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ORIGINAL ARTICLE

The Effect of Doxycycline on Pus Cells and Oxidative Stress in Male Patients with Leukocytospermia

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ABSTRACT

Objective: The World Health Organization (WHO) has defined leukocytospermia as $>10^6$ WBC/ml of semen. However, the clinical significance of leukocytospermia is currently a subject of controversy. Evidence from several recent studies indicates that leukocytospermia could significantly contribute to oxidative stress and male infertility. Several clinical trials have investigated the efficacy of antibiotic therapy to treat patients with pyospermia in an attempt to improve fertility. Currently, doxycycline is the most common antibiotic used to treat pyospermia though larger trials are needed to demonstrate its efficacy in treating pyospermia.

Materials and methods: Hundred male partners with semen analysis showing the presence of significant leukocytospermia (WBC $>1 \times 10^6$/ml), sterile semen culture and satisfying the inclusion and exclusion criteria were enrolled in the study and given doxycycline 100 mg BD for 14 days. Semen analysis for pus cells and oxidative stress (ROS) measurement was done before and after the treatment.

Results: Mean pus cell count before and after treatment with doxycycline was $2.28 \pm 1.26 \times 10^6$/ml and $1.21 \pm 0.58 \times 10^6$/mL respectively, the effect being statistically significant ($p < 0.05$). Resolution of leukocytospermia was seen in 61.4% (54/88) cases after doxycycline treatment. Mean oxidative stress (RLU/sec/million sperms) before and after doxycycline therapy was 79.72 ± 133.9 and 25.44 ± 47.8, the difference being significant ($p < 0.05$).

Conclusion: Study results show that treatment with broad spectrum antibiotic like doxycycline leads to significant decrease in the number of pus cells present in semen thereby significantly decreasing the oxidative stress.

Keywords: Oxidative stress, Male infertility, Leukocytospermia, Doxycycline, Reactive oxygen species.

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INTRODUCTION

Infertility is defined as the failure of conception after at least 12 months of unprotected intercourse.[1] Infertility is a worldwide problem and approximately 8 to 10% of couples within reproductive age group are infertile.[2] It is estimated that globally 60 to 80 million couples suffer from infertility every year, of which probably 15 to 20 million are in India alone.[3] Male factor is the sole cause of infertility in approximately 20% of infertile couples and contributory in 30 to 40% of couples, accounting for infertility in overall 50% of couples.[4] Male infertility management has grown at a slower pace as compared to that of female infertility. The last century has seen rapid advances in the diagnosis and management of male infertility but still the cause eludes us in a majority of cases. Though there has been a lot of research in this area, there is a continued need for more such endeavors to unravel the mystery of male infertility.

While evaluating an infertile couple, the World Health Organization (WHO) recommends performing a semen analysis on the male partner. White blood cells (WBC) are present in most human ejaculates, but abnormally high concentrations of seminal WBC may reflect an underlying pathological condition.[5] The World Health Organization (WHO) has defined leukocytospermia as $>10^6$ WBC/ml of semen.[6] However, the clinical significance of increased leukocyte infiltration in semen, i.e. leukocytospermia, is currently a subject of controversy.[7] Evidence from several recent studies indicates that leukocytospermia could significantly contribute to male infertility.[8] Associations between leukocytospermia and decreased sperm motility and fertilizing ability have been reported by a number of investigators.[9]

The etiology of leukocytospermia in healthy asymptomatic men is not fully understood, but, in some cases, it could represent inflammation resulting from subclinical bacterial infection of the male genital tract.[10] Pyospermia has multifactorial causes, such as infection, inflammation and autoimmunity. The etiology can be classified into several categories: presence of defective sperms, varicocele, chronic prostatitis, smoking, drug abuse like marijuana (social causes), alcohol, exposure to irritants and toxins, use of vaginal products by partner during sexual activity, prolonged abstinence, vasovasostomy,

clomiphene citrate therapy, urethroplasty, genital infections like Chlamydia trachomatis, Gardnerella vaginalis and Ureaplasma urealyticum, genital infections in patients' sexual partners, HIV positive patients having lower CD^{4+} cell counts.[11]

