



Free radicals in female infertility

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OBJECTIVE(S): To know the status of free radicals and their scavengers in the blood and endometrial tissue in infertile women and to develop the base line data in different types of female infertility.

METHOD(S): A prospective study was conducted on 75 women with primary infertility, 20 with secondary infertility and 30 fertile controls. Markers of oxidative stress viz., level of malonyldialdehyde (MDA) for free radicals and antioxidants catalase (CAT) and superoxide dismutase (SOD) as scavenging enzymes were measured in blood and endometrial tissue.

RESULTS : Infertile women had significantly ($P < 0.001$) high MDA levels and significantly ($P < 0.001$) low CAT and SOD levels in both blood and endometrium as compared to those in controls. Unexplained infertility group of patients had significantly ($P < 0.001$) high levels of oxidant (MDA) while antioxidant (CAT and SOD) levels were significantly low ($P < 0.001$).

CONCLUSION(S) : The study favors the significant role of free radicals injury in cases of female infertility, and damage is more at endometrial tissue level in cases of unexplained infertility.

Key words : female infertility, free radicals, scavengers

Introduction

Infertility is defined as one year of unprotected intercourse without pregnancy and is classified as primary in which no previous pregnancy has occurred and secondary in which prior pregnancy has occurred irrespective of its outcome. Recently there is a growing evidence of possible role of highly reactive products of oxygen, termed free radicals, in infertility. A free radical is defined as molecular species capable of independent existence and containing one or more unpaired electrons, making them paramagnetic and relatively active. These are formed as natural byproducts of oxygen metabolism and serve the purpose of burning bacteria and refuse body matter but when out of control, they become toxic and start damaging body tissues by a process called oxidative stress. Substances that neutralize the potential ill effects of free radicals are called antioxidant or free radical

scavengers. Imbalance between these oxidants and antioxidants is responsible for tissue injury and affects fertility.

The aim of this study was to know the status of free radicals and their scavenging enzymes in the blood and endometrial tissue in infertile women and to develop base line data in different cases of female infertility.

Methods

One hundred and twenty-five women were studied. Seventy-five were with primary infertility and 20 had secondary infertility while 30 fertile women attending the hospital with a genital prolapse were taken as controls. Those having male factor of infertility or an associated condition which can alter the level of free radicals viz., hypertension, diabetes mellitus, heart disease, malignancy, and antioxidant therapy were excluded from the study.

Each subject and control was subjected to detailed clinical history and physical examination. The infertile group had undergone baseline investigations of infertility. For specific tests for markers, about 5 mL of venous blood in heparinized syringe and one gram of endometrial tissue in saline were

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taken. Samples were transferred to the biochemistry laboratory at 0-2°C in ice pack flask for analysis of malonyldialdehyde (MDA) for free radicals, and catalase (CAT) and superoxide dismutase (SOD) for scavenging enzymes.

The blood sample was centrifuged at 800 rpm for 10 minutes. Lipid peroxide MDA was estimated from supernatant by Okhawa's method using thiobarbituric acid. Packed RBCs were again washed and centrifuged, from which SOD was measured by McCard Fridovich's method while CAT by Suter's method using hydrogen peroxide phosphate buffer.

Results were expressed as Mean \pm SEM.

Results

Among the different causes of infertility unexplained infertility was the commonest (56/95;58.94%).

In the blood and endometrial tissue the MDA was taken as free radicals and CAT and SOD as scavenging enzymes. In subjects MDA level was significantly ($P < 0.001$) raised in blood as well as in the endometrial tissue as compared to that in controls while CAT and SOD levels were significantly ($P < 0.001$) lowered (Tables 2 and 3).

Table 1. Causes of infertility in subjects (n=95).

Cause of infertility	Primary infertility n=75	Secondary infertility n=20	Total n=95	
			Number	Percent
Endometriosis	11	9	20	21.05
Tubal factor	6	-	6	6.31
Luteal phase defect	13	-	13	13.68
Unexplained infertility	45	11	56	58.94

Table 2. Levels of free radical and scavengers in endometrial tissue (Mean \pm SEM).

Enzymes	Subjects (n=95)	Controls (n=30)	P value
MDA (nmol/g)	3.02 \pm 0.6	1.56 \pm 0.37	< 0.001
CAT (unit/mg)	8.38 \pm 0.70	13.28 \pm 1.42	< 0.001
SOD (unit/mg)	2.88 \pm 0.53	4.97 \pm 0.54	< 0.001

Table 3. Levels of free radical and scavengers in blood (Mean \pm SEM).

Enzymes	Subjects (n=95)	Controls (n=30)	P value
MDA (nmol/mL)	3.78 \pm 0.82	1.51 \pm 0.35	< 0.001
CAT (unit/mL)	15.40 \pm 2.27	27.06 \pm 2.15	< 0.001
SOD (unit / mL)	9.88 \pm 1.43	17.7 \pm 1.79	< 0.001

Although MDA and SOD levels were similar in primary and secondary infertility cases, CAT levels were significantly higher in cases of secondary infertility both in blood and in endometrial tissue ($P < 0.001$) (Table 4).

