

Original Research Article

Evaluation of oxidative stress markers in infertile women

Rajeshwary P^{1*}, M. Nagaprasanth², Salma Mahaboob R³, G. Obulesu⁴


¹Assistant Professor, Department of Biochemistry, Fathima Institute of Medical Sciences, Kadapa, Andhra Pradesh, India

²Assistant Professor, Department of Urology, Chalamada Anandarao Medical College, Karimnagar, Telangana, India

³Assistant Professor, Department of Biochemistry, Fathima Institute of Medical Sciences, Kadapa, Andhra Pradesh, India

⁴Assistant Professor, Department of Microbiology, Fathima Institute of Medical Sciences, Kadapa, Andhra Pradesh, India

*Corresponding author email: prasanthmamilla@yahoo.com

	International Archives of Integrated Medicine, Vol. 3, Issue 10, October, 2016. Copy right © 2016, IAIM, All Rights Reserved. Available online at http://iaimjournal.com/	
	ISSN: 2394-0026 (P)	ISSN: 2394-0034 (O)
	Received on: 09-08-2016	Accepted on: 10-10-2016
	Source of support: Nil	Conflict of interest: None declared.
How to cite this article: Rajeshwary P, M. Nagaprasanth, Salma Mahaboob R, G. Obulesu. Evaluation of oxidative stress markers in infertile women. IAIM, 2016; 3(10): 239-244.		

Abstract

Reproductive failure is a significant public health concern. Recently, ROS have been shown to have an important role in the normal functioning of the reproductive system and in the pathogenesis of infertility in females. Oxidative stress affects both natural and assisted infertility. Treatment that reduces OS may help infertile women with the disease that is caused by imbalance between free radicals and antioxidants. MDA is an end product of lipid peroxidation which can be used as a biomarker to measure the level of oxidative stress. TAC measures the low molecular weight non enzymatic antioxidants. Increase in MDA and decrease in TAC indicates an increase in oxidative stress. In the present study, it was observed that there was a statistically significant increase in serum MDA levels in female factor infertility and unexplained infertility when compared to controls. There was a statistically significant decrease in serum TAC in female factor infertility and unexplained infertility when compared to controls and statistically significant increase in serum LDH in infertile women with unexplained infertility compared to the control group and female factor infertility signifying the apoptotic damage in the unexplained infertility.

Key words

ROS - reactive oxygen species, OS – oxidative stress, MDA- Malondialdehyde, TAC- Total antioxidant capacity, LDH – Lactate dehydrogenase.

Introduction

Infertility is a disease defined as "the inability to conceive following 12 or more months of unprotected sex before an investigation is undertaken unless the medical history and physical findings dictate earlier evaluation and treatment [1]. Approximately 15-20% of couples of reproductive age is infertile which can be attributed equally to both male and female factors. ROS are involved in physiological functions in female reproduction such as are oocyte maturation, ovarian steroidogenesis, ovulation, implantation, and formation of fluid filled cavity, blastocyst, luteolysis and luteal maintenance in pregnancy. ROS acts as mediators of various signaling path ways. Elevated or sustained generation of free radicals lead to imbalance in the intracellular redox homeostasis. Excess levels of free radicals and ROS can be neutralized by antioxidants. Any imbalance between ROS and antioxidants can cause oxidative stress [2]. Oxidative stress involved in various pathologies of female reproductive tract like (PCOS) polycystic ovarian syndrome, endometriosis, tubal factor infertility, unexplained infertility, fibroids, recurrent pregnancy loss, spontaneous abortions. OS also involved in hypothyroidism, hyperthyroidism, fetal teratogenicity, IUGR (intrauterine growth retardation), hydatiform mole, oligospermia, asthenospermia, azospermia . All of which leads to immense burden on maternal, fetal morbidity and mortality [3]. The effects of ROS result from damage to lipids, nucleic acids, proteins and other cellular materials. ROS also causes depletion of ATP levels. Lipid peroxidation is a self propagating reaction unless it is counteracted by antioxidants. Malondialdehyde (MDA) is a stable end product formed from peroxidation of polyunsaturated fattyacids of cell membrane. As hypoxia intensifies the peroxidation and cell membrane disruption, increase in extra cellular activity of Lactate dehydrogenase (LDH). LDH

can be used as a hypoxia marker [4]. The male and female reproductive tracts are rich in both enzymatic and non-enzymatic antioxidants. As it is practically impossible to measure all the biologically active antioxidants in human samples, the concept of a “global” assessment of antioxidant capacity has proved attractive. Total antioxidant capacity (TAC) mainly measures non enzymatic antioxidants [5].