Of the many causes of male infertility, oxidative stress has been identified as one factor that affects fertility status and, thus, has been extensively studied in recent years. Spermatozoa, like any other aerobic cell, are constantly facing the 'oxygen-paradox'.[12] Oxygen is essential to sustain life as physiological levels of reactive oxygen species (ROS) are necessary to maintain normal cell function.[12] Conversely, breakdown products of oxygen, such as ROS, can be detrimental to cell function and survival.[12]

Oxidative stress (OS) is a consequence of an imbalance between the production of ROS and the body's antioxidant defence mechanisms. All cellular components, including lipids, proteins, nucleic acids and sugars are potential targets of oxidative stress. The extent of oxidative stress induced damage depends not only on the nature and amount of ROS involved but also on the duration of ROS exposure and on extracellular factors, such as temperature, oxygen tension and the composition of the surrounding environment, e.g. ions, proteins and ROS scavengers.[11,13-16] Spermatozoa are particularly susceptible to damage induced by ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids[17] and their cytoplasm contains low concentrations of scavenging enzymes.[18] In addition, the intracellular antioxidant enzymes cannot protect the plasma membrane that surrounds the acrosome and the tail, forcing spermatozoa to supplement their limited intrinsic antioxidant defenses by dependence on the protection afforded by the seminal plasma that bathes these cells.[19] Oxidative stress develops when levels of ROS production by leukocytes, spermatozoa or both, become high enough to overwhelm all antioxidant strategies, resulting in lipid peroxidation, impairment of sperm motility and loss of fertilizing potential.[20] ROS attacks polyunsaturated fatty acids (PUFA) in the cell membrane, leading to a cascade of chemical reactions called lipid peroxidation. ROS have a tendency toward chain reactions, proceeding through initiation, propagation and termination. Lipid peroxidation results in loss of membrane fluidity, which is essential for sperm motility and sperm oocyte fusion.[21-26] Oxidative stress is associated with high frequencies of single and double-strand DNA breaks.[27] ROS can cause various types of gene mutations, such as point mutations and polymorphism, resulting in decreased semen quality.[28,29] Other mechanisms, such as denaturation and DNA base-pair oxidation, also may be involved.[27]

While both leukocytes and spermatozoa produce ROS, the concentration of ROS generated by each cell type varies greatly. Several studies have shown that leukocytes are the major source of ROS in semen.[30,31] Leukocytes have been reported to produce 1000 times more ROS compared to spermatozoa engaged in capacitation.[32,33] These disparate levels seem to implicate leukocytes as the offending agents of oxidative injury. It is unclear from the existing literature whether the interaction between leukocytes and spermatozoa implies a direct or indirect stimulatory effect, which may enhance the capacity of spermatozoa to generate excessive ROS.

Several clinical trials have investigated the efficacy of antibiotic therapy to treat patients with pyospermia in an attempt to improve fertility. Haidl[34] reported on improvement of semen quality and decrease of leukocyte and bacteria concentration in seminal plasma as a result of antibiotic treatment in patients with chronic seminal tract infections. Branigan et al[35] combined doxycycline and trimethoprim-sulfamethoxazole successfully for treatment of leukocytospermia. In contrast to reports on positive effects of antibiotic treatment on leukocytospermia and semen quality respectively, other authors have not confirmed these data. In a double-blind prospective study by Comhaire et al[36] found no effect of doxycycline therapy in infertile couples with male accessory gland infection. Neither did Yanushpolsky et al[37] find an effect of doxycycline nor trimethoprim-sulfamethoxazole treatment in asymptomatic infertile men.

Currently, doxycycline is the most common antibiotic used to treat pyospermia though larger trials are needed to demonstrate its efficacy in treating pyospermia.

