BRIEF COMMUNICATION

A PRELIMINARY STUDY OF PROTEIN THIOLS AND SERUM CHOLINESTERASE IN ASSISTED REPRODUCTION

Krishnananda Prabhu, Pratap Kumar*, Muralidhar Pai *, Indrajit Sinha and Anjali Rao

Departments of Biochemistry and *Obstetrics and Gynecology, Kasturba Medical College Manipal, India.

ABSTRACT

In this study we sought to investigate the levels of serum protein thiols and cholinesterase levels before and after intrauterine insemination (IUI). We observed 68% patients showed a decrease in protein thiols and 63% showed a decrease in serum cholinesterase levels after insemination as compared to their respective pre procedure levels. The fall in thiols was statistically significant ($p=0.021$) indicating there is increased oxidative stress after the procedure. However, we could not comment on any reason for the fall in cholinesterase. Whether the changes in above parameters may have some implication on the overall success rate of IUI is yet to be ascertained.

KEY WORDS

Cholinesterase, Intrauterine, Insemination, Thiols

INTRODUCTION

Reactive oxygen species (ROS) influences the entire lifespan of a woman and affects multiple physiological processes like oocyte maturation, fertilization, embryo development and pregnancy (1). Various studies have shown that the process of insemination may itself induce uterine contraction, which may be due to reflex adrenergic/cholinergic stimulation or prostaglandin release (2). Many studies have shown that there is a fluctuation of plasma cholinesterase during pregnancy, which returned to normal pre pregnancy levels after delivery. Cholinesterase involved in non-cholinergic functions such as cellular proliferation, cellular adhesion are found to be transiently expressed in multiple germ line, embryonic and tumor cells. They also appear to play developmentally important role in multiple cell types and amplification and over expression of these genes might affect the fertility (3).

In this study we estimated serum protein thiols and

cholinesterase before and after intrauterine insemination to ascertain any change in their levels.

MATERIALS AND METHODS

Ethical clearance was given by Institutional Ethics Committee. The study was funded by the institution. The subjects were female patients ($n=22$) with primary infertility selected for intrauterine insemination. None of the patients had any liver disease. After obtaining informed consent blood was collected two times once before insemination and once on next day. Cholinesterase was estimated by spectrophotometric method using- Hitachi 911 autoanalyser.

Thiol assay : Reaction mixture contained 900ml 0.2M NaHPO_4 containing 2mM Na_2EDTA , 100 ml plasma, 20 ml 10mM DTNB in 0.2M NaHPO_4 . Absorbance taken at 412 nm 37°C for 5 min and max absorbance was noted. Similarly absorbance of sample blank and reagent blank were measured. Absorbance of sample blank and reagent blank was subtracted from plasma absorbance values to obtain corrected values. The concentration of thiol was calculated using standard curve obtained using glutathione dissolved in phosphate buffer saline (4).

Address for Correspondence :

Dr. Krishnananda Prabhu,
Department of Biochemistry,
Kasturba Medical College, Manipal, India
E-mail : krishnakunj2000@yahoo.com

RESULTS & DISCUSSION

68% patients showed decrease in thiol levels and 63% showed a fall in cholinesterase levels after the procedure compared to that of their pre procedure values respectively (Table 1). The decrease in thiol levels was statistically significant ($p = 0.021$).

The overall pregnancy rate per cycle in assisted reproduction by intrauterine insemination is not very good in spite of extreme precautions and calculations. This indicates that there may be some unknown factor like reflex activation of inflammation or oxidative stress in the uterine cavity by the process of insemination itself leading to poor implantation of fertilized ovum. The changes in serum thiols show there is increased oxidative stress immediately after the procedure which may reflect some changes occurring inside the uterine cavity. Though, cholinesterase levels showed some changes it was difficult to explain the same. However, some studies have shown that exposure to organophosphorous poisoning (cholinesterase inhibitor) in pregnant women had protective effect against low birth weight and abortions (5). This shows there is a role of cholinesterase in female reproductive system. Thus there may be a role for these parameters namely thiols and cholinesterase on overall success rate of insemination. We propose to observe these patients to ascertain as to whether these changes have any impact on the outcome of the procedure.

Table 1: Comparison of pre procedure and post procedure levels of cholinesterase and thiol

Parameter (n =22)	Pre procedure	Post procedure	Significance*
THIOL ($\mu\text{M/L}$)	479 $\pm$ 13.5	452 $\pm$ 18.2	0.021
Cholinesterase (U/L)	8465 $\pm$ 339	8231 $\pm$ 353	NS

*Wilcoxon signed ranks test

REFERENCES

1. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 2003; 79(4): 829-43.
2. Agarwal A, Gupta S, Sharma R.K. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol* 2005; 3: 28.
3. Soreq H, Zakut H. Amplification of butylcholinesterase and acetylcholinesterase genes in normal and tumor tissues: Putative relationship to organophosphorous poisoning. *Pharm Res* 1990; 7 (1): 1-7.
4. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82(1): 70-7.
5. Willis WO, de Peyster A, Molgaard CA, Walker C, MacKendrick T. Pregnancy outcome among women exposed to pesticides through work or residence in an agricultural area. *J Occup Med* 1993; 35(9): 943-9.