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Original Research Article

Effect of omega 3 fatty acid in infertile males with oligozoospermia

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ABSTRACT

Background: Oligozoospermia is the cause of male infertility in 33.3% of cases. Omega-3 fatty acid has been utilized in infertility because of its widespread availability, low cost, and high safety profile. We intended to conduct a study to evaluate the efficacy of omega-3 fatty acid in oligozoospermia.
Methods: A prospective comparative study was conducted in the outdoor of the department of reproductive endocrinology and infertility, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from July 2022 to August 2023. A total of 70 Infertile males with oligozoospermia, were enrolled in the study. The participants were assigned to the omega-3 fatty acid group who was treated with omega-3 fatty acid 1 gm orally twice daily for 12 weeks. The other group was treated with placebo orally twice daily for 12 weeks. Sixty patients completed the 12 weeks of treatment. The changes in sperm count were determined.
Results: The mean age of the participants was 35.6±4.59 years. Following treatment with omega-3 fatty acid significant improvement was observed in sperm count from 11.10±2.81 million/ml to 34.2±31.36 million/ml. Significant improvement in total motile sperm count was also observed. At the end of 12 weeks of treatment 76.7% of participants in the omega 3 fatty acid group and 10.0% in the placebo group had normozoospermia.
Conclusions: Supplementation with omega-3 fatty acid results in improvement of sperm concentration and total motile sperm count in infertile men with oligozoospermia.

Keywords: Oligozoospermia, Omega-3 fatty acid, Sperm concentration, Total motile count

INTRODUCTION

The male factor contributes as a sole cause of infertility in approximately 20% of infertile couples and is an important contributing one in another 20-40% cases.[1] In Bangladesh males are estimated to be responsible for 50% cases of infertility.[2] Unfortunately, like other developing nations, females in Bangladesh are usually blamed for infertility although males are responsible for 60% of the cases. The common phenomena of male infertility include low sperm concentration, abnormal sperm morphology, low sperm motility and related problems. Oligozoospermia or sperm concentration of $<20\times10^6$ spermatozoa/ml, cause couple infertility in 33.3% of cases.[3,4]

Male infertility may result from a variety of causes. Some can be defined accurately while others remain idiopathic reflecting poor understanding of the mechanisms that govern testicular function. It is assumed, this reduction of sperm concentration is closely related to oxidative stress. Environmental pollution, occupational hazards and stressful life style are major contributors for male factor infertility. Antioxidants multivitamins and some stress reliever drugs are being used as empirical treatment for oligospermia. Dietary supplementation and nutritional intervention became popular means of support for our desire for longevity, for being physically and emotionally healthier and smarter. In recent years There is a dramatic

rise in the use of OTC (over the counter) medications to combat infertility and improve semen quality.[5]

Omega-3 fatty acids (ω-3 FA), which we consume through food are 'essential' polyunsaturated fatty acid (PUFA). Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and α-linolenic acid (ALA) are the chief omega-3 PUFAs. Omega-3 FAs are components of all cell membranes. The presence of these PUFAs in cellular membranes is important to maintain the properties of the lipid bilayer.[6] The lipids of the spermatozoa membrane are important for the fluidity and flexibility of spermatozoa and successful fertilization.[7] The second mechanism by which omega-3 PUFAs improve semen quality, involves anti-oxidant activity. Imbalance between antioxidant capacity in seminal plasma and the production of ROS results in oxidative stress. Reactive oxygen species (ROS) significantly and adversely affect sperm function at high concentrations. Balanced seminal plasma antioxidant activity prevents the oxidation of various macromolecules such as DNA, proteins and lipids.[8] Fertile men had higher blood and spermatozoa levels of omega-3 PUFAs compared with the infertile counterparts.[5] Higher intake of omega-3 PUFAs is positively correlated with sperm morphology.[9] A significant improvement in total sperm count and sperm cell density was observed in the infertile men with idiopathic OAT taking omega-3 fatty acids for 32 weeks compared to placebo.[10] Safarinejad et al found significantly lower levels of catalase and superoxide dismutase (SOD) -like activities in the seminal plasma of infertile men compared with fertile men.[5] Catalase and SOD-like activities were significantly positively correlated with sperm density, sperm concentration, sperm motility and sperm morphology.[5] There is at least one meta-analysis which indicate that supplementing infertile men with omega-3 FA improve sperm motility.[11]

The antioxidant activity of omega-3 reduces oxidative stress, has beneficial effects on spermatogenesis and increases the chance of natural pregnancy. Omega-3 FA has excellent safety profiles, is widely available and cost-effective. If effective means of improving sperm parameters are available, expensive interventional ART procedures like IUI and IVF can be avoided. This was the rationale behind conducting this research aimed at evaluation of the efficacy of oral administration of omega 3 fatty acid in improving sperm concentration in infertile male with oligozoospermia.