The purpose of this study was to study the effect of doxycycline on pus cells and oxidative stress in men with leukocytospermia.

MATERIALS AND METHODS

Study Design

Interventional study.

Study Population

All the male partners of the infertile couples attending the infertility clinic of our hospital were subjected to a detailed history, examination and investigations. Detailed history regarding duration of infertility, coital frequency, any sexual dysfunction, previous medical or surgical illness, occupational history, life style factors was taken. Physical examination was performed by a urologist to exclude cases with known factors, such as varicocele, cryptorchidism and endocrine disorders. Men with history of tobacco chewing/smoking, alcohol consumption, prolonged thermal/radiation exposure, prolonged medical illness were excluded from the study.

Sample Size

Hundred male partners with semen analysis showing the presence of significant leukocytospermia (WBC $>1\times10^6$/ml) and sterile semen cultures were included in the study.

SEMEN COLLECTION AND SEMEN ANALYSIS

Semen samples were collected in a sterile plastic container after sexual abstinence of 3 to 5 days. All the samples were examined within one hour of collection. Each sample was incubated at room temperature to allow liquefaction. Semen analysis was performed according to the guidelines of the WHO. The gross appearance, volume, viscosity and pH semen were noted. A wet mount of semen sample was prepared, and microscopic examination was done to measure sperm concentration, sperm motility and sperm vitality. Sperm concentration was determined with an improved Neubauer counting chamber after appropriate dilution. Sperm morphology, specific sperm defects, leukocyte count were determined after staining the smear with papanicolaou stain.

ESTIMATION OF ROS BY CHEMILUMINESCENCE ASSAY

Levels of ROS were measured in a fresh semen sample using a chemiluminescence assay. Four hundred microliter aliquots of the sample was allowed to run in the luminometer to assess basal ROS levels. Ten microliter of luminol (5-amino-2,3,-dihydro-1,4-phthalazinedione) prepared as 5 mM stock in dimethyl sulphoxide, was added to the mixture and served as a probe. A negative control was prepared by adding 10 µl of 5 mM luminol to 400 µl of tap water. The reaction of luminol with ROS results in production of a light signal that is converted to an electrical signal (photon) by a luminometer. Levels of ROS were assessed by measuring the luminol-dependent chemiluminescence with the luminometer for 10 minutes. The results were expressed as RLU/million sperms/second.

Intervention

Cases enrolled were given doxycycline 100 mg BD for a period of 14 days.

Semen samples were evaluated for pus cells and doxycycline before starting and 1 week after administering the last dose of doxycycline.

STATISTICAL ANALYSIS

Number of pus cells and ROS levels before and after treatment were compared using relevant statistical tests. For all analyses, statistical significance was considered as $p<0.05$.

RESULTS

Hundred male partners with semen analysis showing the presence of significant leukocytospermia (WBC $>1\times10^6$/ml), sterile semen culture and satisfying the inclusion and exclusion criteria were enrolled in the study. Detailed history, physical examination was done to rule out any confounding factor. However, only 88 men completed the study with 12 men dropping out of the study. Cases enrolled were given doxycycline 100 mg BD for a period of 14 days. Semen samples were evaluated for pus cells and doxycycline before starting and 1 week after administering the last dose of doxycycline. Table 1 shows the clinical and semen profile of the study group at the initiation of treatment. Table 2 shows that the number of pus cell ($\times10^6$/ml) decreased from 2.28 ± 1.26 to 1.21 ± 0.58 ($p<0.05$) after doxycycline treatment. Oxidative stress decreased from 79.72 ± 133.9 RLU/second/million sperms to 25.44 ± 47.8 RLU/second/million sperms ($p<0.05$) after doxycycline therapy. Table 3 depicts resolution of leukocytospermia was found in 54/88 cases (61.4%) and persistence of leukocytospermia in 34/88 cases (38.6%) after doxycycline treatment.

