The Impact of Genetic variation at TNF-α -308 G/A on their serum production and severity of Asthma disease

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Abstract. Asthma is a chronic respiratory disease leading to morbidity and impaired quality of life worldwide. TNF-α cytokine responsible for the smooth muscle activation and late-phase airway hyper responsiveness. So this study aimed to investigate TNF-α polymorphisms situated at positions –308 G/A with their serum level and asthma severity. A case–control study included 40 asthmatic patients 16 females and 24 males and matched with age and gender 40 healthy subjects as a control group. Blood samples were collected from both groups, genomic DNA was extracted from peripheral blood for further molecular study. The study appeared that TNF-α-308 G/A significantly (P<0.05) high frequencies of homozygous AA, and A allele carrier in asthmatic patients, in contrast homozygous GG genotype and G allele appear at low frequency with patients. The data showed that genetic variants of the TNF-α affect the TNF-α production and associated with the severity of asthma, AA and AG genotype associated with high serum level (58.2 pg/ml and 49.5 pg/ml) respectively and GG genotype associated with low serum level (24.06pg/ml).The study confirmed the distinct role of TNF-α in cases of asthma compared to healthy controls with significantly higher level among patients, mean serum level for patients and control were (50.3 and 5.88 ) pg/ml respectively. Also, mean serum levels of TNF-α were significantly higher in asthmatic patients that suffering from allergic rhinitis(65.39 pg/ml) compared to non-rhinitis patients (34.97pg/ml).

In conclusion, TNF-α-308 GG, genotype contribute to the predisposition of asthma and there was significant association among genotype of this gene and level of cytokine as well as development of the disease. Also observed an association between this gene and serum level of cytokine . AA , AG genotype and allergic rhinitis consider a risk factor of asthma and progress the disease.

Keywords: TNF-α, polymorphism, PCR-SSP, asthma, allergic rhinitis.

Introduction

Asthma is a chronic inflammatory disorder of the airways that considered a major global health problem affecting 1-18% of the population in different countries. It is estimated that the number of people with asthma may be as high as 500 million people worldwide. Asthma and rhinitis are highly prevalent and interrelated diseases and have similar mechanisms to driving inflammatory response. Proinflammatory cytokines involved in allergic inflammation and their levels are increased following local allergen challenge. Cytokines are chemical messengers of both the innate and acquired immune systems. They are secreted by immune cells, epithelial and endothelial cells and play role in intercommunication between them. In other hand participate in modulating inflammatory and immune reaction in many
diseases such as asthma. Asthma is the result of a persistent inflammatory process so immune system plays a crucial role in the pathogenesis of asthma. Tumor necrosis factor-α (TNF-α), are proinflammatory cytokines that play an immune regulatory role in differentiation of B-cells, T-cells, and dendritic cells. In subjects with asthma, an increase in the production of TNF-α after allergen challenge lead to smooth muscle activation, late-phase airway hyper-responsiveness and airway inflammation. The production of cytokines can be controlled by genetic polymorphisms, especially in the promoter regions. A correlation between such polymorphisms and the production levels of a number of cytokines observed in several diseases, IL-10 polymorphism associated with severity of Thalassemia. Polymorphisms in the promoter region of TNF-α 308G/A (rs1800629) involves the substitution of a guanine (G) by an adenine (A) and is associated with an increase in TNF-α expression levels. TNF-α-308 G/A polymorphism is associated with an increased risk of asthma in children. Complex interaction between immune-inflammatory process, cytokines activation and genetic factors is involved in asthma pathogenesis. So present work aimed to investigate the role of TNF-α 308G/A polymorphism with their production of cytokine and progression of disease.

**Subjects and methods:**

The case-control study involved 40 patients with asthma disease (24 males and 16 females) whose ages ranged between 14-50 years attending to allergy specialized center in Al-Sader teaching hospital at AL-Najaf city during the year 2016 after received the Ethical Considerations which approved by the Medical Ethics Committee of the Ministry of Health Al-Sader teaching hospital / Iraq. Polymorphisms of TNF-α-308 G/A promoter gene and serum level of cases were compared with those of 40 individual as a healthy control they included 22 males and 18 females whose ages ranged between 14-48 years. Both physical and clinical examinations were done for each subject and the information was recorded in a data sheet and verbal informed consent was obtained from all participants. Blood samples were collected for estimation of TNF-α serum level by sandwich ELISA, according to Elabscience USA instructions as well as for molecular study.

