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Serum level of interleukins 31 and 33 in polycystic ovary syndrome patients

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ABSTRACT

Level of IL-31 and IL-33 was determined in sera of 30 polycystic ovary syndrome (PCOS) patients and 20 healthy women (controls). The patients were characterized in terms of some syndrome-associated symptoms, which included acne, hirsutism, menstruation and infertility. The results revealed that 40.0% of PCOS patients had acne, while hirsutism was recorded in 73.3% of patients. In addition, 86.7% of patients experienced irregular menstruation. With respect to infertility, 20.0 and 40.0% of the patients were grouped under primary and secondary infertility, respectively. Serum level of IL-31 showed a significant decreased level in total PCOS patients compared to controls (21.1 ± 4.3 *vs*. 24.9 ± 3.1 pg/ml; *p*-value ≤ 0.05). In contrast, IL-33 serum level was significantly increased in patients compared to controls (64.9 ± 7.5 *vs*. 39.8 ± 4.7 pg/ml; *p*-value ≤ 0.001). The level of both cytokines showed some variations in subgroups of patients distributed by syndrome-associated symptoms; acne and infertility for IL-31, and acne and menstruation for IL-33. In conclusion, IL-31 was negatively regulated in PCOS patients, while IL-33 was positively regulated; therefore, both cytokines may play a significant role in pathophysiology of PCOS.

Keywords: Polycystic ovary syndrome, IL-31, IL-33, Symptoms.

1. INTRODUCTION

Polycystic ovary syndrome (PCOS) represents a common reproductive and endocrine disorder in females with an incidence of 5-10%. It is also common among infertile women [1]. Clinically, biochemically and ultrasonographically, the syndrome is characterized by hyperandrogenism, polycystic ovaries, ovarian malfunction and menstrual irregularities [2]. The syndrome can also be presented with some metabolic syndromes; for instance insulin resistance, hyperinsulinemia, dyslipidemia, type 2 diabetes mellitus and obesity [3]. Etiologically, the cause of PCOS is not well-understood; however, it is evident that an interaction between a genetic background and environmental triggers is required to initiate the disorder [4]. Such interaction may lead to immunological irregularities, and a high incidence of anti-histone and anti-dsDNA autoantibodies and C-reactive protein-positive cases in PCOS patients confirm the involvement of immune system in pathology of syndrome [5].

The functions of immune system are regulated by cytokines, which are low molecular weight glycoproteins that participate in all features of innate and adaptive immune responses. Various inflammatory cytokines; for instance interleukin-1 (IL-1), IL-6, IL-18 and tumor necrosis factor-alpha (TNF-α) have been investigated in PCOS patients and the results suggested that the disorder is presented with a chronic low-grade inflammatory condition [6]. IL-31 and IL-33 are two further inflammatory cytokines that may have a role in inflammatory reactions of PCOS, but no evidence is available to support such possibility. However, it has been recently reported that both cytokines are involved in pathogenesis of endometrial cancer, and the authors have gone further to suggest that IL-31 and IL-33 are useful markers in prognosis of disease [7].

IL-31 is a 24 KDa glycoprotein that is expressed by activated CD4+ T cells, preferentially by T cells skewed toward a T helper 2 (Th2) phenotype. It is also produced by mast cells, monocytes, macrophages and monocyte-derived dendritic cells especially in response to oxidative stress [8]. IL-31 is a member of the gp130/ IL-6 cytokine family, which includes IL-6. The latter cytokine has been suspected to have a role in the inflammation of PCOS [9]. The available data suggest that an enhanced expression of IL-31 might be associated with a number of inflammatory diseases [10].

IL-33 is a 30 kDa protein, which was suggested to possess nuclear factor function that is critical for the induction of lymphatic endothelium phenotype, and after that it was identified as a novel member of the IL-1 family [11]. It is characterized as a potent inducer of Th2-associated cytokines (IL-4, IL-5 and IL-13), and functionally, IL-33 has been recognized with a dual function; acting as a pro-inflammatory cytokine and as an intracellular nuclear factor with transcriptional regulatory properties [12]. It is expressed by various types of cells (epithelial cells, endothelial cells, fibroblasts and smooth muscle cells), and epithelial-derived IL-33 has been reported to be an important regulators of innate and adaptive immune responses that are associated with a Th2 cytokine-mediated inflammation [13]. As in IL-31, IL-33 has been discussed to have a role in inflammation of several disease pathologies [14].

The present study is an attempt that examined serum level of IL-31 and IL-33 in Iraqi PCOS patients and correlated such levels with syndrome-associated symptoms.