METHODS

This prospective comparative study was carried out in a University Hospital in Dhaka from July 2022 to June 2023. The ethical clearance was obtained from the institutional review board of the medical university. The study participants were infertile males with oligozoospermia (5-15 million/ml) in at least 2 samples 4 weeks apart. Age was between 20-45 years.

Exclusion criteria were severe oligozoospermia (<5 million/ml), oligoasthenoteratozoospermia, endocrinopathies (FSH>7 mIU/ml, total testosterone <300 ng/dl/<10.40 nmol/L, LH >7 mIU/ml), undescended testis, small testis or epididymal lesion on palpation, genital surgery, systemic diseases like uncontrolled diabetes mellitus, severe kidney disease and liver insufficiency, history of chemotherapy or radiotherapy and antioxidants supplementation in the last three months. A total 70 patients were enrolled according to eligibility criteria. Informed consent was obtained from the participants.

Eligible participants were assigned to either omega 3 fatty acid group or placebo group. Treatment was started after the baseline visit and investigations. Omega-3 fatty acid group included 35 participants who received capsule omega-3 fatty acid 1 gm, (Trumega, Square Pharmaceuticals Ltd.) orally twice daily for 12 weeks. The placebo group included 35 participants who received placebo orally twice daily for 12 weeks. The outcome variables were sperm concentration and total motile sperm count after 3 months of treatment.

The sample size was estimated for 80% power and 0.5 alpha as 30 for each group, 60 in total. Allowing for 15% drop out the final sample size was 35. SPSS (Statistical Package of Social Sciences) version 23 was used for analysis. Socio-demographic and clinical, sonographic characteristics were summarized as frequency for categorical variables, mean±SD or median (interquartile range) as appropriate for continuous variables. Pair wise comparison of outcome variables was done between the treatment arm of omega 3 fatty acid group and the control arm of placebo. Outcome variables were compared between the groups with chi-square test or Fisher's exact test for categorical variables and independent samples t-test (unpaired t test) for continuous variables. A p value of 0.05 or lower was considered statistically significant.

RESULTS

A total of 70 patients were recruited as study participants, 35 in Omega 3 fatty acid group and 35 in placebo group. Before the follow up visit after 3 months, 5 participants dropped out of each group. So, the final analysis was done on 60 patients, 30 in omega 3 fatty acid group and 30 in the placebo group.

Table 1 shows the baseline demographic and clinical characteristics of the study participants. Table 2 summarizes the baseline endocrine parameters. Table 3 describes the baseline sperm parameters of the participants in the two groups. Tables 4 and 5 shows the changes in sperm parameters over 3 months of treatment in the experimental and placebo group. Table 6 shows the difference in post treatment semen parameters between the experimental and placebo group. Figure 1 compares the participants achieving normozoospermia post treatment in the experimental and placebo group.

Table 1: Baseline demographic, clinical and endocrine characteristics of the study participants.

Characteristics		Treatment		P value
		Omega 3 fatty acid (n=35)	Placebo (n=35)	
Age (years)	≤35	19 (54.3)	14 (40.0)	0.231
	>35	16 (45.7)	21 (60.0)	
	Mean±SD	34.57±4.81	36.60±4.02	0.06
	Median (range)	35 (25-44)	37 (30-45)	
Habitat	Urban	21 (60.0)	17 (48.6)	0.337
	Rural	14 (40.0)	18 (51.4)	
Occupation	Service	12 (34.3)	14 (40.0)	0.204
	Businessman	20 (57.1)	21 (60.0)	
	Student	3 (8.6)	0 (0.0)	
Monthly household income (taka)	Low (≤10000)	3 (8.6)	0 (0.0)	0.121
	Middle (>10000-<30000)	18 (51.4)	24 (68.6)	
	High (≥30000)	14 (40.0)	11 (31.4)	
Smoking status	Non-smoker	23 (65.7)	23 (65.7)	0.99
	Smoker	12 (34.3)	12 (34.3)	
Body mass index (BMI)	18-24.9	24 (68.6)	29 (82.9)	0.343
	25-29.9	10 (28.6)	5 (14.3)	
	≥30	1 (2.9)	1 (2.9)	
Type of infertility	Primary	17 (48.6)	14 (40.0)	a0.470ns
	Secondary	18 (51.4)	21 (60.0)	
Duration of infertility	<2	1 (2.9)	3 (8.6)	a0.584ns
	2-5	27 (77.1)	25 (71.4)	
	>5	7 (20.0)	7 (20.0)	

