Original Research Article

Role of Micronutrients in Male Infertile Patient having Abnormal Semen Parameter

ABSTRACT

***Background**: Abnormal semen parameter is the most common cause of infertility in men. Approximately 15% of human couples are infertile and male sub fertility accounts for 50% of cases. About 90% of male factor infertility is idiopathic with no identifiable cause. Affected males has a definite detectable abnormality related to infertility, such as endocrine 1-3% disease, antisperm antibodies 3-13%, varicocele 25.4%, genetic cause 10-15%. A large number of recent studies have focused on the ability of nutraceuticals, to improve the hormonal status and sperm parameters by different mechanisms. Considering the positive effects of micronutrients on sperm motility and count a mixture of micronutrients applied to reverse the sperm parameters.*

***Objectives:** To assess the efficacy of micronutrients regarding improvement of sperm count and motility with abnormal semen parameter.*

***Methods:** It was a prospective observational study. This study was performed in Reproductive Endocrinology and Infertility Unit of Dhaka Medical College Hospital between 06 October, 2019 to 05 October, 2020 (01 year).Study population consist of all the diagnosed case of infertile males having abnormal semen parameter between 25 to 50 years of age at DMCH.A total of 100 infertile males with abnormal semen parameter were selected for this study but finally 70 infertile male were included. A full assessment includes demographic information and baseline semen analysis. Then anti-oxidant was given one tablet in the morning and another at evening for three months and follow up semen analysis was done at prefixed schedule after three months to analysis the changes that was achieved.*

Then pre-treatment and post treatment semen parameters, including sperm count and sperm motility were assessed. Statistical analyses were carried out by using the statistical package for social sciences version 22.0 for windows (SPSS Ins, Chicago, Illinois, USA).

***Main outcome measure(s):** Changes in sperm count and motility.*

***Results**: The mean age was 40.5±5.9 years with ranged from 25 to 50 years. The Mean sperm count was found 12.34±1.84 mill/mL pre-treatment and 15.50±4.69 mill/mL post-treatment which was statistically significant ($p<0.05$). The mean rapid progression was found 28.84±20.77 pre-treatment and 47.44±25.02 post-treatment which was statistically significant ($p<0.05$). The mean non-progressive sperm was found 40.34±21.12 percent before treatment and 28.32±15.47 percent after treatment which was significantly decreased after micronutrient therapy ($p<0.05$). The improvement of semen parameters after treatment was 72.86%.*

***Conclusion**: Majority of the male patients suffer from infertility due to Oligozoospermia and asthenozoospermia. Increasing age, smoking and industrial workers were more common. Sperm count, sperm motility and rapid progression had significantly improved after three months of treatment. Therefore, this study suggests that micronutrients can be helpful to improve the sperm count and motility of infertile male having abnormal semen parameter.*

