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Comparison of Efficacy of Low Dose and High Dose Antioxidants along with Antibiotics in Idiopathic Oligo, Astheno and Oligoasthenospermia

Rashida BM[1,*], Mariya Ehsan[1], Nazia Ehsan[2], Farzana Khan[2], Shahina BSM[2], Azaz B Sharif[1] and Farhana Sharmin[2]

[1]*Department of Medicine, Western University, Ontario, Canada*

[2]*Infertility Care and Research Center, Dhaka, Bangladesh*

Abstract

Objective: The aim of this study was to explore the efficacy of low dose and high dose antioxidants for the treatment of idiopathic oligiospermia, asthenospermia and oligoasthenospermia.

Materials and methods: This prospective quasi experimental study was conducted in Infertility Care and Research Center (ICRC) Ltd, Dhaka, Bangladesh between January 2013 and December 2014. Eighty four patients were the target population for this study. After thorough investigations those patients were diagnosed as idiopathic oligo, astheno and oligoasthenospermia were recruited for this study. Those patients whose female partner had infertility factor except PCOS were excluded from this study. For treatment patients were divided into two groups by lottery. Treatment for group A was Cap Doxycycline 100 mg twice daily for 1 month and tablet Oligocare, low dose antioxidant (Meyer Organic Pvt Ltd, India) 1 tab twice daily for 2 months. Group B was treated by Cap doxycycline 100 mg twice daily for 1 month and combination of micronutrients (High dose antioxidants) for 2 months. Ovulation induction was given to female partner of patient whose semen parameters improved. Results for pregnancy were observed for 6 ovulatory cycles. Data were expressed as mean standard deviation and percentage. Student's t test and χ^2 tests were done for test of significance where appropriate. A p value <0.05 was considered as significant.

Results: In both treatment groups both count and motility were increased significantly after 2 months of treatment. Though mean semen parameter improved significantly in both treatment groups, there was significant difference in number of patient improvement between the groups. In high dose group 79% patients improved after two months of treatment whereas 48% patients improved in low dose group. Similarly pregnancy rate was also higher in high dose group 22% in comparison to 12% in low dose group.

Conclusion: Antioxidants if can be used at a higher dose instead of low dose can give better result in terms of improvement of sperm count, motility and subsequently pregnancy rates.

Keywords: Oligospermia; Asthenospermia; Oligoasthenospermia; Antioxidants

Introduction

Male infertility is a major contributing factor for childlessness. Almost in 50% cases male are responsible for infertility. Although most of the female factors are easy to treat, male factors infertility is sometimes difficult to treat. The most common cause of male infertility is abnormal semen parameters in the form of oligospermia, asthenospermia, oligoasthenospermia, teratospermia and oligoasthenoteratospermia (OATS). For the last few decades sperm parameter has been declining dramatically [1]. Environmental factors, food habit, infections, varicocele, medicines, smoking, alcohol abuse, stress, psychological factors and genetic factor might have some deleterious effect in sperm quality and quantity [2]. The most widely studied evidence of potential environmental reproductive hazard is that sperm counts have been declined in certain industrialized countries [3-6]. Of the many causes of male infertility Oxidative Stress (OS) has been attributed to affect the fertility status of male. Oxidative stress is a result of the imbalance between Reactive Oxygen Species (ROS) and antioxidants in the body.

Reproductive organs are highly susceptible to free radicals or oxidative damage from environmental toxins. Though low level of ROS is essential for fertilization, acrosome reaction, hyperactivation, motility and capacitation [7-11], high level of ROS is considered toxic to spermatozoa. Main source of ROS in human semen is White Blood Cells (WBC) and dead spermatozoa [12]. Cytoplasmic droplets resulting from defective spermatogenesis are a major source of ROS [13]. Increased ROS levels have been correlated with decreased sperm motility [14-16]. It damages the sperm membrane, which in turn reduces the sperm's motility and ability to fuse with the oocyte. It also directly damages sperm DNA, compromising the paternal genomic contribution to the embryo.

Though certain cases of male infertility have specific causes, almost 40-90% cases are due to deficient sperm production of unidentified origin [17]. Spermatogenesis is an energetically demanding process, which requires an optimal intake of antioxidants, minerals and nutrients [18]. A balanced diet and nutritional supplements with high antioxidants content can help reverse some of the oxidative damage from environmental toxins and natural aging. Different micronutrients like Vit C, Vit B12, Vit E, Arginine, Carnitine, Zink, and Selenium have specific role in increasing sperm count and improving function. Antioxidants protect spermatozoa from ROS producing abnormal spermatozoa, scavenge ROS produced by leukocytes, prevent DNA fragmentation, improve semen quality in smokers, block premature

***Corresponding author:** Rashida Begum, Department of Medicine, Western Univesity, Ontario, Canada, Tel: 2263778204; E-mail: rashida_icrc@yahoo.com

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sperm maturation and stimulate spermatogenesis. Antioxidant also improves ART outcome and reduces recurrent pregnancy loss by producing good quality embryo due to improved quality of sperm. ROS generated by WBC in infection causes reduced motility by membrane damage. Upper genital tract infection sometimes remained unrecognized. Subclinical epididymitis can't be diagnosed by culture causes oxidative damage of sperm. So empirical use of antibiotics can help in such cases.

