Interleukin 15 levels in Relation to Intracytoplasmic Sperm Injection Outcome of Women with Poor Ovarian Response

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ABSTRACT

Background: Poor response of the ovary to ovarian stimulation usually indicates a reduction in follicular response, resulting in a reduced number of oocytes retrieved. Cytokines in serum and follicular fluid are subject of extensive researches to identify their role in subfertility with controversial results.

Objectives: To evaluate the relation of Interleukin 15 (IL 15) levels to intracytoplasmic sperm injection outcome in poor responder women undergoing ICSI.

Methods: This cross-sectional prospective study conducted between November 2018 and April 2019 at High Institute of Infertility Diagnosis and Assisted Reproductive Technique in AL-Nahrain University and Baghdad in Vitro Fertilization Private Centre. Which included 80 women divided into two groups: 40 poor responder women without other causes of subfertility and 40 normal responder women with other causes of subfertility. Ovarian hormonal stimulation conducted according to GnRH antagonist protocol, then serum and follicular fluid obtained at the day of oocyte retrieval for measurement of IL 15 concentration measured by ELISA method. Statistical analysis was used to correlate the cytokines concentration with oocyte maturity and biochemical pregnancies rates.

Results: Age, BMI and induction duration did not significantly differ between the two groups, however, mean gonadotropin doses were significantly higher in poor responder group than in control group (2397.6 ±1087.5 vs. 3718.8 ±722.2, p<0.0002) ,while mature oocyte number (9.48 ±2.45 vs. 7.75 ±1.48), being lower in poor responders women compared to control group. On day of oocytes retrieval, there was no significant difference in median levels of serum IL15 and median level of follicular IL-15 between poor response (59.41(17.40) and 61.85(18.00)) and control (56.10(17.75) and 59.40(16.00)) groups.

Conclusion: In spite of higher levels of IL15 in serum and follicular fluid of poor responder women, a relation IL15 with ovarian response could not be established in comparison to control group, however, IL15 may be a predictor of pregnancy among women with poor ovarian response.

Keywords: Cytokines, IL15, Intracytoplasmic sperm injection, Poor responders, Subfertility.

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Women who delay childbearing until their late 30s or early 40s are frequently faced with the distressing fact that their chance of achieving a spontaneous pregnancy is difficult that can jeopardize their hope for successful conception. In Europe at 2010, women undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures in women at age group >40 years represented approximately 16.7% and 17.3%, respectively, of those attending assisted conception clinics[1,2].

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A number of variable factors can affect success rates in assisted conception, and the negative influence of advance in age is one feature that is well recognized. Not only does the response to stimulation steadily reduced, requiring increasing amounts of gonadotropins, but also the cancelation rate in ART cycles is higher, with significant higher rates of miscarriage[3,4]. The chance of pregnancy in addition to live birth rates begins to dramatically decrease after the age of 35 years, and successful managements for these patients continues

to be a great challenge in assisted conception cycles[5,6].

The current strategies of assisted conception aimed at follicular recruitment augmentation, increasing the yield of oocyte, and improve the desired clinical outcomes following IVF among women identified as poor responders[5,6].

Cytokines have been found to be related directly to maintain the delicate balance of the hypothalamo-pituitary-ovarian axis and involved in the maintenance of normal ovarian and menstrual cycles[7,8]. Cytokines are considered the key to reproduction success, creating an immune permissive, embryo receptive environment that optimally support gametogenesis, fertilisation, development of the early embryo, blastocyst implantation, and the growth of the foetus. In spite of their vital role in ovarian function is increasingly being recognised, the understanding of their precise function and interaction remains unclear[9].

There is a limited number of researches available in the literature assessing the role of the inflammatory markers in the poor responder women. Some investigated the possible involvement of inflammatory mechanisms and the role of IL-1 in the ovarian ageing and reduction of ovarian reserve in an animal models and found that IL-1 has a vital role in the age-related exhaustion of ovarian reserve[10]. Others demonstrated that increased levels of follicular fluid (FF) TNF-α were correlated with poor oocyte quality[11]. While other investigators concluded that inhibition of TNF-α improves IVF success rates in subfertile women[12]. The soluble receptor of advanced glycation end-products (sRAGE) in the FF has been recognized as a marker of ovarian reserve and poor ovarian response[13].

Interleukin-15 (IL-15) is recognized as a pleiotropic cytokine that has pivotal roles in stimulating the production of T-helper 1 cells predominant pro inflammatory cytokines, as a result promote T cells and natural killer cells proliferation, also IL15 regulates the differentiation, development, and killing activity of the natural killer cell[14-16].

There are few researchers studied IL15 in women undergoing IVF/ICSI, all of them undergo natural IVF cycles attempts, assessing the relation between FF IL 15 concentrations and age, BMI, follicles size, retrieved oocytes maturity and IVF/ICSI cycle success and birth prediction[17-19].

To our knowledge this the first study investigating the relation of this pleiotropic cytokine (IL15) in serum and FF of poor responder women during stimulated IVF/ICSI cycle.

The aim of this study is to evaluate the relation of serum and follicular fluid IL 15 to ICSI outcome in subfertile women with poor ovarian response.

