Interleukin 10 (−1082 G/A) and (−819 C/T) gene polymorphisms in Egyptian women with polycystic ovary syndrome (PCOS)

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A R T I C L E  I N F O

Article history:
Received 28 March 2016
Revised 2 August 2016
Accepted 2 August 2016
Available online 3 August 2016

Keywords:
PCOS
Cytokines
IL-10
Polymorphism
SNP

A B S T R A C T

Cytokines play critical roles in the pathogenesis of Polycystic Ovarian Syndrome (PCOS). This work was designed to study the implication of IL10 gene polymorphisms (−1082 G/A and −819 C/T) on the susceptibility of Egyptian women to have PCOS. Rotterdam consensus criteria were used to diagnose PCOS patients. Genotyping was performed by single-stranded polymorphism-polymerase chain reaction (SSP-PCR) in 61 PCOS patients and 80 healthy controls, and IL-10 serum levels were measured using Enzyme linked immunosorbent assay (ELISA). The frequency of IL10−1082 G/G (46%) genotype was significantly increased (p < 0.001) while the frequency of −1082 A/A (16%) genotype was significantly decreased (p < 0.05) in PCOS patients compared to controls (14% and 35% for G/G and A/A genotypes, respectively). G allele (65%) is significantly increased (p < 0.01) in PCOS patients while A allele (61%) is significantly increased (p < 0.001) in control subjects. The distribution of IL10−819 T/T genotype was significantly increased (p < 0.05) in PCOS group. G/G genotype (odd ratio (OR) = 5.322) with confidence interval (CI = 2.364–11.982) and the G allele (OR = 2.828 with CI = 1.73–4.61) of −1082 G/G and T/T genotype of −819 C/T (OR = 4.18 with CI = 1.26–13.86) could be considered as risk factors for PCOS. IL-10 levels were significantly lower among PCOS patients (313.42 ± 30.10) compared to normal controls (491.36 ± 303.72). Depending on our preliminary work, IL10−1082 G/G might be considered as a host genetic factor for PCOS susceptibility in Egyptian women. Studies concerning other cytokine gene polymorphisms are required to get a better understanding of the pathogenesis of PCOS disease.

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1. Introduction

Polycystic Ovarian Syndrome (PCOS) is considered as a physiological disorder that causes negative effects involving different body systems, such as the endocrine, metabolic, psychological, and reproductive system. PCOS is estimated to affect 4–12% of women worldwide; being more common among those who are overweight (Sheehan, 2004; Garad et al., 2011). It is most commonly characterized by hyperandrogenism with increased risks of abdominal obesity and insulin resistance. It is also associated with acne, hirsutism, infertility, type 2 diabetes, hypertension, dyslipidemia, high blood pressure, inflammation, and increased risk of cardiovascular diseases (Assuncion et al., 2000; Sam and Dunafain, 2003). The most common irregularities of PCOS include increased serum levels of insulin. Raised insulin levels may upset the balance between follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which is required for follicle development and ovulation by sensitizing granulosa cells to the effects of LH. Granulosa cells from anovulatory women with PCOS are significantly more responsive to LH than granulosa cells of size-matched follicles from normal ovaries (Wills et al., 1998).

Immune dysregulation might play an important role in the pathogenesis of PCOS (Qin et al., 2015). Cytokines are immunomodulatory glycoproteins which control or modulate the activities of cells within the immune system (Urbanek, 2007). Chronic inflammation may be one of the underlying mechanisms for the pathogenesis of PCOS (Qin et al., 2015). Pro and anti-inflammatory cytokines play a key role in several diseases (Moore et al., 2001). The placental and decidual tissues in human from normal women express an array of pro- and anti-inflammatory cytokines (Roth et al., 1996; Piccinini et al., 1998). Low grade chronic inflammation and imbalance between pro- and anti-inflammatory cytokines have been proposed to play a role in the PCOS pathogenesis (Kelly et al., 2001; Söter et al., 2015).

IL-10 an anti-inflammatory and immunosuppressive agent produced within the body, plays a role in the regulation of immune responses (Moore et al., 2001). IL-10 was first described as a product of Th2 cells that inhibited cytokine synthesis in Th1 cells (Fiorentino et al., 1991). IL-10 is now known to be produced by macrophages, dendritic cells (DC), B cells, and various subsets of CD4+ and CD8+ T cells (Friedman et al., 1997; Hatherill et al., 2000; Kamanaka et al., 2006).
The major role of IL-10 is down-regulation of chemokines and cytokines production (De Waal Malefyt et al., 1991; Fiorentino et al., 1991). It stimulates the functions of innate and TH2-related immunity and suppresses TH1-related immune responses (Kamanaka et al., 2006).

