.31	4 (20)	28 ± 0.00 + 20 ± 0.00 + 25 ± 0.00	1 (5)	10.9 ± 7.85 + 21.75 ± 14.21 + 35.00 ± 15.00	4 (20)	NA NA NA	NA NA NA	NA NA NA	.566 .698 .028

Abbreviations: NA, Not Assigned

(IRCT) is IRCT2016081320441N5. As to the ethics, all participants were counseled about the possible efficacy and side effects of spirulina and their consents were obtained.

Study population

This trial was performed from June 2015 to June 2016. All patients with male factor-infertility and poor semen parameters were considered.

Male infertility was diagnosed if one or more standard semen parameters were below the cutoff levels accepted by WHO (1999) (sperm density less than 20 *106/mL, sperm motility less than 50%, and normal morphology less than 30%)[11]. To eliminate possible adverse effects of various factors on spermatogenesis, all the participants had at least two semen analysis performed 3 months apart.

Inclusion and exclusion criteria

Inclusion criteria were the ages between 20-40 years old of participants and their wives, abnormal semen parameters, and documentation of fertile female partner. exclusion criteria were as follows: known medical (varicocele or cryptorchidism) or surgical condition which can result in infertility, a history of cancer chemotherapy, body mass index 30kg/m^2 or greater, a history of alcohol, drug, or other substance abuse, administration of androgens, anti-androgens, and immunosuppressant, severe kidney (serum creatinine greater than 2.0 mg/dL) and liver insufficiency (serum bilirubin greater than 2.0 mg/dL), Azoospermia, and endocrinopathy.

Procedures

A total of 40 patients with idiopathic male infertility were enrolled in the study. Patients were randomized into group A (spirulina group, n=20) and group B (control group, n=20) by simple randomization. All participants were asked to complete occupational and lifestyle questionnaire face to face. Presence of varicocele was determined by doppler ultra-sonography of the scrotum with the valsalva maneuver. Patients in Group A received 2 g spirulina supplement (Far East microalgae Ind. co., Ltd, Taiwan) as well as conventional regimen for the treatment of infertility selected by their physician (220mg/day zinc sulfate,[12] 500mg/day L-carnitine,[13] and 50mg/day clomiphene[14] during 12 weeks of the study, while group B received placebo plus conventional therapy during the study period. Patient's compliance was assessed by comparing the number of pills ingested and the number of days between dispensing visits. The follow up visits were in weeks 4, 8, and 12 after treatment administration, during which the patient's compliance were evaluated. The investigator, responsible to provide drugs for patients and follow-up them, was blinded.

Evaluations

Two standardized semen samples were collected from all patients at baseline and after 12 weeks of treatment. The samples were obtained at home by masturbation into polypropylene containers after 3 to 5 days of abstinence and delivered to laboratory within 1 hour after production. Semen parameters were analyzed blinded by two laboratory technicians at baseline and at the end of the study as a primary endpoint. The secondary endpoint was the rate of pregnancy occurring in the patients ' wives confirmed by a positive blood pregnancy

