WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 01, 123-130. Research Article ISSN 2277–7105

EFFECT OF AGE AND DURATION OF INFERTILITY ON LEVELS OF INTERLEUKIN-6 AND CORRELATION TO PREGNANCY RATE FOR WOMEN UNDERGOING ICSI PROGRAM

Huda Malik Muhammed[1]*, Muhammad-Baqir M-R. Fakhrildin[2] and Ula Muhammed Alkawaz[1]

[1]High Institute of Infertility Diagnosis and Assisted Reproductive Technologies Al- Nahrain University, Baghdad.

[2]Faculty of Medicine, Jabir Ibn Hayyan Medical University, Al-Najaf, IRAQ.

Article Received on
09 Nov. 2017,
Revised on 30 Nov. 2017,
Accepted on 20 Dec. 2017
DOI: 10.20959/wjpr20181-10529

***Corresponding Author**
Dr. Huda Malik Muhammed
High Institute of Infertility Diagnosis and Assisted Reproductive Technologies Al- Nahrain University, Baghdad.

ABSTRACT

Background: Interleukin-6 **(**IL-6) is one of the major proinflammatory cytokine, primarily produced by macrophages and have important role in the chronic inflammatory response. IL-6 is found to be expressed within the human granulosa cells of the Graafian follicle, also in the corpus luteum and ovarian theca cells. Polycystic ovary syndrome (PCOS) is the most common endocrine disorder and chronic low grade inflammation is the important feature in PCOS, that has been suggested to plays an important role in the normal reproductive processes. As a result several inflammatory markers, such as interleukin-6, are increased in PCOS patients compared with the normal women. **Objective:** To study the effect of age and duration of infertility on levels of interleukin-6 in PCOS patients undergoing ICSI. **Subjects, Materials, and Methods.** This prospective study was undertaken in the High Institute of Infertility diagnosis and*Assisted Reproductive Technologies / Al-Nahrain University / Baghdad/Iraq*, during the period from November 2015 to May 2017. A total of sixty infertile patients were recruited (thirty one infertile women with PCOS and non PCOS treated with long agonist protocol and twenty nine women with PCOS and non PCOS treated with antagonist protocol for ICSI technique, all undergoing controlled ovarian stimulation for ICSI technique. Serum and follicular fluid levels of IL-6 were measured at the day of oocyte retrieval by Enzyme linked immune sorbent assay (ELISA). Comparison in serum and follicular fluid IL-6 levels between the groups and their correlation to pregnancy rate was

done to all cases. **Results:** No significant association was found between age of infertile women and serum or follicular fluid IL-6 levels (P>0.05). Effect of duration of infertility on interleukin levels, according to this study demonstrated no significant differences (P>0.05) in the serum and follicular fluid of IL-6 levels with duration of infertility. Regarding the effect of types of ovulation induction protocols on the interleukin levels, there was no significant difference. In addition no significant association was noticed in the levels of interleukin and pregnancy outcome (P>0.05). **Conclusion**: Serum and follicular fluid levels of IL-6 showed no significant association with increase age. In addition, there was neither significant correlation between level of IL-6 and duration of infertility nor with the types of ovulation induction protocols. At the same time, there was no significant correlation between level of IL-6 and pregnancy rate.

KEYWORDS: ICSI, IL-6, age, infertility, pregnancy.

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is one of the common endocrine disorders of uncertain etiology.[1] Although so many factors or conditions are associated with infertility, PCOS is a well recognized cause.[2] In addition to the infertility, it is most frequently associated with insulin resistance which accompanied by compensatory hyperinsulinemia as well as obesity.[3,4]

Over the past few decades, researches on PCOS had focused their attention on the state of chronic low-grade subclinical inflammation and how it related to the metabolic, endocrine, and reproductive disturbances in PCOS patients that compromises multiple aspects of fertility.[5]

Biochemically, IL-6 as the other inflammatory markers is associated with insulin resistance, obesity, polycystic ovary syndrome, metabolic syndrome, and the risk of developing diabetes.[6]

Many regimens of ovulation induction protocols have been described to respond to the needs of patients who range from low, intermediate and high responders. These regimens include different stimulation protocols with gonadotropin releasing hormone agonist or antagonist in combination with exogenous gonadotropins.[7] Therefore, the aim of this study was to

evaluate the effect of age and duration of infertility on levels of interleukin-6 in PCOS patients undergoing ICSI technique.

