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Interleukin-18 serum level and gene polymorphism in Iraqi polycystic ovary syndrome females

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Abstract:

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder among women of reproductive age. Recent research shows that PCOS may have an association with low-grade chronic inflammation and that PCOS may induce an increase in serum interleukin-18 (IL-18) levels.

Methods: To investigate the polymorphisms of the IL-18 gene promoters with PCOS, one single nucleotide polymorphisms (SNPs) in the promoter of the IL-18 gene (at positions -607C/A rs1946518) in 50 Iraqi women with PCOS and 30 controls were evaluated using polymerase chain reaction (PCR). Also, the serum levels hormone profiles and serum level of IL-18 were measured.

Results: No significant differences were found in the genotype distribution, allele frequency and haplotype frequency for polymorphisms of the IL-18 gene between the PCOS and control groups. Comparison to controls, patients with PCOS were more likely to have high levels of IL-18, BMI, FSH, LH, T, and PRL, low level of E2.

Conclusions: The present study concluded that there was no correlation between *IL-18* polymorphisms (at position -607 C/A) and PCOS. The level of IL-18 were highly statistically significant in PCOS comparing to normal women.In comparison to controls, patients with PCOS were more likely to have high levels of, BMI, FSH, LH, T, PRL, and in contrast a low level of E2.

Keyword: IL18, -607C/A, Polymorphism, Polycystic ovary syndrome.

INTRODUCTION

Polycystic ovary syndrome is one of the most common endocrine malfunctions in child-bearing women, which is clinical manifestations of menstrual abnormalities, hair growth, obesity, high blood insulin, and insulin resistance [1]. The etiology of polycystic ovary syndrome is very complication and unclear, and many environmental and lifestyle factors greatly contribute to the pathogenesis of the polycystic ovary syndrome [2-6]. However, hereditary factors play a critical role in the pathogenesis of polycystic ovary syndrome. Currently, many studies have reported an association between genetic factors and the risk of polycystic ovary syndrome [7-11].

Inflammatory cytokines may be important factors in the pathogenesis of polycystic ovary syndrome. There is convincing evidence describing the influence of low-grade inflammation and cytokines in polycystic ovary syndrome [12-16]. IL-18 is an 18 kDa cytokine, which belongs to the Interleukin-1 (IL-1) superfamily [17]. Three previous studies have indicated a significant association between IL-18 polymorphisms and risk of polycystic ovary syndrome in Asian population [18-20]. we aimed to investigate the association of SNPs in the promoter region of IL-18 (IL-18-607C/A rs1946518) with the risk of PCOS in an Iraqi population.

METHODS:

During January 2018 to March 2018, a total of 50 patients with PCOS were selected from AL-Yarmouk teaching Hospital and Baghdad Teaching Hospital, Ministry of Health, Baghdad, Iraq. The diagnosis of PCOS was based on the 2003 Rotterdam ESHRE/ASRM criteria: (1) oligoovulation (cycle intervals > 35 days) and/or anovulation (absence of menstruation for 3 consecutive months); (2) clinical and/or biochemical signs of hyperandrogenism (patients presented with hirsute (Ferriman-Gallwey scale ≥ 6), acne or alopecia, and/or increased circulating levels of testosterone, T ≥ 2.8 nmol/L); (3) polycystic ovaries(12 or more follicles 2-9 mm in diameter and/or increased ovarian volume of more than 10 ml). All other etiologies (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome) were excluded [21]. Simultaneously, a total of 30 healthy individuals without PCOS were randomly selected from AL-Yarmouk teaching Hospital and Baghdad teaching Hospital. All the controls were diagnosed free of PCOS by ultrasonic examination and reproductive hormone tests. The exclusion criteria of controls were those with irregular menstrual periods, malignant tumors, autoimmune diseases and ovarian related diseases. The mean ages of patients with PCOS and controls were 27.9±0.89 and 26.3±1.39y, respectively.

Blood sampling

All subjects underwent a brief physical examination, including height, weight, body mass index [BMI was calculated as follows: weight (kilograms)/height2 (meters)] and blood sampling for hormonal and measurement. Blood samples were obtained between days 2 and 3 of the menstrual cycle. In patients with amenorrhea, bleeding was induced by progestogens, with blood samples taken thereafter. If no bleeding occurred, blood samples were taken after validating the lack of an established pregnancy by a commercially available pregnancy test. Blood was taken from the antecubital vein. Samples were immediately centrifuged, with the serum separated and frozen at -20°C until assayed.

