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Association between interleukin-17 gene polymorphism and rheumatoid arthritis among Egyptians

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ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune systemic inflammatory disorder characterized by synovial inflammation, destruction of joints and systemic manifestations. Interleukin-17 (IL-17) is a pro-inflammatory cytokine that plays an important role in the pathogenesis of RA.
Objective: This study aimed to examine the associations between both IL-17A (197A/G; rs2275913) and IL-17F (7488A/G; rs763780 and 7383 A/G; rs2397084) gene polymorphisms and the susceptibility of RA among Egyptian population.
Methods: IL-17A and IL-17F gene polymorphisms were determined in ninety eight patients with RA and ninety five healthy control subjects using polymerase chain reaction based restriction fragment length polymorphism (RFLP) analysis.
Results: There was no significant difference in the genotype and allele frequencies of IL-17A (rs2275913) and IL-17F (rs763780 and rs2397084) between the controls and the patients with RA ($P > 0.05$).
Conclusion: The results of this study suggested that the IL-17A and IL-17F gene polymorphisms may not be associated with the susceptibility to RA among Egyptian population.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disorder characterized by synovial inflammation, destruction of the affected cartilage and bone (Firestein, 2003). RA has a complex aetiology and it is suggested that both environmental and genetic factors are involved in the disease pathogenesis (Gaffen, 2004; Lee and Weinblatt, 2001; Begovich et al., 2004).

Several studies identified multiple candidate genes that are associated with the susceptibility of RA and the first identified RA susceptibility gene was the human leukocyte antigen HLA-DR4 and HLA-DR1 (Reveille, 2005; Bowes and Barton, 2008; Cornélis et al., 1998; Shiozawa et al., 1998).

It has been evidenced that numerous cytokines are involved in the pathogenesis of RA (Korn et al., 2009). IL-17 is a novel proinflammatory cytokine that contributes in the development and progression of inflammatory and autoimmune diseases. Up to now, six members of IL-17 family were identified [IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F] (Kaabachi et al., 2014; Iwakura et al., 2011). Both IL-17 A and IL-17F are produced by a specific subset of T cells (Th17) and they are located on chromosome 6p12. Both IL-17 A and IL-17 F show high protein sequence homology and bind to the same kind of receptors (Iwakura et al., 2008). Cumulative evidence supported that Th17 cells play a key role in several autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel diseases and multiple sclerosis. One function of IL-17 is to enhance the production of inflammatory cytokines e.g. IL-1 and TNF-α that attract immune cells at the site of inflammation (Shabgah et al., 2014). Signaling by IL-17A increases matrix metalloproteinases and also acts to recruit neutrophils to the peripheral sites through the induction of chemokines and granulocyte colony stimulating factor (Kolls and Anders, 2004).

Research evidences have proved the contribution of Th17 cells and IL-17A in cartilage and bone destructions in RA by binding of IL-17 specific receptor expressed on fibroblasts, endothelial and epithelial cells (Chabaud et al., 2000; Ruddy et al., 2004; Shahrara et al., 2009).

Furthermore, increased expression of IL-17 mRNA and IL-17 protein were detected in joints from RA patients. In IL17 gene, several polymorphisms have been detected which may influence IL-17 expression (Pawlik et al., 2016).

The aim of the present study was to analyze the association between

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IL-17A (rs2275913) and IL-17F (rs763780; His161Arg and rs2397084; Glu126Gly) polymorphisms and the susceptibility to RA among Egyptian population.

2. Materials and methods

2.1. Subjects

The study group consisted of 98 patients with RA and 95 healthy control subjects. All patients were all outpatients attending the Department of Orthopedics and Traumatology, Tanta University Hospital, Tanta, Egypt. Rheumatoid arthritis patients were diagnosed according to American Rheumatism Association (ARA) revised criteria. Patients with diabetes mellitus, other autoimmune diseases and neoplastic diseases were excluded from the study. The control subjects were selected from those who either attended a routine health check at a general practice or at their place at work. Venous blood was collected from all subjects in sterile K_3EDTA (tri-potassium ethylenediaminetetraacetic acid) coated tubes and subjected to low speed centrifugation. White cells were removed from the buffy coat for DNA extraction. Samples were stored at −20 °C till the time of use.

2.2. Ethics

This study was approved by local Ethics Committee of the Faculty of Medicine, Tanta University, Tanta, Egypt and a written informed consent was obtained from all subjects.

2.3. Determination of IL-17A and IL-17F genotypes

DNA was extracted from peripheral blood leukocytes using DNA extraction kit (QIAapm DNA Blood Mini kits; Qiagen, Hilden, Germany) according to the manufacturer instructions. In 25 µl PCR mixture, 100 ng of genomic DNA were used for PCR amplification and 2× PCR master mix was used (Thermo Fisher Scientific, Inc., MA USA).