The gene encoding IL10 is located on the long arm of chromosome 1. The promoter region is highly polymorphic, with 3 Single Nucleotide Polymorphisms (SNPs) at positions $-37.5$ to the range of $0.5 \text{–} 400.0 \text{mIU/ml}$), LH (over the range of $0.5 \text{–} 250 \text{mIU/ml}$) and PRL (over the range of $37.5 \text{–} 8000 \text{mIU/ml}$) in human serum.

2. Materials and methods

2.1. Study design

This case–control study was performed on 61 woman of reproductive age with PCOS and 80 healthy women were recruited at obstetrics – gynecology Department, Said Galal Hospital, Cairo, Egypt from year 2013 to 2015. This study was approved by the Institutional Ethics Committee and informed consent was obtained from all subjects. The onset of the syndrome was defined at the time when the patient fulfilled the diagnostic criteria. PCOS were defined when at least two of the following three features were present: oligo- /amenorrhea (8 menstrual cycles in the presenting year); hyperandrogenism (and/or hirsutism); and polycystic ovaries as Rotterdam consensus criteria to diagnose PCOS (Arosio et al., 2004; Daher et al., 2006; Karadeniz et al., 2008; Stonek et al., 2008; Vural et al., 2010; Wu et al., 2015), we aimed to investigate whether the polymorphisms in the promoter of IL10 gene (−1082 G/A; −819 C/T) could predispose women to PCOS. Very limited data is available about IL10 polymorphism in PCOS women; none of them was performed on the Egyptians.

IL10 SNPs (−1082 G/A and −819 C/T) were genotyped by polymerase chain reaction sequence-specific primer method (PCR-SSP) as described previously (Talaat et al., 2014). DNA of specific region of interest was amplified using specific primers for IL10 promoter region harboring −1082 G/A [FG (sense): 5′-CTACTAAGGCTTCTTTGGGAG-3′, FA (sense): 5′-ACTACTAAGGCTTCTTTGGGAA-3′ and generic primer (antisense): 5′-CAGTGGCAACTGAGATTTGGC-3′]. As internal control the following primers sense: 5′-GCTTCCCAACATCTCCTA-3′ and antisense: 5′-TCACGAGTTCTTGGTGGT-3′ were used. The reaction was done in two tubes, one for each allele, with 25 final reaction volume. The PCR mixtures consisted of DreamTag Green PCR Master Mix (2×) (Fermentas), 10 pmol of each allele-specific primer, 10 pmol of reverse primer, 3.5 pmol of each control primer and 100 ng of DNA. The cycling conditions of PCR were as follows: 94 °C for 2 min [1 cycle], followed by 96 °C for 25 s, 70 °C for 45 s, and 72 °C for 20 s [5 cycles]; followed by 96 °C for 25 s, 65 °C for 50 s, and 72 °C for 45 s [11 cycles]; and finally 96 °C for 25 s, 55 °C for 60 s, and 72 °C for 2 min [15 cycles]. After amplification the PCR products of control primer resulted in an amplicon of 429 bp and the −1082 primers resulted in an amplicon of 258 bp while the −819 primers resulted in an amplicon of 233 bp. By 2% agarose gel, the size of PCR products was determined relative to the migration of a 100 bp step ladder (Fermentas).

2.2. Quantitative determination of follicle-stimulating (FSH), luteinizing hormone (LH) and prolactin (PRL) in serum samples

Serum samples were separated from each patient and control subjects. FSH, LH and PRL were performed using the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Lotus Global Co., LTD, London, UK). The kits have been designed for the quantitative determination of FSH (over the range of 0.5–400.0 mIU/ml), LH (over the range of 0.5–250 mIU/ml) and PRL (over the range of 37.5–8000 mIU/ml) in human serum.

2.3. DNA extraction

Blood samples were collected in ethylene-diamine-tetraacetic acid (EDTA) tube. Genomic DNA was extracted using GeneJETTM Genomic DNA Purification Kit (Fermentas Life Science, Thermo Fisher Scientific Inc., MA, USA) according to the manufacturer’s instructions.

2.4. Genotyping

Plasma was collected from all patients and controls and were separated by centrifugation at 2000 rpm for 15 min at 4 °C, aliquoted, and stored at −80 °C. The total concentrations of IL-10 in plasma samples were measured using a commercial ELISA kit (R&D System, Inc., Minneapolis, MN), according to the manufacturer’s instructions.