Materials and methods

This study was done in a group of 70 women aged 20-40 years with primary infertility. The entire group was further divided in to 2 groups based on the etiology of infertility: Infertility due to female factor (Group 2) and Unexplained infertility (Group 3). The results were compared and correlated with controls. 35 age matched females with history of at least one childbirth were included in this study as controls (group 1). In our study, we excluded infertile women with male factor infertility and other systemic disorders .We collected blood in plain vacutainer tubes and following parameters were analyzed, serum MDA - TBARS (thiobarbituric acid reactive substance) assay, serum LDH - Optimised DGKC, kinetic assay, serum FRAP as a marker of total anti oxidant capacity – Benzie and Strain method [6] . The data was analyzed using SPSS (Statistical package for social science). Unpaired t-Test was used to assess the significance of difference of mean values of different parameters in between the cases and controls.

Results

In the present study, we found that there was significant rise in serum MDA, decrease in TAC and less significant rise LDH in case group 2 when compared to controls. There was significant rise in serum MDA, serum LDH and decrease in TAC in case group 3 when compared to controls. There was significant rise in serum

LDH and decrease in TAC in case group 3 when compared to case group 2 (Table – 1 to 3).

Discussion

Infertility is a common problem experienced by many couples. Numerous treatments are available for infertility. However in some cases, the treatment is empirical in nature because the etiology is not fully understood. Oxidative stress has been implicated in embryo fragmentation, DNA damage, apoptosis and poor pregnancy outcome. Oxidative stress is involved in various gynecological disorders like PCOS, endometriosis, PID, tubal factors, other ovulatory disorders, hyperprolactenemia and hypothyroidism. It has been suggested that

cyclical ROS production may, over time, contribute to oophoritis associated with autoimmune premature ovarian failure, and exacerbated by diminished antioxidant status [7-9]. Macrophages, neutrophils and granulose cells in the graphian follicle are the source of reactive oxygen species, during follicular maturation oocytes are well protected against toxic injury due to oxidative stress by important antioxidants. Total antioxidant capacity measures the low molecular weight non enzymatic antioxidants. Increase in MDA and decrease in TAC indicates an increase in oxidative stress. Results obtained from the present study regarding oxidative stress in female infertility are in agreement with earlier reports of several studies [2, 10-18].

Table – 1: Mean±SEM, SD, p and t values of analyzed parameters in controls and female factor infertility cases.

Parameter	Mean±SEM controls	SD controls	Mean±SEM Cases	SD Cases	t value	P Value
MDA	203.83 ± 8.72	53.43	701.26±28.6	169.09	17.2	< 0.0001
TAC	1154±34.5	204.43	785.48±23.8	141.33	8.78	< 0.0001
LDH	223.15± 9.65	54.14	231.15±8.28	48.86	4.62	0.56
BMI	21.90±0.32	1.87	23.5±0.29	2.5	0.32	0.0001

Table – 2: Mean±SE, SD, p and t values of analyzed parameters in controls and unexplained infertility cases.

Parameter	Mean±SEM Controls	SD controls	Mean±SEM Cases	SD Cases	t value	P Value
MDA	203.83 ± 8.72	53.43	722.63±21.98	131.89	17.06	< 0.0001
TAC	1154±34.5	204.43	526.57±13.2	78.17	16.96	< 0.0001
LDH	223.15±9.65	54.14	272.14±7.17	42.46	7.44	0.0001
BMI	21.20±0.32	1.87	22.43±0.4	2.37	1.85	<0.001

Table – 3: Mean±SEM, SD, p and t values of analyzed parameters in female factor infertility and unexplained infertility cases.

Parameter	Mean±SEM Case 2	SD Case 2	Mean±SEM Case 3	SD Case 3	t value	p value
MDA	704.26±28.6	169.09	722.63±21.98	131.89	2.5	0.6
TAC	785.48±23.8	141.33	526.57±13.2	78.17	9.4	0.0001
LDH	231.15±8.28	48.86	272.14±7.17	42.46	0.15	0.0006
BMI	23.15±0.29	2.5	22.43±0.4	2.37	2.13	0.06

Veena Bhaskar, et al. reported that elevated MDA levels in serum of infertile women, than in fertile women signifies that the oxidative damage in infertile women. Gonzalez et al in their study observed that there is generation of reactive oxygen species from mononuclear cells which occurred in response to hyperglycemia in women with PCOS. This increase in reactive oxygen species was seen both in obese and lean PCOS when compared to matched controls and was independent of obesity [19]. Yilmaz, et al. where significantly lower total antioxidant status was measured in the serum of PCOS women in comparison to healthy controls. Serum malonyldialdehyde, was significantly increased in these subjects. Elevated lipoprotein complexes and lowered antioxidants contribute to excessive growth of endometrial stromal cells implicating the role of OS in infertility associated with endometriosis [14]. Osman Hussein G, et al. proved that the levels of total antioxidant capacity, Catalase and superoxide dismutase were significantly lower in both serum and peritoneal fluid of infertile endometriotic women compared with controls [16].

