

Plasma protein thiols, ceruloplasmin, C-reactive protein and red blood cell acetylcholinesterase in patients undergoing intrauterine insemination

ABSTRACT

OBJECTIVE: To estimate acetylcholinesterase (AChE), protein thiols (PT), ceruloplasmin (CP) and C-reactive proteins (CRPs) to assess any change in their levels following intrauterine insemination (IUI). **MATERIALS AND METHODS:** Forty-two patients aged 31 ± 4.65 years (mean \pm SD) with primary infertility selected for IUI. All of them had induced ovulation with clomiphene citrate 50 mg from day 2 to day 6. After taking the consent, 2 ml of blood was withdrawn before and after 24 h of IUI for biochemical estimations. **RESULTS:** We observed a significant decrease in plasma CP, PT and RBC AChE ($P < 0.001$) following IUI compared with the respective pre-procedure levels. Highly sensitive CRP showed a marginal increase after IUI. **CONCLUSION:** Fluctuations in levels of the above parameters point to their role in the female reproductive system and in the outcome of the IUI.

KEY WORDS: Inflammation, intrauterine insemination, oxidative stress

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INTRODUCTION

Infertility is the inability to conceive after 1 year of unprotected intercourse. It affects 10–15% of couples. Infertility can be due to female factors in 58% and male factors in 25% of the cases or it could be unexplained in 17% of the cases.^[1] Irrespective of the cause of infertility, one of the main modalities of treatment offered to the couples is intrauterine insemination (IUI). Animal and human studies have shown that insemination resulted in an increase in polymorphonuclear leucocytes in the uterine cavity.^[2-4] In addition, animal studies suggested that artificial insemination increases the uterine motility by reflex activation of the cholinergic system.^[5-7] Studies also suggested that oxidative stress could affect assisted reproduction.^[8,9] Hence, we decided to measure antioxidants, factors studied as acute phase reactants and RBC acetylcholinesterase (AChE) to see whether there was any change in their levels following IUI.

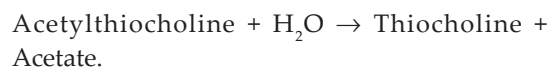
MATERIALS AND METHODS

Institutional ethics committee permission was taken to carry out this study. After taking consent, 2 ml of blood was withdrawn using aseptic precaution in an ethylenediaminetetraacetic

acid vacutainer from 42 patients aged 31 ± 4.65 years (mean \pm S.D) with primary infertility selected for IUI attending the Department of Reproductive Medicine, Kasturba Medical College Hospital, Manipal, during the period from April 2006 to February 2007. Patients having secondary infertility because of some gynecological diseases, patients with any medical diseases requiring medications and patients with neurological/musculoskeletal/autoimmune diseases were excluded from the study. Blood was centrifuged and plasma was separated. RBC was used to estimate AChE. Plasma was used to measure ceruloplasmin (CP), C-reactive protein (CRP) and protein thiols (PT). The method of sperm preparation used for these patients is the standard swim-up technique.

AChE (colorimetric)^[10]

AChE catalyzes the hydrolysis of acetylthiocholine to thiocholine and acetate. The rate of production of thiocholine was measured by the following reaction of thiocholine with 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB). The rate of formation of yellow anion was measured at 410 nm at 25°C in a thermo stated cuvette.



Thiocholine + DTNB → 5-thio-2-nitro benzoic acid + 2-nitrobenzoate-5-mercaptothiocholine (yellow).

Activity was expressed as μmol of substrate hydrolyzed at 25°C /g of Hb or /l of packed cells ($\epsilon = 1.36 \text{ l/mmole/mm}$).

CP (colorimetric)

CP is an oxidase that can catalyze oxidation of some polyamines. When serum is made to react with paraphenyldiamine, it forms a colored complex, which, when read at 546 nm, gives a measure of plasma CP. The normal range varies from 25 to 50 mg/dl.^[11]

PT (colorimetric)

DTNB reacts with accessible SH groups in proteins and reduces to a stable intermediate compound of mixed disulfide, protein-S-S-aromatic compound. The reduced product of DTNB is 5-mercapto 2-dinitrobenzoate. The yellow color developed is measured at the end of 5 min at 412 nm.^[12]

CRP (latex) HS (Roche diagnostics; turbidometry, GmbH, D - 68298 Mannheim, Germany)

Principle: Particle-enhanced immunoturbidometric assay. Anti-CRP antibodies coupled to latex microparticles react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidometrically in a Hitachi 911 autoanalyzer, GmbH, D - 68298 Mannheim, Germany.

