

Original Article

Can blood or follicular fluid levels of presepsin predict reproductive outcomes in ART; a preliminary study

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Abstract: Many stages of COH protocols are considered to potentiate a state of systemic inflammation. The limit beyond which inflammation has negative impacts on the formation of conception and the reproductive outcomes are compromised still remains unclear. Presepsin is a novel biomarker for diagnosing systemic inflammation and sepsis. We aimed to investigate whether plasma and follicular fluid presepsin values on oocyte pick-up (OPU) day, embryo transfer (ET) day and pregnancy test (PT) days could predict reproductive outcomes during IVF treatment in women with UEI. Patients were assigned to two groups according to pregnancy test results; pregnant (Group 1) and non-pregnant (Group 2). From all patients included in the study, 2 cc of venous blood was sampled on the three days and follicular fluid (FF) was collected during oocyte retrieval. Plasma presepsin, CRP and WBC values and FF presepsin values were measured and compared between the 2 groups. There was no significant difference between FF and plasma presepsin levels on the OPU day (298 ± 797.4 ve 352.9 ± 657.1 ; $P=0.701$, respectively). Plasma WBC, CRP and presepsin levels on the OPU, ET and PT days and FF presepsin levels on OPU day were not different between the 2 groups. Plasma presepsin course on the separate 3 days were different between the groups.

Keywords: Presepsin, IVF, C-reactive protein, white blood cells, follicular fluid

Introduction

Today, almost 15% of couples suffer from infertility [1, 2]. Despite the significant advances in the field of reproductive medicine, intended clinical pregnancy rate per embryo transferred has not been attained and the current reported rates can not exceed the level of 33% [3]. Implantation failure has been considered to constitute a significant proportion of treatment failures of which many theories have been employed to explain the underlying pathophysiological mechanism.

Implantation of the embryo is a complex process which involves properly synchronized molecular interactions between hormonally primed endometrium and the conceptus. The molecular interactions are mediated by multiple steroid hormones, cytokines and immunologic factors [4]. In addition to physiological reproductive phenomena including folliculogen-

esis, ovulation and implantation of the embryo, a set of processes employed during the assisted reproductive techniques (ART), including controlled ovarian hyperstimulation (COH), oocyte pick-up (OPU) and embryo transfer (ET), is known to trigger the cascade of complex inflammatory processes. However, the point beyond which physiological inflammation convert to pathological and the reproductive outcomes are compromised still remains unclear. Moreover, human chorionic gonadotropin (hCG) administration in the course of COH protocols is known to stimulate the neutrophil and endothelial activation [5, 6].

Over the past 10 years, several markers have been described to determine the presence and the severity of systemic inflammatory states. C-reactive protein (CRP), procalcitonin (PCT) and lipopolysaccharid-binding protein (LBP) are substantial markers, biological fluid levels of which provide useful information about the

inflammatory response. Presepsin is a novel, 13 kDa molecular weight biomarker for diagnosing systemic inflammation and sepsis. The circulating soluble form of CD14, a cluster of differentiation (CD) marker protein expressed on the surface of various cells including monocytes, macrophages, neutrophils, and B cells, is activated by plasma proteases, generating a 64 aminoacid-residue fragment named sCD14-subtype (sCD14-ST), so-called presepsin [7, 8]. Collected data provide evidence that presepsin may serve as an acute phase reactant, as analogous to CRP [9]. While the biological role of presepsin has not been completely elucidated, it is considered to be a regulatory factor capable of modulating cellular and humoral immune responses by interacting directly with T and B cells [10]. Pathfast assay method appears to be an adequate technique for the determination of presepsin levels in the biological fluids and its sensitivity has been demonstrated to be 100% in the presence of infection [11]. The clinical significance of the acute phase reactant, CRP, in the infertility population has been evaluated by some previous published reports [12, 16].

Even though diagnostic value of determination of presepsin in sepsis cases has been widely accepted, no publications assessing the significance of this peptide in infertility population, are available in the literature. To the best of our knowledge, the effects of presence of presepsin in both plasma and follicular fluid (FF), on the reproductive performance of infertile couples have been investigated for the first time by the present study.

This study aims to investigate whether plasma and follicular fluid presepsin values on oocyte pick-up (OPU) day, embryo transfer (ET) day and pregnancy test (PT) days could predict reproductive outcomes during IVF treatment in women with UEI. Secondary outcome is to detect and to compare plasma and FF presepsin concentrations in a reference infertility population.

