IL-10 and TNF-alpha Gene Polymorphisms in Patients with Celiac Disease

Çölyak Hastalarında IL-10 ve TNF-alfa Gen Polimorfizmeleri

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ABSTRACT

Celiac disease (CD) is an autoimmune disorder characterized by intolerance to ingested gluten. HLA-DQ genes are strongly associated with susceptibility to CD. Non-HLA genes are effective in CD pathogenesis as well as HLA genes. TNF-alpha has a single nucleotide polymorphism (SNP) at position -308 in promoter region, which has been shown to have association with CD in previous studies. The aim of this study is to investigate the association of TNF-alpha and IL-10 cytokine gene polymorphisms with CD and with DQB1*02 status in patients with celiac disease. Thirty three patients and 93 healthy individuals were included in the present study. GG and AA genotypes in position -308 of TNF-alpha gene had a significantly increased frequency in the patient group and in patients with DQB1*02 when compared to the controls. No significant differences could be established for IL-10 gene polymorphisms within the patients and controls. As a result of all these findings, it might be suggested that there is no significant association of IL-10 gene polymorphism with CD and data on TNF-alpha gene polymorphisms are not sufficient enough to clarify the disease pathogenesis thus indicating roles for other genes within or out of MHC gene region.

ÖZET

Çölyak hastalığı (ÇH), glutene toleransından sebep olduğu, incebarsak anormalliliği ile karakterize otoimmün bir hastalıdır. Tüm dünyada yaygın olarak görülüp, genetik, immünolojik ve çevresel faktörler etkendir. İnsan lökosit antijeni (HLA)-DQ genleri ile hastalık arasında güçlü bir birliktelik vardır. HLA genlerinin yanı sıra HLA dışı genler de hastalığın patogenezinde etkili olabilir. Özellikle, TNF-alfa geninin -308 pozisyonundaki polymorfizmin hastalığla ilişkisi kesinleşmiştir. Bu çalışmada IL-10 ve TNF-alpha gen polymorfizmilleri çölyak hastaları ve bu hastalardaki DQB1*02 varlığı/yokluğu ile ilişkisinin tespiti hedeflenmiştir. Çalışmada 33 hasta ve 93 sağlıklı bir heyet dahil edildi. Hasta ve kontrol grubunun periferik kanlarından elde edilen DNA’ları ile PZR-SSP yöntemi kullanılarak sitokin gen polymorfizm tiplerini yapıldı. TNF-alfa -308 GG ve AA genotiplerinin tüm hasta grubu ve DQB1*02 (+) hasta grubunda kontrollere göre anlamlı olarak yüksek olduğu belirlenirken, IL-10 ile hastalık arasında bir ilişki saptanamamıştır. Bu bulgular IL-10 gen polymorfizminin ÇH ile bir ilişkisinin olmadığını ve DQB1*02 varlığının bu hasta grubunda TNF-alfa gen polymorfizmi üzerine bir etkisi olmadığını düşündürdüktedir.

Anahtar Kelimeler: Çölyak Hastalığı, TNF-alpha, IL-10, sitokin gen polymorfizmi, DQB1*02

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INTRODUCTION

Celiac disease (CD) is an autoimmune disorder characterized by abnormalities in the small intestine caused by intolerance to ingested gluten and related proteins in barley and rye. The prevalence of CD is 0.5-1% and 1:150-300 in USA and Europe whereas it is 1% in Turkey. The damage observed in the intestinal mucosa is the result of the interplay between several genes and gluten, the environmental factor which triggers the immune response in patients with CD.

Celiac disease, in 80-90% of the cases, is associated with a specific HLA class II heterodimer, generated by the products of DQ2 and DQ8 molecules. However, the association with DQ alleles cannot explain the wide range of clinical pictures observed in CD. In fact, it has been calculated that HLA region contributes for 40% to the family risk of developing CD.

In a study of Karell et al., it was found that 90% of the patients with celiac disease have HLA-DQA1*0501-DQB1*02 and DQA1*03-DQB1*0302 (DQ8). In addition, non-HLA genes such as cytokine and MICA genes are also reported to play roles in the occurrence of the disease. Among these cytokines, a proinflammatory cytokine with immunomodulatory activity, TNF-α, has an important role in the pathogenesis of CD and a significant association of SNPs (GG, GA, AA) in position -308 of TNF-α gene with celiac disease has been reported.

Another cytokine, IL-10, contains SNPs in the proximal and distal parts of its promoter region. The proximal SNPs are at position -1082 (A/G), -819 (T/C) and -592 (A/C) and these have been reportedly involved in the transcription rate of IL-10 and therefore in the production level of this cytokine. IL-10 polymorphisms in celiac disease have been investigated in different studies. Two of them did not detect any significant difference in the frequency of these polymorphisms between celiacs and controls, whereas one observed a significant lower prevalence of low TNF-α/high IL-10 producers in celiacs with IgA deficiency versus celiacs with normal levels and control.

