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Original Research Article

Serum CRP level in day of ovum pickup and embryo transfer as a predictor for success in patients undergoing IVF/ ICSI

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ABSTRACT

Background: This study was performed to determine the level of serum C-reactive protein (CRP) in day of ovum pickup and embryo transfer as a predictor for success in patients undergoing In-Vitro Fertilization (IVF) and Intracytoplasmic Sperm injection (ICSI).

Methods: This study was a cross sectional study (prospective) that was conducted in assisted reproduction unit in Al-Azhar University Hospital on 50 infertile patients who were candidate for IVF/ICSI, using standard long protocol, blood was drawn two times during cycle, in the day of ovum pickup and the day of embryo transfer.

Results: In (70.6 %) the serum of CRP level was higher in day of embryo transfer than day of ovum pickup. The ratio of CRP level in the day of embryo transfer to the day of ovum pickup was higher (1.54) in patients who became pregnant after ICSI, patients with less than this ratio have not been pregnant.

Conclusions: Controlled ovarian hyper stimulation and puncture of ovaries can potentiate systemic stimulation so increasing serum CRP level in day of embryo transfer rather than day of ovum pickup can predict success in patients undergoing IVF/ICSI.

Keywords: C-reactive protein, Intracytoplasmic sperm injection, In-Vitro Fertilization

INTRODUCTION

C-reactive protein (CRP) is a sensitive marker in inflammatory reactions. The level of this protein has been known to be changed with gender and increase in age.¹

Studies have demonstrated that females at the time of pregnancy have elevated levels of CRP compared to those who are not pregnant however the concentration of this marker doesn't differ between infertile and fertile individuals.² Also, it has been shown that psychological stress causes rise in inflammatory proteins such as CRP, which can result in poor prognosis and pregnancy complications.³

This protein is a sensitive marker in inflammatory process also there is an association between the rise of this

protein and occurrence of atherothrombosis, preterm delivery, low weight of the fetus and pre-eclampsia.^{4,6} This protein enforces the innate immunity and protection against tissue damage from damaged, dead or dying organisms.

Therefore, CRP by increasing the renovation speed of damaged tissues results in healing of these tissues also, it has been shown that psychological stress causes rise in inflammatory proteins such as CRP, which can result in poor prognosis and pregnancy complications.

This protein as a sensitive marker in inflammatory processes rises following hormonal stimulation. CRP doesn't have diurnal alterations. Administration of exogenous estrogen increases its level. Controlled hyperstimulation of the ovary, and specially puncture of the ovaries in in-vitro

fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles is probably associated with some degrees of tissue damage and therefore changes in CRP concentrations.

These changes may affect the success rate of IVF/ICSI, implantation, and pregnancy.^{7,8}

METHODS

This study was a cross sectional study (prospective) that was conducted in assisted reproduction unit in Al-Azhar University Hospital on 50 infertile patients who were candidate for IVF/ICSI in the period from March 2017 to May 2018, using standard long protocol, blood was drawn two times during cycle, in the day of ovum pick up and the day of embryo transfer.

Inclusion criteria

- Age: 24 -36 years
- Normal menstrual cycle 21-35 days with both intact ovaries.
- Normal uterus and fallopian tubes on HSG and/or hysteroscopy.

Exclusion criteria

- Age < 24 or > 36 years.
- Patients with positive CRP before starting IVF/ICSI protocol.
- Hydrosalpinx or intrauterine pathology such as submucous fibroid, adenomyosis, intrauterine septum or adhesions detected by transvaginal ultrasound scan and hysterosalpingogram and/or hysteroscopy.

A written consent was taken from each participant in the study. Embryologists in (ICSI) laboratory, Technicians in the endocrinology laboratory gave the clinical data.

The selected patients who were eligible for the study (n = 50) underwent ovulation stimulation cycle by standard long protocol.

Ovulation stimulation protocol

Patients with mean + SD age of 30.5±4.1 years and mean + SD infertility duration of 4.68±2.01 years underwent treatment by pituitary desensitization using gonadotrophin releasing hormone (GnRH) agonist from day 21 of cycle preceding stimulation cycle, then from second to third day of menstrual cycle, patients received human menopausal gonadotrophin (HMG) injection 150-300 units daily.

When at least three follicles had a diameter exceeding 16-18 mm and estradiol concentration was appropriate, 10,000 IU unit of HCG injection, transvaginal pick up was done.

Then only metaphase II oocytes identified by the presence the first polar body, were chosen for fertilization.

ICSI was performed 3-6 hour after oocyte recovery and 48-72 hour afterwards; fetuses from IVF/ICSI were transferred with labotech embryo transfer catheter.

Serum sampling of IVF/ICSI in different stages

Serum samples of patients who were candidate for IVF/ICSI were drawn in controlled ovarian hyperstimulation (COH) 2 times as follows: The day of ovum pick up (Day – opu) and the day of transfer (Day – transfer)

Elisa method

Fifty µl of antigen (from purified CRP in laboratory) with the concentration of 10 µg per liter was incubated in the sink of ELISA plate for 2 hours at 37 °C.

