



Interleukin-1 β , 27 Level in Stimulated and Non-stimulated PCOS Infertile Women (Cross-Sectional Study)

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Abstract

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BACKGROUND: Polycystic ovary syndrome (PCOS) stands for highly prevalent female endocrine pathology. Females suffering from PCOS have a significantly higher number of inflammatory markers. The IL-1 family members enormously affect the innate immune system.

AIM: Study the changes in the levels of Interleukin-1 β and Interleukin-27 in the stimulated and non-stimulated cycle (by gonadotrophin) in polycystic ovarian syndrome women.

METHODS: Fifty-eight infertile women with polycystic ovary syndrome (PCOS) have been included in this research article. Twenty-nine of them did not undergo ovulation induction (OI) protocols, while the other 29 were under ovulation induction (OI) therapy. Interleukin-1 β and Interleukin-27 measured in both groups, in the stimulated cycle just before administration of HCG and on the 2nd day of the menstrual cycle in the non-stimulated females.

RESULTS: Both interleukins have been significantly more significant in the ovulation stimulated group than the non-stimulated one, with p-value of (0.04) for interleukin -1 β and (0.02) for interleukin-27.

CONCLUSION: The ovulation processes involve an increase in the inflammatory process, as demonstrated by the rise in these interleukins' levels. This increase is more prominent in the process of ovulation induction.

Introduction

Polycystic ovary syndrome (PCOS) represents a highly prevalent endocrine pathology in females with a classic triad of chronic anovulation, hyperandrogenism, and polycystic changes in ovaries [1]. It is remarkably seen in women aged between 18 and 44 years and may manifest itself as early as adolescence [1]. The syndrome is heterogeneous clinically and biochemically. PCOS represents a primary reason for female infertility, and it is responsible for 15–20% of the infertility cases [2]. PCOS is associated with a low-grade of chronic inflammation mainly attributable to the accumulation of visceral fat, although an effect of insulin resistance cannot be excluded [3]. *In vitro* research indicated that pro-inflammatory signals are strong inducers to the steroidogenic enzymes necessary for producing androgens in an ovary's theca cells [4]. This mechanism might be a source of high androgen in the PCOS sufferer and its subsequent hyperandrogenism [5]. Renowned those inflammatory markers, such as high-sensitive C-reactive protein (hs-CRP), interleukin-6 (IL), and TNF- α , are greater in women with PCOS than the general populace. A level

of the above markers has a huge positive correlation with body mass index (BMI). However, the level is further higher in obese women with PCOS than BMI-matched controls [6], [7]. Ovarian dysfunction, insulin resistance, and cardiovascular complications that are frequently observed in patients with PCOS have been attributed to the low-grade inflammatory condition induced by the high level of interleukins [8]. Cytokine polypeptides produced by cells of both the innate and other particular units of the immune system [9]. The IL-1 family members are known to be the major players in the innate immune system [6]. The dual central classic agonist cytokines in this family are IL-1 α , and IL-1 β work similarly through binding to the IL-1 receptor type 1 (IL-1R1) with subsequent expression of an array of pro-inflammatory molecules [10], [11]. The (31 kDa) precursor molecule is shared by both IL-1 α and IL-1 β that is subjected to further processing by specific cellular proteases to form two (17 kDa) molecules [12], [13]. IL-1 β is produced by inflammatory cells and is functional when it reaches maturation by cleavage by caspase-1. Adipocytes, macrophages, and vascular endothelial cells secrete interleukin-1 β (IL-1 β). It acts as a mediator of the inflammatory response and is engaged in cell proliferation, differentiation, and apoptosis [14]. One

of the pro-inflammatory cytokines is IL-27. It is mainly generated from antigen-presenting cells (APCs) [14]. The recent challenge that the infertility arena has encountered is identifying a strategy to exploit IL-27 for therapeutic purposes by modulating the chronic inflammation that is generally observed in PCOS [15].

Cytokines participate in the follicular development, ovulation process, endometrium receptivity, and intercede embryo implantation processes. Humoral immune system interruption may eventually cause disturbance of ovarian folliculogenesis [16]. IL-1 β mechanism of action remains to be clarified. Still, it can be theorized that the increase in the intra-follicular level of IL-1 β after injecting may imitate the local preovulatory events that pave the way to ovulation, which indicates that IL-1 β may have a crucial task in the physiology of equine oocytes through acting on meiosis resumption along with ovarian function through ovulation-inducing. Aim of Study: Study the changes in the levels of Interleukin-1 β and Interleukin-27 in the stimulated and non-stimulated cycle by gonadotrophin in polycystic ovarian syndrome women. Since these two-interleukin related to chronic inflammatory, process which is specific in those women.

Methods

Study subjects

This is a cross-sectional, randomized long-term study carried out from December 2018 to May 2019 in AL-Nahrain University in Baghdad, Iraq, at the consultancy clinical of the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies. The Human Research Ethics Committee of the Institute approved the study. The study cases randomly selected 58 infertile women with polycystic ovary syndrome (PCOS) who consulted the Institute. Their ages ranged from 21 to 35; 29 of them did not undergo ovulation induction (OI) protocol. At the same time, the other 29 were subjected to ovulation induction (OI) therapy protocol.

