

Evaluation of the relationship between superoxide dismutase, estradiol hormone, and the intracytoplasmic sperm injection outcome

Hind Abdulkadim Abdulzahra

Department of Urosurgery & infertility, College of Medicine, Kufa University, Najaf, Iraq.

Correspondence to Hind Abdulkadim Abdulzahra (e-mail: dr.hindabdulkadimd@yahoo.com).

(Submitted: 02 September 2017 – Revised version received: 21 October 2017 – Accepted: 18 November 2017 – Published online: 26 June 2018)

Objectives Multiple factors may be considered as a potential biomarker for assisted reproductive technique success. The objective is to study the connection between superoxide dismutase as antioxidant, and estradiol hormone in patients undergoing assisted reproductive techniques and their effect on the intracytoplasmic sperm injection outcome.

Methods Seventy women presenting with subfertility: male (35), unexplained (6), tubal ($n = 4$) and PCOS ($n = 25$). Blood samples were collected at oocyte retrieval. Blood was analyzed for estradiol hormone by using ELISA. Superoxide dismutase (SOD) was measured in serum by using a calorimetric method by adding serum to tris base and pyrogallol immediately after addition of pyrogallol.

Result There was an insignificant difference between SOD, E_2 in different causes ($P > 0.05$). There was a direct insignificant association between E_2 level and SOD ($r = 0.151$). There was a direct correlation between serum estradiol hormone and total oocyte retrieved, grade I embryos, and total embryos ($P < 0.05$). Also, there was a direct association between serum SOD and grade I embryos ($P < 0.01$).

Conclusion(s) SOD considered as an essential predictor of ICSI outcome. The direct relationship between E_2 and SOD suggests that hormone may play a role in the ovarian metabolism.

Keywords superoxide dismutase, infertility, estradiol, sperm injections, intracytoplasmic

Introduction

One of the free radicals that affect the cell metabolism is superoxide, which eradicates by superoxide dismutase enzyme (SOD).¹ SOD enzyme acts as a defense mechanism by changing of superoxide radicals to hydrogen peroxide (H_2O_2).²

The superoxide ion, as a free radical can damage the cell membranes and destroy the oocyte, sperm, and embryo.³ SOD may play a role in many events like folliculogenesis,³ implantation, and pregnancy.⁴

Estrogens as estradiol had an important function, which act as antioxidant.^{5,6} In high concentration, E_2 may play a defense mechanism to cell lipids against free radicals.⁷ In controlled ovarian hyperstimulation, E_2 will be increased due to increase number of maturing oocytes. The role of E_2 level on outcome of ICSI remain site of discussion.⁸

Materials and Methods

Seventy women presenting with subfertility were taken from the fertility center, in Al Sadder teaching hospital, between March and December 2013. With abnormalities of male, female, tubal, and unexplained types. All patients undergo history and physical examination.

Blood samples were collected at oocyte retrieval. Blood was analyzed for E_2 by using ELISA. SOD was measured in serum by using a calorimetric method by adding serum to tris base and pyrogallol, immediately after addition of pyrogallol at 420 nm.

Tris buffer mM, PH 8.2, which contain tris base (dissolved 0.285 g of tris base in small amount of distilled water (DW)) and EDTA (dissolved 0.111 g of EDTA in small amount of DW)

- Pyrogallol which should prepared freshly by adding 0.0252 g of pyrogallol to 100 ml of DW and 60 ml of HCl.
- Add 50 Mm of serum and 1 ml of tris base, after the addition of pyrogallol, and immediately read the absorbance spectrophotometrically at 420 nm against blank.

Statistical analysis was made by SPSS. Results at $P < 0.05$ was considered significant.

Results

Concerning estradiol hormone and superoxide dismutase enzyme levels in the different etiologies of infertility, there was an insignificant difference in their levels in the different subgroups ($P > 0.05$), as shown in Table 1.

Correlation between estradiol hormone and superoxide dismutase

There was an insignificant direct correlation between serum E_2 and SOD ($P > 0.05$), as shown in Fig. 1.

