ORIGINAL ARTICLE

Role of Interleukin-I β in conception after intracytoplasmic sperm injection

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Abstract

Objective: To identify the role of Interleukin-I Beta(IL- Iβ) in patients undergoing intracytoplasmic sperm injection.

Methods: The quasi-experimental study was conducted at an infertility clinic in Islamabad from June 2010 to August 2011, and comprised couples opting for intracytoplasmic sperm injection. Down regulation of ovaries was followed by calculated stimulation, ovulation induction, oocytes retrieval, intracytoplasmic sperm injection, in vitro maturation of embryos and embryo transfer. Serum Interleukin-I Beta was measured by enzyme-linked immunosorbent assay onovulation induction day. Patients were grouped as non-pregnant with beta human chorionic gonadotropin 5-25 mIU/ml, pre-clinical abortion; beta human chorionic gonadotropin >25 mIU/ml with no cardiac activity and clinical pregnancy with foetal heart confirmation by trans-vaginal scan after 4 weeks of transfer. SPSS 15 was used for statistical analysis.

Results: Of the total 323 patients initially registered, embryo transfer could be carried out in 282(87.30%). Clinical pregnancy was achieved in 101(36%) patients, clinical abortions was the result in 61(22%) cases, while 120(42%) subjects did not conceive at all. Clinical pregnancy was achieved in subjects with high mean Interleukin-I Beta levels; 155.84±51.65 compared to 41.81±11.77and 118.46±35.62pg/mlin non-pregnant, preclinical abortion groups respectively (p=0.001).

Conclusion: The production of Interleukin-I Beta was associated with oocyte maturation, fertilisation, endometrial receptivity and implantation in patients undergoing intracytoplasmic sperm injection.

Keywords: Intracytoplasmic sperm injection, Interleukins, Implantation. (JPMA 65: 49; 2015)

Introduction

Infertility is the biological inability of a couple to enjoy parenthood after unprotected intercourse over a period of at least 1-2 years.[1] Infertility is considered to be an emerging worldwide problem because of social issues and associated psychological upsets likely to occur in both partners. The prevalence of infertility in Pakistan is 21.9% with 3.9% primary and 18.0% of secondary infertility.[2] Data available over the past 20 years reveals that approximately 40 percent of infertility is because of male and female factors each and the remaining occurs due to a combination of factors contributed by both partners.[3]

Intracytoplasmic sperm injection (ICSI) is the best way of assisted reproductive technology (ART) opted by infertile couples when pregnancy fails to occur by simpler reproductive treatment plans.[4] ICSI is carried out over four to six weeks starting from down regulation of ovaries followed by controlled ovarian stimulation (COS), ovulation induction (OI), oocyte pick-up (OPU) and finally embryo transfer (ET).[5] During these extensive, costly and rigorous process, females undergo a number of trans-vaginal scans (TVS), blood tests, hormone analyses and regular check-ups ending up with a maximum success rate of upto 25-30%.

Implantation of ovum after ET occurs in a narrow window during which foeto-maternal cross-talk between invading blastocysts is synchronised with receptive endometrium for a favourable outcome.[6] There is a long list of identified and un-identified maternal and foetal cytokines and growth factors which help in this dialogue that is required for embryo-maternal communication network. Cytokines associated with foetal and maternal membranes play an important role during normal gestation and successful pregnancy. Among the cytokines, Interleukins (IL) are polypeptides initially defined by their action between leukocytes. IL-I is composed mainly of two ligands IL-Iα and IL-Iβ, IL-IR1 and IL-IR2 receptors, and antagonist IL-IRA.[7] IL-I secreted from endometrial epithelium during the luteal phase takes part in apposition, adhesion, attachment and implantation of embryo to the endometrium. The ligand of IL-I (IL-1β) was found to be associated with poor survival of grafted cells after transplantation in heart cells

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and its presence has been documented in human endometrium as well ovarian theca-interstitial cells.[8,9] The aim of the current study was to evaluate the role of IL-1β on pregnancy outcome after ICSI.

Subjects and Methods

The quasi-experimental study was conducted from June 2010 to August 2011 after approval from the institutional ethics review board at a clinic serving infertile couples in Islamabad. Using purposive sampling, the study enrolled consenting couples matching the inclusion criteria which comprised female aged 18-41, duration of infertility more than 2 years, both ovaries present with no morphological abnormalities, normal ovulatory cycle (25-35 days), body mass index (BMI) 18-27 kg/m^2, basal follicle stimulating hormone (FSH) levels on day 2 <10mIU/mL, selected for long protocol with gonadotrophin releasing hormone (GnRH) agonist, stimulated with injection of recombinant follicle stimulating hormone (rFSH; Puregon) and kept on progesterone (P) support with 400mg cyclogestpessaries. Females on GnRh antagonist, short down-regulation with GnRH agonist and ICSI with sperm retrieval by testicular biopsy were excluded.