Table 2: Endocrine parameters compared between groups at baseline (pre-treatment period).

Endocrine parameters		Treatment		P value
		Omega 3 fatty acid (n= 35)	Placebo (n=35)	
Testosterone (nmol/l)	Mean±SD	13.81±2.17	13.23±1.75	[a]0.252ns
	Median (range)	13.29 (10.50-17.80)	13.43 (10.05-16.81)	
FSH (IU/l)	Mean±SD	4.77±1.07	5.00±1.03	[b]0.363ns
	Median (range)	4.77 (3.03-6.68)	5.07 (3.03-6.88)	

[a]Student t test, [b]Mann Whitney U test was done; ns=non-significant.
No significant difference in Testosterone and FSH levels was observed between groups (p value: >0.05)

Table 3: Sperm characteristics compared between groups at baseline (pre-treatment period).

Characteristics		Treatment		P value
		Omega 3 fatty acid (n=35)	Placebo (n=35)	
Sperm concentration (million/ml)	Mean±SD	10.97±2.66	12.09±1.38	[a]0.076ns
	Median (range)	11 (5-14)	12 (10-14)	
Sperm motility (%)	Mean±SD	56.30±7.52	55.07±9.74	[b]0.418ns
	Median (range)	55 (43-70)	51 (45-80)	
Total motile sperm (millions)	Mean±SD	13.37±6.25	15. 61±5.81	[b]0.258ns
	Median (range)	13.01 (1.38-26.40)	13.92 (4.6-29.4)	

[a]Mann Whitney U test, [b] Student t test was done; ns=non-significant.
No significant difference in sperm concentration and sperm motility and total motile count was observed between groups at baseline (p value: >0.05)

Table 4: Difference in sperm characteristics between pre-treatment period and post-treatment period in Omega 3 fatty acid group (n=30).

Sperm parameters		Pre-treatment	Post-treatment	P value
Sperm concentration (millions/ml)	Mean±SD	11.10±2.81	34.2±31.36	<0.001s
	Median (range)	11.5 (16.5-50)	20 (8-150)	
Sperm motility (%)	Mean±SD	56.30±7.52	56.83±6.31	0.391ns
	Median (range)	55 (43-70)	59 (45-67)	
Total motile sperm (millions)	Mean±SD	13.37±6.25	48.38±50.29	<0.001s
	Median (range)	13.01 (1.38-26.40)	25.87 (6.0-216.12)	

Wilcoxon signed rank test was done, s=Significant, ns=non-significant. Data was presented as Mean±SD, Median (range). Significant improvement was observed in sperm concentration and total motile sperm count following treatment with omega-3 fatty acid (p value: <0.001).

Table 5: Difference in sperm characteristics between pre-treatment period and post-treatment period in placebo group (n=30).

Sperm parameters		Pre-treatment	Post-treatment	P value
Sperm concentration (millions/ml)	Mean±SD	12.17±1.37	14.00±12.827	0.855ns
	Median (range)	12 (10-14)	11.5 (5-80)	
Sperm motility (%)	Mean±SD	55.07±9.74	55.57±8.72	0.735ns
	Median (range)	51 (45-80)	51 (44-75)	
Total motile sperm (millions)	Mean±SD	15. 61±5.81	18.53±15.25	0.339ns
	Median (range)	13.92 (4.6-29.4)	14.4 (7.12-90.16)	

Wilcoxon signed rank test was done, s=Significant, ns=non-significant. Data was presented as Mean±SD, Median (range). No significant improvement was observed in sperm characteristics following treatment with placebo (p value: >0.05).

Table 6: Sperm characteristics compared between groups at post-treatment period.

