

Short communication

C-reactive protein level and pregnancy rate in patients undergoing IVF/ICSI

Soheila Arefi^{1,2} M.D., Mohammad Babashamsi² Ph.D., Poneh Shariat Panahi M.Sc.³, Lima Asgharpour Saruiy¹ B.Sc., Hojjat Zeraati⁴ Ph.D.

1 Reproductive Biotechnology Research Center, Avicenna Research institute, ACECR, Tehran, Iran.

2 Monoclonal Research Center, Avicenna Research Institute, ACECR, Tehran, Iran.

3 Payam Nour University, Tehran, Iran.

4 Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Received: 14 November 2009; accepted: 20 May 2010

Abstract

Background: C-reactive protein (CRP) can be increased after hormonal stimulations. The changes of CRP might affect the success of in-vitro fertilization (IVF).

Objective: The aim of this study was to determine the possible relationship between the serum CRP level and outcome of controlled ovarian stimulation, and pregnancy rate in patients undergoing IVF or intra cytoplasmic sperm injection (ICSI).

Materials and Methods: This prospective cross sectional study was performed in Avicenna Infertility Clinic on 70 consecutive infertile patients (Jan 2008-Aug 2009) who were candidate for IVF/ICSI, using standard long GnRH agonist protocol. Blood was drawn 4 times during the cycle, on first day of stimulation, the day of HCG injection, the day of ovum pick up, and the day of embryo transfer.

Results: In 82.2% of cases, the serum CRP level was higher in day of HCG injection than first day of stimulation and also the day of ovum pick up than the day of HCG injection. The ratio of CRP level in the day of transfer to the day of ovum pick up, was significantly higher (ratio ≥ 1.23) in patients who became pregnant after ICSI ($p = 0.001$). All patients with less than this Ratio have not been pregnant.

Conclusion: Controlled ovarian hyper stimulation and puncture of ovaries can potentiate systemic stimulation. Increasing serum CRP level in day of embryo transfer rather than ovum pick up can predict the success in patients undergoing IVF/ICSI.

Key words: C-reactive protein, IVF/ICSI, Pregnancy rate.

Introduction

C-reactive protein (CRP) is a sensitive marker in inflammatory reactions. The level of this protein has known to be changed with gender and increase in age. Studies have demonstrated that females at the time of parturition have elevated levels of CRP

compared to those who are not pregnant; however the concentration of this marker does not differ between infertile and fertile individuals (1). There is an association between the rise of this 110,000-120,000 KD protein and occurrence of atherothrombosis, (2) pre-term delivery (3), low weight of the fetus (2), and preeclampsia (4). This protein enforces the innate immunity and protection against tissue damage through increase in phagocytosis and removing cells and 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

Corresponding Author:

Mohammad Babashamsi, Department of Medical Diagnostic and Biological Products, Monoclonal Research Center, Avicenna Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: babashams@avicenna.ac.ir

inflammatory proteins such as CRP, which can result in a poor prognosis and pregnancy complications (5). This protein as a sensitive marker in inflammatory processes rises following hormonal stimulation (6). CRP does not have diurnal alterations (7), but administration of exogenous estrogen increases its level (8).

Also controlled hyper-ovulation of the ovary, and specially puncture of the ovaries in in-vitro fertilization (IVF) or intra cytoplasmic sperm injection (ICSI) cycles is probably associated with some degrees of tissue damage and therefore changes in CRP concentration (9). These changes may affect the successful 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 (10).

To our knowledge, only a few studies on CRP in assisted reproduction are available in the literature. The purposes of this study were to assess the curve of CRP changes in different stages of IVF/ICSI cycles in patients undergoing treatment of infertility; and the association between these changes with the rate of implantation and pregnancy, which is the first study in this regard.

Materials and methods

Studied patients

This prospective cross sectional study was carried out in Avicenna Infertility Center (Jan 2008-Aug 2009).

To find the required sample size, according to the previous study (Orvieto 2004), the sample size indicated at least 62 by 95% confidence and 5% precision. For accuracy, infertile patients with the age range of 24 to 38 years, who were candidate for IVF/ICSI, were entered to the study. Before recruitment, a complete clinical, radiological evaluation (hystero-graphy), and laboratory work-up consisted of biochemical, spermography, hormonal and hematologic assays were performed for the couples. Those with positive CRP level and severe male factor were excluded from the study. The selected patients who were eligible for the study (n=70) underwent ovulation stimulation cycle by standard long protocol. The characteristics of the studied patients are given in table I.