Different studies showed efficacy of antioxidants in isolated use or in combination with small dose. After using combination of antioxidants with smaller dose we did not get very satisfactory outcome. Studies showed that high intake of antioxidants results in better semen quality, higher sperm numbers and improved motility [19]. So we used combination of high dose antioxidants along with antibiotics to observe the difference if any. So purpose of this study was to explore the differences of efficacy of low dose and high dose antioxidants in the treatment of idiopathic oligo, astheno, and oligoasthenospermia.

Materials and Methods

This prospective quasi-experimental study was conducted in Infertility Care and Research Center (ICRC) Ltd, Dhaka, Bangladesh between January 2013 and December 2014. Eighty four patients were the target population for this study.

Initial assessment and recruitment: All patients' semen analysis was done by single observer in accordance with the WHO 2010 criteria [20]. If any parameter either count, motility, or both motility and count were below the normal level of WHO 2010 criteria was considered as abnormal semen parameter. Though abnormal morphology is also included in abnormal semen parameter, we did not include the teratospermia cases in this study. In case of abnormal semen parameter patient was examined thoroughly to exclude any abnormality in genital system. Following investigations were done to diagnose the cause of abnormal semen: Antisperm Antibody (ASA), semen culture, hormone analysis FSH, LH Testosterone and prolactin, testicular, prostatic and seminal vesicle ultrasonography to detect any abnormality, colour Doppler ultrasonography for varicocele detection. Y chromosome microdeletion testing was done in severe oligospermia (≤5 million) cases to exclude deletion of any segment of AZF region of Y chromosome. If no cause was found a repeat semen analysis was done after 4 weeks as there may be abnormality due to incorrect sample collection, time lapse between collection and analysis or due to unknown reason. After two consecutive abnormal reports patients were recruited for the study and divided into two groups, group A and group B. For two groups patients were selected by lottery and two different treatment schedules were offered to those patients according to lottery. Before recruitment female partner was evaluated thoroughly to exclude any infertility factor. The patient whose female partner has infertility factor except PCOS was excluded from the study. Those patients who have already received some antioxidants were excluded from this study. So inclusion criteria were only idiopathic oligo/astheno and oligiasthenospermia who did not receive any treatment before.

Treatment

Treatment for group A was Cap Doxycycline 100 mg twice daily for 1 month and tablet Oligocare (Meyer Organic Pvt Ltd, India) 1 tab twice daily for 2 months. Composition of Oligocare has shown in Table 1. There are multiple nutrients with smaller dose. Group B was treated by Cap doxycycline 100 mg twice daily for 1 month and combination of micronutrients for 2 months, which has shown in Table 2. Numbers of nutrients were less with higher dose.

After 2 months of therapy semen analysis was done. Ovulation induction was given to female partner (irrespective of PCOS and non-PCOS) of those patients whose semen parameters improved. As maintenance therapy oligocare was continued in group A and only Vit E was continued in group B. Vit E scavenges all the three types of free radicals [10]. To observe semen parameter's stability one semen analysis was done after 3 months of starting ovulation induction. Results for pregnancy were observed for 6 ovulatory cycles. As both regimens had been using for treatment purpose previously and for group B regimen ethical permission was taken before [21], for this study ethical permission did not seek for. Informed verbal consent was taken from all patients. Data were expressed as mean standard deviation, percentage. Student's t test and χ^2 tests were done for test of significance where appropriate. A p value <0.05 was considered as significant.

Results

Table 3 shows the characteristics of the patients of both groups. There was no difference in both groups. In group A, 4 patients had severe oligospermia whose count was 5 million/ml and others count was between 8-12 million/ml. In 18 patients total motility was between 15-25% and others had 30-35%. But mostly were non-progressive. Thirty four patients' Fast Forward (FF) motility was 10-15% and others had 20-25%. In group B, 2 patients had severe oligospermia (5 mil/ml) and others count was between 7-12 mill/ml. In 13 patients total motility was 15-25% and others had 30-35%. Most of the patients (28) patients' FF motility was 10-15% and others had 20-25%. In both treatment groups both count and motility were increased significantly after 2 months of treatment (Table 4). Though mean semen parameter improved significantly in both treatment groups, there was significant difference in number of patient improvement between the groups. In high dose group 79% patients improved after two months of treatment whereas 48% patients improved in low dose group ($p<0.05$). Average increment of count in group A was 4 times, which was 5.5 times in group B. Increment of total motility was 2 times FF motility was 2.5 times in group A, which were 2.5 times and 3 times in group B respectively.