***Keywords:** Oligozoospermia, asthenozoospermia, Infertility, micronutrients, antioxidant.*

INTRODUCTION

The concept of male subfertility has evolved rapidly since 2000 [1]. About 90% of male factor infertility is idiopathic with no identifiable cause. Only a minority of affected males have a definite detectable abnormality related to infertility. Associated factors are endocrine 1-3% disease, anti-sperm antibodies 3-13%, varicocele 25.4%, and genetic cause 10-15% [2]. Approximately 15% of human couples are infertile and it is generally recognized that male sub-fertility plays a contributory role in up to 50% of cases [3, 4]. Male subfertility falls into two categories either, productive or obstructive. Oligozoospermia is a productive cause of male infertility [3]. Male infertility is a syndrome of the male reproductive function, characterized by the inability to produce a sufficient amount of sperm for fertilization of an oocyte. Male infertility has been disregarded and under-explored as conception was previously observed as a female substance [39,40]. The World Health Organization (WHO) classified oligozoospermia, as "sperm count below 15 million per millilitre (ml) of semen as average" and asthenozoospermia as "sperm motility rapid progression <32 or rapid progression +non-progressive <40". Reproductive organs are highly susceptible to free radicals or oxidative damage from environmental toxins like pesticides, insecticides and heavy metals [2]. A large number of studies have focused on the ability of many substances, generally termed nutraceuticals, to improve the hormonal status and sperm parameters by different mechanisms. These studies found that micronutrients play an important role in improving abnormal semen parameters [5]. Deterioration of semen parameters may be due to exposure to the environmental toxicant which has detrimental effects on reproductive hormones, spermatogenesis or sperm function. The most widely studied evidence of environmental factors is associated with occupational pollutants, changes in lifestyle, exposure to toxic agents and changes in dietary habits [6,7]. All India Institute of Medical Sciences reported that the majority of men, who were exposed to high temperatures at their workplaces such as welders, dyers, blast furnace workers and those employed in cement and steel factories were more prone to infertility. This is due to excessive environmental heat which increases the temperature of the scrotum, causing a negative effect on sperm production [8]. Lifestyle plays an important role in poor semen quality [5]. Cigarette smoke contains well-known somatic cell mutagens and potent carcinogens. The exact mechanism by which sperm damage occurs is not clear but may be related to an increase in the vulnerability of spermatozoa to oxidative stress given these cells [9,10]. Ageing is an important factor responsible for the decline in semen quality, as sperm concentration decreases with increased age [11]. A balance nutritional supplement with anti-oxidant content can help reverse some of the oxidative damage from environmental toxins and natural ageing. Different micronutrients like vitamin C, vitamin B12, vitamin E, arginine, carnitine, zinc and selenium have specific roles in increasing sperm count and improving function [12-15]. Results of supplementation with folic acid and zinc are particularly relevant because they appear to work synergistically and results in a >70% increase in sperm concentration when taken under study conditions [16]. Selenium supplementation has been shown to yield a significant dose-dependent increase in total sperm count after 26 weeks, although toxicity was reported at excessive doses [17]. When vitamin C intake increases its concentration in seminal plasma prohibits DNA damage [18]. Vitamin E is a fat-soluble antioxidant that neutralizes free radicals and protects cellular membranes against O2 free radicals. It also prevents lipid peroxidation and therefore improves the functions of other antioxidants [19]. Vitamin E also inhibits the production of ROS in infertile males [20]. L-carnitine (LC) or 3-aminobutyric acid is a naturally occurring compound and also a semi-essential vitamin-like substance required for human metabolism. Findings show a positive relationship between initial sperm movement and increased LC in epididymis and L-acetyl in sperm [21, 22]. CoQ10 also known as ubiquinone is an antioxidant. After 6 months of therapy, CoQ10 increased in the semen of patients who received CoQ10, and sperm motility was improved in these individuals [23]. Zinc supplementation normally protects the spermatozoa against bacteria and also prevents damage to chromosomes [24]. Zinc plays an important role in testicular development and sperm maturation [25]. On the basis of published scientific literature, individual micronutrient has a positive role in male infertility with abnormal semen parameter. Considering the individual benefit of different micronutrients, a blend of micronutrients was used in this study to reverse sperm motility and count in case of abnormal semen parameters. The aim of the study was to assess the efficacy of micronutrients regarding improvement of sperm count and motility with abnormal semen parameter.

METHODOLOGY

This is a prospective observational study. A total of 70 patients were enrolled and analyzed in this study. The study was conducted at the "Reproductive Endocrinology and Infertility Unit", Dhaka Medical College Hospital (DMCH). The study period was 1 year from 06 October 2019 to 05 October 2020. Male Infertile patients having abnormal semen parameters (oligozoospermia and asthenozoospermia) attending the "Reproductive Endocrinology and Infertility Unit" of DMCH Purposive sampling. The sample was selected by fulfilling the inclusion and exclusion criteria.

Inclusion criteria:

- 25-50 years of age oligozoospermic infertile man whose sperm count (is between 10 to 15 million/ml).
- 25-50 years of age asthenozoospermic infertile man whose sperm motility:
- Rapid progression <32.
- Rapid progression and non-progressive <40.
- Infertile male with abnormal semen parameters with a normal endocrine profile.

Exclusion criteria:

- Medical co-morbidity DM, chronic hypertension, epilepsy, chronic depressive disorder.
- History of any endocrinological disorders that affect semen parameters such as hypothyroidism and

hyperprolactinemia.
- Present or past history of drug addiction, smoking and alcoholism.
- History of pelvic surgery–varicocelectomy.
- Present and past history of genital tuberculosis and mumps orchitis.
- Present and past history of taking drugs affecting the semen parameter.
- Present and past history of tumour and trauma.
- Testicular failure with high Follicular Stimulating Hormone.