Ovulation stimulation protocol

Patients with mean±SD age of 30.96±3.39 years and mean±SD infertility duration of 8.61±2.87 years underwent treatment by pituitary desensitization using gonadotrophin releasing hormone (GnRH) agonist (Superfact, Aventis Pharma, Germany) from day 21 of the cycle proceeding the stimulation cycle. Then from the second to the third day of the menstrual cycle, patients received human menopausal gonadotrophin (HMG) injection (Merional, IBSA, Switzerland) 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 was administered. At 34-36 hours after HCG injection, transvaginal ultrasound guided ovum pick up was done. Then, only metaphase II oocytes, identified by the presence of the first polar body, were chosen for fertilization. ICSI was performed 3–6 hr after oocyte recovery and 48-72 hr afterwards; fetuses from IVF/ICSI were transferred with Labotech embryo transfer catheter.

Serum sampling of IVF/ISI cases in different stages

Serum samples of patients who were candidate for IVF/ICSI were drawn in controlled ovarian hyperstimulation (COH) four times as follows: the day of ovulation stimulation start (Day-S); the day of HCG injection (Day-HCG); the day of ovum pick-up (Day-OPU); and the day of transfer (Day-Transfer).

Also, on ovum pick-up day, a sample of clear and not bloody follicular fluid was obtained. All samples were centrifuged for 10 minutes at 1000 g, and were stored at -20°C until the final assay. The gathered samples were evaluated by comparative enzyme-linked immunosorbent assay (ELISA) method for determining the level of CRP.

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 antibody 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 with concentration of 62 µg/ml without anti-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 45 minutes. After repeated washing, 50 µl of substrate was added to each sink, and 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, standard curve of CRP concentration changes was determined in the samples.

Statistical analysis

For data analysis paired t-test, Mann-Whitney U test, Wilcoxon, Friedman, and Pearson correlation coefficient were used. Analyses were done by SPSS software for Windows (version 11.5). The significance level was set at 0.05.

Results

The characteristics of studied patients are presented in Table 1. The causes of infertility were ovulatory (28.9%), male factor (31.8%), tubal and peritoneal (13.1%), endometriosis (11.8%) and idiopathic (14.4%).

The rate of ovarian hyperstimulation, miscarriage and successful pregnancy in the patients were 2.8%, 8.6% and 42.9%, respectively. The pre-IVF data and clinical parameters of the

two groups, pregnant and non pregnant women are summarized in table II.

In the present study there, were no significant differences in age, duration of infertility and number of oocytes and number of transferred embryo between these two groups. Also there was no significant relationship between hyperstimulation (two moderate and one severe) and CRP changes. The CRP level in different stage of COH has been shown in table III. In all patients who underwent COH, CRP level on Day-HCG elevated significantly compared to the day of ovulation stimulation (4.94 ± 1.92 mg/L vs. 3.97 ± 1.92 mg/L), ($p < 0.001$). This elevation continued significantly till OPU day ($p < 0.001$). Mean \pm SD of CRP on the day of fetus transfer was 6.61 ± 4.16 mg/L. Although there was a significant increase in Day- transfer comparing to OPU day ($p < 0.01$), this rise was found in only 54.3% of patients, and in remaining subjects a decreased CRP level was detected. Patients, whose CRP level decreased on transfer day, had a lower chance of pregnancy. Whereas, in 68.4% of patients whose CRP level elevated on fetus transfer day, applied treatment resulted in pregnancy. The rate of pregnancy in those, whose CRP decreased was only 12.5% ($p < 0.001$).

The CRP level was not significantly different between pregnant women and those who did not become pregnant in any stages except for transfer day ($p < 0.001$) (Table III).

Assessment of the pattern of CRP changes was done using the Friedman test. It was observed that a significant increase was present in CRP level ($p < 0.001$). Also, ratio of CRP in each stage comparing to the previous stage was calculated. In spite of a reverse relationship (with estradiol increase, the rate of CRP changes decreased) in all cases, this relationship was not statistically significant.

Table I. Characteristics of studied patients.

