Determination of IL17 Plasma Levels and IL-17A gene Polymorphisms in Patients with Allergic Rhinitis

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Abstract

Aim: The present study aims to determine whether the level of serum interleukin -17 and IL-17 receptors (IL-17A) gene variant increases or decreases susceptibility to Allergic Rhinitis. **Method:** In this case-control study fifty patients with rhinitis and fifty apparently healthy persons as a control in Babylon province / Iraq were enrolled in this study. **Results**: the results of this study were shown significant difference in frequency of IL-17 A (rs2275913) A>G polymorphism between Rhinitis patients and controls .Also , there were significant difference in frequency of IL-17A (rs 2275913) between rhinitis patients and controls and there were significant increase in patient compared with control. **Conclusion:** The bpresent study suggests that patients with AA allele for IL-17A (rs2275913) have increased susceptibility to rhinitis compared with GG allele , Also the present study suggests that IL-17A (rs2275913) is considered risk factor for Allergic Rhinitis. This study suggests that the level of IL-17 patients with rhinitis highly significant compared with healthy.

Keywords: *Allergic Rhinitis,IL-17,IL-gene polymorphism*

Introduction

Allergic Rhinitis a type of inflammation in the nose which occurs when the immune system overreacts to allergens in the air [1]. Signs and symptoms include a runny or stuffy nose, sneezing, red, itchy, and watery eyes, and swelling around the eyes [2]. Some people may develop symptoms only during specific times of the year, often as a result of pollen exposure [3] . Allergic rhinitis is typically triggered by environmental allergens such as pollen, pet hair, dust, or mold [4]. Inherited genetics and environmental exposures contribute to the development of allergies [5].Cytokines play an essential role in mediating allergic inflammation. The importance of the T helper cell (Th) 2 cytokines in both the development of allergic sensitization and pathology of allergic inflammation is well established [6]. While healthy subjects are predominated by Th1-type cells, nasal mucosa and epithelial tissues of AR subjects are dominated by Th2-type lymphocytes[7].The recent discovery of another T lymphocyte subset, namely Th-17 cells, has been fundamental to our understanding of how Th-1 cells can actually mediate inflammatory events by producing IFN-c [8] . T helper 17 cells are characterized by the production of various cytokines, including IL-17, IL-6, tumor necrosis factor-a and IL-22 [9]. Another Th subgroup of regulatory T cells (Tregs) has restrictive influences on both Th1 and Th2 cell-mediated inflammation. The lack of Tregs causes the emergence of allergic inflammation along with the increase in Th2 cells[10]. The Tregs fetch the allergic inflammation under control by synthesizing IL-10 and the changment growth factor-β (TGF-β)[11].The patient with Allergic Rhinitis showed an allergen-specific functional defect in Treg that promotes Th2 polarization and consequently IgE synthesis [12]. An increasing number of studies have demonstrated relationship between IL-17 and neutrophilic inflammation, implicating IL-17 as a potential candidate gene in predicting allergic rhinitis susceptibility. Interestingly, an analysis which reported by Chinese study showed that IL-17A rs 2275913(−197G/A) were

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found to be commonly associated with allergic rhinitis [13]. Thus, the aim of the present study was to investigate the impact of IL-17A rs2275913, genes polymorphisms on IL-17A plasma levels together with their influence on susceptibility as well as its severity.

Ethical statements:

The study design was reviewed and approved by the ethics committee of Hilla Teaching Hospital and Imam Sadiq Hospital in Babylon province in Hilla city. All persons participated in this study was agreed to participate and signed an informed consent.

Material and Methods

Study Design:

This study design was a case-control study. Patient with diabetes mellitus, Patient with hypertention, Smokers, Patients with rheumatoid arthritis, Pregnancy, Any inflammatory diseases, Any autoimmune diseases. This study included 100 individual , the age was ranged between (18 - 70) years ,All samples were collected from 1st of September 2019 till 1st of January 2020. The practical side of the study was performed at the laboratory of biochemistry department in College of Medicine / University of Babylon. We collected blood samples from healthy subjects, patients during rhinitis.(5 ml) blood were drawn,(2ml) EDTA tubes immediately frozen at -20 °C for further assay. and (3ml) gel tubes, clotted at room temperature for 10-20 minutes and centrifuged for 20 minutes at 3000 rpm. Serum samples were immediately frozen at -20 °C for further assay.

Genotyping analysis

Genomic DNA was isolated from EDTA peripheral blood samples of all patients and extracted by standard salting-out procedure [19]. The identification of the IL-17A rs2275913 (A/G -197) polymorphisms were performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) using specific primers: [(IL-17A*F: 5'-AACAAGTAAGAATGAAAAGAGGACATGGT-3'; IL-17A*R: 5'-CCCCCAATGAGG TCATAGAAGAATC-3') respectively followed by a digestion of the amplification products using EcoNI enzymes respectively.

Measurement of serum IL-17 levels

Serum IL-17 was measured by commercial enzyme-linked immunosorbent assay (ELISA) using reagent kits Elabscience (China)

The presence of DNA extracted was detected by using agarose gel electrophoresis technique ,then the genomic DNA was amplification by PCR technique, After PCR product was digested with restriction ECON1 enzyme and the products were analyzed by 5% polyacrylamide gel electrophoresis., The PCR fragment was divided into 102 bp, 68 bp and 34bp fragments.