Biochemical analysis

Basal serum levels of Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), Testosterone (T), prolactin (PRL) and Estradiol (E2) were determined on menstrual cycle day 3 by Using of Electrochemiluminescence immunoassay technique "ECLIA" on Cobas e411 immunoassay analyzers. While serum level of IL-18 was determined by Using Enzyme-linked immunosorbent assay (ELISA).

DNA extraction

Genomic DNA was extracted from venous blood samples using Wizard® Genomic DNA Purification Kit (Promega, USA) as per the manufacturer's instructions and stored at -20°C until use. The DNA concentration was determined by using the Nanodrop. DNA quality also can assess by simply analyzing the DNA by Agarose gel electrophoresis [7]. The DNA fragments were separated in agarose gels in different concentration, 1% was used for whole-genome extracted DNA while 2% using for visual checking of a PCR product. A 1X TBE electrophoresis buffer was used in DNA electrophoresis, and the run was for 80 minutes at 100v/mAmp. The agarose gel was stained with Ethidium bromide 0.5 g/ml, the DNA bands were visualized by U.V transilluminator at 365 nm wavelength. Then the gel documentation system was used for document the bands.

IL18 SNP:

In the current study, the single nucleotide polymorphism (SNP) was genotyped which was *IL18* (-607 C/A, rs1946518) SNP. The Allele-specific primer technique (ASP) was employed in the present study in order to genotyped this SNP.

Primers:

The specific primers were designed for the -607 region of *IL18*. Primers were supplied by Alpha DNA Company (USA) as a

lyophilized product of different picomoles concentrations and dissolved in sterile deionized water to have the final concentration of 10 pmol/µl. The details of these primers which including sequence and their PCR Product size are presented in table (1).

PCR Components

The components of PCR reaction and conditions were shown in Table 2. Also, the optimization of PCR reaction was accomplished after several trials for the precision of DNA and primers concentrations. The PCR reaction was carried out as shown in table 2.

Statistical analysis

The data were expressed as a mean ± standard error, independent t-test and ANOVA table were used to express the probability (two-tailed) at the level 0.05 and 0.001 by using the commercial computer program IBM SPSS version 25.0. The genotyping and alleles frequency were calculated by Hardy-Weinberg calculator. While, OR, X^2, 95%CI and Fischer exact probability (two-tailed).

RESULT

Clinical and biochemical characteristics in patients and controls

In comparison to controls, patients with PCOS were more likely to have significantly increased levels of IL-18, BMI, FSH, LH, T, PRL, and significantly decreased level of E2, as shown in Table 3.

IL18 gene polymorphism

Polymorphisms at positions -607 in the promoter of the *IL-18* were analyzed by a conventional PCR. At the polymorphic site, a common reverse primer and two sequence-specific forward primers were used, resulting in two PCR reactions performed for every individual DNA. The specific PCR products from homozygous individuals showed one DNA segment, while heterozygous individuals exhibited two specific fragments, as expected. As shown in Figure 1, there were CC, CA and AA genotypes at position -607.

***IL18* SNPs in the studied groups**

PCOS patients and controls genotyping and allele frequencies of *IL-18* polymorphisms were summarized in Table 4. There was no significant variation between the observed and expected genotype frequencies of the PCOS patients' group. Accordingly, there was no deviation from the equilibrium. In contrast, there was a significant difference between the observed and expected genotype frequencies of the controls (Table 4). Also, *C* allele frequency was 54% in PCOS patient's group, while 53% in controls. Such, the frequency of *A* allele in the PCOS patient's group was 46%, while in controls was 47%. According to the current results, the frequency of *the C* allele has an attributed fraction, while the *A* allele has a protective fraction.

Table 1: Specific primer sequence for C/A alleles and their PCR product sizes for positions −607 in the promoter of the IL-18 gene

Primer	Sequence (5'→ 3')	PCR Product size
Common reverse	TAACCTCATTCAGGACTTCC	301
forward primers 1	GTTGCAGAAAGTGTAAAAATTATTAC	196
forward primers 2	GTTGCAGAAAGTGTAAAAATTATTAA	196
Control forward	CTTTGCTATCATTCCAGGAA	301

Table 2: PCR reaction of *IL18* gene components and conditions [18].