Variable	Mean \pm SD	5-95 percentile
Age (years)	30.96 \pm 3.39	25.55-36.45
Infertility duration (year)	8.61 \pm 2.87	4-14
Serum estradiol on the day of HCG injection (pg/mL)	1856.14 \pm 623.77	930.5-3034
Mean number of oocytes retrieved (n)	12.01 \pm 4.68	5-20.45
Mean number of fetuses transferred (n)	3.53 \pm 0.68	2-4

Table II. Pre-IVF treatment data and clinical parameters for the two groups, pregnant and non pregnant women.

	Pregnant (n=30)	Not pregnant (n=40)	Mann-Whitney test (p-value)
Age (years)	31.04±2.99	29.96±1.94	NS
Duration of infertility (years)	9.91±2.59	7.30±3.15	NS
Serum estradiol on the day of HCG injection (pg/ml)	2061.29±954.9	1701.27±815.34	NS
Mean number of oocytes retrieved (n)	13.71±5.48	10.31±3.9	NS
Mean number of fetuses transferred (n)	3.1±0.9	2.9±1.13	NS
Hyperstimulation syndrome (n)	1	2	NS

All data are expressed as mean standard deviation.
NS: Non Significant

Table III. CRP levels in different stages in pregnant and not pregnant groups.

	Pregnant (n=30)	Not pregnant (n=40)	Mann-Whitney test (p-value)
First day (mg/L)	4.36 (1.92)	3.68 (1.88)	NS
HCG day (mg/L)	5.10 (1.84)	4.82 (1.99)	NS
OPU day (mg/L)	5.90 (2.58)	5.27 (1.98)	NS
Transfer day (mg/L)	8.98 (4.18)	4.84 (3.18)	< 0.001

All data are expressed as mean standard deviation.
NS: Non Significant

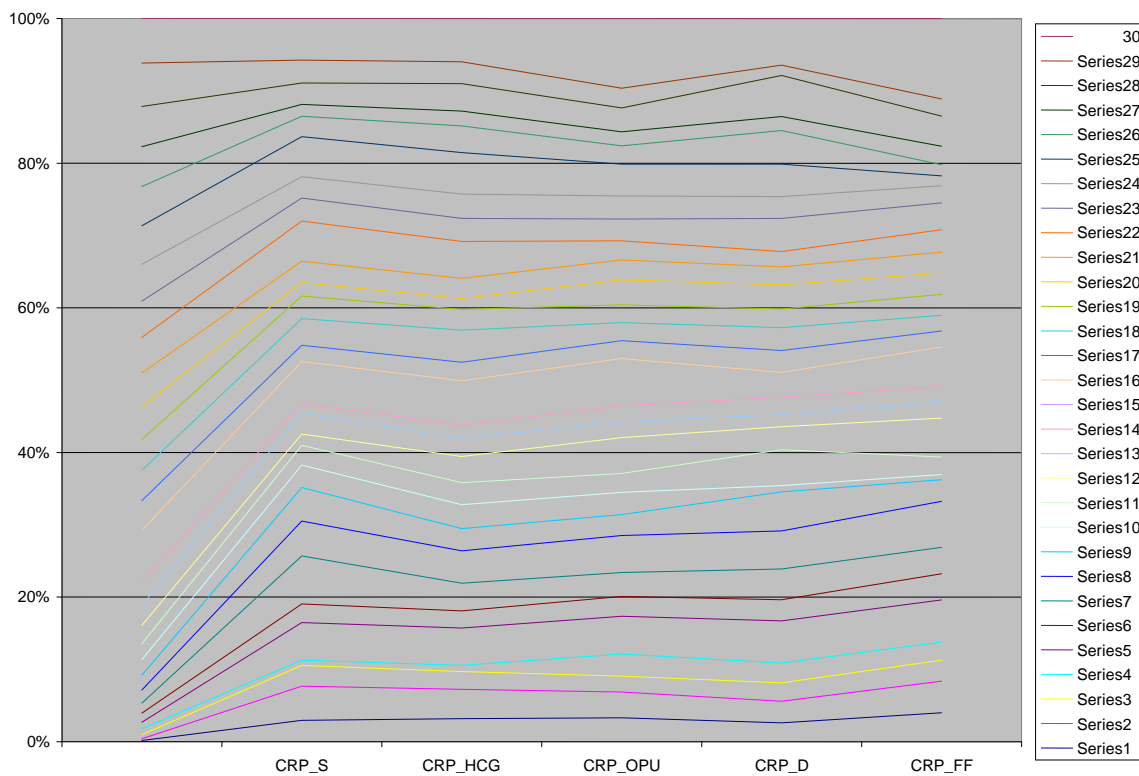


Figure 1. The chart of CRP level changes in the pregnant group.