DISCUSSION

In the present study, mean pus cell count before and after treatment with doxycycline was $2.28\pm1.26\times10^6$/ml

Table 1: Clinical and semen profile of the study group

Parameter	*Mean ± SD*	*Range*
Age (years)	29.23 ± 4.65	21-42
Duration of infertility (years)	5.05 ± 2.79	1.5-15
Total sperm concentration (million/ml)	60.40 ± 33.58	0.06-185
Motility (%)	46.19 ± 17.46	5-80
Morphology (%)	43.87 ± 17.89	10-95
No. of pus cells ($\times10^6$/ml)	2.28 ± 1.26	1.25-8
Oxidative stress (ROS/million sperms/second)	79.72 ± 133.96	11.34-79.72

ROS: Reactive oxygen species; SD: Standard deviation

Table 2: Effect of doxycycline on pus cells and oxidative stress

	Pretreatment	*Post-treatment*	*p-value*
No. of pus cells ($\times10^6$/ml)	2.28 ± 1.26	1.21 ± 0.58	<0.05 Significant
Oxidative stress (ROS/million sperms/second)	79.72 ± 133.9	25.44 ± 47.8	<0.05 Significant

ROS: Reactive oxygen species

Table 3: Effect of doxycycline on pus cells (N = 88)

	N	*Percentage (%)*
No. of patients showing resolution of pus cells	54	61.4
No. of patients showing persistence of pus cells	34	38.6

and 1.21 ± 0.58 × 10^6/ml respectively, the effect being statistically significant ($p < 0.05$). Resolution of leukocytospermia was seen in 61.4% (54/88) cases after doxycycline treatment. Cardoso EM et al found significant decrease ($p < 0.0001$) in pus cells after doxycycline therapy.[38] Significant reduction in the number of pus cells ($p < 0.001$) was observed by Skau and Folstad in their meta-analysis.[39] Vicari observed that while WBC values remained unmodified in matched controls, they were significantly lowered in the subjects treated with doxycycline.[40] Branigan et al found 36 (68%) responded to treatment with doxycycline and 17 (32%) failed to respond.[35] Yanushpolsky et al did not find any significant effect of doxycycline on resolution of leukocytospermia. They found eight out of 13 cases showed resolution of pus cells and five out of 13 cases showed persistence of leukocytospermia even after doxycycline therapy, the difference being statistically insignificant ($p = 0.44$).[37] Berger et al found significant decrease in the number of leukocytes/ml of semen after doxycycline therapy ($p < 0.05$).[41]

Doxycycline was chosen as it is a broad spectrum antibacterial against a variety of Gram-negative, Gram-positive pathogens as well as Chlamydia trachomatis and Ureaplasma urealyticum which have commonly been implicated as cause of leukocytospermia and are difficult to culture from semen. Successful treatment of leukocytospermia with doxycycline implies a bacterial etiology of the WBCs. However, since all the subjects did not show resolution of leukocytospermia post-treatment, the etiology of leukocytospermia must be multifactorial. Besides subclinical bacterial infection, inflammation, viral infections, presence of defective sperms, chemical irritants may also lead to leukocytospermia. Hence, efforts should be made to find the cause and give directed treatment in cases of persistent leukocytospermia.

In our study, mean oxidative stress (RLU/sec/million sperms) before and after doxycycline therapy was 79.72 ± 133.9 and 25.44 ± 47.8, the difference being significant ($p < 0.05$). Vicari observed significant reduction in WBC-specific ROS ($p < 0.05$) and the chemiluminescence ROS ($p < 0.01$) following treatment with doxycycline.[41]

While both leukocytes and spermatozoa produce ROS, the concentration of ROS generated by each cell type varies greatly. Several studies have shown that leukocytes are the major source of ROS in semen.[30,31] Leukocytes have been reported to produce 1000 times more ROS compared to spermatozoa. Several studies have shown that leukocytes are the major source of ROS in semen. Besides directly producing (ROS), leukocytes have also been shown to lead to increased oxidative stress by causing adverse changes in spermatozoa. Morphologically abnormal sperms, especially those with cytoplasmic retention, have been shown to generate high levels of ROS. As expected decrease in the number of leukocytes led to decrease in the oxidative stress in both the groups following treatment with doxycycline.

