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EFFECT OF AGE AND DURATION OF INFERTILITY ON LEVELS OF INTERLEUKIN-18 AND CORRELATION TO PREGNANCY RATE FOR WOMEN UNDERGOING ICSI PROGRAM

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ABSTRACT

Background: Interleukin-18 (IL-18) is a cytokine which belongs to the interleukin-1 (IL-1) superfamily. Although initially thought to be synthesized mainly by the macrophage and adiposity, now it has been found that IL-18 mRNA is expressed in a variety of cells that include Kupffer cells, T and B cells, osteoblasts and dendritic cells. It is essential to follicular growth and oocyte maturation and it was observed that concentration of IL-18 in serum and follicular fluid increased with raised body mass index. Polycystic Ovarian Syndrome (PCOS) is heterogeneous disorder. They hypothesized that metabolic and inflammatory biomarkers might be increased in PCOS women and PCOS offspring at a young age. **Objective:** To study the effect of age and duration of infertility on levels of interleukin -18 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 patients with PCOS and non PCOS treated with long agonist protocol and twenty nine patients with PCOS and non PCOS treated with antagonist protocol) all undergoing controlled ovarian stimulation for ICSI technique. Serum and follicular fluid levels of IL-18 were measured at the day of oocyte retrieval by Enzyme linked immune sorbent assay (ELISA). Comparison in serum and follicular fluid IL-18 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 level of IL-18 levels (P>0.05), while significant association was found with its follicular fluid level (P<0.05). According to this study significant difference (P<0.05) in the serum IL-18 levels with the duration of infertility but not in its follicular fluid level. In addition, there was no significant difference in its levels with the two ovulation induction protocols. Also no significant association was noticed in the levels of interleukin- 18 and pregnancy outcome (P>0.05). **Conclusion**: Serum level of IL-18 showed no significant association with increase age. While its follicular fluid level was significantly associated. In addition, there was no significant correlation between follicular fluid levels of IL-18 and duration of infertility but significant correlation was found with its serum level. At the same time, there was neither significant correlation between levels of IL-18 and the two types of ovulation induction protocols nor with the pregnancy rate.

KEYWORDS: ICSI, IL-18, Ovulation, Pregnancy, Infertility.

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is considered as a complex and heterogeneous disorder affecting about 5% to 10% of women ages between 18 and 44 years.[1, 2] It is a well recognized cause of infertility[3], which characterized by the association of chronic an ovulation, hyperandrogenism, and polycystic ovaries on ultrasound.[4] In PCOS, inflammation is found in patients who are obese as well as non-obese. Different stimulation protocols was described according to the patients needs, included the long agonist protocol, the antagonist protocol in addition to the gonadotropins.[5]

Cytokines are considered to be important mediators of inflammation and there are produced by a broad range of non immune cell types, including the normal ovarian cells.[6] IL-18 had been suggested to favor ovarian folliculogenesis.[7] A positive correlation was reported between follicular fluid IL-18 levels and the number of retrieved oocytes and implantation success in women with various etiologies of infertility.[7, 8, 9] This inflammatory marker was associated with insulin resistance, obesity, polycystic ovary syndrome, metabolic syndrome, and the risk of developing diabetes.[10]

SUBJECTS, MATERIALS, AND METHODS

This prospective study was 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 cause of her infertility), from these sixty patients thirty one treated with long agonist protocol and twenty nine patients treated with antagonist protocol.

Infertile women with thyroid dysfunction, hyperprolactinoma, hypogonadotropic hypogonadism and Cushing's syndrome all were excluded from this study, then all patients were subjected to full medical history taking and complete physical examination. Measurement of hormones level (FSH, LH, Prolactin, Testosterone, TSH, and Estradiol(E_2) in the cycle day two. Suppression of the endogenous luteinizing hormone surge was done with either GnRH agonist triptorelin (Decapeptyl; 0.1mg Ferring Co, Kiel, Germany) or antagonist (cetrotide injection in a dose of 0.25 mg). Controlled ovarian stimulation (COS) was initiated with injectable gonadotropins, and the starting gonadotropin dose was selected on the basis of age, early follicular FSH and estradiol (E_2) levels and the number of antral follicles. Serial monitoring of ovarian response was assessed by transvaginal ultrasound and serum E_2 assays. Follicular maturation was triggered with recombinant hCG (rhCG) given subcutaneously in a dose of 6500 IU(Ovitrelle®; Merck Serono**)** when two or more follicles >17 mm were achieved. Transvaginal ultrasound guided 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*. Fertilization was evaluated 12 to 20 h after insemination. The presence of two pronuclei confirmed normal fertilization. Embryo transfers were performed on Day 2 or Day 3 post-insemination using a flexible catheter (Gynetics®, Belgium). Luteal support was provided with intramuscular injections of progesterone in oil (50 mg daily). Positive serum hCG tested 12 days after embryo transfer was considered as evidence of implantation. Clinical pregnancy was defined as intrauterine gestational sac visible on transvaginal ultrasound.