The sinks were washed three times, each time three minutes, with buffering washing solution. Then, for blocking, 150 µl of BSA 1% was added to each sink and placed for 45 minutes at 37 °C environment. The washing was again done in three stages. Purified antibiotic against CRP with 1/80 dilution was prepared. Twenty-five µl of this preparation was added to each sink. Afterwards, serial dilution of 62 µg/ml of CRP protein was prepared and was added to the sinks. For negative control, in one sink only CRP, and for positive control anti-CRP with 1/80 dilution was considered in one sink.

For measuring CRP concentration of the serum samples of patients, 25 µl of the sample was mixed with 25 µl of 1/80 diluted antibody and the mixture was incubated at 37 °C for one hour.

After washing, 50 µl sheep anti-human-HRP 1/1500 was added to each sink and the solution was incubated at 37 °C for 15 minutes.

The reaction was terminated with adding 15 µl of sulfuric acid 20%, and light absorption at 492 nm wavelength was measured by ELISA reader. With the aid of serial dilution prepared of CRP concentration changes was determined in the samples.

RESULTS

This study included 50 infertile cases who were candidate for IVF/ICSI, 37 cases (74%) complaint of 1ry infertility and 13 cases (26%) complaint of 2ry infertility (Table 1).

Table 1: Types of infertility.

Type of infertility	Frequency (n=50)	Percentage
Primary	37	74.0
Secondary	13	26.0

The age of cases ranged from 20-36 years, the (Mean age±SD) was 30.5±4.1 years with median 31.5, the duration of infertility ranged from 1-9 years, the (Mean±SD) was 4.68±2.01 years with median 4.5 (Table 2).

Table 2: Percentage of age and duration of infertility

	Range	Mean±SD	Median
Age	20-36	30.5±4.1	31.5
Duration of infertility	1-9	4.68±2.01	4.5

The number of oocyte which was picked up ranged from 3-22 oocytes, the (Mean±SD) was 9.96±3.43 with median 9.5 and number of embryos transferred ranged from 1-4 embryos, the (Mean±SD) was 3.2±1.03 with median 4 (Table 3).

Table 3: Percentage of no of oocytes pickup and No of embryo transfer.

	Range	Mean±SD	Median
No Of oocytes pickup	3-22	9.96±3.43	9.5
No Of embryo transfer	1.0-4.0	3.2±1.03	4

Pregnancy rate was 58% (29 cases) (Table 4).

Table 4: Percentage of pregnancy.

Pregnancy	Frequency (n=50)	Percentage
+VE	29	58.0
-VE	21	42.0

CRP level on day of ovum pickup (mg/l) ranged from 3.15 - 19.20, the (Mean±SD) was 8.08±3.93 with median 7.27 and CRP level on day of embryo transfer (mg/l)

ranged from 3.30 - 39.80, the (Mean±SD) was 9.93±7.15 with median 6.81 (Table 5).

Table 5: CRP level on day of ovum pickup and day of embryo transfer.

	Range	Mean ±SD	Median
CRP level on day of ovum pickup (mg/l)	3.15-19.20	8.08±3.93	7.27
CRP Level on day of embryo transfer (mg/l)	3.30-39.80	9.93±7.15	6.81

CRP level was increased in 68% of cases (34 cases) and decreased in 32% of cases (16 cases) (Table 6).

Table 6: Percentage of CRP change.

CRP change	Frequency (n=50)	Percentage
Increased	34	68
Decreased	16	32

In all patients who underwent controlled ovarian hyperstimulation (COH).

There was statistically significant difference on comparing CRP levels between pregnant and non-pregnant cases on day of ovum pick up.

In pregnant woman CRP level on day of ovum pick up (mg/l) Mean + SD was 7.38±3.63 with median 6.48 and CRP level on day of embryo transfer (mg/l) Mean + SD was 11.37±8.27 with median 9.10 (Table 7).

Table 7: Comparison regarding CRP level.

	Pregnancy		P value
	+ve	-ve	
CRP level on day of ovum pickup (mg/l)			
Mean±SD	7.38±3.63	9.05±4.12	0.03 ^S
Median	6.48	9.05	
CRP Level on day of embryo transfer (mg/l)			
Mean±SD	11.37±8.27	7.94±3.05	0.2 ^{NS}
Median	9.10	8.25	

S: Significant, NS: Non-Significant

In non-pregnant women CRP level on day of ovum pick up (mg/l) Mean + SD was 9.05±4.12 with median 9.05 and CRP level on day of embryo transfer (mg/l) Mean + SD was 7.94±3.05 with median 8.25 (Table 7).