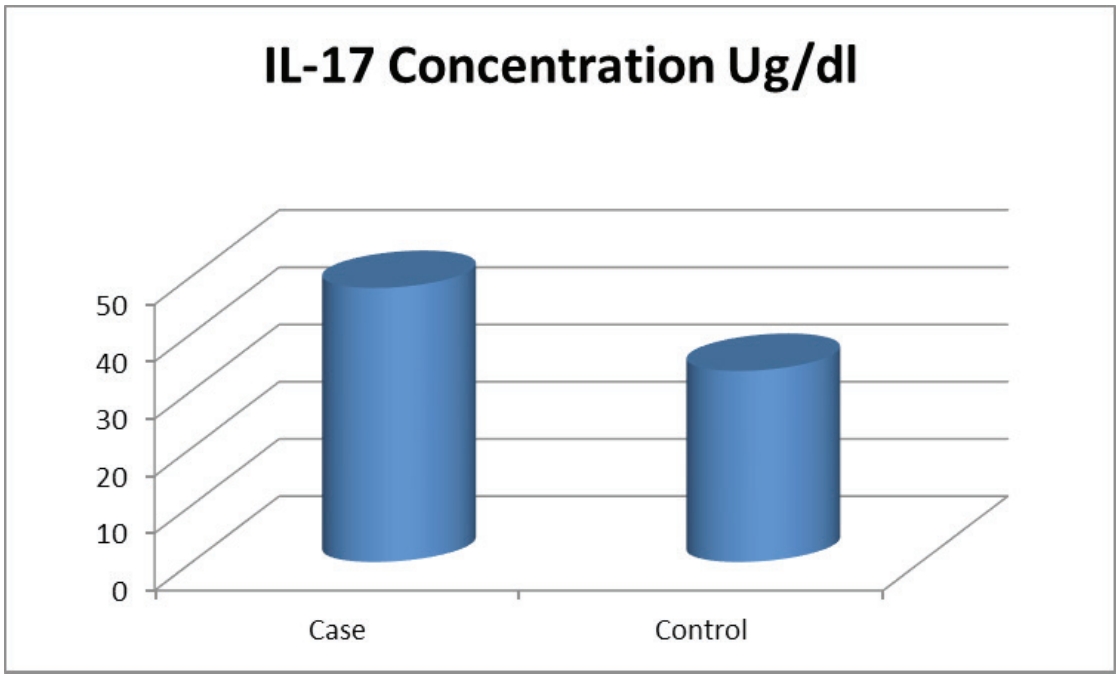
Statistical Analysis

Data were analyzed by the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Normally distributed data were expressed as the mean±SD and non-normally distributed data were expressed as the median (25-75th percentiles). The Student paired t-test was used to compare normally distributed data between two groups, and the rank sum test was applied for nonnormally distributed data between two groups. The $\chi 2$ test was used to compare frequencies. All reported p values were two-sided, and $p < 0.05$ was considered statistically significant.

Results:

The demographics and clinical characteristics of the healthy control subjects and patients with allergic rhinitis are shown in Table 1. significant difference in age, sex and family history was observed between patients and healthy control. As expected, patients with attacks showed (mean±SD: 46.3 ± 3 more than 40 year vs. 26.5 ± 3 for less than 40 years, $p< 0.05$) compared to healthy control subjects (Table-I). However, the FEV1%pred levels were found higher in patients during remission than during asthma attack ($p< 0.05$).

Serum IL-17 (Fig.1) level was significantly elevated in patients during patient with allergic rhinitis attack and remission ($p< 0 .000$) compared with healthy control subjects. In addition, serum IL-17 levels was much higher during rhinitis attack than those during remission ($p< 0.05$).



(Figure 1): IL-17 concentration in serum of case and control in allergic

Analysis of IL-17A rs2275913 polymorphism

There were significant differences in genotypes and alleles frequencies between patients and controls (Table :1):

(Table: 1). Results of IL-17A rs2275913 genotyping in patients and controls

IL-17A genotype	Patient n=	Control n=	P value	OR(95% CL)
A	58%	52%	P<0.05	OR: 5.892*; CI 95%** 1.77-19.57
G	42%	48%		

Otherwise, the IL-17A studied polymorphism was associated to the activity of the AR in our patients group.

Discussion

The present study showed significantly increased serum IL-17 level in patients with allergic rhinitis compared to healthy subjects, lately, serum IL-17 levels were found higher in rhinitis patients than healthy subjects[14]. These results proposed that serum IL-17 levels play an important role and linked to the severity of the rhinitis[15]. In the present study was significant association was found for IL-17A rs2275913 and disease susceptibility, $p < 0.05$. The subject result was showed the relationship between RS2275913 gene polymorphisms and allergic rhinitis ,this result was agreement with F Fatahi et al in Iranian study [16]. Also in Spain study was showed RS2275913 gene polymorphisms was relationship with allergic rhinitis. the IL-17A*A allele conferred a role for AR risk, OR = 5.892*; 95% CI 1.77-19.57. Therefore, the risk conferred by IL-17A rs2275913 G allele in AR predisposition might be weak and that could explain the absence of association reported in the mainstream of published studies as well as the present study.

Acknowledgments: Authors would like to thank the staff of the Biochemistry Department at College of Medicine, University of Babylon, Iraq, for their efforts and facilities to complete the task of this study. Also, authors would like to thank the staff of labrotary at Merjan Teaching Hospital, Hilla city, Iraq, for their

assistance in the collection of samples.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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