Each Oligocare tablet contains (Group A)	
Vit A: 375 mg	L-Arginine: 10 mg
Vit C: 75 mg	L-Carnitine Tartarate: 50 mg
Vit E: 12.5 mg	Elemental iron: 5 mg
Vit D3: 12.5 mcg	Elemental Zink: 7.5 mg
Methylcobalamine: 750 mg	Elemental Copper: 1 mg
Co-Enzyme Q 10: 2.5 mg	Elemental Manganese: 2 mg
Lycopene: 2 mg	Elemental Selenium: 100 mcg
Folic acid: 1.5 mg	Pyridoxyl Hydrchloride: 5 mg
	Glutathionine: 2.5 mg

Table 1: Ingradients of oligocare tablet.

Dose of each micronutrient (Group B)
L- Carnitine: 2.64 gm daily in four divided doses (each tab contains 330mg)
Vit C: 1 gm daily
Vit E: 800 IU daily in two divided doses (each cap contains 400 IU)
Vit B 12: 1000 mcg daily
Zink: 60 mg in three divided doses (each tab contains 20 mg)

Table 2: Combination of micronutrients.

Parameters	Group A Mean ± SD	Group B Mean ± SD	Significance
Age	37.3 ± 2.5	35.3 ± 3.1	0.314
Duration of infertility	5.1 ± 2.9	4.89 ± 2.8	0.201
Hormones			
FSH mU/ml	6.54 ± 1.1	5.85 ± 0.9	0.98
LH mU/ml	5.42 ± 0.8	5.46 ± 1.12	0.184
Prolactin ng/l	18.42 ± 1.8	17.64 ± 1.21	0.598
Testosterone ng/ml	356.77 ± 91.2		
Pretreatment count (mill/ml)	10.20 ± 11.12	11.24 ± 14.32	0.896
Pretreatment (%) motility			
Total motility	30.12 ± 15.42	34.23 ± 18.75	0.489
Progressive motility	15.68 ± 11.98	18.45 ± 10.65	0.524

Table 3: Patients characteristics.

Groups	Pre Treatment Mean ± SD	Post Treatment Mean ± SD	Significance
Group A			
Count (mill/ml)	10.20 ± 11.12	39.89 ± 25.64	0.000
Total motility (%)	28.12 ± 15.42	55.21 ± 13.56	0.004
Progressive motility (%)	15.68 ± 11.98	38.14 ± 9.89	0.009
Group B			
Count (mill/ml)	11.24 ± 14.32	62.28 ± 24.42	0.000
Total motility (%)	30.23 ± 18.75	75.13 ± 14.34	0.008
Progressive motility (%)	16.45 ± 10.65	48.34 ± 8.76	0.001

Table 4: Pretreatment and post treatment difference in count and motility in two treatment groups.

Similarly pregnancy rate was also higher in high dose group 22% in comparison to 12% in low dose group. Though statistically it was not significant but numerically it was almost double.

Discussion

Most environmental toxicants have been shown to impair testicular spermatogenesis by inducing ROS [22]. Male reproduction does not only require normal sperm parameters in terms of sperm concentration and motility but also the integrity of the genetic constitution of the spermatozoa. ROS and other noxious substances causes increased number of mitochondrial DNA mutations, nuclear DNA fragmentation, impair sperm membrane integrity and therefore motility and also affect the genetic constitution of the sperm [23,24]. Several studies showed that diminished DNA integrity in the sperm affects the developmental competence of the embryo [25,26]. DNA integrity is a prerequisite, not only for the fertilization of an oocyte, but also for oocyte activation and achievement of an ongoing pregnancy [27]. DNA integrity can be considered as a sperm function that is independent from sperm count, motility, and morphology [28,29]. Disturbances in sperm DNA integrity have been described in patients with varicoceles, testicular tumours, genital tract infections/inflammations, and patients with 'idiopathic' infertility [27,30,31].

In idiopathic oligospermia and asthenosermia, where there is no specific reason for lifestyle changes, a simple balanced nutritional diet and nutritional supplements with antioxidants can help reverse some of the oxidative damage from environmental toxins and natural aging. In men certain nutrients are essential for formation of healthy sperm. Micronutrients like vitamin C, vitamin E, L-carnitine, zinc, vitamin B complex have positive effect on both sperm count and motility [32-41]. In addition of high intake of fruits, vegetables, whole grains and nuts, supplements are also needed for noticeable effects on sperm quality. In some cases bacterial infection may cause oxidative damage but infection remains unrecognized even by culture and sensitivity. So empirical antibiotic in some cases may reduce oxidative damage of sperm and improve semen quality.