Most of the polymorphisms in cytokine genes affect gene transcription influencing both the individual response to infections and the individual susceptibility to several immune-mediated or inflammatory diseases, including also celiac disease. In this regard, we aimed at investigating association of TNF-α and IL-10 cytokine gene polymorphisms in patients with celiac disease according to the presence of DQB1*02.

MATERIALS- METHODS

A total of 33 (mean age: 32±8 years; range:19-47 years; F/M: 21/12) patients with celiac disease, unrelated to one another and 93 healthy, unrelated individuals with a mean age of 35±9 years (range:18-57 years; F/M:46/47) were included in the study. All the subjects enrolled in this study, patients and control subjects, were Caucasians living in Turkey. The control group was in Hardy Weinberg equilibrium. The study was approved by the Local Ethical Committee of Dr. Lutfi Kirdar Kartal Education and Research Hospital.

Genomic DNA was isolated from peripheral blood of all patients and controls. DNA quality was measured by spectrophotometry at 260 nm and 280 nm, respectively. Samples with 260/280 ratio lower than 1.5 were excluded from the study. Following DNA isolation, genotyping for IL-10 (-1082, -819 and -592) and TNF-α (-308) was performed by PCR-SSP method (One Lambda Cytokine Genotyping Tray, OneLambda, Canoga Park, CA, USA) due to the superiority of SSP method in means of accuracy, reliability and sensitivity on restriction fragment length polymorphism (RFLP).

Statistical analyses were performed using SPSS software (version 12). Allele and genotype frequencies were obtained by direct counting and compared to those in the control group using Fisher’s exact and Pearson chi-square test. The p values were corrected by the Bonferroni method multiplying the p-value by the number of alleles compared for each cytokine. A corrected p-value less than 0.05 was considered as significant.

RESULTS

Concerning IL-10 at position (-1082,-819,-592), no significant allele and genotype differences were observed between CD patients and the controls (Table 1). For TNF-α, increased GG (49% vs. 16%; p=0.0007, p<0.0001) and AA (9% vs. 0%; p=0.016, p<0.0001) and decreased GA genotype frequencies (42% vs. 84%; p<0.0001, p<0.0003) were
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observed in patients compared to controls. However, the allele frequencies did not differ between the patients and the controls (Table 2).

When patients were divided into 2 groups (with or without DQB1*02), no significant differences were observed for IL-10 within 2 groups (with DQB1*02 (n:19, 57.5%), without DQB1*02 (n:14, 42.5%)). However, for TNF-α, increased frequency of GG genotype (47% vs. 84%, p:0.001, pc:0.003) was found in patients with DQB1*02 compared to the controls. In patients without DQB1*02, the frequency of TNF-α GG genotype was increased (57% vs. 16%, p:0.001, pc:0.003) while the frequency of GA genotype was decreased (36% vs. 84%, p:0.002, pc:0.006) when compared to the healthy controls (Table 3).

**Conclusion**

IL-10 affects many aspects of inflammatory and immune responses and acts on both hematopoietic and non-hematopoietic cells. Several polymorphisms have been observed in human IL-10 gene flanking sequence. Studies with IL-10 gene polymorphisms reported several associations of IL-10 gene polymorphisms with SLE, RA, and Type I Diabetes. Although no significant correlation has been established between celiac disease and IL-10 gene polymorphisms in most of the studies, one study by Garrote et al. reported the significantly high frequency of GCC haplotype in celiac patients with DQB1*02. Interestingly, IL-10 polymorphisms with a low production phenotype have been associated with a more severe form of juvenile rheumatoid arthritis, disease severity in multiple sclerosis, and rate of progression in primary glomerulonephritis. In all cases, no differences in polymorphism prevalence were observed between the patient and control populations, suggesting that these polymorphisms do not represent the causative gene but contribute to modifying the phenotype. We could not find any significant association between IL-10 gene polymorphisms and CD.

It has been shown that the susceptibility to CD development is strongly influenced by MHC genes, particularly the alleles of HLA-DQB1*02. It has also been shown that A allele of TNF-α at position -308 accompany this susceptibility although this may vary for CD patients from different populations. We could not establish a significant difference for the frequency of A allele for TNF-α at position -308. However, there was a significant difference with an increased frequency of GG genotype (low producer genotype) (49% vs. 16%; p:0.0007, pc:0.002) and decreased GA genotype (high producer genotype) frequency (42% vs. 84%; p<0.0001, pc<0.0003) in patients compared to healthy controls. The higher frequency of AA genotype in the patients was at the border of insignificance after Bonferroni correction (pc:0.048). These results for decreased TNF-α GA and increased GG genotypes in patients remained significant when patients with DQB1*02 were compared to the controls.
We must mention that our group of patients were too small to obtain results with high level of evidence. In addition, our control group was not matched for age and gender to the patients and this might also influence the statistical results. Despite these facts, it might be speculated that a complex interaction of immune related genes within or out of MHC gene region might be contributing to the development of immune reactions in patients with CD varying from one population to another.

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