2. MATERIALS AND METHODS

2.1 Patients

The Committee of Medical Ethics at the Iraqi Ministry of Health approved the study, in which 30 Iraqi women with PCOS were investigated. The patients were referred to Al-Alwayia Hospital for Gynecology and Obstetrics in Baghdad during the period February 2015- January 2016 for diagnosis and treatment, and their age ± standard deviation (SD) at the time of diagnosis was 25.5 ± 5.9 years. The diagnosis was made by the consultant medical staff at the hospital. It was based on clinical, ultrasonic and laparoscopic examinations [2]. The patients were characterized in terms of some syndrome-associated symptoms, which included acne, hirsutism, menstruation and infertility. The patients were firstly diagnosed and none of them was under medication at the time of investigation. In addition to patients, 20 apparently healthy women (control sample) were also included and their age mean was 32.6 ± 7.1 years. They were health personnel at the hospital, and none of them experienced PCOS or other related diseases; for instance, endometriosis.

2.2 Methods

Blood samples were collected from participants in plain tubes, and after clotting, the samples were centrifuged (3000 rpm) for 15 minutes. Serum was collected, aliquoted and frozen at -20 ºC until assessment for levels of cytokines (IL-31 and IL-33).

Serum level of IL-31 and IL-33 was assessed using commercial kits (Peprotech, United Kingdom), which are based on a sandwich enzyme-linked immunosorbent assay (ELISA) designed for quantitative measurement of natural and recombinant human IL-31 or IL-33 in serum, plasma and other biological fluids, in which anti-human IL-31 or IL-33 coating antibody (Capture Antibody) was adsorbed onto wells of 96-well plate. IL-31 or IL-33 present in sample or standard binds to antibodies that were adsorbed to the wells. A biotinylated anti- IL-31 or IL-33 antibody is added and binds to the cytokine captured by the first antibody (Detection Antibody). Following incubation, unbound biotinylated anti-IL-31 or -IL-33 antibody is removed during a wash step, and avidin horseradish peroxidase (HRP) conjugate is then added and binds to the biotinylated anti-cytokine antibody. Following incubation, unbound avidin-HRP conjugate is removed during a wash step, and a substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of IL-31 or IL-33 present in the sample or standard. The color development was monitored with ELISA plate reader and absorbance was measured at a wavelength of 405 nm. A standard curve was prepared from standard dilutions and IL-31 or IL-33 serum level was determined from a curve fitting equation.

2.3 Statistical analysis

Data were given as a percentage frequency or mean ± SD. For differences between means, the significance was assessed by Analysis of Variance (ANOVA), and a *p*-value ≤ 0.05, 0.01 or 0.001 was considered significant. The statistical analyses were carried out using the statistical package SPSS version 13.

3. RESULTS AND DISCUSSION

It was observed that 40.0% of PCOS patients had acne, while hirsutism was recorded in 73.3% of patients. In addition, 86.7% of patients experienced irregular menstruation. With respect to infertility, 20.0 and 40.0% of the patients were grouped under primary and secondary infertility, respectively (Table 2).

Serum level of IL-31 showed a significant decreased level in total PCOS patients compared to controls (21.1 ± 4.3 ± 4.5 *vs*. 24.9 ± 3.1 pg/ml; *p*-value ≤ 0.05) (Table 1). Such profile was almost similar in the patients distributed by presence or absence of hirsutism, as well as menstruation (regular and irregular). However, acne and infertility groups were exceptions. Patients who had acne showed a significant decreased level of IL-31 compared to patients who had no acne (18.4 ± 3.2 *vs*. 22.8 ± 3.6 pg/ml; *p*-value ≤ 0.01). Infertility subgroups were the second exception, and patients in primary and

secondary subgroups recorded a significant increased level of IL-31 compared to unmarried (single) patients (22.3 ± 4.9 and 22.2 ± 2.7, respectively *vs*.19.2 ± 4.3 pg/ml; *p*-value ≤ 0.05) (Table 2). For IL-33, a different observation was made, and the serum level was significantly increased in patients compared to controls (64.9 ± 7.5 *vs*. 39.8 ± 4.7 pg/ml; *p*-value ≤ 0.001) (Table 1). In the case of patients distributed by acne, a significant decreased level of IL-33 was observed in patients who had acne compared to patients without acne (59.6 ± 5.4 *vs*. 68.5 ± 6.7 pg/ml; *p*-value ≤ 0.01). In addition, patients with regular menstruation had a significantly lower level of IL-33 compared to patients with irregular menstruation (53.7 ± 2.5 *vs*. 66.6 ± 6.5 pg/ml; *p*-value ≤ 0.01) (Table 2).