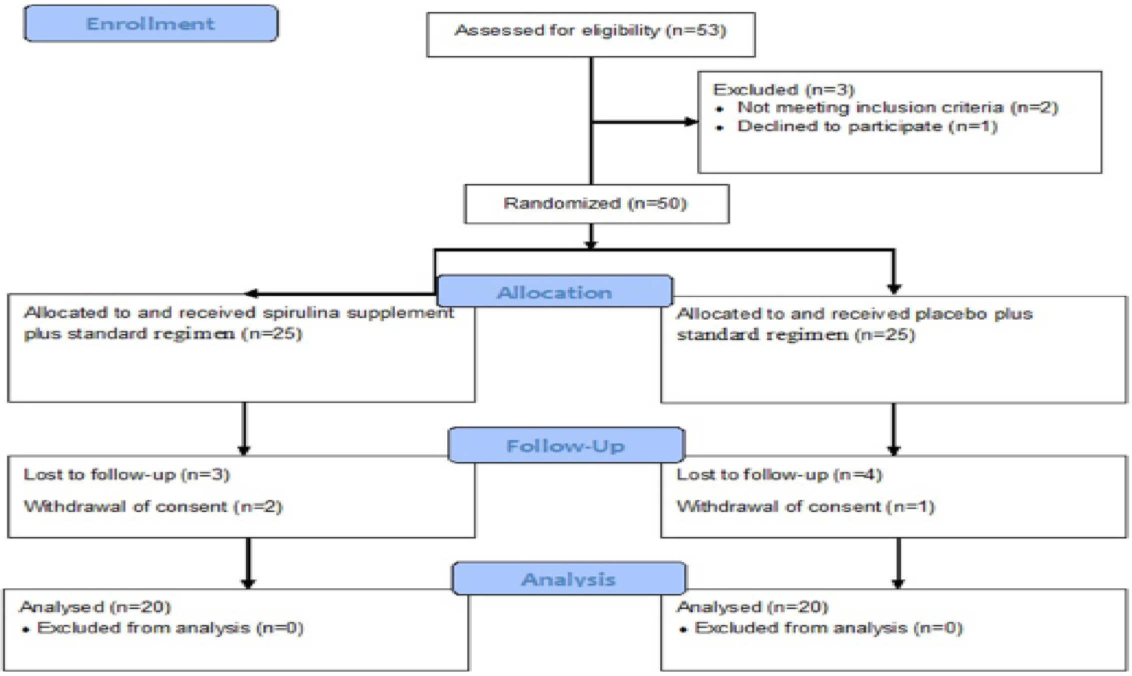

Figure1. Flow diagram of the trial

test.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences software, version 22 (SPSS Inc, Chicago, USA). Variables were tested for normality by Kolmogorov-Smirnov test. Categorical variables were described with absolute and relative (percentage) frequencies. Continuous variables were expressed as mean ± standard deviation (SD). Student t-test and paired t-test were applied for statistical analysis of continuous variables. Differences in proportions were tested by Pearson chi-square when assumptions were met; if not, the Fisher's exact test was used. *P*-value< 0.05 was considered as the significance level.

RESULTS

The CONSORT diagram of the clinical trial is reported in **Figure 1**. During the follow up period, 5 patients in each group were excluded from the analysis due to the loss of follow up and withdrawal of consent.
Evaluation of patients before/after treatment in the control group revealed that the average sperm motility in patients with one disordered factor was the only measure that was significantly increased after the treatment with conventional regimen (33.04 ± 9.72 vs. 48.70 ± 13.82, *P* = .008) (**Table 1**).
Assessment of participants before/after treatment in the experimental arm showed that both the average sperm motility in isolated motility disorder (37.30 ± 10.97 vs 51.00 ± 7.12, *P* = .01) and the average sperm morphology in isolated morphology problem (5.5 ± 0.50 vs 47.50 ± 10.5, *P* = .02) were recovered completely after treatment and these differences were statistically significant. However, in participants with all three disordered factors of the sperm count, sperm morphology, and sperm motility, the only factor that was statistically improved after the treatment was sperm morphology (*P* = .02) (**Table 1**). In groups in which there was only a single outlier participant with a specific disorder, their given statistics were not calculated.
According to our results, the average sperm count, morphology, and motility were not significantly different between the study groups at the end of the study period (**Table 1**).
Our results showed a pregnancy rate of 5% in the partner of patients in the spirulina group versus 0% in the control group.

DISCUSSION

We found no significant differences with regards to semen parameters between the study groups. On the other hand, significant improvement in the sperm morphology and motility was reported after the treatment with spirulina (compared to pre-treatment specimen); while motility was the only variable that was improved in the control group (compared to pre-treatment specimen).
Although the exact etiology of male infertility is unknown, it is attributed to several environmental factors such as exposure to certain chemicals, heavy metals, pesticides, electromagnetic radiation, smoking, alcohol abuse, chronic stress, and inflammation in the male reproductive system.[15-18] Most of these factors ultimately cause oxidative stress. The resulting excessive free radicals cause a pathological response that can lead to reduced sperm count, decreased sperm motility, and development of abnormal sperm morphology.[19]
The semen protective antioxidant system consists of enzymetic and non-enzymatic factors. Vitamin A, E, C, and B complex, glutathione, pantothenic acid, carotenoids, coenzyme Q10, carnitine, and minerals such as zinc, selenium, and copper are efficient anti-oxidants that help to protect the body from oxidative damage.[19] As a result, various clinical trials have been per-

formed to evaluate the possible beneficial effects of these agents on improvement of the sperm parameters in males as well as pregnancy rates in their partners in patients with idiopathic male infertility.[20-21]

In a study, the effects of selenium supplementation in males with infertility were considered. Results showed that a low dosage of selenium could improve the sperm motility and increase the chance of successful conception. However, not all participants responded to treatment in this study and only 56% of them showed a positive response to this treatment.[5] Our results also showed that treatment with spirulina could significantly increase the sperm motility based to baseline-12 weeks of treatment sub-analysis in spirulina-treated patients.