Hypothyroidism is one of the common endocrine disorder causing female infertility. Hypothyroidism can affect fertility due to anovulatory cycles, luteal phase defects, hyperprolactinemia, and sex hormone imbalance [20, 21]. Das, et al. study found that an overall negative correlation between reactive oxygen species in follicular fluid and embryo quality and the association of lower total antioxidant capacity with decreased fertilization potential [22]. In the present study mean value of serum total antioxidant capacity levels is significantly low in unexplained infertility compared to women with female factor infertility. Polak, et al. reported that total anti oxidant status was found to be lower in peritoneal fluid of women with idiopathic infertility compared to fertile controls and individuals with tubal infertility, they hypothesized that peritoneal fluid diffuses into the fallopian tubes where it may cause damage to sperm, which are known to be sensitive to oxidative stress [23]. In the present study it was

observed that statistically significant increase in serum LDH in infertile women with unexplained infertility compared to the control group and female factor infertility signifying the apoptotic damage in the unexplained infertility. Hypoxia intensifies the lipid peroxidation and cell membrane disruption, increase in extra cellular activity of lactate dehydrogenase. In the present study, it was observed that statistically significant increase in mean values of BMI in infertile women with female factor infertility and unexplained infertility compared to controls .Obesity affects ovulation, response to fertility treatment, pregnancy rates and outcome A.M. Clark observed that weight reduction improves the chances of conception, fertility rates in infertile women irrespective of their infertility diagnosis [24 -32]. Robker, et al. [17] observed that adipocyte hypertrophy can increase cytokine, adipokine production, hypoxia and oxidative stress which can contributes to infertility in obese women.

Conclusion

Reactive oxygen species appears to have both physiological and pathological role in the female reproductive tract. Oxidative stress can affect female fertility potential in a number of ways. It may affect ovulation, fertilization, implantation and embryo development. The present study aims to assess the predictive values and correlation between oxidative stress markers (malondialdehyde, total antioxidant capacity, lactate dehydrogenase). Hence the present study confirms that presence of increased lipid peroxidation and oxidative stress in infertile women. Thus administration of antioxidant vitamins and minerals like vitamin C, vitamin E, Zn, Se, taurine, hypotaurine, glutathione and beta-carotene may benefit in the treatment of infertility.

References

1. ASRM. Definition of "infertility". Fertil Steril., 2004; 82(Suppl1): S206.
2. Veena Bhaskar S, Sharmila Upadhyya, et al. Evaluation of oxidative stress, antioxidants and prolactin in infertile

- women. *Indian Journal of Clinical Biochemistry*, 2008; 23(2): 186-190.
3. Agarwal Ashok, Sajal Gupta, Anjali Chandra, et al. Oxidative Stress and its Role in Female Infertility and Assisted Reproduction: Clinical Implications. *International Journal of Fertility and Sterility*, 2009; 2(4): 147-164.
 4. Predrag Jovanović, et al. Lactate dehydrogenase and oxidative stress activity in primary open angle glaucoma aqueous humour. *Bosnian Journal of Basic Medical Sciences*, 2010; 10(1): 83-88.
 5. Teitz text book of clinical biochemistry, fourth edition: a) Pituitary function; pages 1976-1980. b) Enzymes: pages 601-602. c) Vitamins and trace elements: pages 1077-1078.
 6. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovarian syndrome. *Hum Reprod.*, 2004; 19: 41-47.
 7. Bedaiwy MA, Falcone T, Sharma RK, Goldberg JM, Attaran M, Nelson DR, et al. Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. *Hum Reprod.*, 2002; 17: 426-431.
 8. Bedaiwy MA, Goldberg JM, Falcone T, Singh M, Azab H, et al. Relationship between oxidative stress and embryotoxicity of hydrosalpingeal fluid. *Hum Reprod.*, 2002; 17: 601-604.
 9. Behrman HR, Kodaman PH, Preston SL, Gao S. Oxidative stress and the ovary. *J Soc Gynecol Investig.*, 2001; 8(Suppl 1 Proceedings): S40-S42.
 10. Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. *Curr Opin Obstet Gynecol.*, 2006; 18: 325-332.
 11. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence?. *Lancet*, 1994; 54: 485-500.
 12. Yeon Lee, Sajal Gupta, Ashok Agarwal. Role of Oxidative Stress in Polycystic ovary Syndrome. *Current Women's Health Reviews*, 2010; 6: 96-107.
 13. Gonzalez F, Rote NS, et al. Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. *J Clin Endocrinol Metab.*, 2006; 91: 336-340.
 14. Foyouzi N, Berkkanoglu M, Arici A, Kwintkiewicz J, Izquierdo D, Duleba AJ. Effects of oxidants and antioxidants on proliferation of endometrial stromal cells. *Fertil Steril.*, 2004; 82 Suppl 3: 1019-1022.
 15. Cemil Kaya, Aycan Fahri Erkan, S. Dinçer Cengiz, et al. Advanced oxidation protein products are increased in women with polycystic ovary syndrome: relationship with traditional and non traditional cardiovascular risk factors in patients with polycystic ovary syndrome. *Fertility and sterility*, 2009; 92(4): 1372-1377.
 16. Osman Hussein G, El-Refaey Abdel Aziz A., et al. Leptin and antioxidant profile in infertile women with endometriosis, 2010; 2(3): 135 – 143.
 17. Robker, et al. Inflammatory pathways linking obesity and ovarian dysfunction. *J Reprod Biol.*, 2011; 88: 142-148.
 18. Yilmaz M, Bukan N, Ayvaz G, A Karakoc, Toruner F, Cakir N, et al. The effects of rosiglitazone and metformin on oxidative stress and homocysteine levels in lean patients with polycystic ovary syndrome. *Hum Reprod.*, 2005; 20: 3333-3340.
 19. Gonzalez F, Rote NS, et al. Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. *J Clin Endocrinol Metab.*, 2006; 91: 336-340.