SUBJECTS, MATERIAL AND METHODS

The present study was prospective conducted during the period starting from: November 2015 to May 2017*. Sixty infertile female patients were recruited in Baghdad from the Infertility clinic in "The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ AL Nahrain University'' all undergoing controlled ovarian stimulation for ICSI technique and then divided into three groups: twenty PCOS patients treated with metformin, twenty PCOS patients not received metformin, and twenty non PCOS patients (male factor as acuase of her infertility), from these sixty patients thirty one treated with long agonist protocol and twenty nine patients treated with antagonist protocol.

Patients with thyroid dysfunction, hyperprolactinoma, hypogonadotropic hypogonadism, and Cushing's syndrome all were excluded from the study. Then after full medical history taking and complete physical examination*, measurement of hormones level (FSH, LH, Prolactin, Testosterone, Estradiol (E_2) and TSH) was done in day two of the cycle. The endogenous luteinizing hormone surge was suppressed with either GnRH agonist triptorelin (Decapeptyl; 0.1mg Ferring Co, Kiel, Germany) or antagonist (cetrotide injection 0.25mg). Controlled ovarian stimulation (COS) was started with injectable gonadotropins, and the starting gonadotropin dose was selected according to the patients age, previous response of ovulation induction and the number of antral follicles. Individualized step-down or step-up protocols were instituted and the ovarian response was assessed by transvaginal ultrasound and serum E_2 assays. Then triggering of ovulation was done with recombinant hCG (rhCG) given in a dose of 6500 IU (Ovitrelle; Merck Serono**)** subcutaneously when two or more follicles reached the size of >17 mm. Oocyte retrieval was performed 34-36 h following the hCG injection. Retrieval of oocytes was followed by insemination by ICSI as per clinical practice at our center. Then 12 to 20 h after insemination, the fertilization was evaluated. The presence of two pronuclei usually confirmed normal fertilization. Day two or Day three post-insemination embryo transfers were performed using a flexible catheter*(Gynetics®, Belgium). Luteal support was done using 400mg/bid of vaginal progesterone (Cyclogest, Actavis). Positive serum hCG tested 14 days after embryo transfer was considered as evidence of implantation. Clinical pregnancy was considered when intrauterine gestational

sac visible on transvaginal ultrasound. Luteal support with progesterone was continued until documentation of the fetal cardiac activity, and subsequently tapered off.

Assessment of serum and follicular fluid IL-6

Serum and follicular fluid were collected at the time of oocyte retrieval, the follicular fluid usually collected from follicles ≥ 14 mm; avoiding contamination with the flushing medium, follicular fluid for each patient was pooled, centrifuged at 3000 rpm for 10 min and the supernatant was stored at −20°C until assay using diagnostic kit for interleukin-6 assessment in this study (Shanghai Yehua Biological Technology, China) uses enzyme-linked immune sorbent assay based on biotin double antibody sandwich technology to assay Human IL-6.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to evaluate effect of difference factors in study parameters. Least significant difference –LSD test (ANOVA)*or T-test was used to significantly compare between means in this study. Values were expressed as mean ± standard error of the mean. Statistical significance was defined as ($P < 0.05$) and highly significant defined as ($P<0.001$).[8]

RESULTS

Low mean serum IL-6 was associated with younger age although not significant. No significant association was found between age of infertile women and follicular fluid IL-6 ($P> 0.05$). As demonstrated in table (4-1), IL-6 shows increment in their serum levels with the increased age, as higher levels were seen in patients age more than 35 years compared to the younger age 35 years or less. Although the difference not significant (serum IL-6 270.39 ± 24.35 vs. 343.67 ± 49.45, $P>0.05$). In contrast, inverse relation was seen in their follicular fluid level with the maternal age, as the follicular fluid level of IL-6 tend to be lower in older age group although the difference was not significant (185.37 ± 28.15 vs. 130.71 ± 21.79, $P> 0.05$).

