Original Article

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To Evaluate the Efficacy of Combination Antioxidant Therapy on Oxidative Stress Parameters in Seminal Plasma in the Male Infertility

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Introduction: Infertility is defined as inability to conceive after 1 year of unprotected intercourse and it affects 7% of male population and 8–10% of couples. According to estimates WHO, 13-19 million couples in India are infertile. Oxidative stress is the causative factor in 25% of infertile males.

Aim: To study the efficacy of antioxidant therapy on oxidative stress parameters in seminal plasma of infertile male.

Materials and Methods: Forty patients of male infertility were enrolled in study after two abnormal semen analyses reports at 2-3 weeks interval, of oligozoospermia and/or asthenozoospermia, as per WHO guide line 1999. First semen sample was collected at a time of enrollment of study and second semen sample was collected three months after combined antioxidant therapy. Semen samples from the infertile male (the second confirmatory sample of oligoasthenozoospermia) were taken and after liquefaction semen sample were utilized for various analyses, 0.5 ml of sample for standard semen analysis, 1.2 ml sample for separation of seminal plasma to evaluate Oxidative stress (OS) parameters like Malondialdehyde (MDA), Protein Carbonyl (PC) and antioxidant capacity by Glutathione (GSH). We followed the patient for three months after completion of the treatment.

Results: *Semen parameters* – Out of 40 patients recruited in the study group 7 patients had only oligospermia (1 to 20 million/ml) and 31 patients had oligoasthenozoospermia (motility range 0-50%) and 2 patients had oligoasthenoteratozoospermia. There was no patient with asthenospermia alone as abnormal semen parameters. After the three months treatment with combined antioxidants the semen parameters like count (mean SD = -1.70±1.44) and motility (mean +SD= -9.56±9.05) were significantly increased (p-value=0.000).

Oxidative Stress Assessment – The level of MDA which is a marker of oxidative stress was significantly lower after the three months therapy of antioxidants (p-value=0.002) whereas another marker which is denoted by PC was also lower after the treatment but not statistically significant (p-value=0.584). The level of antioxidants GSH also significantly increased after the treatment (p-value=0.000). After the treatment out of 40, five patients conceived (16.7%).

Conclusion: As we have seen through this study antioxidant dramatically reduced the oxidative stress markers and enhancing the antioxidant enzymes. They should be used on routine basis in case of male infertility.

Keywords: Manoldialdihyde, Protein Carbonyl, Reactive oxygen species

INTRODUCTION

The incidence of infertility is approximately 15%, it affects the population psychologically and medically [1]. Out of 15% infertile patients 50% are male and 50% are females, so male factor is also equally contributing factor [2]. In approximately 25% of infertile males no identifiable cause is found, and oxidative stress has been attributed to affect the fertility state [3]. As it affects a huge no. of patients so has been extensively studied in recent years [4].

The imbalance between pro-oxidants and antioxidants is called as oxidative stress [5]. It can either be produced by decreasing the antioxidants defense mechanism or increasing the pro-oxidants like reactive oxygen or nitrogen species [6-8]. However, certain amount of ROS is needed for oxidation for a cell to function [9]. But excessive production can hamper body's natural antibody defense mechanism and lead to multiple disorders including male and female infertility [10].

It has been proved by various studies that high levels of ROS is present in unexplained male infertility, increasing levels of ROS causes chemical and structural modifications to sperm DNA, protein, lipids in mitochondria and plasma both [11-13]. Plasma membrane is more susceptible to oxidation and other chemical reactions because it is richly supplied by polyunsaturated fatty acids. An increased level of ROS disrupts fluidity of sperm plasma membrane, causing impaired motility which further effects on fusion events such as acrosome reaction and sperm oocyte fusion [14]. It also causes DNA damage to mitochondria and nuclear genomes of spermatozoa [15].

Endogenous ROS in semen are generated by leukocytes and abnormal spermatozoa [16]. In leukocytes mainly neutrophils and macrophages are major contributory factor of reactive oxygen intermediate like O_2 and H_2O_2.

Antioxidants such as vitamin C and E, folate, zinc, selenium, carnitine, and carotinoids are the scavengers of ROS and their use has been studied as a treatment to reverse the adverse impact of high concentration on semen parameters.

Zinc, a trace element used as an antioxidant and has a membrane stability activity, which in turns destroys hydrogen peroxide molecule. Carnitine is involved in the transport of long chain fatty acids into mitochondrial matrix for beta oxidation and exert antioxidant activity via increasing expression of antioxidants such as heme oxygenase and endothelial nitric oxide synthetase. Astaxanthine, a carotenoid extract from algae with a high no. of conjugated double bonds, rendering it a more potent antioxidant. Ubidecarenone, acts by inhibiting hydrogen peroxide formation in seminal fluid, a membrane stabilizer. Lycopene, a potent antioxidant inactivates hydrogen peroxide and nitrogen dioxide thereby preventing free radical damage caused by ROS.