Assessment of serum and follicular fluid IL-18

At the time of oocyte retrieval, in addition to the serum, the follicular fluid was collected from the follicles measured ≥ 14 mm for each patient, then centrifugation at 3000 rpm for 10 min was done and the supernatant was stored at −20°C until assay using diagnostic kit for interleukin-18 assessments 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-18.

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).[11]

RESULTS

The interleukin- 18 did not show any significant difference in its level in both serum and follicular fluid, although higher level was seen in the serum in comparison to the follicular fluid (43.18 ± 7.91 vs. 32.37 ± 8.05, P>0.05). IL-18 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 was not significant (serum level of IL- 18 38.44 ± 6.13 vs. 51.23 ± 15.06, P>0.05). In contrast, inverse relation was seen in their follicular fluid levels with the maternal age as the follicular fluid level of IL-18 tend to be significant (P<0.05) lower in older age group(36.23 ± 6.34 vs. 18.95 ± 4.75). This study demonstrated significant difference (P<0.05) in the serum IL-18 levels with the duration of infertility but not in its follicular fluid level, as higher serum level seen in patients with short duration of infertility that is 1-5 years.

Regarding the effect of types of ovulation induction protocols on the interleukin levels, there was no significant difference as illustrated in table (4-4). Meanwhile, the higher sera and follicular fluid levels of interleukin were noticed with the long agonist protocol although the difference was not significant. However, no significant (P>0.05) association was noticed in the levels of interleukin -18 and pregnancy outcome, nevertheless, 22 patients with pregnancy positive test demonstrated lower levels of serum and follicular fluid IL-18 as compared to 38 patients with pregnancy negative test.

Table 4-1: Mean serum and follicular interleukins levels.

Type of interleukin	Levels of interleukin		T-Test value
	Serum	Follicular fluid	
IL- 18 (ng/L)	43.18 ± 7.91	32.37 ± 8.05	15.744 NS
NS-non significant, IL-18= interleukin -18			

Table 4-2: Effect of age on levels of interleukins.

Types of interleukin		Age groups (Years)		T-Test
		≤ 35 *n*= 48	>35 *n* = 12	
IL-18 levels	Serum	38.44 ± 6.13	51.23 ± 15.06	21.598 NS
	Follicular	36.23 ± 6.34	18.95 ± 4.75	16.350 *
* (P<0.05), NS: Non-significant, IL-18=interleukin -18, n= number				

Table 4-3: 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-18 levels	Serum	48.59 ± 9.05	35.05 ± 7.67	19.82 ± 4.09	28.639 *
	Follicular	33.44 ± 6.92	35.81 ± 9.65	14.56 ± 0.80	24.332 NS
* (P<0.05), NS: Non-significant.					

IL-18=interleukin -18, n =number.

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

Types of interleukin		Types of OIP		T-Test value
		Agonist *n*= 31	Antagonist *n* = 29	
IL-18 levels	Serum	43.83 ± 8.85	37.97 ± 7.27	17.928 NS
	Follicular	39.06 ± 9.19	26.05 ± 4.34	15.232 NS
NS: Non-significant				

IL-18=interleukin -18, OIP= ovulation induction protocol, n= number.

Table 4-5: **Mean interleukin levels according to pregnancy outcome.**

Types of interleukin		Outcome of pregnancy		T-Test value
		Positive *n*= 22	Negative *n*= 38	
IL-18 levels	Serum	34.44 ± 8.62	44.80 ± 7.56	17.288 NS
	Follicular	31.37 ± 9.96	33.59 ± 5.99	14.688 NS
NS: Non-significan				

IL-18=interleukin -18, n= number.

DISCUSSION

This study showed that the serum level of IL-18 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 in the serum level as seen in the table (4-2). This agrees with Marciniak A, et al[12] and disagree with study of Radwan, et al[13] who observed no significant correlation has been found between the age of the patients and follicular levels of IL-18,

propably, the small sample size of patients can be the considered as possible causes for discrepancy in this study.

In addition, this study elucidated significant higher serum levels of IL-18 in patients with short duration of infertility that is 1-5 years, while its follicular fluid levels showed no significant difference with the duration of infertility and in the study of Morin-Papunen L, et al[14] showed no significant differences between the groups in regard to the duration of infertility. In addition, no significant association was found between type of ovulation induction and serum and follicular fluid IL-18; however, agonist type was associated with higher levels of interleukin-18 as shown in the table (4-4). Veronika Günther, et al[15] reveled that the correlation between IL- 18 levels in serum and the ovarian stimulation response was not statistically significant whereas the correlation between IL-18 levels in follicular fluid and the ovarian stimulation response was significant. However, this discrepancy can be related to the differences between the used types of ovulation induction protocols and/or to their doses and duration of treatment. On the other hand, the present data identify adverse influences of serum and follicular fluid levels of IL-18 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(4-5) this is in line with study of Altun T, et al[16], and Sessions et al.[17]

CONCLUSION

From the results of the present study there was inverse correlation between follicular fluid level of IL-18 and age. In addition, its serum level decreased with the increased duration of infertility. On the other hand, there is neither significant correlation between level of IL-18 and the types of ovulation induction protocols nor with the pregnancy rate, as the patients with positive pregnancy showed lower level of IL-18 than those with negative pregnancy test.

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