Characteristics		Treatment		P value
		Omega 3 fatty acid (n=30)	Placebo (n=30)	
Sperm concentration (million/ml)	Mean±SD	34.2±31.36	14.00 ±12.82	<0.001s
	Median (range)	20 (8-150)	11.5 (5-80)	
Sperm Motility	Mean±SD	56.83±6.31	55.57±8.72	0.255ns
	Median (range)	59 (45-67)	51 (44-75)	
Total motile sperm (millions)	Mean±SD	48.38±50.29	18.53±15.25	<0.001s
	Median (range)	25.87 (6.0-216.12)	14.4 (7.12-90.16)	

Mann Whitney U test was done, s=Significant, ns=non-significant. Significant difference in sperm concentration and total motile sperm count was observed between groups at post-treatment period (p value: <0.001).

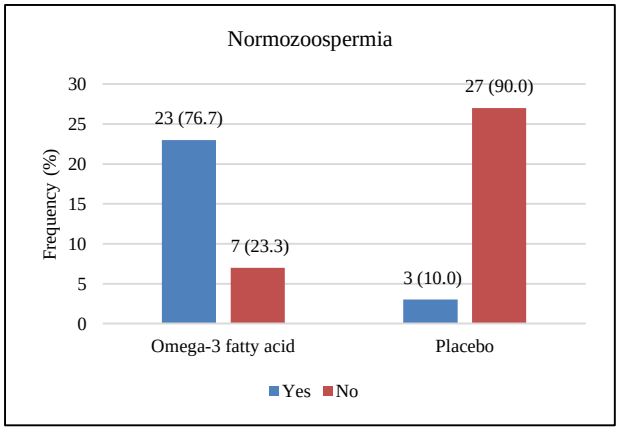

Figure 1: Distribution of post-treatment normozoospermic participants between groups.

After being treated 23 (76.7%) participants became normozoospermia, whereas in the placebo group only 10% became normozoospermia.

DISCUSSION

The issue of male infertility is a significant concern in global health, which has profound effects on the psychological and physiological well-being of couples.[12] A prevalent factor contributing to male infertility is reduced sperm count defined as oligozoospermia, which accounts for around 15% of all cases of male infertility.[13] In recent times, there has been a significant focus on the use of omega-3 polyunsaturated fatty acids (PUFAs) as a form of supplementation in the treatment of mental, neurologic, and cardiovascular disorders.[14] The inclusion of omega-3 polyunsaturated fatty acids (PUFAs), such as

alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), in dietary patterns has been observed to have positive impacts on fertility as well. Given their favorable safety profiles, widespread availability, cost-effectiveness, and observed positive impact on spermatogenesis in human studies, omega-3 polyunsaturated fatty acids (PUFAs) could be regarded as a potential nutritional supplement for enhancing semen quality. According to Almujaydil et al, there is evidence suggesting that diets abundant in omega-3 fatty acids have an impact on sperm quality by preserving the integrity of the sperm membrane and maintaining the stability of the mitochondria.[15] Several previous researches have been undertaken and have reported on the efficacy of omega-3 fatty acid in the treatment of oligozoospermia. Nevertheless, the findings exhibit inconsistency and are subject to limitations. The objective of this study was to investigate the potential impact of omega-3 fatty acids on the treatment of oligozoospermia.

Our study reveals that sperm concentration, total motile sperm count increase significantly after three months of oral supplementation with omega 3 fatty acid in infertile males with oligospermia. This finding is supported by several other previous studies.[10,16] In this study, the age of the male participants was 35.6±4.59 years. And the majority of them were suffering from secondary infertility. The mean age reported by Mehmud et al for males suffering from infertility was 35.4±6.6 years.[17] The study conducted by Bashed et al also reported the mean age to be 36 years of the infertile male patients.[18] Their studies also had secondary infertility in the majority.

A notable enhancement in sperm concentration was detected in the group receiving omega-3 fatty acids, whereas no substantial improvement was reported in the placebo group. In a study conducted by Safarinejad, the administration of omega-3 fatty acid supplements resulted in a considerable enhancement of the overall sperm concentration in men with idiopathic OAT.[10] There were low levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exhibited pretreatment. The integration of omega 3 fatty acid into the cell membrane of spermatozoa and the antioxidant properties might be the reason of the impact of omega-3 in the process of spermatogenesis and the significant rise in the number of sperm count in omega-3 fatty acid group.[5] Hosseini et al conducted an evaluation of the impact of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on various sperm parameters, such as total sperm concentration and sperm motility.[11] But the interventions involving DHA and EPA supplementation did not result in any significant alterations in sperm concentration when compared to the control group.