**DNA isolation and PCR**

Genomic DNA was extracted from fresh peripheral blood cell (2 ml in EDTA) using a commercially available kit according to protocol of Geneius™ Micro gDNA Extraction Kit, Geneaid, USA, and then stored at -20°C till use. Single nucleotide polymorphisms (SNPs) related to the TNF-α-308 G/A were determined using PCR with sequence-specific primers (PCR–SSP) in two reactions employing one common forward and two reverse primers 5’-CTG CAT CCC CGT CTT TCT CC-3’ (forward), 5’-ATA GGT TTT GAG GGG CAT CA3’ (reverse one) and 5’-ATA GGT TTT G AG GGG CAT CA3’ (reverse tow) which amplify of 836bp of the promoter region of gene. The reaction mix was done in 25μl volumes include 5μl of template DNA, GoTaq®Promega Green Master Mix12.5 μl. Primers (forward 2 μl and 1 μl of each reverse) and Nuclease Free water 3.5 μl (Applied PCR system, USA), and PCR conditions for TNF-α gene are initial denaturation at 96°C for 5 min, followed by 96°C for 45s, 55°C for 80s and 2min of extension at 72°C, with a final extension of 3 min at 72°C. The result of PCR products were resolved by electrophoresis on agarose gel stained with 4μl (0.5% concentration) from
ethidium bromide, the run lasted for 1 hour for 100 V. The gel was then photographed on UV light(320 nm) and scored for the presence or absence of an allele specific band.

**Statistical analysis**

Data were expressed as mean ± standard deviation, to show significantly the data of TNF-α serum levels in patients and healthy controls by using student t-test. Allele frequencies were estimated using the gene-counting method. Differences of genotype and allele frequencies between groups were analyzed using the χ² test. Pearson coefficient and odds ratios (ORs) for the risk of asthma and their 95% confidence intervals (CIs) were calculated using logistic regression analysis. All statistical analyses were performed by Microsoft excel and the Graph Pad software (prism version 6). The difference was considered significant if p < 0.05.

**Results**

1- Asthma’ Risk Factors

The characteristics of asthma patients as compared to healthy controls are shown in Table(1). Among the studied risk factors, positive history family showed a significant association with the incidence of asthma, with 67% of patients and 43% of controls, and allergic rhinitis recorded 72.5% in asthmatic patients.

Table 1

| Risk Factors         | Cases N=40 | Control N=40 | P-value | OR (95%CI) |
|----------------------|------------|--------------|---------|------------|
| Mean age ± SD (years) | 34.5±10.9  | 31.9±11.3    | 0.183[NS] |            |
| Gender               |            |              | 0.2502[NS] | 1.500      |
| Male                 | 24(60%)    | 22(55%)      |         |            |
| Female               | 16(40%)    | 18(45%)      |         |            |
| Family history       |            |              |         |            |
| Positive             | 62.3%(25)  | 25%(10)      |         |            |
| Negative             | 37.5%(15)  | 75%(30)      |         |            |
| Rhinitis             |            |              |         |            |
| Yes                  | 26(65%)    |              |         |            |
| No                   | 14(35%)    |              |         |            |

2- Distributions of TNF-α -308 G/A Genotypes and Alleles in Patients and healthy control

The distribution TNF-α -308 G/A polymorphism was detected by PCR -SSP technique, at this locus three genotype; GG, GA and AA with band sizes of 836 bp Figure (1).
The results revealed that homozygous genotype AA frequency was higher significantly in asthmatic patients 17.5% when compared to the healthy controls 5%, GA genotype 67.5% in the patients, whereas GG frequency record 15% only as shown in Table (1), the “A” allele was shown to be more prevalent in the asthmatic patients 51.25% as compared to controls 36.25% and was strongly associated with the disease.

Table (2): Frequency and distribution of TNF-α -308 G/A genotype and allele frequencies in Asthmatic patients and controls.

| Allel | Control | Cases | P value | Odd ratio | (95% CI) |
|-------|---------|-------|---------|-----------|----------|
|       | N=40    | N=40  |         |           |          |
| AA    | 2(5%)   | 16(40%) | 0.0384* | 4.030     | 0.7821-20.77 |
| GA    | 14(35%) | 20(50%) | 0.3196  | 1.246     | 0.4962-3.130 |
| GG    | 24(60%) | 4(10%)  | 0.0330  | 0.3665    | 0.1230-1.092 |
| G/allele | 51(63.75%) | 39(48.75%) | 0.0279 | 0.5409 | 0.2873-1.018 |
| A/allele | 29(36.25%) | 41(51.25%) | 0.0279* | 1.849 | 0.9821-3.480 |

OR=Odd ratio, P( <0.05) , CI =confidence interval

3-Concentration of TNF-α in Serum of patients and healthy

The present study indicated that the level of TNF-α has increased significantly (P ≤ 0.05) in the serum of asthma patients 50.3 pg/ml in comparison with mean concentration in control group 5.88 pg/ml, figure(2).
The results showed that TNF serum levels were significantly higher in the asthmatic patients. TNF-α levels at position −308 were significantly (p < 0.05) different among the asthmatic patients with genotypes AA and AG the mean serum levels were (58.2 and 49.5 pg/mL), respectively. In contrast GG genotype showed a low TNF-α serum level 24.06 pg/mL, figure (2).