AIM

To evaluate the efficacy of antioxidant therapy on oxidative stress parameters in seminal plasma of infertile male.

MATERIALS AND METHODS

Study was conducted in Department of Obstetrics and Gynaecology and Department of Biochemistry during December 2012-December 2013 after institutional ethical committee clearance.

Study Population - It was a prospective interventional study done in the infertility clinic of Department of Obstetrics and Gynaecology, couples were evaluated for the factor of infertility. Male partners of women with normal fertility work-up viz. confirmed ovulation, patent tubes and absence of any endometrial or adnexal pathology formed the study group. Two abnormal semen analyses reports at 2-3 weeks interval, of oligozoospermia and/or asthenozoospermia, as per WHO guide line 1999, were considered essential for inclusion in study group (sperm density <20 million/ml, motility <50%). Azoospermia, severe oligospermia, and asthenospermia were excluded from the study.

Forty patients were enrolled in study. First semen sample was collected at a time of enrollment of study and second semen sample was collected three months after antioxidant therapy.

Antioxidant therapy (Tablet Fertisure M) twice a day was given for three months. This tablet contains Astaxanthin-8 mg, Ubidecarenone-50mg, L-carnitine-340mg, Zinc-5mg and Lycopene-2.5mg.

The female partner was followed for up to three months after completion of the treatment, for conception.

Sample Collection

Semen samples from infertile male and controls were obtained by masturbation, without the use of lubricants after an abstinence of 3-5 days. The sample was collected in the semen collection room of Hospital Laboratory Services block of the hospital, in a sterile wide mouthed plastic container. Semen samples from the infertile male (the second confirmatory sample of oligoasthenozoospermia) were taken and after liquefaction semen sample were utilized for various analyses, 0.5 ml of sample for standard semen analysis, 1.2 ml sample for separation of seminal plasma to evaluate oxidative stress parameters.

Semen Analysis

After liquefaction, 0.5 ml sample was taken for semen analysis. All samples were processed and the conventional seminal parameters were analyzed according to the "World Health Organization (WHO) guidelines for the examination of human semen" within 1 hour of receiving the samples. The following conventional parameters were documented: volume, sperm density, motility, morphology and presence of pus cells with the help of microscope, macular chamber and slides.

Measurement of MDA was Done by the Same Protocol as Cited by Chowdhary et al., [17]

0.1 ml of seminal plasma +0.9 ml of distilled water (DW) + 0.5 ml of TBA reagent. Heated for one hour in boiling water bath. After cooling centrifuged for 10 minutes at 4000rpm. The absorbance of the supernatant was read on a spectrophotometer at 534nm.

Optical density vs volume in nmol/ml was plotted on the standard graph and the concentration of malonedialdehyde (MDA) was expressed in nmol/mL serum.

**TBA reagent = 0.67 gm of 2 thiobarbituric acid + 100ml of DW+0.5gm of NaOH+ 100ml of glacial acetic acid.*

Measurement of protein carbonyl was done by the same protocol as cited by D Yeni et al., [18]

Content was calculated based on the molar extinction coefficient of DNPH (C2.2x10^4/M), and expressed as nano moles per milligram of protein.

Measurement of GSH was done by same protocol as cited by AR Chowdhary [17]

0.2ml of seminal plasma + 1.8ml of DW+ 3ml of precipitating solution*, Stand for 5 minutes filtered.

A 1ml of clear filtrate + 4 ml of freshly made disodium hydrogen phosphate (4.6g/l solution of 0.5ml of DTNB reagent 5, 5 dithiobis -2 nitrobenzoic acid: 20mg in 100ml of citrate buffer). Absorbance of yellow colour read in spectrophotometer at 412nm.

The optical density was plotted against volume on standard graph and the concentration of GSH was expressed as milligram per deciliter of semen.

**Precipitating solution: metaphosphoric acid 1.67g, disodium EDTA 0.2g Nacl 30g in 100ml of DW.*

RESULTS

Semen Parameters

A total of 40 patients recruited in the study group 7 patients had only oligospermia (1 to 20 million/ml) and 31 patients had oligoasthenozoospermia (motility range 0-50%) and 2 patients had oligoasthenoteratozoospermia. There was no patient with asthenospermia alone as abnormal semen parameters.

After the three months treatment with antioxidants the semen parameters like count (mean SD = -1.70+ 1.44) and motility (mean +SD= -9.56+9.05) were significantly increased (p-value=0.000) [Table/Fig-1].

