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Immunological and molecular detection of Interleukin-1β(IL-1β) in Iraqi Women with Polycystic Ovarian Syndrome

Running title: *Detection of IL-1β in Iraqi Women with Polycystic Ovarian Syndrome*

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ABSTRACT

Background: Interleukin-1 considered as a cytokine type multifunction and it is a feature that causes inflammation. **Aim and Objective**: to detect and study the immunological and molecular affect of Interleukin-1β(IL-1β) in Iraqi Women with Polycystic Ovarian Syndrome. **Materials and Methods**: 250 Iraqi women, their ages were from 20 to 50 years, they were divided into two groups: study group (n=125, Polycystic Ovary Syndrome PCOS) while the other group (n=125 control group mainly normal women), blood samples weretaken in the second menstruate cycle day, the concentration of IL-1β wascalculated for each group, and after this determine the IL-1β SNP (IL-1β rs16944 A/G) by using real time PCR. **Results**: PCSO patients show a noticeable difference compared with other group. The mean level of IL-1β was 69.66±93.39 (Pg /ml), 3.52E2±139.15 (Pg /ml) in PCOS and healthy control respectively. The levels were ranging between 0 and 598 Pg/ml. The GG genotype (mutant gene) was detected in 94/125 (75.2%) of PCOS, and the AG genotype was detected in 31/125 (24.8%) of PCOS. **Conclusions:** the results showed high significant association of G allele in IL-1β rs16944 gene SNP (A/G) with PCOS, (P<0.0001), this increasing may related to ovulation lack, while the polymorphism in IL-1β-511A/G, can be a predisposeaspect for the PCOS weakness.

Keyword: Interleukin-1β(IL-1β), Iraqi Women, Polycystic Ovarian Syndrome

Introduction

Interleukin-1 considered as a cytokine type multifunction and it is a feature that causes inflammation which eventually affect ovulate, implant, and fertilization processes. The 2q12-13 chromosomes clustered (IL-1 genes) which associated with three genes, IL-1RN, IL-1 alpha, and IL-1 beta[(1, 2)].

Generally, (IL-1α)polymorphism can occurs due to the PCSO, this was the finding of an investigation carried out in 2007 by Kolbus and his coworker, and consequently it affects the percent of LH/FSH hormones [(3)]. Zangeneh and his coworkers explained that the immunity play an important role in fertilizing and implanting the egg inside the uterus (4), while Escobar-Morreale and his coworkers find the factors that affect the pro-inflammation and related it to the correlation between IL-1 and the HPA (HypothalamousPituotary Adrenal) that control the steroid genesis of the adrenal [(5)].

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The current study was carried out to study the immunological and molecular affect of Interleukin-1β(IL-1β) in Iraqi Women with Polycystic Ovarian Syndrome.

Materials and methods

A total of 250 women cases were studied, their ages were between 20 and 50 years, they were divided in 2 equal groups, the first one diagnosed with PCSO, while the other one was consist of normal women and considered as control group. Sample were taken in the second menstruate cycle day; the IL-1β was concentrated to find out the IL-1β SNP (IL-1β rs16944 A/G) by using real time PCR

Results

The level of IL-1 β showed significant difference in both groups (PCOS and healthy). The mean level of IL-1β was 69.66±93.39 (Pg /ml), 3.52E2±139.15 (Pg /ml) in PCOS and healthy control respectively. The levels were ranging between 0 and 598 Pg/ml,as shown in table 1.

Table 1: Serum interleukin-1 β level in PCOS patients and healthy control groups

Serum IL-1 (Pg /ml)	Number	Mean	Std.Deviation	P-value
Patients	125	69.66	93.39	<0.0001
Controls	125	3.52E2	139.15	

**E2: ×100*

Detection of the interleukin-1beta SNP (IL1β-511 (A/G) rs16944), the DNA of interleukin 1-β was take out of thesample, then enlargedby the means of PCR and specific for IL-1 β; A is the wild allele and G is the mutant allele.

The GG genotype (mutant gene) was detected in 94/125 (75.2%) of PCOS, and the AG genotype was detected in 31/125 (24.8%) of PCOS, in comparison to healthy controls, GG genotype was detected in 7/125 (5.6%) of healthy controls, and the AG genotype was detected in 118/125 (94.4%) of healthy controls with odds ratio of GG genotype being associated with PCOS at 51.1, P<0.0001) as shown in figure 1, and table 2.