Infertile males with abnormal semen parameters attending the study centre, DMCH during the study period were included as the study population. Data were collected chronologically by interview, physical examinations and laboratory investigations using a structured questionnaire containing all the variables of interest according to the inclusion and exclusion criteria. A full assessment includes demographic information, clinical presentation, smoking habits, and medical, surgical, and drug histories with a physical examination (height, weight, blood pressure) done. Semen analysis reports (two times at three months intervals), oral glucose tolerance test (OGTT), serum Follicular stimulating hormone, serum Luteinizing hormone, serum Prolactin and serum testosterone were done. All hormonal tests were done at the Institute of Nuclear Medicine, Dhaka medical college hospital. Hormonal tests were done to exclude endocrinological factors related to infertility. For semen analysis, each man provided a semen sample by masturbation into a wide-mounted sterile plastic container labelled with name and ID, in a room close to the laboratory the period of abstinence (3-5 days) was recorded and the semen sample was analyzed by Neubauer counting chamber according to World Health Organization guideline (2010). All semen analysis was done under a controlled condition in one lab by the same Embryologist. The analysis was performed before treatment and three months after starting the first dose.

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean values were calculated for continuous variables. The quantitative observations were indicated by frequencies and percentages. Paired t-test was used for continuous variables. P values <0.05 was considered statistically significant.

RESULT

This is a prospective observational study which was carried out in the Reproductive Endocrinology and Infertility Unit, Department of Gynecology and Obstetrics, Dhaka Medical College Hospital, Dhaka, between 06 October 2019 to 05 October 2020. A total of 70 infertile males with abnormal semen parameter patients were included in this study maintaining inclusion criteria. Table 1 shows that the majority (40.0%) of patients belonged to age 41-45 years. The mean age was found 40.5±5.9 years with a range from 25 to 50 years. More than one-third (34.3%) patients were industrial workers, and 23(45.7%) patients had monthly family income <10000 BDT. Mean sperm count was found 12.34±1.84 mill/mL before treatment and 15.50±4.69 mill/mL after treatment. Sperm counts were significantly increased after micronutrient therapy ($p<0.05$) (Table 2). The mean rapid progression was found 28.84±20.77 before treatment and 47.44±25.02 after treatment. Rapid progression was significantly increased after micronutrient therapy ($p<0.05$) (Table 3). The mean non-progressive sperm was found 40.34±21.12 before treatment and 28.32±15.47 after treatment. Non-progressive sperm was significantly decreased after micronutrient therapy ($p<0.05$) (Table 4). The mean Immotile was found 17.64±16.18% before treatment and 16.67±16.22% after treatment. Immotile were decreased after micronutrient therapy but were not statistically significant ($p>0.05$) (Table 5). Figure 1 shows the improvement of semen parameters after treatment, there was 72.86% improvement.

Table 1: Distribution of the study patients by socio-demographic characteristics (N=70).

Socio-demographic characteristics	Frequency	Percentage
Age groups (years)		
25-30	6	8.6
31-35	8	11.4
36-40	13	18.6
41-45	28	40
46-50	15	21.4
Mean ± SD	40.5± 5.9	
Occupational status		

Industrial worker	24	34.3
Private service	17	24.3
Farmer	11	15.7
Gov. service	8	11.4
Abroad	6	8.6
Other	4	5.7
Family income (Taka)		
<10000	32	45.7
10000-30000	24	34.3
>30000	14	20

Table 2: Mean sperm count difference between pre-treatment and post-treatment.

Variables	Micronutrients are taken		p-value
	Pre Treatment (n=70)	Post Treatment (n=70)	
	Mean±SD	Mean±SD	
Sperm count (mill/mL)	12.34±1.84	15.50±4.69	0.000^{s}
Range	10.0-15.0	5.0-25.0	

Table 3: Mean rapid progression difference between pre-treatment and post-treatment.

Variables	Micronutrients are taken		p-value
	Pre Treatment (n=70)	Post Treatment (n=70)	
	Mean±SD	Mean±SD	
Rapid progression	28.84±20.77	47.44±25.02	0.000^{s}
Range	(2-90)	(2-90)	

Table 4: Mean non-progressive sperm difference between pre-treatment and post-treatment.

Variables	Micronutrients are taken		p-value
	Pre Treatment (n=70)	Post Treatment (n=70)	
	Mean±SD	Mean±SD	
Non-progressive sperm	40.34±21.12	28.32±15.47	0.03^{s}
Range	(8-85)	(8-62)	

Table 5: Mean non-progressive sperm difference between pre-treatment and post-treatment.