A significant difference (p<0.05) in the count, and motility were observed before and after treatment.

Variables	Mean	Mean±SD	p-value
Sperm count (before)	10.93	-1.70±1.44	0.00
Sperm count (after)	12.63		
Sperm motility (before)	28.66	-9.56±9.05	0.00
Sperm motility (after)	38.23		

[Table/Fig-1]: Count and motility before and after treatment.

Biochemical Analysis

Oxidative Stress Assessment

The level of MDA which is a marker of oxidative stress was significantly lower after the three months therapy of antioxidants (p-value -0.002) whereas another marker which is denoted by PC was also lower after the treatment but not statistically significant (p-value-0.584). The level of antioxidants GSH also significantly increased after the treatment (p-value-0.000) [Table/Fig-2].

Pregnancy Rate

After the treatment out of forty, five patients conceived (16.7%).

Variables	Mean	Mean± SD	p-value
MDA(before)	4.053	0.980± 1.52	0.002
MDA(after)	3.073		
PC(before)	0.666	0.0189±0.18	0.584
PC(after)	0.647		
GSH(before)	20.406	-32.39±5.60	0.00
GSH(after)	52.80		

[Table/Fig-2]: Oxidative stress parameters before and after treatment

DISCUSSION

The present data shows a significant difference in the semen parameters like count and motility after three months of antioxidant therapy and also the biochemical parameters like MDA and PC were significantly decreased and antioxidants were significantly increased after the treatment. As the duration of the treatment used in our study cover the spermatogenic cycle, so, it is clearly visible that the damage at any level of spermatogenesis can be easily corrected by three months of antioxidants therapy.

As it is well known fact that spermatogenic cycle completes in three months and this is the idea of present study to give antioxidants for three months to cover the sperm damage at any level of spermatogenesis.

Similar to our study, improved in semen concentration and motility has been described after 6 months of treatment with vitamin E alone [19], and similarly Scott et al., reported improvement in sperm motility after three months of combination of vitamin A, E, C, and selenium [20].

Contradictory to our study, Greco et al., not found any difference in the parameters like count and motility after three months of antioxidants therapy [21], but in contrast to basic semen parameters, but they also witnessed positive results on DNA fragment index after antioxidant therapy. In another study by Menzo et al., that combination of antioxidants including vitamin C, E, Zinc, Selenium, and beta carotene in 57 patients for 90 days, decreases DNA fragmentation index by 19% [22].

In present study we found high levels of MDA in infertile male as compared to fertile controls. Same results have been found in several studies like Nabil et al., Hsieh YY et al., Freczek et al., [23-25], but our results are in contrast with Suleiman et al., he demonstrated that MDA concentration in seminal plasma was not related with sperm count and motility [19].

Heidar et al., found an inverse correlation between total activity of catalase [26], superoxide dismutase and glutathione peroxidase with total content of malonaldehyde (marker of lipid peroxidation) in seminal plasma from normozoospermic samples. In asthenozoospermic samples there was no significant correlation between the activities of these enzymes and MDA content. An intrinsic defect of plasma membrane of sperms in asthenospermic samples which does not allow antioxidant enzymes to adsorb and act. Another metanalysis revealed strong evidence in support of antioxidants when sperm motility was evaluated particularly in asthenospermic patients. However, the authors pointed out inconsistent outcome variable with pregnancy related outcomes studied in only 50% studies of which 60% showed positive results.

The pregnancy rate in our study was 16.7% similar to other studies like Comhaire et al., p=0.028, and Cavallini et al., p=0.001 showed significantly higher pregnancy rates in the treatment group as compared to controls [27,28].

In present study, we have chosen combinations of antioxidants in the form of commercially available drugs, as it takes care of sperm damage at various stages of spermatogenesis induced by oxidative stress.

The improvement is not consistent and there is wide variation in the treatment regimens used. Therefore, further large randomized controlled studies were needed to see the effect at standardized doses of specific antioxidant on spontaneous as well as assisted reproductive techniques.

LIMITATION

Sample size was small and follow-up period was also short. It is not feasible to do oxidative stress parameters like MDA, PC, GSH in routine laboratory.

CONCLUSION

As oxidative stress is increasing because of life style factors causing end number of diseases as our study showed oxidative stress markers MDA and PC were significantly high and GSH was significantly low in infertile male as compared to fertile control. We also observed increased pregnancy rate after antioxidant therapy. Thus, OS parameters and antioxidant may be consider as routine procedure where ever facility available. As antioxidant are not having any major side effects and it is very effective in cases with oxidative stress therefore we conclude it should be routinely recommended in treatment of male infertility.