Correlation between estradiol hormone, superoxide dismutase, and intracytoplasmic sperm injection outcome

Table 2 shows that there was a significant direct relation between serum E_2 and total oocyte, grade I embryos and total embryos ($P < 0.05$), and highly significant direct relation between serum SOD and grade I embryos ($P < 0.01$).

Estradiol hormone and superoxide dismutase levels and pregnancy

Table 3 demonstrates estradiol hormone and superoxide dismutase enzyme levels regarding pregnancy. Which were higher in a pregnant woman than normal. The increase of SOD in subfertile patients is associated with the increase odds ratio for pregnancy (Odds = 1.926, $P = 0.011$).

The area under the corresponding ROC curve for SOD and E_2 were compared between pregnant women and normal, for the confirmation of pregnancy (Fig. 2). SOD had the largest area under the curve (AUC) 0.820, representing that a level of 4 (μ IU/ML) gave sensitivity of 80% and specificity 67%, whereas E_2 level had (AUC: 0.633), indicating that a threshold of 1300 ng/dl gave sensitivity of 100% and specificity 47%.

Table 1. Estradiol hormone and superoxide dismutase enzyme in the different etiologies of infertility

Cause	Estradiol (ng/dl)	SOD (μ IU/ML)
Male	1901.14 ± 964.26	3.85 ± 1.92
Tubal	1761.20 ± 774.13	4.59 ± 1.12
PCOS	2397.29 ± 673.05	4.48 ± 2.16
Unexplained	1387.15 ± 1106.15	1.85 ± 1.11
P value	0.213	0.554

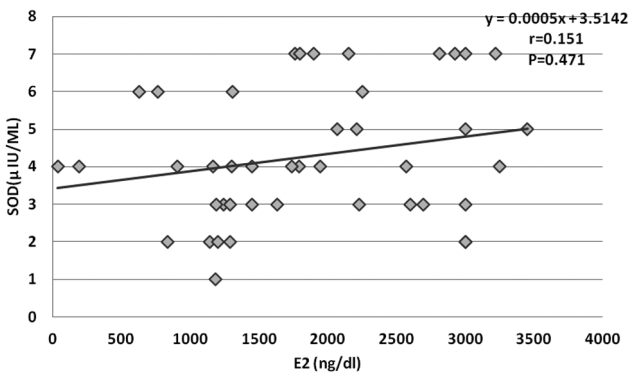


Fig. 1 Association of estradiol hormone and superoxide dismutase.

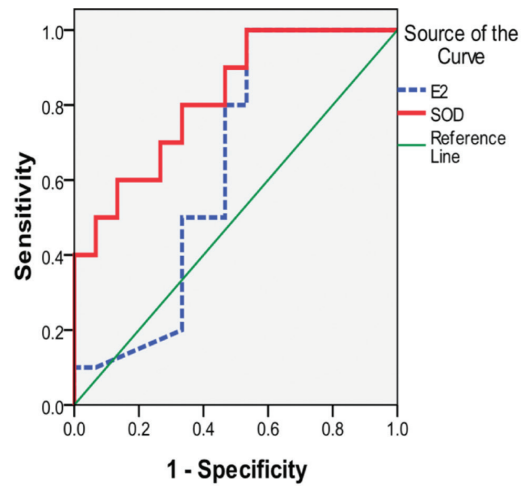
Table 2. Correlation between estradiol hormone, superoxide dismutase, and intracytoplasmic sperm injection outcome

ICSI characteristics		Estradiol hormone	Superoxide dismutase
oocyte retrieval	r	0.267*	0.274
	P	0.036	0.167
MII	r	0.214	0.132
	P	0.095	0.512
Pronucleus (PN)	r	0.237	0.082
	P	0.063	0.683
Fertilization rate (FR)	r	0.007	0.052
	P	0.958	0.795
Cleavage rate (CR)	r	0.083	0.054
	P	0.524	0.788
Embryo grading	Grade I r	0.306*	0.488**
	P	0.016	0.010
	Grade II r	0.172	0.109
	P	0.182	0.587
Grade III	r	-0.038	-0.240
	P	0.772	0.228
	Grade IV r	0.122	-0.365
	P	0.344	0.061
No. of total embryos	r	0.309*	0.137
	P	0.015	0.494

MIl metaphase II; *Correlation is significant at the $P < 0.05$; r correlation coefficient.