The enrolled subjects were down-regulated with GnRH agonist by daily injection of Deca Peptyl from day 21 of previous cycle followed by COS by gonadotrophins (Inj Puregon intra-muscularily [IM] or subcutaneously [SC]) from 2nd to 3rd day of cycle for 14 days. Maturity of follicle (20mm) was assessed by series of TVS starting from the 5th day of COS till decision of OI on which day, venous sample was taken for IL-Iβ estimation, and measurement of endometrial thickness by TVS was done before IM injection of hCG (Pregnyl 10,000 IU).

OPU was performed 36 hours after OI by vaginal ultrasound probe with 16G adapter and double lumen oocyte aspiration needle on 14th, 15th or 16th day of COS. All eggs collected were treated and then transferred to the incubator for about 1-2 hours prior to insemination by ICSI procedures. Semen analysis was performed by strict Kruger's criteria and film was prepared by Silselect gradient.[10] ICSI by micro injections of spermatozoa was performed at right angles to the position of polar body under the microscope. Fertilised embryos (presence of two pronuclei; 2PN) were assessed and graded daily for their developmental characteristics in vitro; cleavage till differentiation into distinct cell types with formation of fluid filled cavity (blastocysts). ET of blastocysts was done five to seven days after OI by Sims-Wallace Embryo Replacement Catheter under ultrasound guidance. Luteal support was maintained by P vaginal pessaries (Cyclogest 400mg) twice a day from the day of OPU. Fertilisation rate (FR) was defined as the proportion of oocytes resulting in 2PN formation.[11] Mean implantation rate (IR) was the proportion of embryos transferred resulting in an intrauterine gestational sac. A clinical pregnancy (CP) was defined as the presence of one or more gestation sacs by ultrasound.[12]

The subjects were grouped on the basis of human chorionic gonadotropin (hCG)in serum samples (βhCG) and TVS performed 2 and 4 weeks after OPUrespectively.[4] Patients were grouped as non-pregnant with beta hCG 5-25 mIU/ml, preclinical abortion; beta hCG >25 mIU/ml with no cardiac activity and CP with foetal heart confirmation by TVS after 4 weeks of transfer.

Data was analysedusing SPSS 15. Clinical characteristics were summarised in terms of frequencies and percentages for qualitative variables (age group), and mean standard deviation (SD) standard error of mean (SEM) for continuous/quantitative variables. Statistical comparison of mean IL-Iβ of three groups was performed by using one-way analysis of variance (ANOVA). P<0.05 was considered significant. Association of reproductive rates, including CP rate (CPR), IR, oocyte maturity rate (OMR), FR and endometrial thickness with IL-Iβ was analysed by using multiple logistic regression analysis.

Results

Of the total 323 patients initially registered, ET was carried out in 282(87.30%) and cycle characteristics of such patients were noted (Table-1). Among them,

Table-1: Cycle characteristics of patients.

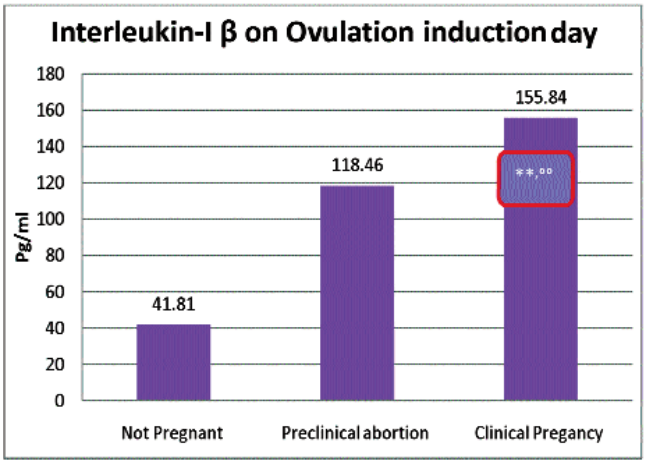
Variable	Mean±SD
Age	31.55±4.62
duration of infertility	7.48±3.68
Body mass index	23.55±3.86 kg/m^2
Endometrial thickness	8.58±3.41
Total number of puregons used /patient	60±3.07
Puregons used/day	4.00±0.21
Oocytes retrieved	19.35±0.52
Oocyte recovery rate	97.21±6.8
Number of oocytes fertilized	15.07±0.49
Fertilization rate	61.01±1.71
Number of cleaved embryos	10.57±0.42
Cleavage rate	53.05±22.95
Number of blastocyst transferred	31.20±2.99)

SD: Standard Deviation

Table-2: Multiple logistic regression.

Independent Variable	Odds Ratio	95% C .I. for Odds Ratio		P Value
Implantation rate	1.012	1.005	1.020	0.001
Clinical Pregnancy rate	1.053	1.022	1.085	0.001
Oocyte Maturity Rate	1.024	1.001	1.047	0.042
Fertilization Rate	1.024	1.001	1.047	0.042
Endometrial thickness	1.017	1.008	1.026	0.001

Multiple logistic regression applied on categorical variables of reproductive rates<50% and ≥ 50% and endometrial thickness,< and ≥ 8 mm.
CI: Confidence Interval.



Mean values expressed in pg/ml
Groups compared by one way analysis of variance, **p value < 0.001 for significant results of clinical pregnancy with preclinical abortion, °°p value < 0.001 for significant results of clinical pregnancy with non-pregnant group.