2.5. Measurement of plasma IL-10 by enzyme-linked immunosorbent assay (ELISA)

Plasma was collected from all patients and controls and were separated by centrifugation at 2000 rpm for 15 min at 4 °C, aliquoted, and stored at −80 °C. The total concentrations of IL-10 in plasma samples were measured using a commercial ELISA kit (R&D System, Inc., Minneapolis, MN), according to the manufacturer’s instructions.

2.6. Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 19 (LEAD Technology Inc.). Data were presented as means with corresponding standard deviation (SD). Comparisons among different groups were performed by independent t-test. Each polymorphism was examined in the control population to confirm that the distribution of the genotypes confirmed to Hardy–Weinberg expectations (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The online tool SNPstats (http://bioinfo.iconologia.net/SNPstats) performed the haplotype analyses and calculated the LD parameters (D′ and r2). The genotype, allele, and haplotype frequencies were compared between cases and controls using a chi-square test ($\chi^2$). The odds ratio (OR) and 95% confidence intervals (CI) were calculated to assess the risk associated with particular allele, genotype or haplotype. Correlation between variables was determined using Spearman’s correlation test. A p-value of $<0.05$ was considered significant.

3. Results

3.1. Patient’s characteristics

Both PCOS women (30.23 ± 7.2) and controls (26.16 ± 5.14) are age matched. In the PCOS group, LH (9.12 ± 1.38) and FSH (6.86 ± 0.41) were significantly elevated (p < 0.001) in comparison with healthy
women (4.87 ± 0.33 and 5.23 ± 0.20 for LH and FSH, respectively). PCOS is positively correlated with the elevation in FSH (r = 0.341; p < 0.001) and LH (r = 0.334; p < 0.001). LH:FSH ratio might be elevated as a result of oral contraceptive pills. Subjects were non-obese, no significant difference in BMI was observed (28.0 ± 3.8 and 30.28 ± 5.70, for control and PCOS subjects, respectively).

3.2. Association between IL-10 polymorphisms and PCOS

The frequency of genotypes for IL10 (−1082 G/A) did not deviate significantly (p > 0.05) from the Hardy–Weinberg equilibrium (HWE) for genotypes GA, GG and AA (11%, 41%, 18% (observed) vs. 12.4%, 38.1%, 27.8% (predicted) for controls and PCOS; respectively). The values predicted by assumption of the HWE were different to those observed for IL10 genomic polymorphisms and haplotypes (predicted) for controls and PCOS; respectively). The values predicted for IL10 (−819 C/T) genotype CC, CT and TT [32%, 18%, 11% (observed) vs. 27.6%, 26.8%, 6.5% (predicted)] (p < 0.01) in PCOS patients while it was unsignificantly changes (p > 0.05) in controls [43%, 33%, 4% (observed) vs. 44.25%, 30.49%, 5.25% (predicted)].

The genotype frequencies of the two SNPs in patients and controls with IL10 (−1082 G/A and −819 C/T) genotypes and allele frequencies in PCOS and controls are shown in Table 1. Analysis of IL10 (−1082 G/A and −819 C/T) SNPs revealed that there was a significant increase in the frequency of −1082 GG genotype (p < 0.001) and significant decrease in the frequency of −819 AA genotype (p < 0.05) in PCOS patients compared to control. G allele is significantly increased (p < 0.01) in PCOS patients while A allele is significantly increased (p < 0.001) in control subjects. Both the GG genotype (OR = 5.322 with CI = 2.364–11.982) and the G allele (OR = 2.828 with CI = 1.73–4.61) could be considered risk factors for PCOS. Moreover, G/G-A/A vs AA was significantly increased in PCOS patients (OR = 2.74 with CI = 1.21–6.22) while G/A-A/A vs GG was significantly increased in control subjects (OR = 0.19 with CI = 0.08–0.42).

There was no significant difference between the distribution of −819 CC and CT genotypes between PCOS patients and controls. In contrast, TT genotype frequency was significantly increased (p < 0.05) in PCOS patients compared to their control counterparts which might be considered as a risk factor for the disease (OR = 4.18 with CI = 1.26–13.86). Moreover, C/C-C/T vs TT was insignificantly changed between both groups while C/T-T/T vs CC was significantly increased in control subjects (OR = 0.23 with CI = 0.07–0.79).