CONCLUSION

Bacterial and viral infections are postulated to be important etiological factors for male infertility. However, despite of extended diagnostic efforts providing highly specific and sensitive methods for detection of most of the bacterial and viral infections, the causal link between infection and male infertility cannot be established in most of the cases. Hence, broad spectrum antibiotics are routinely and empirically prescribed to patients with leukocytospermia. Study results show that treatment with broad spectrum antibiotic like doxycycline in these infertile men with leukocytospermia led to significant decrease in the number of pus cells present in semen thereby significantly decreasing the oxidative stress. However, a few patients had persistent leukocytospermia even after treatment with doxycycline, it is therefore recommended that further detailed evaluation of such patients should be done to find out its cause and administer an appropriate treatment.

REFERENCES

1. Rowe PJ, Compaire FH, Hargereave TB. WHO manual for standard investigation and diagnosis of the infertile couple. Cambridge: Cambridge University Press; 1993. p. 1-92..
2. World Health Organization. Recent advances in medically assisted conception. Report of a WHO Scientific group. WHO Tech Rep Ser 1992;820:1-111.
3. World Health Organization. The World Health Report 1996 fighting disease, folstering development. WHO, Geneva; 1996.
4. Thonneau P, Marchand S, Tallec A, et al. Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988-1989). Hum Reprod 1991;6(6):811-816.
5. El Demiry MI, Hargreave TB, Busuttil A, et al. Immunocompetent cells in human testis in health and disease. Fertil Steril 1987;48(3):470-479.
6. World Health Organization. WHO laboratory manual for the examination of human sperm and semen—cervical mucus interaction. 4th ed. New York, NY: Cambridge University Press; 1999. p. 128.
7. Thomas J, Fishel SB, Hall JA, et al. Increased polymorphonuclear granulocytes in seminal plasma in relation to sperm morphology. Hum Reprod 1997;12(11):2418-2421.
8. de Bellabarba GA, Tortolero I, Villarroel V, et al. Nonsperm cells in human semen and their relationship with semen parameters. Arch Androl 2000;45(3):131-136.
9. Maruyama DK Jr, Hale RW, Rogers BJ. Effects of white blood cells on the in vitro penetration of zona-free hamster eggs by human spermatozoa. J Androl 1985;6:127-135.
10. Baratt CLR, Bolton AE, Cooke ID. Functional significance of white blood cells in the male and female reproductive tract. Hum Reprod 1990;5(6):639-648.