PCR Components		Volume	
Go Taq® Green Master Mix		10µl	
Control forward primer		1µl	
Forward Primer		1µl	
Common reverse primer		1µl	
Nuclease-free water		4µl	
DNA template		3µl	
Final Volume		20µl	
Steps	**Temperature**	**Time**	**No. of cycle**
Initial denaturation	94ºC	2:00	1
Denaturation	94ºC	00:20	7
Annealing	64ºC	00:40	
Extension	72ºC	00:40	
Denaturation	94ºC	00:20	25
Annealing	57ºC	00:40	
Extension	72ºC	00:40	
Final extension	72ºC	7:00	1
Hold	10ºC	10:00	

Table 3: Clinical and biochemical characteristics of PCOS patients and controls

Parameters	PCOS patients (n = 50)	Controls (n = 30)	Probability
IL-18 pg/ml	609.73±26.58	477.67±10.25	P < 0.001
BMI kg/m^2	29.48±0.76	24.12±0.88	P < 0.001
LH mIU/ml	8.32±0.45	6.18±0.44	P < 0.05
FSH mIU/ml	5.80±0.35	5.11±0.26	P < 0.05
T ng/ml	0.54±0.13	0.05±0.01	P < 0.001
PRL ng/ml	18.25±2.81	14.23±1.35	P < 0.05
E2 pg/mL	81.53±8.79	93.49±6.69	P < 0.05

Results described as means ±SE; PCOS, polycystic ovary syndrome; BMI, body mass index; LH, luteinizing hormone; FSH, Follicle-Stimulating Hormone; T, Testosterone; PRL, prolactin; E2, estradiol.