Table 1

| Cytokine gene       | Control (N = 80) | PCOS (N = 61) | p     | OR (95% CI)       |
|--------------------|-----------------|--------------|-------|-------------------|
| **IL-10 (−1082 G/A) genotype** (N, %) |
| G/G               | 11 (14%)        | 28 (46%)     | p > 0.001 | 5.322 (2.364–11.982) |
| G/A               | 41 (51%)        | 23 (38%)     | NS    | 0.76 (0.29–1.13)  |
| A/A               | 28 (35%)        | 10 (16%)     | p > 0.05 | 0.36 (0.16–0.82)  |
| G/A-A/A           | 69 (86.2%)      | 33 (54.1%)   | p > 0.001 | 0.19 (0.08–0.42)  |
| G/G-G/A           | 52 (65%)        | 51 (83.6%)   | p > 0.05 | 2.74 (1.21–6.22)  |
| Allele frequency  |                 |              |       |                   |
| G                 | 63 (39%)        | 79 (65%)     | p > 0.01 | 2.82 (1.73–4.61)  |
| A                 | 97 (61%)        | 43 (35%)     | p > 0.001 | 0.35 (0.21–0.57)  |
| **IL-10 (−819 C/T) genotype** (N, %) |
| C/C               | 43 (54%)        | 32 (52%)     | NS    | 0.94 (0.48–1.85)  |
| C/T               | 33 (41%)        | 18 (30%)     | NS    | 0.59 (0.29–1.21)  |
| T/T               | 04 (05%)        | 11 (18%)     | p > 0.05 | 4.18 (1.26–11.86) |
| C/T-T/T           | 37 (46.2%)      | 29 (47.5%)   | NS    | 1.05 (0.54–2.05)  |
| C/C-C/T           | 76 (95.0%)      | 50 (82.0%)   | p > 0.05 | 0.23 (0.07–0.79)  |
| Allele frequency  |                 |              |       |                   |
| C                 | 119 (74%)       | 82 (67%)     | NS    | 0.70 (0.42–1.106) |
| T                 | 41 (26%)        | 40 (33%)     | NS    | 1.415 (0.843–2.377) |

NS (not significant); p < 0.05 (significant); p < 0.01 (moderate significant); p > 0.001 (highly significant); OR (odd ratio); CI (confidence intervals).

The frequency of IL10 (−1082/−819) haplotypes in PCOS patients and healthy controls is shown in Table 2. The AC and AT were the most frequent haplotype in controls group while the GC and GT were the most frequent haplotype in PCOS patients. A significant increased (p < 0.01) in GT haplotype coincides with significant reduction (p < 0.01) in AT haplotype in PCOS was demonstrated. The linkage disequilibrium (LD) pattern between the −1082 G/A and −819 C/T SNPs showed a non-significant LD, with a D’ value of 0.1578 and r2 value of 0.1009.

3.3. Plasma levels of IL-10 in PCOS patients

The mean plasma concentration of IL10 was significantly decreased (p < 0.001) in PCOS patients compared to controls (313.426 ± 30.103 versus 4914.362 ± 303.726) was demonstrated. The PCOS disease was significantly correlated (r = 0.818; p < 0.001) with the reduction in the IL10 secretion level.

3.4. Differential expression of IL-10 in patients and controls according to polymorphisms and haplotypes

As shown in Table 3, the mean plasma concentration of IL10 was comparable in PCOS patients with different genotypes and haplotypes, and the same trend was also observed in controls. Thus, no particular genotype or haplotype could be responsible for the significant reduction in the IL10 level between PCOS patients and controls.

3.5. Differential expression of FSH, LH and PRL in patients and controls according to polymorphisms

As shown in Table 4, the mean concentration of all tested hormones in IL10 (−1082 G/A) and −819 C/T was comparable in PCOS patients with different genotypes, and only IL10−1082 AA genotype has a significant (p < 0.05) increase in FSH, LH and PRL levels.

4. Discussion

The etiology of PCOS involves multiple genetic and epigenetic alterations that are still indistinct, but polymorphisms in cytokine genes may play an important role (Merino et al., 2009) especially the risks for PCOS susceptibility and functional SNPs. A variety of molecular epidemiological studies have been focused on the association between cytokine (TNF-α, IL-1α, IL-1β, IL-6, IL-10, or IL-18) gene polymorphisms and PCOS risk. However, the results from different studies have been inconsistent (Wu et al., 2015). IL-10 is a product of monocytes and lymphocytes that has been regarded as one of the extremely important anti-inflammatory immune-regulating cytokines, as it effectively regulates down-regulator pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α (Fernandez-Real and Ricart, 2003). As imbalance between pro- and anti-inflammatory cytokines may participate in PCOS, the polymorphic gene of IL-10 can be an important biomarker for predisposition to PCOS. Therefore, we conducted this study to better clarify the association between two polymorphisms −1082G/A and −819C/T in the IL10 promoter and the risk of PCOS.