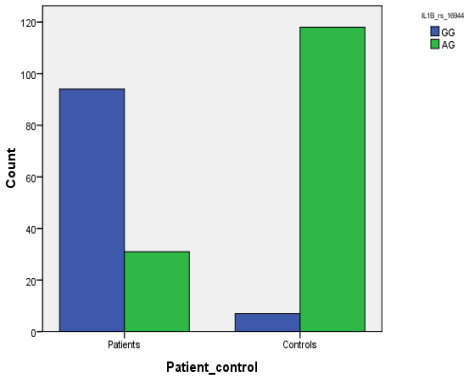

Figure 1: Genotype polymorphism of IL-1β rs16944 for control and PCOS patients group

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Table 2: Genotype polymorphism of IL-1β rs16944 for the PCOS patients and healthy control

P<0.0001 Odds ratio=51.1,P<0.0001		IL1B_rs_16944		Total
		GG	AG	
Patients	Count	94	31	125
	% within Patient control	75.2%	24.8%	100.0%
	% within IL1B_rs_16944	93.1%	20.8%	50.0%
Healthy controls	Count	7	118	125
	% within Patient_control	5.6%	94.4%	100.0%
	% within IL1B_rs_16944	6.9%	79.2%	50.0%
Total	Count	101	149	250
	% within Patient control	40.4%	59.6%	100.0%
	% within IL1B_rs_16944	100.0%	100.0%	100.0%

Additionally, the results showed highlsignificant association of G allele in IL-1β rs16944 gene SNP (A/G) with PCOS, (P<0.0001), as shown in table (3).

Table 3:Allele polymorphism of IL-1β rs16944 for the PCOS patients and healthy control

P<0.0001 Odds ratio=6.31,P<0.0001		IL1B_rs_16944		Total
		G allele	**A allele**	
Patients	Count	219	31	250
	% within Patient_control	87.6%	12.4%	100.0%
	% within IL1B_rs_16944	62.4%	20.8%	50.0%
Healthy controls	Count	132	118	250
	% within Patient_control	52,8%	47.2%	100.0%
	% within IL1B_rs_16944	37.6%	79.2%	50.0%
Total	Count	351	149	500
	% within Patient control	70.2%	29.8%	100.0%
	% within IL1B_rs_16944	100.0 %	100.0 %	100.0%

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Discussion

Polycystic ovarian syndrome is clinically important not because of decreased fertility, but also because of long term metabolic and cardio-vascular disease. PCOS is commonly identified through polycystic ovaries, hirsutism, and anovulation.Often, PCOS is conversely affected with the insulin confrontation, obesity, and dyslipidemia; moreover, it carries animportantthreatto develop the metabolic squeal and the cardiovascular, this includes the metabolicsyndrome as well as the diabetes [(6)].

In our study, the increasing in IL-1β may related to the the short of ovulate in PCSO women which match a study carried out by Zangenehand his coworkers[(4)].

The IL-1α inhibit estradiol production through the granulose cells of the ovary, while the beta type stimulate the secretion of basal progesterone in theca cells and granulose in follicles [(6)]. Three associated genes are consist in 2q12-13 chromosome, and they are 1L-1R, 1L-1 alpha, and 1L-1 beta which promote at -511 position which correspond to PCSO protein[(7)], Which can be a predispose reason to the susceptibility of PCOS, this was found in Korean women as explained by in 2014 by Kim and his coworkers [(8)].

The distribution of genotypes of IL1β-511 (A/G) between patients and health controls revealed very interesting results. There were very significant differences in IL1β-511 (A/G) between patients and controls with the majority of patients with GG genotypes being 75.2%, while most of the healthy controls carrying the AG genotype were 94.4% and most importantly, only small proportion of health controls have GG genotype were found in 5.6% and only 24.8% of PCOS patients with AG genotype, while AA genotype not detectible in both PCOS patients and healthy control groups. These results clearly indicate that the GG genotype is a risk factor for polycystic ovarian syndrome. Interestingly, the findings of this study indicated that individuals with GG IL1β-511 are 51 times more likely to have PCOS than AG genotype. This actually can be one of the valued genetic predictors for PCOS even many years before its onset[(9, 10)].

A recent study conducted in 2020 in Saudi Arabia showedhighly comparable results in regard to IL-1β-511GG genotype and PCOS [(11)].

Conflicts of interest

The author declares that there are no conflicts of interest.

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Ethic statement

The researchers already have ethical clearance from all required institution and laboratories.

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