In another study, the efficacy of folic acid and zinc sulfate on semen parameters was evaluated in infertile and subfertile men. Results revealed that treatment with these supplements could significantly increase the total sperm count (74%) and also lead to minor increase in abnormal spermatozoa (4%) in both subfertile and fertile men. However, pre-intervention concentrations of folate and zinc in the blood and seminal fluid did not significantly differ between fertile and subfertile men.[12] The improving effects of zinc sulfate on the semen parameters have been studied in another trial in infertile men. This study reported strong linear associations between the sperm count and normal sperm morphology with seminal zinc concentrations.[20] In our study, there was a trend toward an increase in the level of sperm count after 12-week treatment period in spirulina-treated patients (5.42 vs. 16.43, $P = .076$). Though, this improvement was not statistically significant which may be due to a significant lower baseline sperm count in this group compared to controls and a limited sample size in both groups, therefore; it deserves further evaluation in the clinical setting.

A trial analyzing the efficacy of coenzyme Q10 supplementation on semen parameters showed a significant improvement in the semen morphology, density, and motility in infertile men. This study mentioned a positive correlation between treatment duration with coenzyme Q10 and improvement in semen parameters. Coenzyme Q10 also significantly decreased the serum follicle stimulating hormone and luteinizing hormone at the 26-week treatment phase.[21] Another trial was performed on 228 men randomly distributed in two groups of placebo and coenzyme Q10 treatment (200mg/day for 26 weeks). At the end of the trial, the sperm density, motility, and morphology increased significantly in the men treated with coenzyme Q10.[22]

With regard to the protective effects of vitamins and minerals in the improvement of spermatogenesis in men with idiopathic infertility and the fact that spirulina is full of minerals, vitamins and caretinoids, in this study, we evaluated the hypothesis that this supplement may have beneficial effect on the semen parameters in infertile men.[7] The efficacy of spirulina on animal fertility has been extensively studied. Its beneficial effects on improving the reproductive performance and reducing teratogenicity in albino mice were addressed previously. An increase in fertility rate from 77.5% to 82.5% and a 33.7% decrease in stillbirth rate were shown. This agent also improved the survival rate of off-springs in diabetic mice from 83.61% to 88.9%.[23] In another study, spirulina was shown to be safe as a supplement used in laying hens' diets with a significant improvement on reproductive and productive performances.(24) In a pilot study carried out on 6 boars, it was revealed that the addition of spirulina to the main diet for 40 days could increase the volume of ejaculation (30 mL) as well as the spermatozoa concentration (27mln/mL). The sperm motility also had a 30% increase in this study.[25]

Despite these promising experimental studies, we did not find any clinical study in the literature to assess the efficacy of spirulina on human reproductive function. Therefore, we designed this pilot study with randomized trial design to analyze the value of this natural product on the semen parameters in comparison with conventional treatment in men with infertility and abnormal semen analysis.

According to the WHO definition, abnormality in any of the sperm count or motility or morphology can lead to infertility. This study showed that the addition of 2 g spirulina per day to the conventional treatment did not significantly improve the semen parameters and sperm function in all subgroups. In addition to routine analysis mentioned in previous studies, to better assess the results, we evaluated the efficacy of treatments in different subgroups (i.e. patients with isolated versus multiple abnormalities in their semen analysis). In the spirulina-treated group there were significant differences between baseline and post-treatment sperm motility and morphology in patients with isolated motility or morphology disorders. Interestingly, the positive effect of spirulina on improvement of the sperm morphology was also observed in patients with combined oligospermia, asthenospermia, and teratospermia disorders. Therefore, with attention to one case of confirmed pregnancy in the spirulina arm, it sounds that it exerts its benefit on fertility mainly by improving the morphology of sperm.

Study limitations

We admit that the small sample size of this pilot study in the subgroups can be a limitation for a thorough statistical analysis. Improved semen quality and quantity does not certainly translate into an improved pregnancy rate. Although we followed all the patients for assessing pregnancy occurrence during the study period, there were 7 single males in the control group and 4 in the spirulina group that made this assessment impossible for all participants.

CONCLUSIONS

This pilot randomized trial provides initial evidence on the possible beneficial effects of spirulina mainly in patients with impaired sperm motility or morphology. Due to the limited sample size, further larger randomized trials not only at the level of semen parameters but at the scope of paternity are required to confirm these potential benefits.

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CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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