Material and methods

The present is a prospective cross-sectional study conducted with the patients who admitted to the Division of Assisted Reproduction, Department of Obstetrics and Gynecology, Zeynep Kamil Training and Educational Hospital

due to the desire of having a child between June 2013 and December 2013. Approval of the ethics committee was obtained prior to the initiation of the study.

This study was included primary or secondary infertile women with major indications for IVF, married for at least 3 years, who were non-smokers, age 23-39 years, body mass index (BMI) 18-28 kg/m², regular menstrual cycles ranging from 25 to 35 days, normal basal serum FSH (≤ 10 IU/l) and estradiol (E2 ≤ 85 pg/ml) levels determined on day 3 of the cycle, no uterine (fibroids, adenomyosis, mullerian malformations), ovarian (endometrioma, polycystic ovaries), or adnexal (hydrosalpinx) abnormalities assessed by transvaginal ultrasonography which were treated with the classical agonist long protocol or the GnRH antagonist fix protocol. Additionally, all semen analyses of male partners were normal according to the World Health Organization criteria for normality [17].

Previous trials in which ≤ 4 oocytes were retrieved or cycles in which no embryo was obtained for transfer, women with any significant systemic disease, endocrine, or metabolic disorders and those with any demonstrable infertility etiologies were excluded. Demographic characteristics, basal hormone profile levels, transvaginal sonographic findings, ovarian stimulation characteristics and treatment outcomes of the patients included have been recorded into the database constructed for the study.

The starting gonadotropin dose was individualized according to age, body mass index (BMI), ovarian reserve determined by antral follicle count and basal FSH, and experience from previous cycles. Gonadotropin stimulation was achieved by either rFSH or hMG. All patients were administered acetyl-salicylic acid 100 mg daily and folic acid 400 mcg daily simultaneously with the start of the protocol.

Serial ultrasonographic controls and E2 level measurements were made until 3 follicles ≥ 17 mm and a serum E2 level >500 pg/ml were detected. Choriogonadotropin alpha 250 μ g s.c. (Ovitrelle®; Merck Serono, Italy) was administered to induce final follicular maturation. Transvaginal ultrasound-guided oocyte retrieval was performed 35-36 hours after hCG administration. All patients were implemented

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Table 1. Demographic, stimulation and treatment characteristics of the groups

Characteristics	Group 1 (n=12)	Group 2 (n=41)	P
Age, y	32.9±4.1	31.1±3.8	0.155 ^a
Infertility duration, y	6.3±3.2	6.6±3.8	0.999 ^a
Antral follicles on day 1, n	14.2±3.2	14.2±4.0	0.865 ^a
D3 FSH, IU/l	7.3±1.2	6.7±1.5	0.322 ^a
D3 estradiol, pg/ml	52.0±16.2	50.8±16.8	0.924 ^a
GnRH agonist/antagonist protocol, n	6/6	18/23	0.965 ^b
Induction duration, d	9.3±1.6	8.6±1.7	0.297 ^a
Average used gonadotrophin, IU	2561.4±1134.3	2388.4±1098.2	0.742 ^a
Endometrium on HCG day, mm	10.8±1.6	10.0±1.6	0.141 ^a
Serum E2 on HCG day, pg/ml	2448.6±1005.3	1904.2±785.9	0.109 ^a
MII oocytes, n	7.7±3.9	5.8±3.5	0.091 ^a

Data are presented as mean ± SD. ^aMann-Whitney test. ^bχ² test.

Table 2. Plasma WBC, C-RP and presepsin, and FF presepsin values of the groups

	Group 1 (n=12)	Group 2 (n=41)	P
Plasma WBC values on OPU day	9.1±2.4	8.5±2.1	0.279 ^b
Plasma WBC values on ET day	14.1±3.9	11.9±3.6	0.092
Plasma WBC values on PT day	9.4±1.8	7.9±2.2	0.019
Plasma C-RP values on OPU day	0.47±0.3	0.55±0.4	0.199 ^b
Plasma C-RP values on ET day	0.57±0.6	0.48±0.3	0.594
Plasma C-RP values on PT day	0.62±0.5	0.69±0.9	0.387
Plasma Pr values on OPU day	242±115.4	385.4±743.5	0.603
Plasma Pr values on ET day	296.3±145.1	306±140.3	0.877
Plasma Pr values on PT day	280.6±237.4	250.4±125.1	0.781
OPU FF presepsin values	243.6±531.1	314.3±866.5	0.055 ^a