RESULTS

All the values were expressed as mean \pm standard error of mean. A Wilcoxon signed ranks test was used to assess the significance between the groups. There was a significant decrease in plasma CP, PT and RBC AChE ($P < 0.001$) following IUI compared with the respective pre-procedure levels. Highly sensitive CRP showed a marginal increase after IUI [Table 1].

DISCUSSION

Essentially, all the plasma sulfhydryl groups are protein associated and behave as powerful extracellular antioxidants. Proteins containing reactive sulfhydryl groups under oxidative stress are converted to mixed disulfides with attached glutathione (S-thiolation), leading to a fall in their

levels. These thiolated proteins are very early products of protein oxidation during oxidative stress, occurring within seconds after generation of oxygen radicals.^[13]

CP is a blue-colored α_2 globulin synthesized by the liver. It binds to copper and is an important antioxidant in the plasma. Its concentration is altered in various disease conditions.^[14,15]

We observed a significant decrease in CP and PT ($P < 0.001$) following IUI, indicating that there could be increased oxidative stress.

AChE are enzymes present in nerve endings and RBC and act on acetylcholine liberated from pre-synaptic vesicles and inactivate it. Studies have shown that levels of AChE in seminal plasma have an inverse relationship with sperm motility, indicating that acetylcholine has a role in sperm motility.^[16,17]

Human and animal studies have shown that as compared with normal mating, artificial insemination increases the uterine motility resulting from reflex activation of cholinergic nerve fibers, which was most prominent at 5–10 min after IUI and are sustained for 30 min.^[5,6] Animal studies also showed that intramuscular injection of acetylcholine immediately after natural mating significantly increased the frequency and amplitude of uterine contraction leading to an increased number of sperms recovered from the vagina after 1 hour.^[7] Similar studies have shown that AChE levels in RBC were significantly elevated during pregnancy and returned to normal pre-pregnancy levels at 6th post-partum week. This increased activity of AChE probably suppressed the action of acetylcholine on the uterus; thus, protecting the pregnancy.^[18,19] We found a significant decrease in AChE levels following the procedure ($P < 0.001$), indicating that IUI may have caused an increase in the cholinergic activity (similar to that seen in animal studies).^[5-7] This led to an increased acetylcholine release and hence more AChE was used up to destroy the acetylcholine, leading to a fall in its levels.

Endometrial inflammation is well known to adversely affect the fertility and implantation of the fertilized ovum^[20] and hence this concept has been exploited in the action of intrauterine devices acting as contraceptives. Animal studies have shown that as compared with normal mating, artificial

Table 1: Comparison of variables before and after intrauterine insemination in patients

Variable	Pre-IUI [†] (mean \pm SEM) [‡]	Post-IUI [†] (mean \pm SEM) [‡]	P-value*
Acetylcholinesterase (IU/l)	9434.3 \pm 332.7	6838.6 \pm 586.3	<0.001
Thiols ($\mu\text{mol/l}$)	287.3 \pm 10	249.7 \pm 16	<0.001
Ceruloplasmin (mg/dl)	19.9 \pm 0.5	15 \pm 0.8	<0.001
C-reactive protein (mg/l)	1.4 \pm 0.8	1.8 \pm 1.1	NS

*Wilcoxon signed ranks test; [†]IUI = intrauterine insemination; [‡]SEM = standard error of mean, NS = Non significant

insemination triggered an acute inflammatory response, indicated by intrauterine fluid accumulation containing polymorphonuclear neutrophils.^[2-4] Thus, we measured the acute phase reactant highly sensitive CRP, which showed a marginal increase after IUI, indicating some acute inflammatory response, which may have adversely affected the implantation of the fertilized ovum. However, if we measured the same in the uterine fluid after the procedure, it could have been more specific. But, this was not possible ethically as a sampling attempt could have damaged the implantation of the fertilized ovum. Thus, fluctuations in the levels of the above parameters show their possible role in the female reproductive system and in the outcome of IUI. Serial measurements of these in every cycle may be valuable in predicting the outcome of the IUIs.

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