Table (4-2) illustrates the effect of duration of infertility on interleukin levels, according to this study no significant differences ($P>0.05$) were declared in the serum and follicular fluid of IL-6 levels with duration of infertility. However, the patients with duration of infertility (1-5) years associated with significantly ($P >0.05$) higher levels of serum IL-6 (315.26 ± 30.94, 247.61 ± 35.46, 264.40 ± 55.86), same result seen with its follicular fluid level (188.26 ± 31.95, 165.00 ± 40.86, 129.40 ± 14.34, $P>0.05$).

Regarding the effect of types of ovulation induction protocols on the interleukin levels, there was no significant difference as illustrated in table (4 -3). Meanwhile, the higher sera and follicular fluid levels of interleukin -6 were noticed with the long agonist protocol although the difference was not significant (P>0.05).

Table (4-4) shows that no significant association was noticed in the levels of interleukin and pregnancy outcome (P>0.05), nevertheless, 22 patients with pregnancy positive test demonstrated lower levels of serum and follicular fluid IL-6 as compared to 38 patients with pregnancy negative test.

Table 4-1: Effect of age on levels of interleukin.

Types of interleukin-6		Age groups (Years)		T-Test
		≤ 35 *n*=48	>35 *n*=12	
IL-6 levels	Serum	270.39±24.35	343.67 ± 49.45	76.071 NS
	Follicular	185.37±28.15	130.71 ± 21.79	87.242 NS
* (P<0.05), NS: Non-significant				

IL-6=interleukin-6,n=number.

Table 4-2: Effect of duration of infertility on levels of interleukin.

Types of interleukin		Duration of infertility			LSD value
		1 – 5 *n*= 32	6 – 10 *n* = 23	≥ 11 *n* = 5	
IL-6 level	Serum	315.26±30.94	247.61±35.46	264.40±55.86	100.87 NS
	Follicular	188.26±31.95	165.00±40.86	129.40±14.34	115.68 NS
LSD=least significant difference * (P<0.05), NS: Non-significant.					

IL-6=interleukin-6, n= number.

Table 4-3: Mean interleukin levels classified according to the types of ovulation induction protocols.

Types of interleukin		Types of OIP		T-Test value
		Agonist *n*= 31	Antagonist *n* = 29	
IL-6 levels	Serum	296.64±36.33	272.65 ± 24.21	63.143 NS
	Follicular	208.67±41.62	137.84 ± 15.44	72.416 NS
NS: Non-significant, IL-6=interleukin-6, OIP= ovulation induction protocol.,				

Table 4-4: Mean interleukin levels according to pregnancy outcome.

Types of interleukin		Outcome of pregnancy		T-Test value
		Positive *n*= 22	Negative *n*= 38	
IL-6 levels	Serum	247.95 ± 36.08	306.52 ± 27.49	60.89 NS
	Follicular	167.63 ± 42.90	178.37 ± 27.03	69.83 NS
NS: Non-significant.				

IL-6=interleukin-6,n=number.

DISCUSSION

This study showed that the serum level of IL-6 increased with the increased age of the patients, while it is follicular fluid level showed inverse correlation with age, although the difference was not significant as seen in the table (4-1). This agrees with Marciniak A, et al[9] and in the study of Altun T, et al[10] they showed that positive correlation was noticed between follicular fluid IL-6 level and advancing age. In addition, this study elucidated no significant difference in the both serum and follicular fluid levels of IL-6 in respect to the duration of infertility, as this finding agree with the study of Morin-Papunen L, et al[11] in which no significant differences between the groups in regard to the duration of infertility was observed. In addition, no significant association was found between type of ovulation induction and serum and follicular fluid IL-6, however, agonist type was associated with higher levels of interleukin-6 as shown in the table (4-3). Veronika Günther, et al[12] reveled that the correlation between IL-6 levels in serum and the ovarian stimulation response was not statistically significant whereas the correlation between IL-6 levels in follicular fluid and the ovarian stimulation response was significant. However, the small sample size of patients, differences in the doses of the two types of ovulation induction protocols, can be the considered as possible causes for discrepancy in this study.