Methods

This cross-sectional prospective study was conducted between November 2018 and April 2019 at Infertility clinic in High Institute of Infertility Diagnosis and Assisted Reproductive Technologies at Al- Nahrain University and from Baghdad IVF private centre.

Inclusion criteria were as follows: body mass index (BMI) 18-28 kg/m^2, no uterine (leiomyoma, endometriosis, adenomyosis, congenital abnormality of genital tract), ovarian (endometrioma, polycystic ovaries), or adnexal (hydrosalpinx) abnormalities detected by transvaginal ultrasonograghy and/or hysteroscopy and/or laparoscopy and normal semen analyses according to the World Health Organization criteria for normality[1,2].

Patients were divided into two groups according to the Bologna criteria for poor ovarian response. Group 1 includes 40 women who were normoresponder and Group 2 include 40 poor responder women. Two out of three of the following criteria were essential in order to classify a patient as poor ovarian responder: (i) advanced maternal age (≥40 years) or any other risk factor for poor ovarian response; (ii) a previous poor ovarian response (≤3 oocytes

with a conventional stimulation protocol); or (iii) an abnormal ovarian reserve test (AFC <5-7follicles or AMH <0.5-1.1 ng/ml)[6]. Written informed consent was obtained from all women.

Ovarian stimulation was achieved according to flexible GnRH antagonist protocol which performed with recombinant FSH or human menopausal gonadotropin, gonadotrophins were started on day 2 or 3 of menstruation, administration of gonadotrophin was individualized according to women age, baseline serum FSH concentration on day 3 and BMI followed by adjustment based on individual ovarian response until triggering. During ovarian hyperstimulation, monitoring done with serial transvaginal sonography for assessment of: follicular growth, endometrial thickness and pattern, in addition to serum estradiol (E2) level.

When the leading follicles reached a diameter of 14 mm, 0.25 mg of GnRH antagonist was added daily until day of trigger. When 2 -3 leading follicles reached a mean diameter of ≥17mm, a 250 µg of recombinant human chorionic gonadotrophin hCG (Ovitrel, Merck-Serono) was administered subcutaneously 35 hours before transvaginal oocyte retrieval. Oocytes were retrieved by transvaginal ultrasound-guided oocyte aspiration, which was done approximately 34-36 hours after hCG administration.

ICSI was performed, 2-4 h later to oocyte collection. Fertilization assessment for the presence of two pro-nuclei (2PN) stage was conducted 16-18 h of ICSI. Embryo transfer done under ultrasound guidance. The number of embryo transferred was limited to two or three in order to prevent multiple pregnancies. All patients received luteal support using vaginal progestron (Crinon 8% gel, Serono, UK). Biochemical pregnancy was assessed based on the serum beta hCG after 14 days of embryo transfer.

A clean and transparent human FF samples were collected during oocyte retrieval. The FF samples from individual women were pooled, centrifuged at 3000 rpm for 10 minutes and collected in labelled vial then the supernatant was stored at -20°C until assayed. Venous blood samples were taken at the same day of oocyte retrieval.

Serum and FF that were obtained on day of oocyte retrieval were estimated for cytokines levels by enzyme-linked immunosorbent assay (ELISA) technique using diagnostic kit for IL-15 (Komabiotech, Seoul, Korea).

Data were collected, summarized, analysed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Quantitative variables (number and percentage) were presented as mean ± standard deviation values and compared using the independent Samples t test and Mann–Whitney U test. Qualitative variables (number and percentage) were compared with Fisher's exact or Pearson chi square tests. Numeric variables that are not normally distributed (IL15 levels) were expressed as median (an index of central tendency) and inter-quartile range (an index of dispersion). Statistical significance was assumed with a probability error of $p < 0.05$.

Results

There was no significant difference between group 1(normoresponder) and group 2 (poor responder women) regarding age, BMI, and induction duration, (Table 1).

Mean gonadotropin dose required for ovarian stimulation was significantly higher in Group 2 than in Group 1 (2397.6 ±1087.5 vs. 3718.8 ±722.2, p<0.0002). The number of MII oocytes was significantly lower in Group 2 than in Group 1 (8.2 ±3.8 vs. 3.6 ±2.4; p<0.0001). There was no significant difference in pregnancy rates between both groups (24.6% and 19.6%; p value=0.660), (Table 2).

Serum and FF IL 15 median (interquartile range) levels were not significantly different between Group 1 patients 56.10(17.75) and 59.40(16.00) in comparison to group 2 (59.41(17.40) and

61.85(18.00)) (P=0.070 and 0.305, respectively), (Table 3).

Both groups were further divided into two subgroups as, patients who conceived and those who did not. Table 4 demonstrates serum and FF IL 15 median (interquartile range) levels did not significantly differ between pregnancy positive and the pregnancy negative women in Group 1 normoresponder women (58.66(18.85) and 60.26(19.23); P=0.055and 62.56(20.34) and 64.11(19.44); p value=0.067) respectively). While table 5 demonstrates serum and FF IL 15 median (interquartile range) levels among group 2 poor responder women where there was a significant difference between pregnancy positive and the pregnancy negative women (60.04 (23.01) and 66.32 (24.66); P=0.004 and 56.22 (18.45) and 59.14(19.01); p value=0.003, respectively).