Table 1: Serum level of IL-31 and IL-33 in total polycystic ovary syndrome patients.

Groups	Mean ± SD (pg/ml		*p*-value	95% Confidence Interval of Difference	
	PCOS Patients	Controls		Lower	Upper
IL-31	21.1 ± 4.3	24.9 ± 3.1	≤ 0.05	-5.9	-1.8
IL-33	64.9 ± 7.5	39.8 ± 4.7	≤ 0.001	21.6	28.6

PCOS: Polycystic ovary syndrome (No. of patients = 30); No. of controls = 20.

Table 2: Serum level of IL-31 and IL-33 in polycystic ovary syndrome patients distributed by syndrome-associated symptoms.

PCOS-Associated Symptoms	Number (%)	Mean ± SD (pg/ml	
		IL-31	IL-33
Acne			
Present	12 (40.0)	18.4 ± 3.2**	59.6 ± 5.4**
Absent	18 (60.0)	22.8 ± 3.6	68.5 ± 6.7
Hirsutism			
Present	22 (73.3)	20.4 ± 4.2	64.1 ± 7.7
Absent	8 (26.7)	22.6 ± 3.3	67.1 ± 7.2
Menstruation			
Regular	4 (13.3)	18.6 ± 3.3	53.7 ± 2.5**
Irregular	26 (86.7)	21.4 ± 4.1	66.6 ± 6.5
Infertility			
Primary	6 (20.0)	22.3 ± 4.9	65.6 ± 8.9
Secondary	12 (40.0)	22.2 ± 2.7	67.1 ± 5.1
Unmarried (Single)	12 (40.0)	19.2 ± 4.3*	62.4 ± 8.7

*p-value ≤ 0.05, **p-value ≤ 0.01 (compared to corresponding subgroup)

The two investigated cytokines showed an opposite profile in sera of PCOS patients; IL-31 level was significantly decreased, while IL-33 level was significantly increased. Such observation may suggest that IL-31 was down-regulated in patients, but there has been no direct evidence that can support such observation. However, inflammation has been suspected in PCOS and various inflammatory biological markers have been assessed in women with this syndrome, and one of these markers is IL-6 [15]. A recent meta-analysis of 20 articles that investigated IL-6 serum level in PCOS patients demonstrated that such cytokine was significantly increased in patients compared to controls, but the authors suggested that a high serum level of IL-6 is not an intrinsic feature of PCOS due to a significant heterogeneity among included studies [16]. IL-31 is a member of the IL-6 cytokine family [9], and therefore it might be subjected to the same heterogeneity of IL-6 in PCOS. However, it is not possible to understand the role of IL-31 in pathogenesis of PCOS from one study, and further studies are certainly required.

For IL-33, the obtained results suggest that its serum level was up-regulated in PCOS patients. In agreement with such findings, it has been recently demonstrated that IL-33 serum level was significantly increased in PCOS patients [17]. Both observations confirm the recognized key role of IL-33 in innate and adaptive immunity, and its contribution to inflammatory process in tissues. In this context, it has been demonstrated that IL-33 released through a cell damage mediated by environmental agents, can be associated with an induction of inflammatory responses as in PCOS, and this may cause damage to local tissues in the ovary [18]. It has been suggested further that IL-33 role in inflammatory disease may arise from a failure of regulatory responses driven by IL-33, in which T-regulatory cells are involved [19]. In addition, it has also been demonstrated that IL-33 is regulated by sex hormones, which are normally disturbed in PCOS patients [20].

The presence of chronic inflammation in PCOS has been demonstrated in the peripheral blood and ovaries of patients. This evident by increased serum level of inflammatory mediators; for instance C-reactive protein, TNF-α, IL-1, IL-6 and IL-18 [6], and probably IL-31, as suggested by the present study. These markers are well-known for their contribution to chronic inflammation. Furthermore, ovarian tissues of PCOS have been reported to harbor more macrophages and lymphocytes compared to controls. These cells secrete inflammatory cytokines, which in turn, activate more immune cells to enhance a further production of cytokines [21], and IL-33 may be one of them. This suggestion is confirmed by the reported association of IL-33 with pathogenesis of some autoimmune

(rheumatoid arthritis and systemic lupus erythematosus) and inflammatory (inflammatory bowel disease) diseases [18]. It possible to conclude that IL-33 might be in involved in the inflammatory response observed in PCOS patients, while IL-31 may encounter such effects. However, further studies that target such subject are encouraged to understand the role of IL-31 and IL-33 in pathogenesis of PCOS.

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