Variables	Micronutrients are taken		p-value
	Pre Treatment (n-70)	Post Treatment (n=70)	
	Mean±SD	Mean±SD	
Immotile	17.64±16.18	16.67±16.22	0.619^{ns}
Range	(2-80)		

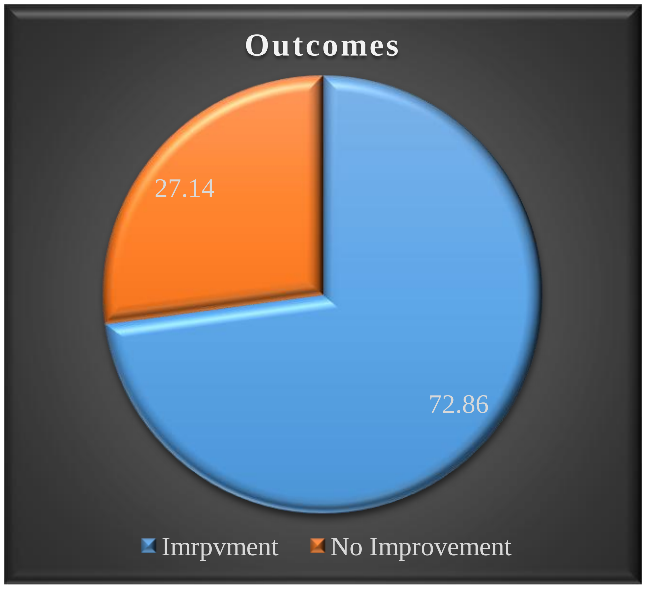

Figure 1: Outcome of the treatment of the study respondents (N=70).