Inclusion criteria

The diagnostic criteria have been done based on the Rotterdam criteria for Polycystic Ovary Syndrome (2003 consensus ESHRE/ASRM) [15].

Exclusion criteria

Other androgen excess etiologies and ovulatory causes of infertility, endometriosis, tubal factor infertility, anatomy, and pathology have been excluded from the study. Information about the category and period of infertility, hirsutism, and pattern of menstrual cycle was

obtained. For each infertile woman, the entire history and examination 8 were made. Ethical approval was taken from the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies Ethical committee at AL-Nahrain University. All contributors were knowledgeable about the study aim and procedures and obtained written consent from them.

Body mass index (BMI)

It was measured by dividing women's weight in kilograms by the height in meters squared (kg/m^2). It was categorized as follows: average weight (18.5–24.9), overweight (25.0–29.9), and obesity with a body mass index equivalent to or higher than 30.0 kg/m^2 .

Blood sampling

For 2nd day of the menstrual cycle (CD2), peripheral venous blood samples have drawn for non-ovarian stimulated patients and on ovulation triggering day very soon before the injection of human chorionic gonadotropin (hCG) for the ovarian stimulated patients. When blood samples clotted, spins at 2500 rpm for 15 min were done, and sera were obtained and kept at -20°C until the time of analysis. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), prolactin (PRL), and total testosterone were measured for all study patients on the menstrual cycle day 2 (CD2). The hormones were measured in the participants' sera using automated mini-VIDAS machines (BIOMERIEUX/France) using its costume kits. Measuring IL-1 β and IL-27 levels were performed using ELISA technology with kits supplied by (CUSABIO/China) and the tests were done according to the kit supplier instructions.

Ovulation induction

Twenty-nine women have been given an injectable FSH (Gonal-f[®]) (Merck Serono S.A./Schweiz) as a protocol for ovulation induction till 2 dominant follicle obtained at least, and triggering was done by human chorionic gonadotropin (hCG) injection (OVITRELLE[®]).

Statistical analysis

We utilized the SAS (Statistical Analysis System-version 9.0) software to perform statistical analysis. $p < 0.05$ was considered statistically significant.

Results

In the mean BMI of these two groups, there has been no significant difference. Furthermore, no

Table 1: Type and duration of infertility

Parameter	PCOS without ovulation induction no. (%)	PCOS with ovulation induction no. (%)	p-value
Primary infertility	23 (79.31)	19 (65.52)	0.378
Secondary infertility	6 (20.69)	10 (34.48)	
Duration of infertility	3.83 \pm 2.36	4.34 \pm 2.84	0.45
Duration of primary infertility	4.17 \pm 2.39	5.05 \pm 2.95	0.3
Duration of secondary infertility	2.50 \pm 1.87	3 \pm 2.16	0.63
Normal BMI (18–24.9 kg/m ²)	4 (13.8)	2 (6.9)	0.494
Overweight BMI (25–29.9 kg/m ²)	13 (44.8)	9 (31.03)	
Obesity BMI (>30 kg/m ²)	12 (41.4)	18 (62.07)	

p < 0.05 statistical significant, PCOS: polycystic ovary syndrome women; BMI: Body mass index.

significant difference in type and duration of infertility between the dual groups was perceived in Table 1. Table 2 illustrates the hormonal level in cycle day two between both groups, which show no significant difference.

Table 2: Hormonal parameters between the two groups

Hormonal parameter	PCOS without ovulation induction (mean \pm SD)	PCOS with ovulation induction (mean \pm SD)	p-value
FSH	5.51 \pm 1.74	4.79 \pm 1.33	0.08
LH	8 \pm 6.52	5.53 \pm 2.62	0.06
LH/FSH ratio	1.40 \pm 0.73	1.21 \pm 0.65	0.31
E2	65.26 \pm 62.16	57.85 \pm 22.56	0.55
PRL	19.19 \pm 8.72	19.20 \pm 8.31	1.00
Total Testosterone	0.59 \pm 0.20	0.66 \pm 0.20	0.21
Elevated Testosterone	0.78 \pm 0.06	0.80 \pm 0.07	0.48

p < 0.05 statistical significant; pcos: Polycystic ovary syndrome women, FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; PRL: Prolactin.

There has been a significant difference in IL-1 β and IL-27 between the stimulated group and the non-stimulated group. Figure 1 shows that the level of IL-1 β was significantly higher (p-value 0.04) in PCOS stimulated group if compared with non-stimulated other (90.60 \pm 51.67 and 68.38 \pm 21.26, respectively), also regarding the status of IL-27, figure (2) shows that its level was significantly higher (p = 0.02) in stimulated PCOS group compared to non-stimulated PCOS (13.14 \pm 8.6 and 8.27 \pm 6.27, respectively).