Figure: Comparison of Interleukin-1β in outcome groups.

clinical pregnancy was achieved in 101(36%) patients, clinical abortions was the result in 61(22%) cases, while 120(42%) subjects did not conceive at all.

Female cause of infertility was found in 70(25%) patients, 36(13%) had unexplained infertility, male infertility was found in 113(40%) cases, while both were responsible in 63(22%). The mean IL-Iβ on OI day (hCG administration) was150.76±57.63, median 147.45and interquartile range (IQR) of 78.45 pg/ml. The CP group showed statistically significant levels of IL-Iβ compared to the other groups (Figure). The presence of IL-Iβ increased the chance of being pregnant by 1% of patient undergoing ICSI cycle. It increased all the rates including IR, CPR, OMR and FR together with endometrial thickness by 1%which facilitated the conception (Table-2).

Discussion

The role of cytokines in reproductive processes such as follicular development, ovulation, fertilisation, implantation and embryo development has been well documented. IL-Iβ is one of the important cytokines involved in pre-implantation embryo development, protection of embryo, endometrium nourishment, successful communication, affirmative implantation and positive pregnancy outcome with subsequent continuation of pregnancy.[13] Probable mechanism of successful implantation by the IL-Iβ is to increase expression of adhesive protein integrins at the implantation site in the endometrium which helps in embryonic attachment with endometrium.[14]

In our study, the CP group showed statistically significant levels of IL-Iβ compared to the other groups. Results indicated that the presence of IL-Iβ increased the chance of being pregnant by 1% of patient undergoing ICSI cycle. It increased all the rates including IR, CPR, OMR and FR together with endometrial thickness by 1% which facilitated the conception. The detection of IL-Iβ in serum samples of women undergoing ICSI-ET cycles is supported by researchers.[15,16]

It has been found that during the process of ovulation, series of inflammatory reactions occur under influence of prostaglandin E2 (PGE2), P, gonadotropin, growth factors and cytokines in the ovary.[16] IL-1β secreted by humangranulosa and theca cells invitro acts as a paracrine factor in the sequence of events that lead to ovulation and has been detected in follicular fluid of animal studies.[17]

The outcome of increase in OMR by 1% with IL-Iβ in our study is supported by literature.[18] Oocyte maturity requires higher concentrations of growth hormone, P, IL-1β and tumour necrosis factor (TNF)alpha which facilitates fertilisation.[19] The synthesis of proteases, plasminogen activator and prostaglandins required for fertilisation is also regulated by IL-1β.[19] In our study, patients who had higher content of this cytokine had better FR. Once the oocyte is fertilised, it needs to discover the implantation window during which the endometrium is prepared under the influence of P, PGE2, locally-produced cytokines, growth factors, home box transcription factors and cyclooxygenase derived prostaglandins.[9,20] It is well documented that specific proteins, growth factors and cytokines induce receptors for PGE2 as well as P which cause increase in uterine receptivity.[12,21] The increased levels of IL-1β on OI day in patients of CP with increased number of retrieved,

mature and fertilised oocytes supports its role in ovulation and oocyte maturation which has also been observed by other researchers.[17] The role of IL-1β in the facilitation of implantation and CP is supported by literature as well.[9,16]

It is quite evident that the quality of embryos and endometrial receptivity determine the foeto-maternal crosstalk for implantation, placentation and continuation of pregnancy.[22] Inadequate uterine receptivity accounts for more than 60% procedure failures which are credited to lack of optimal concentrations of hormones, collapse of pinopods over the micro villi and inadequate time synchronisation in development of blastocysts and pinopods.[23] The role of IL-Iβ as a mediator of the materno-foetal relationships and a regulator of uterine receptivity has been investigated and proved in different species of vertebrates.[23,24] The endometrial thickness of females with high IL-Iβ in our study was more than 8mm which indicates its influence on the endometrium in paracrine manner to make it more receptive for implantation.[14,25]

In our study the range of detection of IL-Iβ was low; 26.2 to 272.5 pg/ml compared to 53.3 pg/ml to 290.4 pg/mL identified earlier.[12] The detection of hormones and cytokines on day of OI gives an insight of pre-ovulatory phase of ovarian cycle, administration of hCG causes ovulation to be released of prostaglandins and inflammatory mediators that can disturb the results. In our study, serum samples for IL-Iβ were taken before hCG injection, whereas they were taken after hCG injection on the day of oocyte retrieval by earlier study.[16]

The current study is limited due to small sample size. Besides, it did not look for IL-Iβ in the samples of follicular fluid and focused on a single cytokine in serum samples. Yet, it is the first study done in Pakistan to identify the role of immune mechanisms in terms of oocyte parameters and uterine receptivity.

Conclusion

IL-Iβ increased number of retrieved, mature and fertilised oocytes together with increase in endometrial thickness and reproductive outcome rates in patients undergoing ICSI. Presence of IL-Iβ is thus an important predictive factor for the implantation of fertilised ovum and finally a positive pregnancy outcome after ICSI. There is need for detection of follicular cytokines and growth factors along with their impact on reproductive outcomes after assisted delivery to provide new options for the best treatment of infertility.

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