Table 3. Estradiol hormone and superoxide dismutase enzyme and pregnancy

Test	Pregnant	Non-pregnant	P-value	Odd's ratio
Estradiol hormone (ng/dl)	2092.03 ± 623.16	1837.89 ± 964.93	0.343	1.003
Superoxide dismutase (μ IU/ML)	5.43 ± 1.53	3.62 ± 1.72	0.011*	1.926



	AUC	Cutoff point	Sensitivity	Specificity	95% CI	
					Lower bound	Upper bound
E ₂	0.633	1300	100%	47%	0.411	0.856
SOD	0.820	4.00	80%	67%	0.655	0.985

Fig. 2 Receiver operating characteristics curve analysis for estradiol hormone and superoxide dismutase enzyme levels in pregnant and non-pregnant woman.

The increase of SOD levels is associated with more positive pregnancy success.

Discussion

Table 1 shows the estradiol hormone and SOD enzyme levels in the different etiologies of infertility. There was an insignificant difference in their levels in the different subgroups ($P > 0.05$).

Many researches were found an insignificant association between antioxidant and the cause of subfertility in ART,⁹ while others like Pekel and his co-workers conflict with their findings. They reveal that SOD levels were higher in some types like PCOS than control group.¹⁰

Increased SOD level associated with increased success rate especially in male.¹¹ Also, SOD plays a role in quality of oocyte and ultimately play a role in achievement of ART.¹²

Correlation between superoxide dismutase and estradiol hormone

There was an insignificant direct relation between E₂ and SOD ($r = 0.151$, $P > 0.05$), Fig. 1. This result was agreed with Younis et al. (2012). SOD may had a role on E₂ hormone production.¹³

Correlation between superoxide dismutase, estradiol hormone, and ICSI outcome

As mentioned in Table 2, there was a significant direct relation between serum E₂ and total oocyte, grade I embryos and total embryos ($P < 0.05$), and the highly significant direct relation between serum SOD and grade I embryos ($P < 0.01$).

High levels of free radicals are seen in follicular fluid of woman with controlled ovarian stimulation than those who is not^{14,15}. Bedaiwy et al. found that free radicals affect on different conception aspect like fertilization rates.¹⁶ Bedaiwy et al. found that high level of SOD associated with higher fertilization rate and decrease destruction of embryo.¹⁶

Some researcher found a direct relation between free radicals level and level of destruction of embryo^{17,18}.

As for the association between serum E₂ level and ICSI outcome, some researcher not found a connection between E₂ and achievement of pregnancy.¹⁹⁻²¹

Other studies found the higher E₂ associated with higher pregnancy rates achieved²²⁻²⁴. Estrogen is associated with the increase of retrieved oocyte, grade I embryos, and endometrial quality.²⁵

Superoxide dismutase and estradiol and pregnancy

Table 3 shows estradiol hormone and SOD enzyme levels regarding pregnancy, which were higher in pregnant woman

than normal. The increased level of SOD in subfertile patients is associated with the increased odds ratio for pregnancy (Odds = 1.926, $P = 0.011$). This agrees with other investigators who evaluated a variety of oxidative stress biomarkers in serum and found direct relationship between antioxidants activity and pregnancy.^{9,26,27} This result also agreed with Younis et al. (2012), who found that SOD was higher in pregnant group, whereas peak E₂ was not different in the two groups.¹²

Some studies even suggested that E₂ by itself has antioxidant property and that adverse oxidative stress is associated with lower E₂ levels.⁸ SOD is connected with good quality oocyte and improved ART outcome. The area under the corresponding ROC curve for SOD and E₂ were compared to confirm the pregnancy (Fig. 2). Some researchers established that fertilization was confirmed by antioxidant concentration.¹²

Younis et al. revealed that SOD associated with success ART¹³ and free radicals associated with gametes damage.¹⁵

Some studies have suggested that high E₂ negatively affect the ART results.²⁸ Others show E₂ had no effect on endometrium²⁹ or pregnancy³⁰.

Conflict of Interest

None. ■

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