Table 2

| Haplotype       | Control (N = 80) | PCOS (N = 61) | p     | OR (95% CI)        |
|-----------------|-----------------|--------------|-------|-------------------|
| GC              | 54 (38.3%)      | 61 (42.9%)   | -     | 1.00              |
| AC              | 51 (36.1%)      | 35 (24.4%)   | NS    | 0.70 (0.38–1.28)  |
| AT              | 34 (24.5%)      | 15 (10.9%)   | P < 0.01 | 0.23 (0.09–0.63)  |
| GT              | 2 (1.1%)        | 30 (21.8%)   | P > 0.01 | 28.36 (2.82–285.45) |

NS (not significant); p < 0.01 (moderate significant); OR (odd ratio); CI (confidence intervals).
As shown in our results, there was a significant increase in the frequency of IL10 (−1082 G/G) genotype and significant decrease in the frequency of IL10 (−1082 A/A) genotype in PCOS patients. G allele is significantly increased in PCOS patients while A allele is significantly increased in control subjects. It is obvious from our results (significant increase in G/G-G/A as AA with OR = 2.74) that the association of the disease with G allele is strong even with its presence on one chromosome only.

IL10 (−1082) A allele frequency in our controls was similar to those reported in Daher et al. (2006) in Brazilian population, but lower than that reported in Italy (Arosio et al., 2004) and Austria (Stonek et al., 2008). In contrast to our data, the genotype and allele frequencies for IL10 genotypes between PCOS and controls (Karadeniz et al., 2008). No notable differences were spotted in allele frequencies for IL10 genes between PCOS and controls (Karadeniz et al., 2008). In contrast to our data, the genotype and allele frequencies for IL10 gene polymorphism of the PCOS patients has no effect on FSH, LH and PRL levels. Vural et al. (2010) stated that IL10 (−1082) genotype did not influence clinical/laboratory parameters in PCOS.

In conclusion, this preliminary study indicated that IL10 (−1082 and −819) genotypes may play a role in PCOS in Egyptian patients. The significant reduction in the level of IL-10 between patients and control was independent of any particular genotype/haplotype in IL10 (−1082 G/A) and (−1082 C/T). Our results provide help to Gynecologists for better individual therapeutic strategy taking into consideration patient's genetic repertoire that may influence its therapeutic response. The sample size of the present association study is likely insufficient for detecting all the associations between SNPs and PCOS. Larger prospective studies are needed to confirm our findings.

5. Conclusions

In conclusion, this preliminary study indicated that IL10 (−1082 and −819) genotypes may play a role in PCOS in Egyptian patients. The significant reduction in the level of IL-10 between patients and control was independent of any particular genotype/haplotype in IL10 (−1082 G/A) and (−1082 C/T). Our results provide help to Gynecologists for better individual therapeutic strategy taking into consideration patient’s genetic repertoire that may influence its therapeutic response. The sample size of the present association study is likely insufficient for detecting all the associations between SNPs and PCOS. Larger prospective studies are needed to confirm our findings.

Conflict of interest

The authors declare no conflict of interest.

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Table 3

| Genotype | Control (N = 80) (Mean ± SD) | PCOS (N = 61) (Mean ± SD) | p |
|----------|-------------------------------|---------------------------|---|
| IL10 (−1082 G/A) |                               |                           |   |
| G/G (11, 28) | 5974.3 ± 1231.9 | 296.1 ± 44.6 | <0.001 |
| G/A (41, 13) | 4755.4 ± 439.8 | 281.8 ± 47.9 | <0.001 |
| A/A (28, 10) | 5044.7 ± 432.5 | 289.6 ± 32.3 | <0.001 |
| G | 4887.0 ± 345.6 | 308.3 ± 41.1 | <0.001 |
| IL10 (−819 C/T) |                               |                           |   |
| C/C (43, 32) | 5304.6 ± 502.9 | 316.6 ± 42.4 | <0.001 |
| C/T (33, 18) | 4983.7 ± 485.2 | 249.4 ± 48.7 | <0.001 |
| T/T (4, 11) | 2590.8 ± 730.5 | 349.4 ± 79.9 | <0.001 |
| C | 5165.2 ± 352.2 | 292.4 ± 32.3 | <0.001 |
| T | 4725.0 ± 454.8 | 287.3 ± 42.9 | <0.001 |

Table 4

| Genotype | FSH (Mean ± SE) | LH (Mean ± SE) | PRL (Mean ± SE) | P |
|----------|----------------|----------------|-----------------|---|
| IL10 (−1082 G/A) |                               |                           |   |
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