In our study, there was statistically significant enhancement in total motile sperm count among participants given omega-3 fatty acid. Safarinejad studied a cohort of 211 infertile men with idiopathic oligoasthenoteratozoospermia.[10] They exhibited pre-treatment low levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The administration of omega-3 fatty acids resulted in an improvement in the overall motility of sperm. Hosseini et al found that the administration of omega-3 therapies resulted in a considerable enhancement in sperm motility.[11] González-Ravina et al showed that the administration of omega 3 (DHA) supplements results in a considerable enhancement in sperm motility and that a higher dosage yields a more immediate improvement.[16] There are prior researches which explored the impact of omega-3 fatty acids on sperm motility with inconclusive results. In a study conducted by Knapp, it was observed that the administration of menhaden oil, which included DHA + EPA, did not have any significant impact on sperm motility.[19] Conquer et al found that the supplementation of DHA in males with asthenozoospermia did not have any significant impact on the parameters related to sperm quality.[20] There was significant difference in nature or cause of infertility among male participants in above mentioned studies. Variation in omega-3 fatty acid dose formulation and duration of supplementation (10 weeks/3 months/8 months) explains the difference of our findings with other studies.

Omega- 3 fatty acid usually contains alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In our study we used omega 3 fatty acid (1gm) which is the combination of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The potential discrepancy in the outcomes of the impact of omega-3 on infertile patients may arise from the variations in the proportion of EPA and DHA within omega-3, as well as differences in its purity. Among the studies mentioned above different omega-3 preparations with different levels of purity were used. Conquer et al used either 400 or 800 mg of a DHA-preparation containing 38.6% DHA equivalent to a daily DHA dose of, respectively, 154 or 309 mg.[20] Purity of the omega-3 supplements used in the other RCTs were considerably higher. González-Ravina et al used a formula with minimum 90% omega-3 (percentages of DHA and EPA not specified) and Martínez-Soto et al used a preparation with 76.2% DHA.[16,21] Percentages of omega-3 FA in the supplement used by Safarinejad were not specified, but the author states that the daily supplement consisted of 1.12 gm EPA and 0.72 mg DHA.[10]

Supplementation with omega-3 capsules, specifically those containing DHA or a combination of EPA and DHA, has been shown to improve at least one fertility marker in semen.[10,16,21] In our study, the supplementation was administered for a duration of only 3 months or 12 weeks. The study conducted by Safarinejad in 2011 had a duration of 32 weeks and discovered improvements in four distinct semen quality parameters.[10] In contrast, studies with shorter durations, ranging from 10 weeks to 3 months, conducted by all González-Ravina et al, Conquer et al and Martínez-Soto et al, found improvements in only one or two semen parameters, or no improvement at all.[19,21] This

might explain our findings of only observing improvement in sperm count or concentration and motility.

Although we compared the improvement in semen quality parameters, it is important to note that study population of others was diverse. They included individuals with conditions such as idiopathic oligoasthenoteratozoospermia, asthenozoospermia, and men undergoing evaluation for infertility. We compared the effectiveness solely in patients with oligozoospermia which posed a challenging task. However, as inferred from the preceding discourse, it is evident that omega-3 fatty acids have a significant role in enhancing sperm count or concentration and motility. Therefore, it is recommended that physicians can consider suggesting this supplementation for patients with oligozoospermia.

There were many limitations of the study. The study was conducted in a single center with relatively small sample size due to time constraints, so it limits generalizability. Only one dose of omega-3 fatty acid was used in this study, so effect of different doses of omega-3 on different sperm parameters could not examined. Several studies provide omega-3 for longer duration and reported significant impact of it in majority of the sperm parameters, whereas we had the opportunity to explore the effectiveness after 3 months only. Side-effects after taking omega-3 fatty acid was not evaluated. Studies with different dose of omega-3 fatty acid and for longer periods. The studies could incorporate the effects on sperm DNA fragmentation.

CONCLUSION

The administration of omega-3 fatty acid for 3 months results in significant increase in the number of sperm and the number of total motile sperm, when compared to placebo. More than three-fourth of the infertile males with oligozoospermia had normozoospermia after three months supplementation.

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