11. De Lamirande E, Gagnon C. Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effect. Hum Reprod 1995;10 (Supp 1):15-21.
12. Sies H. Strategies of antioxidant defense. Eur J Biochem 1993; 215(2):213-219.
13. Agarwal A, Prabakaran SA. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. Indian J Exp Biol 2005;43(11):963-974.
14. Aitken RJ. Molecular mechanisms regulating human sperm function. Mol Hum Reprod 1997;3(3):169-173.
15. Griveau JF, Le Lannou D. Reactive oxygen species and human spermatozoa: physiology and pathology. Int J Androl 1997; 20(2):61-69.
16. Sikka SC. Relative impact of oxidative stress on male reproductive function. Curr Med Chem 2001;8(7):851-862.
17. Alvarez JG, Storey BT. Differential incorporation of fatty acids into and peroxidative loss of fatty acids from phospholipids of human spermatozoa. Mol Reprod Dev 1995; 42(3):334-346.
18. Storey BT, Alvarez JG, Thompson K. Human sperm glutathione reductase activity in situ reveals limitation in the glutathione antioxidant defense system due to supply of NADPH. Mol Reprod Dev 1998;49(4):400-407.
19. Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil Steril 1992;57: 409-416.
20. Sikka SC, Rajasekaran M, Hellstrom WJG. Role of oxidative stress and antioxidants in male infertility. J Androl 1995;16(6): 464-468.
21. Aitken RJ. Free radicals, lipid peroxidation and sperm function. Reprod Fertil Dev 1995;7(4):659-668.
22. Kodama H, Kuribayashi Y, Gagnon C. Effect of sperm lipid peroxidation on fertilization. J Androl 1996;17(2):151-157.
23. Aitken J, Fisher H. Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. Bioessays 1994;16(4):259-267.
24. Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biol Reprod 1989;41(1):183-197.
25. Aitken RJ, Harkiss D, Buckingham DW. Analysis of lipid peroxidation mechanisms in human spermatozoa. Mol Reprod Dev 1993;35(3):302-315.
26. Makker K, Agarwal A, Sharma R. Oxidative stress and male infertility. Indian J Med Res 2009;129(4):357-367.
27. Kodama H, Yamaguchi R, Fukuda J, et al. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. Fertil Steril 1997;68:519-524.
28. Sharma RK, Said T, Agarwal A. Sperm DNA damage and its clinical relevance in assessing reproductive outcome. Asian J Androl 2004;6(2):139-148.
29. Spiropoulos J, Turnbull DM, Chinnery PF. Can mitochondrial DNA mutations cause sperm dysfunction? Mol Hum Reprod 2002;8(8):719-721.
30. Eggert-Kruse W, Bellman A, Rohr G, et al. Differentiation of round cells in semen by means of monoclonal antibodies and relationship with male infertility. Fertil Steril 1992;60:1069-1075.
31. Tomlinson MJ, Barrat GLR, Cooke ID. Prospective study of leukocytes and leukocyte sub-populations in semen suggests that they are not a cause of male infertility. Fertil Steril 1993; 60:1069-1075.
32. Fisher H, Aitken R. Comparative analysis of the ability of precursor germ cells and epididymal spermatozoa to generate reactive oxygen metabolites. J Exp Zool 1997;277(5):390-400.
33. Shekarriz M, Sharma RK, Thomas AJ, et al. Positive myeloperoxidase staining (Endtz test) as an indicator of excessive reactive oxygen species formation in the semen. J Assist Reprod Genet 1995;12:70-74.
34. Haidl G. Macrophages in semen are indicative of chronic epididymal infection. Arch Androl 1990;25(1):5-11.
35. Branigan EF, Spadoni LR, Muller CH. Identification and treatment of leukocytospermia in couples with unexplained infertility. J Reproduc Med 1995;40(9):625-629.
36. Comhaire FH, Rowe PJ, Farley TM. The effect of doxycycline in infertile couples with male accessory gland infection: a double blind prospective study. Int J Androl 1986;9(2):91-98.
37. Yanushpolsky EH, Politch JA, Hill JA, et al. Antibiotic therapy and leukocytospermia: a prospective randomized controlled study. Fertil Steril 1995;63:142-147.
38. Cardoso EM, Santoianni JE, De Paulis AN, et al. Improvement of semen quality in infected asymptomatic infertile male after bacteriological cure. Medicina (B Aires) 1998;58(2):160-164.
39. Skau PA, Folstad I. Do bacterial infections cause reduced ejaculate quality? A meta-analysis of antibiotic treatment of male infertility. Behav Ecol 2003;14(1):40-47.
40. Vicari E. Effectiveness and limits of antimicrobial treatment on seminal leukocyte concentration and related reactive oxygen species production in patients with male accessory gland infection. Hum Reprod 2000;15(12):2536-2544.
41. Berger RE, Karp LE, Williamson RA, et al. The relationship of pyospermia and seminal fluid bacteriology to sperm function as reflected in the sperm penetration assay. Fertil Steril 1982; 37:557-564.