In the present study we compared the effect of low dose and high dose micronutrients along with antibiotics in the treatment of oligospermia, asthenospermia and oligoasthenospermia. Among two treatment Groups, group A contains a number of antioxidants with smaller doses and group B contains higher dose of antioxidants but lower in number (Tables 1 and 2). Though in both groups there was significant increment of mean sperm count and motility (Table 4), we found improvement of higher number of patients' semen parameters after using high dose antioxidants (Table 5). There was a significant difference in number of patients who got benefit from the treatment. Almost 79% patients' semen parameters improved after getting high dose antioxidants, whereas which 48% was in low dose group. Although there was no statistically significant difference in number of pregnancy, it was also almost double in high dose group, 22% vs. 12% in low dose group. As pregnancy is the combined result of male and female parameters so improvement of semen parameters is not the sole indicator of a couples' fertility potential. Moreover, trial was given only for six ovulatory cycles after improvement of semen parameters.

Different studies showed the positive effect of direct antioxidants in improving sperm count and motility [32-41]. Some studies showed positive effect of using vitamin E in high doses (600 and 800 IU daily) [42,43]. However, a small double blind trial found no benefit from high doses of vitamin E and C [44]. In our study we found profound positive effects in combination of high dose therapy. Vitamin B12 deficiency in men can lead to reduce sperm counts and lowered sperm motility particularly with elevated homocystine release due to oxidative stress. Vit B12 increases the enzymatic efficiency of the Methelenetrihydrofolate Reductase (MTHFR), and cystathionine betasynthase enzyme, which are responsible for removing homocystine from the circulation and

Parameters	Group A; N%	Group B; N%	Significance
Improvement of semen Parameters	20; 47.62	33; 78.57	0.003*
Pregnancy	5; 11.90	9; 21.43	0.241

χ^2=8.6403* χ^2=1.3714.

Table 5: Outcome of treatment of two groups.

improves sperm count and motility [45].

Different trials have demonstrated positive effects of oral L-carnitine and zinc in high doses to improve sperm function [35-37,46-48]. A double blind placebo controlled study showed the significant improvement of sperm count and motility by administration of zinc in high doses in infertile men [49]. Zink has antioxidative properties and is cofactor of the ROS scavenging enzymes glutathione peroxidases. Decreased zink concentration in seminal plasma was found in infertile men [50]. In the present study zinc and carnitine are also used in high doses along with vitamins and the effect of a combination drug is significant. In spite of high doses there was no reported side effects. Antioxidants also improve the outcome of IVF/ICSI in terms of better fertilization and implantation by improving sperm's fine morphology and DNA integrity. It also reduces the miscarriage rate, so useful in recurrent pregnancy loss [51-53].

What should be the duration of the treatment? In-spite of positive effect in a large number of study a few investigators reported no effect [54,55] and sometimes detrimental [56,57]. So there is a debate and controversy of long -term use of antioxidants. Though, previously it was used for 80-90 days now it is recommended to use the combination for 60 days only [58]. So we used combination of high dose only for 60 days. After that to maintain scavenging effect we continued Vit E only. After 6 months trial we recommended alternate treatment for all couples.

Studies showed that percent motility and progressive motility of sperm increased significantly after using antioxidants [59,60]. Cochrane databases showed both clinical pregnancy and live birth rate increased significantly after using antioxidants in subfertile men. Though dose schedule were varied in different studies net outcome is comparable with our study [61,62].

Limitation of the study was that we could not perform MSOME (Motile Sperm Organelle Morphology Examination) to detect sperm head vacuoles due to DNA damage. As antioxidant can correct this abnormality for clinical purpose this assessment should not affect the treatment approach. We also did not do karyotyping in any case, so chromosomal abnormality might present in some cases. We could not perform Y chromosome micro-deletion test in all cases due to economic constrains. In group A 15 and in group B 17 patients had sperm concentration <5 million/ml. We did micro-deletion test only for those cases and all were found with normal AZF segment of Y chromosome.

Treatment of male infertility is a challenge for fertility specialists. Various nutritional strategies have been presented, which have a beneficial impact on sperm count, motility and ultimately fertility. For idiopathic infertility, dietary supplementation with a combination of micronutrients is effective, well tolerated and safe even in high doses for a short duration. Before going for expensive and unpredicted treatment like IVF/ICSI a short trial can be given with antioxidants, which might improve the sperm quantity and quality and help achieving pregnancy of some couples.

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