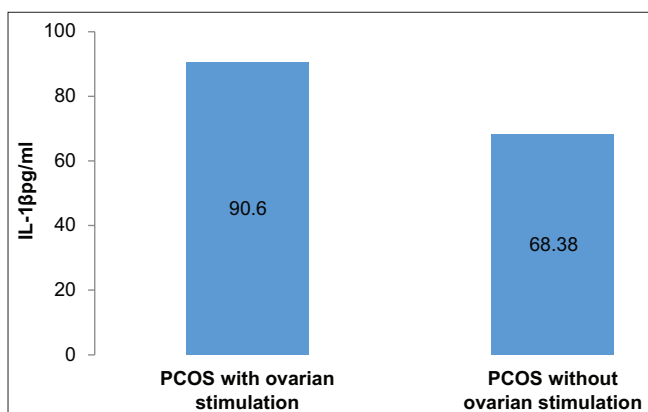


Figure 1: Level of IL-1 β in both stimulated and non-stimulated PCOS groups

Discussion

Interleukins have a well-documented beneficial role in the female reproductive physiological process such as ovulation, fertilization, follicular development, implantation, and typical

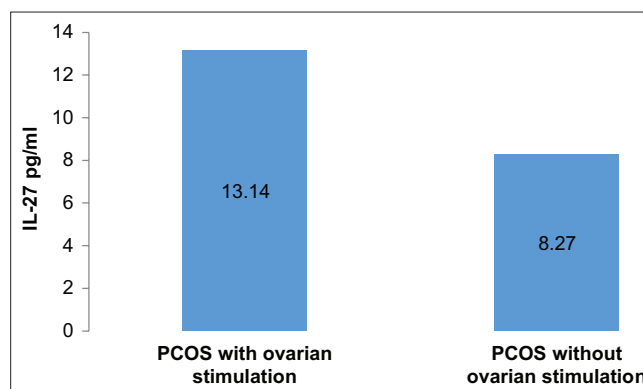


Figure 2: Level of IL-27 in both stimulated and non-stimulated PCOS groups

pregnancy [17], [18]. Endogenous E2 and P4 are sensitive to ovarian stimulation and show dramatic and fast change, different from the normal menstrual cycle variation. However, there is limited knowledge about the effect of ovarian stimulation on the level of serum cytokines [19]. Promotion of steroidogenesis, recruitment, follicular growth, and activation of leukocytes essential for ovulation, and tissue remodeling throughout ovulation, luteinization, and luteolysis by action of interleukin on the ovary [20]. Immunological defects have been concerned with female reproductive failure [21]. Another study revealed that polycystic ovarian syndrome women have significant overproduction in inflammatory mediators [21]. It recognized that ovarian stimulation affected circulating interleukin levels [19]. Another study showed that the level of interleukins is influenced by exposure to recombinant follicle-stimulating hormone (r-FSH) therapy [22]. A lot of evidence proposes that an interleukin-1 system is implicated in periovulatory events. The previous work established that interleukin-1beta (IL-1 β) in the mare increases the ovulatory rate of metaphase II oocytes. *In vitro* investigations have revealed several cellular doings of granulosa and theca cells, like steroidogenesis and the synthesis of proteases and prostaglandins synthesis regulated by IL-1 β [23]. This study showed a marked increase in cytokines, IL-1 β non stimulated and stimulated groups, higher in the stimulated group. As observed in this study, the peak value of plasma IL-1 β may be attributed to high level of FSH given in the stimulated group which leads to an increase in the production of reactive oxygen species that upregulates the expression of the gene [24]. Furthermore, it supports the facts that had been recognized previously in the role of interleukins in steroidogenesis and ovulatory process. In ovulation induction, many follicles will recruit and mature, causing such an increase in the levels. According to Russell and Robker (2007), LH acts on the ovary and stimulates the synthesis of IL-1 β , which has been shown to mimic gonadotropin activities at the preovulatory stage in the mare [25]. A previous study showed that clomiphene citrate treated rats at the estrus phase have a high plasma concentration of IL-1 β , suggesting the later

involvement in ovulation processes. Therefore, the effectiveness of clomiphene citrate may be attributed to its ability to stimulate the synthesis of IL-1 β [26]. An interventional, *in vivo* study which concerned injection of 1 microgram of IL-1 β in the mare follicle resulted in coincided ovulations [24]. In this study, the level of IL-27 was found to be higher significantly in the stimulated group, which is in agreement with another study that recognized a significant increase in the level of IL-17 and IL-27 in women with unexplained infertility undergo ovulation induction determined instantaneously before ovulation triggering by giving HCG injection if compared with the control fertile women group [17]. As mention in other study that conclude, IL-27 is upregulated by estrogen and progesterone which promotes decidualization possibly through a STAT3-dominant pathway [27]. In other study, there was a relationship between detectable serum IL-1 β at the start of an IVF cycle and increasing levels of IL-1 β over time and successful IVF outcome [28].

Conclusion

The ovulation processes involve an increase in the inflammatory process, as demonstrated by the rise in these interleukins' levels. This increase is more prominent in the process of ovulation induction.

Author Contributions

All authors have sufficiently contributed to the study, and agreed with the results and conclusions.

Ethical Statement

Informed consent was taken from each participant. The study methodology was reviewed and approved by the consultancy clinical of the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies in AL-Nahrain University in Baghdad, Iraq.

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