Patients whose CRP level decreased on transfer day (11 cases), had lower chance of pregnancy, whereas patients

whose CRP level elevated on embryo transfer day had high chance of pregnancy (24 cases) (Table 8).

Sensitivity of CRP level testing was 82.8% while Specificity was 52.4% (Table 9). Positive predictive value was 70.6% while Negative predictive value was 86.8% (Table 9).

Table 8: Testing the ability of increased CRP level at day of embryo transfer to predict pregnancy

	Observed pregnancy		Total
	+ve	-ve	
CRP change (Expected pregnancy)			
Increased (+ve)	24	10	34
Decreased (-ve)	5	11	16
Total	29	21	50

Table 9: Values of CRP testing.

Sensitivity	Specificity	PPV	NPV
82.8%	52.4%	70.6%	86.8%

PPV: Positive predictive value, NPV: Negative predictive value

DISCUSSION

C-Reactive Protein (CRP) is a sensitive marker in inflammatory reaction. The level of this protein has known to be changed with gender and increase in age.¹

females at the time of pregnancy have elevated levels of CRP compared to those who are not pregnant. However, the concentration of CRP doesn't differ between infertile and fertile individuals.² There is an association between the rise of this 110.000-120.000

KD protein and association of atherothrombosis, preterm delivery, low weight of fetous, and preeclampsia. This protein enforces the innate immunity and protection against tissue damage through increase in phagocytosis, removing cells and damaged, dead or dying organisms.⁴⁻⁶

Controlled hyperstimulation of the ovary and especially puncture of the ovaries in in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles is probably associated with changes in CRP concentrations. these changes may affect the success rate of IVF/ICSI, implantation, and pregnancy, also administration of human chorionic gonadotropin (HCG) regardless of the response rate of the ovaries, causes activation of endothelial cells and neutrophils.⁷⁻⁹

The level of CRP increases in ovulation stimulation cycle with standard long protocol. Wunder et al in 2005 showed that leptin and CRP in ovulation stimulation cycle increase until the day of puncture, 9 Orvieto et al showed the same results. This elevation from induction to puncture was 60%.¹⁰

Levin et al reported that the average CRP level in hyperstimulated patients was significantly higher than patients who aren't hyperstimulated or the control group.¹¹ Ovulation induction is an inflammatory process leading to increased level of CRP, but with different patterns. These patterns of changes can be used as markers of successful outcome of IVF treatment. Almagor et al, found that serum CRP levels measured on

day of embryo transfer to be correlated with the outcome of in-vitro fertilization (IVF) treatment patients, whose CRP level decreased on transfer day, had lower chance of pregnancy. While patients whose CRP level elevated on embryo transfer day, applied treatment resulted in pregnancy.¹²

The current study was prospective cross-sectional study measuring level of C-Reactive protein (CRP) in day of ovum pick up and day of Embryo transfer as a predictor for success in patients undergoing in-vitro fertilization (IVF)/Intracytoplasmic sperm injection (ICSI).

Fifty (50) infertile patients with the age range of 20 to 36 years, levels of CRP were measured in day of ovum pick up and day of embryo transfer.

In this study, it was found that serum CRP levels was higher in day of Embryo transfer than day of ovum pick up in infertile patients who became pregnant and serum CRP level was lower in day of Embryo transfer than day of ovum pick up in infertile patient who were not pregnant.

The receiver operating characteristic (ROC) curve showed that specificity (SP) was 52.4%, sensitivity (SV) was 82.8%, positive predictive value (PPV) was 70.6%, negative predictive value (NPV) was 86.8% and accuracy was 70 %.

In another study that was conducted at Department of Epidemiology and Biostatistics, school of Public Health Tehran University of Medical Sciences, Tehran, Iran, this prospective cross sectional study was performed in Avicenna infertility clinic on consecutive infertile patients who were recruited from Jan. 2008 to Aug. 2009, Infertile patients with the age range of 24 to 38 years, who were candidate entered the study, before recruitment, a complete clinical, radiological evaluation and laboratory work up were performed. Those with positive CRP level and sever male factor were excluded from the study.

The selected patients who were eligible for the study underwent ovulation stimulation cycle by standard long protocol. It was found that patients whose CRP level decreased on transfer day, had lower chance of pregnancy while patients whose CRP level elevated on embryo transfer day, applied treatment resulted in pregnancy.⁷

CONCLUSION

C-reactive protein (CRP) is a sensitive marker in inflammatory reactions. Controlled hyperstimulation of the ovary especially puncture of the ovaries in in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles was probably associated with changes in CRP concentration, these changes may affect success rate of IVF/ICSI implantation and pregnancy.

Patients whose CRP level decreased on transfer day, had lower chance of pregnancy, whereas patients whose CRP level elevated on embryo transfer day had high chance of pregnancy.

Further studies are warranted to investigate and explain the correlation between successful implantation and serum CRP around the time of embryo transfer.

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Ethical approval: Not required

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