Comparison TNF-α level between asthmatic patients with rhinitis and without rhinitis

The present study has indicated that TNF-α has been increase in the serum of asthmatic patients with allergic rhinitis (69.39 pg/mL) in comparison with asthmatic patients without allergic rhinitis with mean concentration (44.65 pg/mL) P ≤ 0.05 figure(3).

Discussion

The study clarify that family history and Acute rhinitis (AR) were recorded a significant association with asthma. Gaidan et al., illustrated that a positive correlation between incidence of asthma and family history and emphasis that first relative with asthma increased the incidence of disease compared to controls who had such relative. The results observed that the asthma and AR were associated and affected with each other. AL-Samarai et al., 2009 confirmed that AR and asthma are strongly associated with each other. Also, the American Academy of Allergy, Asthma, and Immunology estimated that up to 78% of patients with asthma have nasal symptoms.
and 38% of patients with AR have asthma. The present study revealed that homozygous genotype AA and heterozygote genotype GA frequency was higher in asthmatic patients when compared to the healthy controls. The TNF-α gene promoter polymorphisms affect the susceptibility or severity of autoimmune diseases and seems to be highly associated with the development of these diseases. Wilson et al. identified the associations between TNF-α gene polymorphisms and asthma susceptibility and illustrated that TNF-α at position -308G>A, a candidate gene for asthma. TNF-α were associated with an increased risk of asthma and showed that TNF-α -308 AA polymorphism is significantly associated with chronic asthma in Iraqi patients. The results found that the A allele and AA genotype in asthmatic patients were higher, about 2-fold than the control, with a significant association. In contrast, the “G” allele and GG genotype have a rather preventive or protective role. The frequency of TNFA-308G allele-containing genotype in asthmatics was much lower than normal controls, so GG genotype may have a protective role in asthma pathogenesis. In same line, Mathanraj et al., explain that the SNP at position _308G/A TNF-α A allele was reported to be associated with a high production of TNF-α and A allele was present at a higher frequency in asthmatics as compared to controls. Zedan et al., found that TNF-α-308 GA genotypic polymorphism may be a contributing factor to the susceptibility and severity of asthma in Egyptian children. The present study indicated that the level of TNF-α has increased significantly in the serum of asthma patients in comparison with the control group. TNF-α plays a significant role in the inflammatory disease affecting the lung, and the high levels of TNF-α are directly linked to asthmatic complications characterized by severe, persistent asthma. Berry et al. demonstrated that the TNF-α may contribute to the dysregulated inflammatory response seen in the asthmatic airway which is raised by the findings of the increased TNF-α mRNA and protein in the airway of patients with asthma. Aoki et al., indicated that the presence of a G-to-A polymorphism at position -308 in the promoter region of the TNF-α gene could increase levels of TNF-α 6-7-fold in plasma and Bronchoalveolar lavage fluid from asthmatic airways. Puthothu, indicated that significantly elevated risks were associated with the A allele in Asians and demonstrated that the A allele of the -308A/G polymorphism could increase the susceptibility to asthma. Ruan and Lv revealed asthmatic subjects with genotypes carrying at least one A allele (AA and AG genotypes) exhibited significantly higher TNF-α serum levels and increased the risk of asthma more than those of GG genotype. Similarly, Yang et al. explains that individuals carrying the GA genotype have higher amounts of TNF-α mRNA, and serum protein levels, than individuals with the G/G genotype. Also, added that other promoter polymorphisms within IL-10 *1082G/A, gene, had been influencing IL-10 plasma levels during the disease and stated an significant association among genotype and level of cytokine as well as development of asthma disease. Iwasaki et al., observed that the lack of TNF-α inhibited the development of acute rhinitis (AR) in mice. This suggests that TNF-α may play a role in the pathogenesis of AR. Acharya et al., observed a significant upregulation of TNF-α levels in nasal lavage fluid following allergen challenge in patients with AR. The increased expression levels of TNF receptor on nasal epithelial cells from patients with AR have been reported by. TNF-α an important mediator in the induction and maintenance of inflammation in allergic rhinitis. Also, Christine et al., revealed that allergic patients have significantly higher levels of proinflammatory cytokines IL-1.
IL-6, IL-12, and TNF-α. Minhas et al., found that the patients carrying the TNF-α A allele of the TNF-α –308 promoter region more commonly had AR with higher circulating levels of TNF-α in Pakistani Patients.

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