Discussion

Acute inflammation occurs within several hours after infection or tissue damaging. Hepatocytes increase the synthesis of large number of proteins under the effect of interleukin-1. Concentration of CRP, with a molecular weight of 110,000-120,000 KD, increases 1000 times after invasion and tissue damage. It activates complement and can attach to activated lymphocytes, invasive organisms, and damaged tissues. CRP can act as a nonspecific opsonin to increase phagocytosis, removing cells and damaged, dead or dying organisms, reinforce innate immunity, and protection against tissue injury. Therefore, CRP by increasing the renovation speed, results in healing of damaged tissues. Studies have shown that CRP correlates with age and is higher in females (1). There was no significant difference regarding CRP level between infertile and fertile not pregnant individuals in Wood study (1). Also we didn't show any significant differences between initial CRP level in pregnant women and those who did not become pregnant. Stork *et al* showed that estrogen usage in menopause women is associated with the elevation of serum CRP (9). Also, it has been noted that exogenous estrogen and progesterone is associated with increased and decreased CRP concentration, respectively. Ten-times increase in endogenous estrogen and progesterone in menstrual cycles is related to 29% decrease and 23% increase in CRP level, respectively (10). In this study, CRP was measured by comparative ELISA method with designed antigen, which is quantitative and more precise than latex method. This study demonstrated that the level of CRP increases in ovulation stimulation cycle with long protocol. Wunder *et al* in 2005 showed that leptin and CRP in ovulation stimulation cycle increases until the day of puncture (10). Also, Orvieto *et al* showed the same result. This elevation from induction to puncture was 60% (8).

The relation between estradiol and CRP levels was assessed in our study. In spite of a reverse relation (with estradiol elevation, the rate of CRP changes was decreased) in all cases, this relationship was not statistically significant. Also it has not been shown any significant relation between estradiol and CRP levels in Wander study (11). According to the study of Orvieto it might be due to more prominent effect of HCG than estradiol on the neutrophil and endothelial activation which affect on CRP level (12).

Levin *et al* reported that the average CRP level in hyperstimulated patients was significantly

higher than patients who were not hyperstimulated or the control group (13). In our study, there was no significant relationship between hyperstimulation (two moderate and one severe) and CRP changes. All studied patients underwent long protocol and received HCG. Studies have shown that in ovulation stimulation cycles in IVF, the use of GnRH agonist instead of HCG is associated with a lower level of inflammation, and with this modality, CRP changes during the cycle decreases by 96% (14).

Our study showed that patients, whose CRP level decreased on transfer day, had a lower chance of pregnancy. Whereas, in 68.4% of patients whose CRP level elevated on fetus transfer day, applied treatment resulted in pregnancy. The present study is the first to provide information on changes on CRP levels during COH and the rate of pregnancy. In the study of Sacks *et al*, CRP levels have been measured before IVF stimulation and 14 days after egg collection. Women pregnant after IVF had significantly higher CRP levels compared to non-pregnant women 14 days after egg collection (15). Also Almagor *et al* found serum CRP levels, measured on the day of embryo transfer to be correlated with the outcome of *in vitro* fertilization (IVF)-treatment (16). The reason of this correlation might be due to the another inflammatory marker, leukocyte selectin (L-selectin), which was even found to be decreased during controlled ovarian hyperstimulation until peak estradiol was reached, and then significantly increased after hCG administration as showed by orvieto (17). However, we couldn't find any other related reason to show why pregnancy rate is less in lower CRP levels in the day of embryo transfer. More studies are warranted to investigate and explain the correlation between successful implantation and serum CRP level around the time of embryo transfer.

Conclusion

In the presented study, we showed that ovulation induction is an inflammatory process leading to increased levels of CRP, but with different patterns. These patterns of the changes can be used as markers of successful outcome of the IVF treatment.