For IL-17A (rs2275913) polymorphism, PCR was performed as described previously (Najafi et al., 2014). The primers used were: forward 5′-TCT CCA TCT CCA TCA CCT TTG-3′and 5′-GTC CAA ATC AGC AAG AGC ATC-3′ (as a reverse primer). The PCR cycling conditions consisted of a denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 57 °C and extension for 1 min at 72 °C. A final extension for 5 min at 72 °C was done (BIORAD, T100 Thermal Cycler, BioRad Laboratories, Inc.). The PCR product was digested by the restriction enzyme, XagI (Thermo Fisher Scientific, Inc., MA USA) according to manufacturer instructions and analyzed in 2% agarose gel stained with ethidium bromide and visualized by UV light. The amplified PCR product size was 815 bp. Restriction digestion for the homozygous GG genotype gave 286, 259 and 270 bp fragments; while the homozygous AA genotype produced 286 and 529 bp fragments.

For IL-17 F gene polymorphisms, the PCR was performed as described before (Paradowska-Gorycka et al., 2010). Two primers were used, forward: 5′-GTG TAG GAA CTT GGGCTG CAT CAA T-3′ and: 3′-AGC TGG GAATGC AAA CAA AC-3′ (as a reverse primer). The PCR conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 30 s; 55.2 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 10 min. The amplified PCR product size was 470 bp. 10 µl of the PCR products were digested with 1 µl of Eco47I (Thermo Fisher Scientific, Inc., MA USA) for the Glu126Gly polymorphism, and with Hin1II (Thermo Fisher Scientific) for the His161Arg polymorphism at 37 °C and separated by size on 2% agarose gel. Digestion of PCR product with Eco47I gave 470 bp for genotype AA and 75 and 395 bp for genotype GG. On the other hand, restriction cut of PCR product with Hin1II gave 52, 130 and 288 bp for genotype AA, whereas genotype GG gave 52 and 418 bp fragments.

Table 1
Genotype distribution and allele frequency of IL-17A (rs2275913) and IL-17F (rs763780 and rs2397084) in subjects with RA and control group.

IL-17 polymorphism	Control (n = 95)		RA (n = 98)		χ^{2a}	*P* value*
	No	%	No	%		
IL-17A (rs2275913)						
Genotypes:						
AA	22	23.16	19	19.39	0.599	0.321
AG	43	45.26	40	40.81	0.563	0.316
GG	30	31.58	39	39.8	0.293	0.149
Alleles:						
A	87	45.78	78	39.79		0.251
G	103	54.21	118	60.2		
IL-17F (rs763780)						
Genotypes:						
AA	40	42.1	38	38.78	0.222	0.662
AG	39	41.1	40	40.82	0.001	0.970
GG	16	16.8	20	20.4	0.404	0.525
Alleles:						
A	119	62.63	116	59.18		0.525
G	71	37.37	80	40.82		
IL-17F (rs2397084)						
Genotypes:						
AA	56	58.95	61	62.24	0.661	0.374
AG	31	32.63	28	28.57	0.639	0.324
GG	8	8.42	9	9.19	0.852	0.527
Alleles:						
A	143	75.26	150	76.53		0.707
G	47	24.73	46	23.46		

[a] Chi-square analysis between patients with RA and healthy controls.
* *P*-value < 0.05 was considered as significant.

2.4. Statistical analyses

Data were analyzed using SPSS for Windows version 20.0 (SPSS Inc., Chicago. IL, USA). Student's *t*-test was used to compare mean values of continuous variable in cases and control, whereas χ^2 analysis was used to compare categorical data.

3. Results

3.1. Genotype distribution and allele frequency of IL-17A (rs2275913)

The genotype distribution of IL-17A (rs2275913) polymorphisms was in Hardy-Weinberg equilibrium in both the RA and control groups. Table 1 shows the genotype and allele frequencies in both RA and control groups. The genotypes AA, AG and GG were 19.39%, 40.81% and 39.8 respectively in RA group and were 23.16%, 45.26% and 31.58% respectively in the control group.

There was no significant difference in the genotype and allele frequencies between patients with RA and healthy controls ($P > 0.05$).

3.2. Genotype distribution and allele frequency of IL-17F (rs763780 and rs2397084)

The genotype distribution of IL-17F (rs763780) and (rs2397084) polymorphisms were all in Hardy-Weinberg equilibrium in both the RA and control groups. For IL-17F (rs763780), the frequencies of genotypes AA, AG and GG were 38.78%, 40.82% and 20.4% in patients with RA, and they were 42.1%, 41.1% and 16.8% in control subjects respectively. For IL-17F (rs2397084), the frequencies of genotypes AA, AG and GG were shown in 62.24, 28.57% and 9.19% in patients with RA and were 58.95%, 32.63% and 8.42% in control subjects respectively (Table 1). There was no significant difference in the distribution of genotypes and allele frequencies of both IL-17F (rs763780) and (rs2397084) between patients with RA and healthy controls ($P > 0.05$).