Data are presented as mean ± SD. OPU; oosit pick-up, ET; embryo transfer, PT; pregnancy test, WBC; White Blood Cells (mcl), C-RP; C-reactive protein (mg/L), P; presepsin (pg/mL). Statistical analysis was performed using Mann-Whitney test.

a single dose of cefazolin sodium (Sefazol, Mustafa Nevzat İlaç San., Turkey) 1 gr, i.m. at the course of OPU procedure and were given doxycycline 100 mg capsule (Tetradox capsule, Fako İlaç, Turkey) twice daily and methylprednisolone 16 mg capsule (Prednol tablet, Mustafa Nevzat İlaç San., Turkey) once daily and continued for 4 days. Luteal support was initiated on the night of oocyte retrieval and continued until the day of pregnancy testing. If the test was positive, progesterone treatment was continued up to 9th gestational weeks.

Fertilization was assessed at 16-18 h after ICSI and one or two embryos with the best morphological grade were transferred into the uterine cavity under ultrasound guidance (GE Logic alfa

200). Serum pregnancy test was performed 12 days after the embryo transfer. hCG levels >20 mIU/ml was considered as a positive pregnancy test. Patients were assigned to two groups according to pregnancy test results; pregnant (Group 1) and non-pregnant (Group 2). From all patients included in the study, 2 cc of venous blood was sampled in to ethylenediaminetetraacetic acid (EDTA) containing tubes, on three separate days; OPU day, ET day and PT days, immediately before the procedure. Body temperatures of all patients were measured at the time of each blood sampling and those with a temperature ≥37°C were excluded. Besides, during oocyte retrieval, the FF was collected from the first mature (>17 mm) follicle at the first entry without contamination. FFs contaminated with blood or that do not contain oocyte were not used. Venous blood

samples were centrifuged for 2 minutes at 1300 x g. The separated plasma was transferred into eppendorf tube to store at -40°C until the assaying time. Aspirated FFs were centrifuged for 10 minutes at 2000 x g at room temperature and separated supernatant fluids were transferred in the cold chain immediately to the laboratory within the eppendorf tubes to be stored at -80°C until performing the assay. On the assaying day, within 4 hours after the samples are thawed, presepsin levels were measured by using the Pathway® Presepsin assay (Mitsubishi Medience, Tokyo, Japan). Plasma and FF presepsin values were compared between the 2 groups. Additionally, WBC and C-RP values were obtained in the blood samples, and compared between the groups.

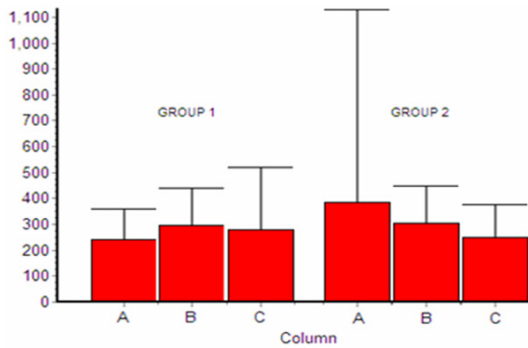


Figure 1. The course of plasma presepsin levels on OPU, ET and PT days within the groups. A: OPU day, B: ET day, C: PT day. OPU, oosit pick-up; ET, embryo transfer; PT, pregnancy test.

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows 15.0 software (SPSS, Chicago, IL, USA). Descriptive statistics were given as mean and standard deviation. Parametric comparisons were performed using Student's t-test, and non-parametric comparisons were performed using Mann-Whitney U test. Categorical data were evaluated by using χ^2 test. Statistical significance was defined as $P < 0.05$.

Results

Between June and December 2013, 53 couples with UEI were included in the study among 560 patients. Group 1 and 2 consisted of 12 and 41 patients, respectively. No patients had fever after OPU or ET and no OHSS occurred after cycles. There was no difference between the groups regarding demographic and stimulation characteristics and treatment outcomes, as presented in **Table 1**.

In the total of 53 included patients, we did not detect any significant difference between FF and venous plasma presepsin levels on the OPU day (298 ± 797.4 vs 352.9 ± 657.1 ; $P = 0.701$, respectively). The comparisons of venous plasma WBC, C-RP and presepsin values on OPU, ET and PT days and FF presepsin values on OPU day between the 2 groups are presented in **Table 2**, which revealed no statistically significant difference between the groups regarding any of the measurement parameters. However, both plasma and FF presepsin levels on OPU day were lower in the group 1 than in the group 2. Further, plasma presepsin levels in the group 1 first increased followed by a reduc-

tion on OPU, ET and PT days, respectively, whereas in the group 2 the levels demonstrated a continuous decline trend (**Figure 1**).