Acknowledgment

We would like to express our sincere appreciation to experts in Avesina Research

Institute and Farzan Institute for Research and Technology and for their technical assistance.

References

1. Wood WG, Lüdemann J, Mitusch R, Heinrich J, Maass R, Frick U. Evaluation of a sensitive immunoluminometric assay for the determination of C-reactive protein (CRP) in serum and plasma and the establishment of reference ranges for different groups of subjects. *Clin Lab* 2000; 46: 131-140.
2. Trevisanuto D, Doglioni N, Altinier S, Zaninotto M, Plebani M, Zanardo V. High-sensitivity C-reactive protein in umbilical cord of small-for-gestational-age neonates. *Neonatology* 2007; 91: 186-189.
3. Lohsoonthorn V, Qiu C, Williams MA. Maternal serum C-reactive protein concentrations in early pregnancy and subsequent risk of preterm delivery. *Clin Biochem* 2007; 40: 330-335.
4. Garcia RG, Celedón J, Sierra-Laguado J, Alarcón MA, Luengas C, Silva F, et al. Raised C-reactive protein and impaired flow-mediated vasodilation precede the development of preeclampsia. *Am J Hypertens* 2007; 20: 98-103.
5. Coussons-Read ME, Okun ML, Nettles CD. Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. *Brain Behav Immun* 2007; 21: 343-350.
6. de Maat MP, Madsen JS, Langdahl B, Bladbjerg EM, Tofteng CL, Abrahamsen B, et al. Genetic variation in estrogen receptor, C-reactive protein and fibrinogen does not predict the plasma levels of inflammation markers after long term hormone replacement therapy. *Thromb Haemost* 2007; 97: 234-239.
7. Meier Ewert HK, Ridker PM, Rifaei M, Price N, Dinges DF, Mullington JM. Absence of diurnal variation of C-reactive protein level in healthy human subjects. *Clin Chem* 2001; 47: 426-430.
8. Störk S, Bots ML, Grobbee DE, van der Schouw YT. Endogenous sex hormones and C-reactive protein in healthy postmenopausal women. *J Intern Med* 2008; 264:245 -253.
9. Wunder DM, Dretschmer R, Bersinger NA. Concentration of leptine and C-reactive protein in Serum and follicular fluid during assisted reproductive cycles. *Hum Reprod* 2005; 20: 1266-1271.
10. Orvieto R, Chen R, Ashkenazi J, Ben-Harush A, Bar J, Fisch B. C- Reactive protein level in patients undergoing controlled ovarian hyperstimulation for IVF cycle. *Human Reprod* 2004; 19: 357-359.
11. Wander K, Brindle E, O'Connor KA. C-reactive protein across the menstrual cycle. *Am J Phys Anthropol* 2008; 136: 138-146.
12. Orvieto R, Schwartz A, Bar Hava I, Abir R, Ashkenazi A, Lamarka A, et al. Controlled ovarian hyperstimulation –a state of endothelial activation. *Am J Reprod Immunol* 2000; 44: 257-260.
13. Levin I, Gamzu R, Pauzner D, Rogowski O, Shapira I, Maslovitz S, et al. Elevated level of CRP in Ovarian hyper stimulation syndrome: an unrecognized potential hazards? *BGOJ* 2005; 112: 952-955.
14. Orvieto R, Zagatsky I, Yulzari-Roll V, La Marca A, Fisch B. Substituting human chorionic gonadotropin by gonadotropin-releasing hormone agonist to trigger final follicular maturation, during controlled ovarian hyperstimulation, results in less systemic inflammation. *Gynecol Endocrinol* 2006; 22:437.440.
15. Sacks GP, Seyani I, Lavery S, Trew G. Maternal C-reactive protein levels are raised at 4 weeks gestation. *Hum Reprod* 2004; 19, 1025–1030.
16. Almagor M, Hazav A, Yaffe H. The levels of C-reactive protein in women treated by IVF. *Hum Reprod* 2004; 19, 104–106.
17. Orvieto R, Ben-Rafael Z, Schwartz A, Schwartz A, Abir R, Fisch B, et al. Soluble L-selectin levels during controlled ovarian hyperstimulation. *Gynecol Endocrinol* 2001; 15, 29–33.