DISCUSSION

This study observed that the majority of the patients (40.0%) belonged to age 41-45 years. The mean age was found 40.5±5.9 years with a range from 25 to 50 years. More than one-third (34.3%) of patients were industrial workers, and 23(45.7%) patients had monthly family income <10000 BDT. Begum et al also reported that the mean age was found 34.96±3.6 years.2 Imhof et al. also reported the mean age of men taking the active compound was 34 years (min/max: 18-43 years) [26]. This study showed that the mean sperm count was found 12.34±1.84 million/mL before treatment and 15.50±4.69 million/mL after treatment. The mean rapid progression was found 28.84±20.77 before treatment and 47.44±25.02 after treatment. The mean non-progressive sperm was found 40.34±21.12 before treatment and 28.32±15.47 after treatment. Sperm count and rapid progression were significantly increased after micronutrient therapy (p<0.05) but non-progressive sperm was significantly decreased after micronutrient therapy (p<0.05). Begum et al reported mean count was found 15.67±15.15 million/mL in pre-treatment and 32.63±21.18 million/mL in post-treatment. Mean total motility was found 39.65±22.34 in pre-treatment and 60.30±11.29 in post-treatment. After treatment, there was a significant improvement both in count and motility [2]. Lenzi et al evaluated the effects of a therapeutic formulation, Proxeed Plus, on sperm parameters in oligo asthenozoospermia men. This prospective, randomized, double-blind, placebo-controlled clinical trial involved 175 males (19-44 years) with idiopathic oligoasthenozoospermia. Males received Proxeed Plus or a placebo for 3 and 6 months. Sperm volume, progressive motility and vitality significantly (p<0.001) improved after 6 months compared to baseline [27]. Imhof el al conducted a comparative pilot study at the fertility center IMI, Vienna, Austria. A total of 132 sub-fertile males (active treatment group) took two capsules daily of the active component for a three months period between the first and follow-up semen analysis. Sub-fertile men received no active treatment and served as control. A main outcome measure was the standardized semen analysis [26]. All parameters evaluated by semen analysis significantly increased after three months of treatment with the active compounds. Median ejaculatory volume, sperm cell density, sperm motility (progressive and total) and normal morphology increased by 33.3%, 215.5%, 83.1%, 36.4% and 23.0% respectively. These increments were significantly higher than those observed among controls. A total of 34 pregnancies were reported after six months of follow-up whereas eleven were reported control group [26]. PM Gopinath et al conducted a Placebo-controlled, Double-blind, randomized, Parallel three-arm, Multicentric trial. Compared to placebo, a statistically significant improvement was seen in sperm count (14.8-26.35 in arm 1 and 14.37-24.8 million/ml in arm 2, p < 0.0001), and sperm total motility (39.2-51.6% in arm 1 and 38.4-50.1% in arm 2, p < 0.0001), at 90 days, and treatment further improved these parameters at day 180. No intergroup difference was seen between arm 1 and arm 2.36 Mahammad K. Moslemi et al included 690 infertile men with idiopathic asthenoteratospermia who received supplemental daily Se (200 µg) in combination with vitamin E (400 units) for at least 100 days. They observed 52.6% (362 cases) total improvement in sperm motility, morphology, or both, and 10.8% (75 cases) spontaneous pregnancy in comparison with no treatment (95% confidence interval: 3.08 to 5.52). No response to treatment occurred in 253 cases (36.6%) after 14 weeks of combination therapy. The mean difference between semen analyses of cases before and after treatment was 4.3% with a standard deviation of 4.29. On the basis of paired t-test results, combination therapy with oral Se and vitamin E was effective for the treatment of oligozoospermia or asthenozoospermia or induction of spontaneous pregnancy ($P \leq 0.001$) [37]. Orv Hetil et al conducted a clinical trial with l00 males with low sperm quality (sperm count 5-20 million/mL, motility 10 -40%, and abnormal morphology 30-50) where he found that dietary supplements statistically and clinically significantly improved sperm count and motility. In 74 cases this dietary supplement demonstrated a beneficial effect on sperm quality (more than 10% increase in sperm count, or quality of motility, or shape); in 16 cases the improvement exceeded 30%. No adverse effect could be accounted for by this treatment [28]. A systemic review of randomized studies was conducted to evaluate the effects of oral antioxidants on sperm quality and pregnancy rate in infertile men. Despite the methodological and clinical heterogeneity, 14 of the 17(82%) trials showed an improvement in either sperm quality or pregnancy rate after antioxidant therapy [29]. In 2008, Paradiso Galatioto et al used a multi-drug therapy including several antioxidants (consisting of daily administration of vitamin A 0.06 IU, vitamin C 3 mg, vitamin E 0.2 mg, N-acetyl-cysteine (NAC) 10 mg, zinc 0.01 mg, thiamine 0.4 mg, riboflavin 0.1 mg, pyridoxin 0.2 mg, nicotinamide 1 mg, pantothenate 0.2 mg, biotin 0.04 mg, cyanocobalamin 0.1 mg, calciferol 8 IU, calcium 1 mg, magnesium 0.35 mg, phosphate 0.45 mg, iron 0.2 mg, manganese 0.01 mg, copper 0.02 mg) in 42 oligozoospermic subjects: the treatment group had a 20-fold higher chance of having a normal sperm count than untreated men, and a non-significant increase in the chance of achieving pregnancy [30]. An observational study was conducted to evaluate the effect of multiple micronutrient therapy is sub-fertile males. This study included 103 sub-fertile men. Here 42 patients received 2 tablets of oligocare daily and 61 received 2 tablets with other concomitant therapy. The result of this study suggested that multiple micronutrient monotherapy is equally effective in the management of sub-fertility or idiopathic oligozoospermia in male patients as compared to combination therapy along with multiple micronutrients [31]. The present study showed that for 51(72.86%) patients, there were improvements in semen parameters and for 19(27.14%) there were no improvements in semen parameters. Begum et al. observed 69.55% of patients showed improvement after treatment in terms of count and motility [2]. All studies which examine the effect of multiple antioxidants in a supplementation showed an improvement in semen parameters after therapy [30,32-36]. Above mentioned study's findings are consistent with this study. One study showed a significant improvement in sperm concentration after combination therapy without improvement in motility and morphology [37]. Henkel et al found that the risk of consuming excessive dosages of micronutrient supplements, which may be toxic and results in a phenomenon termed an antioxidant paradox. It may lead to reductive stress, which is reported to be as dangerous to cells as oxidative stress and can be the cause of diseases such as cancer or cardiomyopathy. Therefore, there is a need for more elaborate research to establish the clear benefits and risks involved in antioxidant therapy for male infertility [38].

Limitations of the study: The present study had some limitations. The following should be kept in mind while deciding on the implications of the findings of the study: The study population was selected from one selected tertiary hospital in Dhaka city. So, the results of the study may not reflect the exact picture of the entire community. The small sample size was also a limitation of the study. The study was conducted for a short period of time.

CONCLUSION AND RECOMMENDATIONS

This study was undertaken to evaluate the effect of micronutrients on sperm count and motility in infertile males with abnormal semen parameters. Sperm count and motility had significantly improved after three months of treatment with a micronutrient supplement. Therefore, this study suggested that micronutrients can be helpful to improve sperm count and motility. Improvement of seminal quality may improve the fertility outcome of an infertile couple. Even though a recent study concluded the positive effect of sperm parameters, there is a need for further investigation with randomized control studies to confirm the efficacy and safety of antioxidant supplementation. There is also a need to determine the ideal dose of each compound to improve semen parameters, fertilization rates and pregnancy outcomes. Larger trials with high-quality controls and randomization must be performed to establish clinically relevant guidelines for supplementations.

Ethical approval: *The study was approved by the Institutional Ethics Committee.*

Consent: *As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).*

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