Discussion

It has hitherto not been clarified which mechanisms might interfere with the fertility outcomes in UEI patients. However, it has been hypothesized that the impaired inflammatory response might play a role at any stage of the cascade of complex mechanisms in the development of conception; thus compromising the reproductive performance. In this context, adverse fertility outcomes obtained in the low-grade, chronic, systemic inflammatory states, such as obesity, smoking and endometriosis, appear to be in support of this theory [18, 19]. An acute phase reactant, CRP, levels of which provide information regarding the presence and the levels of the inflammation within the systemic environment, has been investigated in assisted reproduction [12-16, 20], however, the results are controversial. Though some authors suggested that higher CRP levels during IVF cycles were associated with failure of conception [12], others reported higher blood CRP values in women who could conceive [20]. Some other authors postulated that CRP measurements were not a predictive marker of IVF success [15, 16]. Recently, levels of another acute phase protein, serum amyloid-A, has been investigated in FFs of women undergoing COH protocol and it was reported that elevated follicular levels were associated with decreased pregnancy rates and, thus, might signify lower reproductive performance [21]. Presepsin is a novel biomarker used for diagnosis of systemic inflammatory processes, such as sepsis, severe sepsis and septic shock, with a high diagnostic capacity [22, 23]. However, to our knowledge no studies assessing presepsin use in IVF treatments exist in the literature. The present study is the first to compare IVF patients who conceived and who did not regarding plasma and FF presepsin levels. Furthermore, WBC and CRP levels on the OPU, ET and PT days, were compared in this study. Plasma WBC levels were not significantly different between the groups on the three separate days. CRP levels in our study did not significantly differ between pregnant and non-pregnant women, in accordance with the results of previous studies indicating that the predictive value of this marker on the reproductive outcomes in IVF treatments

was insufficient [15, 16]. Similar to CRP level results, measurement of plasma presepsin levels on three separate days did not reveal any significant difference between the women who conceived and who did not. Moreover, although FF presepsin levels did not significantly differ from plasma presepsin levels on the OPU day ($P=0.701$), assessing FF presepsin levels on the OPU day revealed that levels in the women who did not conceive were higher than those in women who conceived and that the difference was close to the significance border, but not ($P=0.055$).

It has been accepted that not only the levels but also the trend of the inflammatory markers at the different stages of reproductive processes are of pivotal clinical significance. Indeed, literature assessment demonstrated that the variation pattern of plasma CRP levels on different days in women undergoing IVF has been investigated by several studies. Almagor et al. [13] indicated in their study that concentrations of CRP in blood increased significantly during the first week following oocyte retrieval and that clinical success was associated with a relative small increment in CRP on the day of embryo transfer. Orvieto et al. [14] reported that levels of CRP gradually increased on the suppression day, hCG day and OPU day, respectively, and they, hereby, concluded that COH potentiated a state of systemic inflammation. Wunder et al. [15] also reported a similar pattern of CRP increase during the course of COH. In the present study, the variation pattern of plasma presepsin levels differed between women who conceived and who did not. Blood levels of presepsin in patients who conceived, increased on the ET day when compared to the level on the OPU day, however, it slightly declined on the PT day. Per contra, in the absence of pregnancy, presepsin reached its highest value on the OPU day, which was followed by a gradual decrease in the subsequent ET and PT days.

Our study appears to be the first in terms of examining the relationship between blood levels of presepsin and IVF outcomes. Though our study results did not signify any difference between women who conceived and who did not, regarding blood presepsin levels at the different stages of COH process, FF presepsin levels on the OPU day were higher, close to the significance limit, in those who did not con-

ceived. It seems possible to obtain beneficial results with more comprehensive studies with extensive case series.

In conclusion, this study is insufficient to determine whether measurement of presepsin levels in plasma and/or FF could be a reliable marker in predicting reproductive outcomes. The pattern of plasma presepsin course is different in patients who achieve pregnancy and who do not. However, it has been demonstrated that there exists no difference between FF and plasma levels of presepsin in the infertility population. Although presepsin can be detected in the follicular fluid, these preliminary results indicate that it cannot be used as a diagnostic marker.

Disclosure of conflict of interest

None.

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