20. Antonio Mancini, Elena Giacchi, et al. Hypothyroidism, Oxidative Stress and Reproduction. *Hypothyroidism – Influences and Treatments*, 1991; 7: 118-131.
21. Baskol G, Atmaca H, Tanriverdi F, et al. Oxidative stress and enzymatic antioxidant status in patients with hypothyroidism before and after treatment: *Experimental and Clinical Endocrinology & Diabetes : Official Journal, German Society of Endocrinology [and] German Diabetes Association*, 2007; 115(8): 522-526.
22. Das S, Chattopadhyay R, Ghosh S, et al. Reactive oxygen species level in follicular fluid–embryo quality marker in IVF? *Hum Reprod.*, 2006; 21: 2403–2407.
23. Polak G, Koziol-Montewka M, Gogacz M, Blaszkowska I, Kotarski J. Total antioxidant status of peritoneal fluid in infertile women. *Eur J Obstet Gynecol Reprod Biol.*, 2001; 94: 261–263.
24. Thornley B., A.M.Clark, et al. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. *Human Reproduction*, 1998; 13(6): 1502–1505..
25. De Moraes- Ruehsen M, Blizzard RM , Garcia – Bunuel R, et al. Autoimmunity and ovarian failure. *Am J.*, 19724; 120: 257-263.
26. Droge W., et al. Free radicals in the physiological control of cell function. *Physiol Rev.*, 2002; 82: 47–95.
27. Dumollard R, Duchen M, Carroll J. The role of mitochondrial function in the oocyte and embryo. *Curr Top Dev Biol.*, 2007; 77: 21–49.
28. Dunaif A, et al. Insulin resistance and the polycystic ovarysyndrome: mechanism and implications for pathogenesis. *Endocr Rev.*, 1997; 18: 774-800.
29. Eiben B, Bartels I, Bahr – Porsch S, et al. Cytogenetic analysis of 750 spontaneous abortions with the direct –preparation method of chorionic villi and its implications for studying genetic causes of pregnancy wastage. *Am J Hum Genet.*, 1990; 47: 656-663.
30. Elizabeth H. Ruder, Terryl J., et al. Oxidative stress and antioxidants: exposure and impact on female fertility. *Human Reproduction Update*, 2008; 14(4): 345–357.
31. Filicori M, Butler JP, Crowley WF Jr. Neuroendocrine regulation of the corpus luteum in the human: evidence for pulsatile progesterone secretion. *J Clin Invest.*, 1984; 73: 1638-1647.
32. Frisch RE, Mc Artthur JW. Menstrual cycles: fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science*, 1974; 185: 949-995.

Infertility is a complex issue and different factors such as stress oxidative can be involved in this problem. So, any attempt to neutralize oxidative stress... results in that in infertile women, the level of melatonin and TAC was lower in comparison with fertile women. As it has been previously described, human fertility is directly and indirectly controlled by OS. Based on these documented data, we can. MDA level in fertile women was significantly lower than infertile women ($p < 0.001$) and the level of TAC in fertile women was significantly higher than infertile women ($p < 0.001$). Spearman test revealed a significant and direct correlation between melatonin and TAC in fertile and infertile women and a significant but reverse correlation between melatonin and MDA in infertile and fertile women. Conclusion: Differences in the level of oxidative stress biomarkers in fertile and infertile individuals have been reported. This study revealed a significant correlation between melatonin and oxidati...