



Reactive Oxygen Species in peritoneal fluid has association with idiopathic infertility in females

*D Anil Kumar¹, V Shanmuga Priya², J Jaiprabhu², R Swaroopa³

¹Associate Professor, ²Assistant professor, Department of Biochemistry, Vinayaka Missions Medical College, Karaikal, Pondicherry, India.

³Associate Professor, Department of Pathology, Vinayaka Missions Medical College, Karaikal, Pondicherry, India.

Abstract

We have designed this prospective study to determine whether reactive oxygen species (ROS) in the peritoneal fluid might be a factor contributing to idiopathic female infertility. This study was carried out at Advanced Research Centre in association with department of Obstetrics & Gynecology of Vinayaka Mission Medical College and Hospital, Pondicherry with tertiary care facilities. Present study population includes 50 women with idiopathic infertility posted for laparoscopy and 50 controls include fertile women posted for laparoscopic sterilization. Aspiration of peritoneal fluid is done for estimation of ROS levels, presence of polymorphonuclear granulocytes and leukocyte distribution. Reactive oxygen species were observed in the peritoneal fluid of patients with unexplained infertility. Levels of ROS in cell free fluid were low when compared to that of fluid with cell pellet. Our study concludes that reactive oxygen species in the peritoneal fluid may contribute directly/indirectly to idiopathic infertility.

Keywords: Infertility, Peritoneal fluid, reactive oxygen species, Oxidative stress

*Corresponding Author: Dr D Anil Kumar, Associate Professor, Department of Biochemistry, Vinayaka Mission Medical College and Hospital, Pondicherry, India. E-mail: munna_dr_74@rediffmail.com

Received: June 22, 2014 Accepted: September 7, 2014. Published: September 20, 2014. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Infertility is a common problem experienced by many couples. Numerous treatments are available for female infertility. However, in some cases the treatment is empirical in nature because the etiology of infertility is not fully understood. Recently, reactive oxygen species

(ROS) have been shown to have an important role in the normal functioning of reproductive system and in the pathogenesis of female infertility. Reactive oxygen species most likely to play a regulatory role in ovarian steroidogenesis, folliculogenesis, oocyte maturation, and luteolysis. Reactive oxygen species may also play a role in other female reproductive organ diseases such as endometriosis.

Materials and Methods

Peritoneal fluid (PF) was aspirated during laparoscopy from all the 50 women subjects without contamination with blood in a sterile container. Wang Y et al. 1997 followed a method to estimate ROS. [1] Aliquots of PF were centrifuged at 500Xg. ROS measured in both cell

free supernatant and pellet using chemiluminescence assay. 10 µl of 5mmol/L Luminol (5-amino-2, 3 dehydro-1, 4 naphthalazinedione; Sigma Chemical Co., St Louis, MO, USA) in dimethyl sulfoxide (Sigma Chemical Co) added to 500 µl of both PF samples. Biggers, Whitten, and Whittingham (BWW) medium was used as blank. 10 µl of 5mmol/L luminol added to BWW medium was used as negative control. Levels of ROS were determined by measuring chemiluminescence in the integrated mode for 15 min and results were expressed as $\times 10^4$ counted photons per minute (c.p.m.). Pellets of PF was smeared and stained using Wright's stain to count macrophage, neutrophils, monocytes and lymphocytes. data presented as medians and values of ROS from supernatant and pellet PF were compared. Spearman's rank order correlation was used to assess the ROS values and the patient's age and PF volume.

Results

The average age and BMI of the patients were 22.5 years and 26.5. Levels of ROS in supernatant and pellet PF sample from idiopathic infertile patients showed no correlation with age and peritoneal fluid volume. Women with idiopathic infertility had elevated levels of ROS values in both supernatant and pellet PF samples. But reactive oxygen species were high in cellular peritoneal fluid when compared to cell free fluid. (Figure 1) The distribution of macrophages in Pellets of PF sample show normal ($21\% \pm 10\%$) and similarly monocytes, neutrophils and lymphocytes showed normal pattern.

Discussion

Infertility is a common problem experienced by many couples. Numerous treatments are available for female infertility. However, in some cases, the treatment is empirical in nature because the etiology of infertility is not fully understood. Recently, reactive oxygen species (ROS) have been shown to have an important role in the normal functioning of reproductive system and in the pathogenesis of infertility in