On the other hand, the present data identify adverse influences of serum and follicular fluid levels of IL-6 on successful ICSI outcome, as suggested by the observed decline in the pregnancy rate with increasing levels of these markers although not significant, as shown in table(4-4) this is in line with study of Altun T, et al[10], and Sessions et al.[13] While in study of Younis A, et al[14] demonstrated no association between serum IL-6 and pregnancy rate. Other previous studies have identified detrimental implications of elevated follicular fluid IL-6 levels on the outcome of ICSI. Tugba et al 2011 concluded that the increased follicular fluid IL-6 levels may adversely influence endometrial receptivity. However, this assumption needs further studies in future. In addition, the differences in the cultured media used, sperm

parameters, and environmental factors, all these factors played major role on the outcome of ICSI.

CONCLUSION

From the results of the present study, there is neither significant correlation between level of IL-6 and duration of infertility nor with the types of ovulation induction protocols. And the level of serum IL-6 increased with increase age, while it is follicular fluid level decreased with increased age, although the difference was not significant. In addition, although there is no significant correlation between level of IL-6 and pregnancy rate, the patients with positive pregnancy showed lower level of IL-6 than those with negative pregnancy test.

REFERENCES

1. Kabel AM. Polycystic ovarian syndrome: insights into pathogenesis, diagnosis, prognosis, pharmacological and non-pharmacological treatment. Pharmaceutical Bioprocessing, 2016; 4(1).
2. Winston Crasto, Pallavi Rao and Helena Gleeson. The Effect of Metformin on Reproduction–A Short Review Journal of Endocrinology, Diabetes, and Obesity, 28 May 2014.
3. Yildiz BO. Approach to the patient: contraception in women with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism, 2015 Mar 1; 100(3): 794-802.
4. Conway G, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Franks S, et al. The polycystic ovary syndrome: a position statement from the European Society of Endocrinology. European journal of endocrinology, 2014 Oct 1; 171(4): P1-29.
5. Zhang Y, Hu M, Meng F, Sun X, Xu H, et al. Metformin Ameliorates Uterine Defects in a Rat Model of Polycystic Ovary Syndrome. EBio Medicine, 2017 Apr 30; 18: 157-70.
6. Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya M. Contribution of macrophage polarization to metabolic diseases. Journal of atherosclerosis and thrombosis, 2016 Jan 6; 23(1): 10-7.
7. Pacchiarotti A, Selman H, Valeri C, Napoletano S, Sbracia M, et al. Ovarian stimulation protocol in IVF: an up-to-date review of the literature. Current pharmaceutical biotechnology, 2016 Mar 1; 17(4): 303-15.
8. Motulsky HJ. Common misconceptions about data analysis and statistics. British journal of pharmacology, 2015 Apr 1; 172(8): 2126-32.

9. Marciniak A, Nawrocka Rutkowska J, Brodowska A, Wiśniewska B, Starczewski A Cardiovascular system diseases in patients with polycystic ovary syndrome - the role of inflammation process in this pathology and possibility of early diagnosis and prevention. Ann Agric Environ Med, 2016 Dec 23; 23(4): 537-541.
10. Altun T, Jindal S, Greenseid K, Shu J, Pal L. Low follicular fluid IL-6 levels in IVF patients are associated with increased likelihood of clinical pregnancy. Journal of assisted reproduction and genetics, 2011 Mar 1; 28(3): 245-251.
11. Morin-Papunen L, Rantala AS, Unkila-Kallio L, Tiitinen A, Hippeläinen M, et al. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): a multicenter, double-blind, placebo-controlled randomized trial. The Journal of Clinical Endocrinology, 2012 Mar 14; 97(5): 1492-500.
12. Günther V, Alkatout I, Fuhs C, Salmassi A, Mettler L, et al. The Role of Interleukin-18 in Serum and Follicular Fluid during In Vitro Fertilization and Intracytoplasmic Sperm Injection. Bio Med research international, 2016 Sep 22; 2016.
13. Sessions-Bresnahan DR, Carnevale EM. Age-associated changes in granulosa cell transcript abundance in equine preovulatory follicles. Reproduction, Fertility and Development, 2015 Jul 29; 27(6): 906-13.
14. Younis A, Hawkins K, Mahini H, Butler W, Garelnabi M. Serum tumor necrosis factor-α, interleukin-6, monocyte chemotactic protein-1 and paraoxonase-1 profiles in women with endometriosis, pcos, or unexplained infertility. Journal of assisted reproduction and genetics, 2014 Nov 1; 31(11): 1445.