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REFERENCES

[1] Vaid NB. Female infertility: A diagnostic challenge. *JIMSA*. 2003;16:54–55.
[2] Snarlip ID, Jarop JP, Belker AM. Best practice policies for male infertility. *Fertil steril*. 2002;77:873–82.
[3] Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in Male Factor infertility: An update. *Am J Repro Immun*. 2008;59:2–11.
[4] Agarwal A, Sushil P. Oxidative stress and antioxidant in male infertility: a difficult balance. *Iranian J Reprod Med*. 2005;3(1):1–8.
[5] Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int J Biochem Cell Biol*. 2010;42:1634–50.
[6] Burton GJ, Jauniaux E. Oxidative Stress. *Best Pract Res Clin Obstet Gynaecol.* 2010;25:287–99.
[7] Cindrova-Davies T, Yung HW, Johns J, Spasic-Boskovic O, Korolchuk S, Jauniaux E. Oxidative stress, gene expression, and protein changes induced in the human placenta during labor. *Am J Pathol.* 2007;171:1168–79.
[8] Ruder EH, Hartman TJ, Goldman MB. Impact of oxidative stress on female fertility. *Curr Opin Obstet Gynaecol.* 2009;21:219–22.
[9] Chandra A, Surti N, Kesavan S, Agarwal A. Significance of oxidative stress in human reproduction. *Arch Med*. 2009;5:528–42.
[10] Webster RP, Roberts VH, Myatt L. Protein nitration in placenta - functional significance. *Placenta*. 2008;29:985–94.
[11] Shen HM, Chia SE, Ong CN. Evaluation of oxidative DNA damage in human sperm and its association with male infertility. *J Androl*. 1999;20:718–23.
[12] Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: an update. *Am J Reprod Immunol*. 2008;59:2–11.
[13] Aitken RJ, De Iuliis GN. On the possible origins of DNA damage in human spermatozoa. *Mol Hum Reprod.* 2010;16:3–13.
[14] Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. *Biol Reprod.* 1989a;41:83–197.
[15] Sawyer DE, Mercer BG, Wiklendt AM, Aitken RJ. Analysis of gene-specific DNA damage and single-strand DNA breaks induced by pro-oxidant treatment of human spermatozoa in vitro. *Mutat Res*. 2003;529:21–34.
[16] Sakkas D, Seli E, Bizzaro D, Tarozzi N, Manicardi GC. Abnormal spermatozoa in the ejaculate: abortive apoptosis and faulty nuclear remodelling during spermatogenesis. *Reprod Biomed Online*. 2003;7:428–32.
[17] Chaudhari AR, Das P, Singh R. Study of oxidative stress and reduced glutathione levels in seminal plasma of human subjects with different fertility potential. *Biomed Res*. 2008;19(3):207-10.
[18] Yeni D, Gundogan M, Cigerci IH, Avdatek F, Fidan AF. Seasonal variation of oxidative stress parameters in ram seminal plasma. *J Animal Vet Adv*. 2010;9:49-54.
[19] Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxi- dation and human sperm motility: p rotective role of vitamin E. *J Androl.* 1996;17:530–37.
[20] Scott R, MacPherson A, Yates RW, Hussain B, Dixon J. The effect of oral selenium supplementation on human sperm motility. *Br J Urol*. 1998;82:76–80.
[21] Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl*. 2005;26(3):349-53.
[22] Ménézo YJ, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie P, et al. Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. *Reprod Biomed Online*. 2007;14(4):418-21.
[23] Nebil H, Moemen LA, Abu Elela MH. Studying the Levels of malondialdehyde and antioxidant parameters in normal and abnormal human seminal plasma. *Australian J Basic Appl Sci*. 2008;2:773-78.
[24] Hsieh YY, Chang CC, Lin CS. Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility. *Int J Biol Sci*. 2006;2(1):23-29.
[25] Fraczek M, Szkutnik D, Sanocka D, Kurpisz M. Peroxidation components of sperm lipid membranes in male infertility. *Ginekol Pol*. 2001;72(2):73-79.

[26] Tavilani H, Goodarzi MT, Vaisi-raygani A, Salimi S, Hassanzadeh T. Activity of antioxidant enzymes in seminal plasma and their relationship with lipid peroxidation of spermatozoa. *Int Braz J Urol*. 2008;34(4):485-91.
[27] Comhaire FH, El Garem Y, Mahmoud A, Eertmans F, Schoonjans F. Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial. *Asian J Androl*. 2005;7(3):257-62.
[28] Cavallini G, Ferraretti AP, Gianaroli L, Biagiotti G, Vitali G. Cinnoxicam and L-carnitine/acetyl-L-carnitine treatment for idiopathic and varicocele-associated oligoasthenospermia. *J Androl*. 2004;25(5):761-70.

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