females. [2-4] Reactive oxygen species may also play a role in other reproductive organ diseases of women such as endometriosis. Oxidative stress develops when there is an imbalance between the generation of ROS and the scavenging capacity of antioxidants in the reproductive tract. It affects both natural and assisted fertility. Oxidative stress develops when there is an imbalance between the generation of ROS and the scavenging capacity of antioxidants in the reproductive tract. It affects both natural and assisted fertility. Because assisted reproductive techniques are used extensively in the treatment of infertility, it is critical to understand the in-vitro conditions that affect fertilization and embryo development. [5-6] Treatments that reduce oxidative stress may help infertile women with diseases that are caused by this imbalance. Such strategies include identifying the source of excessive generation of ROS, treating the primary cause, and in-vitro and in-vivo supplementation of antioxidants. Treatments that reduce oxidative stress may help infertile women with diseases that are caused by this imbalance. Such strategies include identifying the source of excessive generation of ROS, treating the primary cause, and in-vitro and in-vivo supplementation of antioxidants. Reactive oxygen species are a double-edged sword, which have been localized in the female reproductive tract in various animal and human studies. Research is in progress to identify the mechanisms that are involved in the etiology of female reproductive diseases caused by ROS, and to create effective strategies that can counteract oxidative stress. [7-9] Oxidative stress influences a host of reproductive processes in a woman's life. The role of oxidative stress is becoming increasingly important as there is new cumulative evidence which suggests that oxidative stress is involved in conditions such as abortions, preeclampsia, hydatidiform mole, fetal embryopathies, preterm labor and intrauterine growth retardation, all of which lead to an immense burden of maternal and fetal, morbidity and mortality. Hence reactive oxygen has their direct and indirect role in infertility.

Conclusion

Idiopathic infertility accounts for 3% to 18% of all female infertility. In the present study the extracellular level of reactive oxygen species in the peritoneal fluid was clinically more important because it can impair sperm in the vicinity. Levels of reactive oxygen species were statistically significant in patients with idiopathic infertility when compared to controls. Hence this difference indicates that high levels of reactive oxygen species may contribute to infertility in patients with idiopathic infertility. Reduced antioxidant status may be responsible for the increased levels of reactive oxygen species. The screening of antioxidant status and reactive oxygen species in female patients with idiopathic infertility may be a useful factor.

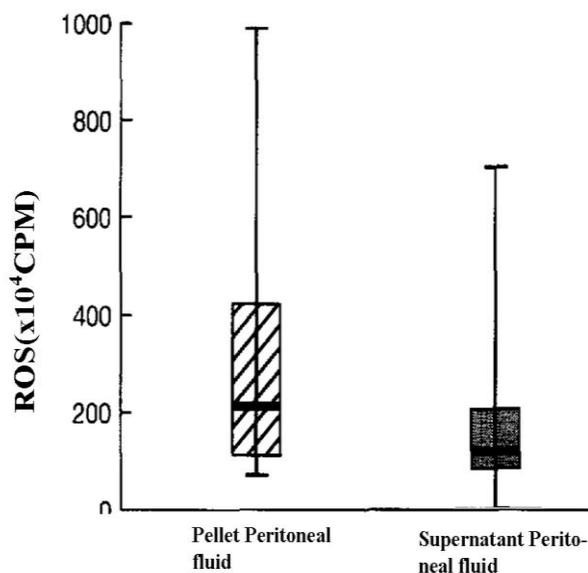


Figure 1: Comparison of reactive oxygen species in cell free and cellular pellet peritoneal fluid. Central horizontal line represents the median and vertical line represents ranges of ROS values.

References

1. Wang, Y., Sharma, R. K., Falcone, T., Goldberg, J. Agarwal, A. Importance of reactive oxygen species in the peritoneal

- fluid of Women with endometriosis or idiopathic infertility. *Fertil Steril*, 1997; 68: 826-30.
2. Ho, H. N., Wu, M. Y., Chen, S. U., Chao, K. H., Chen, C. D. Yang, Y. S. Total antioxidant status and nitric oxide do not increase in peritoneal fluids from women with endometriosis. *Hum Reprod*, 1997; 12: 2810-5.
3. Dong, M., Shi, Y., Cheng, Q. Hao, M. Increased nitric oxide in peritoneal fluid from women with idiopathic infertility and endometriosis. *J Reprod Med*, 2001; 46: 887-91.
4. Liu, Y., Luo, L. Zhao, H. Levels of lipid peroxides and superoxide dismutase in peritoneal fluid of patients with endometriosis. *J Tongji Med Univ*, 2001; 21: 166-7.
5. Bedaiwy, M. A., Goldberg, J. M., Falcone, T., Singh, M., Nelson, D., Azab, H., Wang, X. Sharma, R. Relationship between oxidative stress and embryotoxicity of hydrosalpingeal fluid. *Hum Reprod*, 2002; 17: 601-4.
6. Murphy, A. A., Palinski, W., Rankin, S., Morales, A. J. Parthasarathy, S. Macrophage scavenger receptor(s) and oxidatively modified proteins in endometriosis. *Fertil Steril*, 1998; 69: 1085-91.
7. Murphy, A. A., Santanam, N. Parthasarathy, S. Endometriosis: a disease of oxidative stress? *Semin Reprod Endocrinol*, 1998; 16: 263-73.
8. Zeller, J. M., Henig, I., Radwanska, E. Dmowski, W. P. Enhancement of human monocyte and peritoneal macrophage chemiluminescence activities in women with endometriosis. *Am J Reprod Immunol Microbiol*, 1987; 13: 78-82.
9. Wang, Y., Sharma, R. K., Falcone, T., Goldberg, J. Agarwal, A. Importance of reactive oxygen species in the peritoneal fluid of women with endometriosis or idiopathic infertility. *Fertil Steril*, 1997; 68: 826-30.