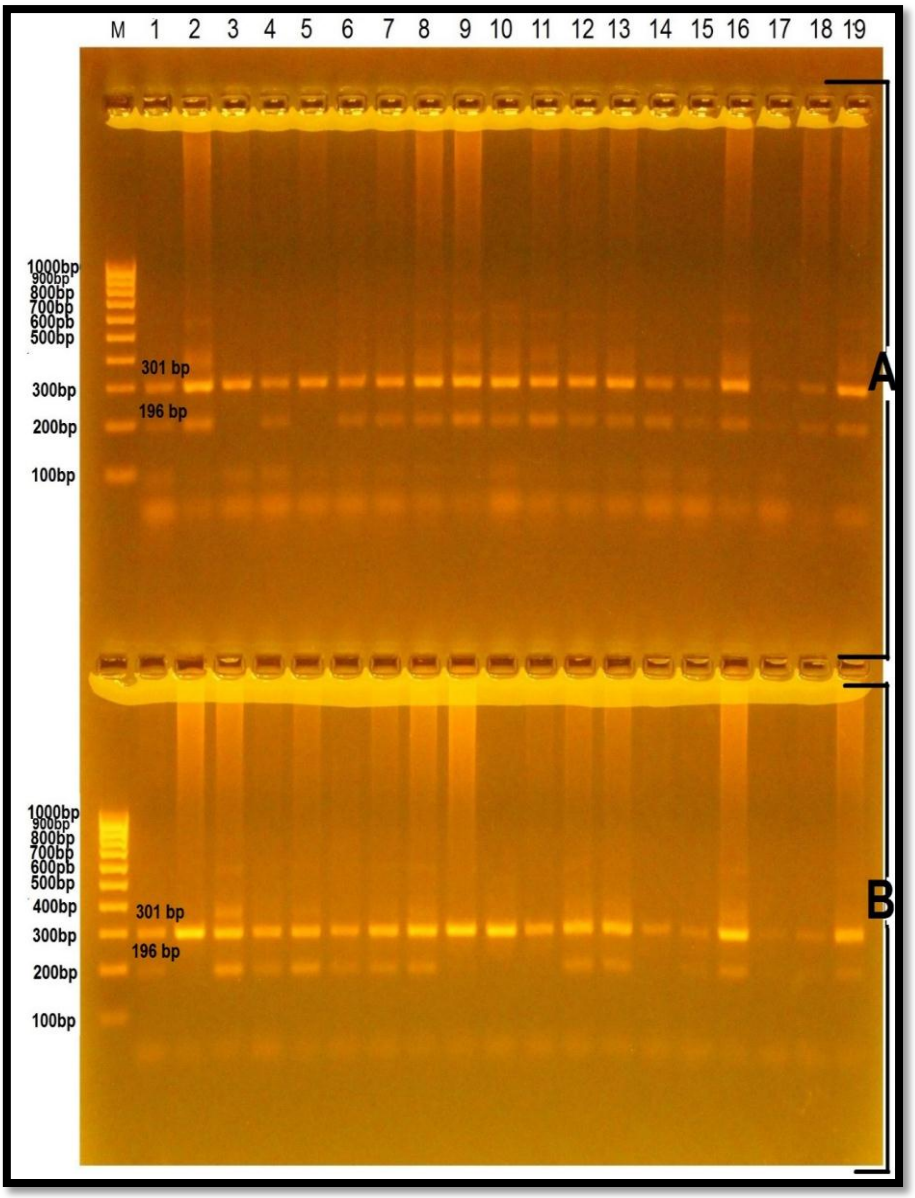

Figure 1: Gel electrophoresis of *IL-18* SNP (rs1946518) on 2% agarose gel stained with ethidium bromide. The molecular size of the amplified PCR product was 196 bp and the molecular size of the reference gene was 301 bp. L lane: DNA ladder (100 bp); Lanes 1, 2, 3, 4, 5 ------19: tested samples. Homozygous genotype (AA or CC) is presented with a single band in one line for each sample in A or B, while heterozygous genotype (CA) is presented by a single band in two adjustments lines for each sample (two lanes) in A and B. lanes 1,2,3: CA, CC, and AA.

Table 4: *IL-18* genotyping and allele frequencies of rs1946518 in the studied groups

Genotype	PCOS Patients (Numbers = 50)				Controls (Numbers = 30)				OR (95% CI)	X^2	*P*
	Observed		Expected		Observed		Expected				
	No.	%	No.	%	No.	%	No.	%			
CC	12	24.0	14.58	29.16	5	16.67	8.53	28.44	1.58 (0.50-4.95)	0.60	0.58
CA	30	60.0	24.84	49.68	22	73.33	14.93	49.78	0.55 (0.21-1.44)	1.47	0.33
AA	8	16.0	10.58	21.16	3	10.00	6.53	21.78	1.71 (0.43-6.89)	0.57	0.52
Total	50	100	50	100	30	100	30	100			
X^2	2.16				6.72						
P-HWE	0.14				**0.01**						
Allele frequency											
C	0.54				0.53						
A	0.46				0.47						

P-HWE: Probability of Hardy-Weinberg Equilibrium, OR: Odd ratio, 95% CI: 95% confidence interval, X^2: Pearson's chi-square test, *P*: Fisher's exact probability (two-tailed).

DISCUSSION

IL-18 is a proinflammatory cytokine that induces the production of TNF-a [23], IL-6 [24], and C-Reactive Protein (CRP) [25]. Similar to IL-6 and CRP, IL-18 is considered a strong risk marker for cardiovascular death [26]. Females with PCOS have chronic decreased level of the inflammation [27, 28], often associated with obesity and a subsequent increased risk for type 2 diabetes [29-31]. Recently, a study has shown that PCOS induces the increase in IL-18 serum level, which was also associated with several indexes of global and visceral adiposity, and with insulin resistance [32]. The human *IL-18* is located on chromosome 11q22.2-q22.3; it is composed of six exons and five introns [33]. Other studies described three SNPs at positions -656G/T, -607C/A and -137G/C in the promoter of the first exon of the *IL-18* [34, 35]. These promoter SNPs have been implicated as susceptibility loci for various diseases, including asthma [36], pulmonary tuberculosis [37], inflammatory bowel disease [38], Parkinson's disease [39], polycystic ovary syndrome [18], type I diabetes [40], and allergic disorders [41]. In the present study, the results demonstrated that IL-18-607C/A polymorphism was not associated significantly with the increased risk of PCOS. Chronic low-grade inflammation is reported to be correlated with abnormal metabolism in PCOS patients, and it results in abnormal endometrium implantation [42, 43]. A previous study performed the association between IL-18 serum level and PCOS, and there was a correlation between the increased level of IL-18 in PCOS patients and each of insulin resistance, obesity, and hyperandrogenism [18]. Another study in Greeks patients performed that IL-18 has elevated in lean patients, increased with the presence of obesity and insulin resistance [44]. Also, another previous study performed a meta-analysis with eighteen studies, found there was no association between IL-18-607C/A polymorphisms and the risk of PCOS [45]. However, some studies reported inconsistent results, it found that IL-18-607C/A polymorphism was significantly associated with the increased risk of PCOS [45]. While the present results demonstrated that no correlation between IL-18-607C/A and PCOS.

CONCLUSIONS:

- The present study concluded that there was no correlation between *IL-18* polymorphisms (at position -607 C/A) and PCOS.
- The level of IL-18 were highly statistically significant in PCOS comparing to normal women.
- In comparison to controls, patients with PCOS were more likely to have high levels of, BMI, FSH, LH, T, PRL, and in contrast a low level of E2.

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