Table 1: Demographic characteristics of both groups.

Characteristics	Group 1 Mean(±SD)	Group 2 Mean(±SD)	P- value
Age (year)	31.3±7.8	34.4±4.6	0.361
BMI (kg/m^2)	26±2	25±2	0.451
Induction duration (day)	8.7±1.4	8.6±1.9	0.337

Table 2: Stimulation characteristics and ICSI outcomes of both groups.

Characteristics	Group 1 Mean(±SD)	Group 2 Mean (±SD)	P- value
Gonadotropin dose (IU)	2397.6 (±1087.5)	3718.8 (±722.2)	<0.0002
MII oocytes, n	8.2 (±3.8)	3.6 (±2.4)	<0.0001
Pregnancy test positive (%)	22.6%	19.6%	0.660

Table 3: Serum and FF IL 15 median (interquartile range) levels of both groups.

Characteristics	Group 1	Group 2	P- value
Serum IL 15 (pg/mL)	56.10(17.75)	59.41(17.40)	0.070
FF IL 15 (pg/mL)	59.40(16.00)	61.85(18.00)	0.305

Table 4: The association of serum and FF IL15 median (interquartile range) levels with pregnancy outcome among Group 1 women.

Group 1: Normal responder women			
IL15 Levels	Pregnancy positive	Pregnancy negative	P value
Serum IL15	58.66(18.85)	60.26(19.23)	0.055
FF IL15	62.56(20.34)	64.11(19.44)	0.067

Table 5: The association of serum and FF IL15 median (interquartile range) levels with pregnancy outcome among Group 2 women.

Group 2: Poor responder women			
IL15 levels	Pregnancy positive	Pregnancy negative	P value
Serum IL 15	60.04(23.01)	66.32(24.66)	0.004
FF IL15	56.22(18.45)	59.14(19.01)	0.003

Discussion

A favourable response to controlled ovarian stimulation is of utmost importance in assisted conception. It has been recognized that both very low and very high ovarian responses are associated with increased cancellation rates of ICSI cycles and reduced pregnancy rates[1,2,20]. Although various biochemical and sonographic tests have been found to predict response of the ovary, in the clinical practice, there is no single accurate and highly predictive test to assess response of the ovary and no precise screening test available to identify poor ovarian response. Hence; the diagnosis of poor responder may revealed only during ovulation induction[21-23].

It is well known that inflammation is a hallmark of many processes in reproductive physiology, including ovulation, menstruation, and implantation[24]. However, uncontrolled inflammation might negatively affect hormone balance, ovulation[25], and fertility by deteriorating ovarian reserve and ovarian response[26-28]. In many researches, the reduced ovarian reserve was reported to accelerate in the inflammatory states, such as diabetes mellitus, Behçet's Disease, Takayasu arteritis and myotonic dystrophy[29-32]. In addition, endometriosis, a systemic chronic inflammatory disorder, is associated with a reduced ovarian response to gonadotropin stimulation and with lower pregnancy rates[33].

The relationship of the immunological profile in FF and oocyte developmental potential and implantation outcome has been uncovered[7,8]. To improve assisted conception results, one essential problem that needs to be studied is to precisely predict oocyte and embryo developmental potential. Because the FF affects oocyte development, its composition has been studied as a valuable predictor of oocyte and embryo quality[9].

This study on IL15 measurement in serum and FF and clinical characteristic of women undergoing ICSI in medically stimulated cycles, including poor responder women indicated for ICSI. Increased concentrations of IL-15 in FF and serum have been associated with reduced biochemical pregnancy within the same group of poor responder women undergoing ICSI. From the foregoing, it is possible that the increased concentration of IL 15 in FF and serum is directly and/or indirectly associated with growth inhibition of the developing follicle and eventually affecting the oocyte maturity and its potential for fertilization and developing a good quality embryo. Thus, it might be related negatively with the outcome of the IVF process[14,15].

Moreover, in this study the association of increased serum and follicular IL15 levels with reduced pregnancy rates in poor responder subgroup may supports the role for IL15 in implantation in previous studies which found that IL15 regulation in the human endometrium is complex and IL15, as a possible stimulator for natural killer cells, and found to be expressed at human endometrium and first trimester decidua. Many authors recommend that a greater understanding of IL15 regulation within human endometrium is important in identifying key events of human reproduction and its role in regulating the function of uterine natural killer cells[16,20].

This study conclude serum and FF IL 15 levels were not statistically different between the normoresponder and poor responder infertility patients. Additionally, achieving pregnancy did not make any significant difference among control group while there was significant difference in poor responders group were significantly lower levels of IL 15 detected in women who conceived in comparison to women who did not conceived. Consequently, serum and FF IL 15 seems to be a reliable marker in predicting pregnancy in poor responder subfertile women, more comprehensive researches assessing any possible vital relationship between diminished ovarian reserve and IL15 are needed.

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