In both primary and secondary infertility cases, highest mean MDA levels in endometrial tissue were seen with unexplained infertility and lowest with endometriosis. CAT and SOD activity was lowest in unexplained infertility. Serum MDA, CAT and SOD levels did not differ significantly between different causes of infertility (Figure 1).

Discussion

Oxidative stress due to free radicals precipitates a range of pathological changes that affect the reproductive functions in both men and women. Said et al ¹ support the role of NADPH in oxidative stress mediated DNA damage in abnormal sperm morphology. Agarwal et al ² suggested the role of reactive oxygen species (ROS) in infertility with endometriosis and in unexplained infertility. We studied the levels of MDA (marker of oxidative stress), and CAT and SOD (antioxidant protective enzymes) in endometrial tissue and blood and had similar findings. Polak et al ³ found significantly higher concentration of MDA in peritoneal fluid from patients with

Table 4. comparison between primary and secondary infertility.

Marker	Endometrial tissue			Blood		
	Primary Infertility	Secondary infertility	P value	Primary infertility	Secondary infertility	P value
MDA	3.07 ± 0.61	2.85 ± 0.54	0.22	3.73 ± 0.81	3.99 ± 0.80	0.19
SOD	2.87 ± 0.49	2.95 ± 0.64	0.73	10.0 ± 1.40	9.46 ± 1.48	0.03
CAT	8.24 ± 0.76	8.93 ± 0.64	< 0.001	15.1 ± 2.39	16.4 ± 1.36	< 0.001

Values are given as Mean ± SEM

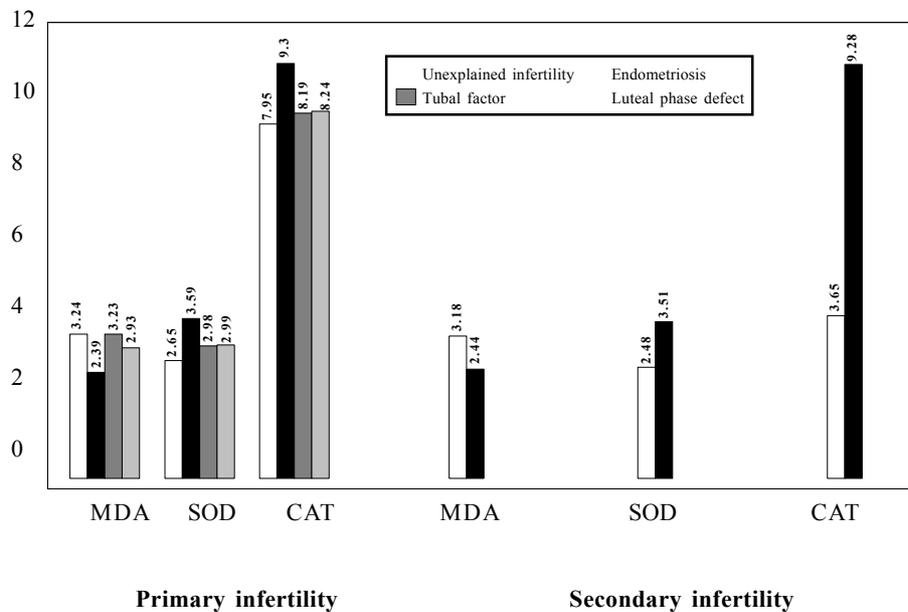


Figure 1. Markers in endometrial tissue in different causes of infertility

unexplained infertility and endometriosis compared to that in controls which is in accordance with our results. The highest MDA levels are found in unexplained infertility cases in our study.

Dong et al ⁴ have shown significantly high levels of nitric oxide in peritoneal fluid of patients with unexplained infertility and endometriosis.

SOD and CAT are primary defences against oxidative stress and are found to be significantly low in endometrium in cases of infertility compared to those in controls. CAT levels are found significantly higher in women with secondary infertility when compared to those in primary infertility. The lowest levels were found in cases of unexplained infertility in our

study. Similarly Polak et al ⁵ found that total antioxidant status (TAS) was significantly lower in peritoneal fluid from women with unexplained infertility. In another study, Polak et al ⁶ found peritoneal TAS was significantly lower compared to plasma TAS. We found that the endometrial tissue antioxidant levels are much lower than plasma levels.

Conclusion

Our study supports etiological role of free radicals in female infertility. It also shows that damage is more pronounced at endometrial tissue in unexplained infertility, as levels of MDA are highest and of CAT and SOD are lowest in the endometrium. Damage is more at local endometrial tissue level as compared to that seen in blood.

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