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Influence of tumor necrosis factor alpha gene promoter polymorphisms and its serum level on migraine susceptibility in Egyptian patients

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ABSTRACT

Background: Migraine is a common chronic neurological disorder with still largely unknown pathogenesis. We aimed to explore the possible role of tumor necrosis factor alpha (*TNF-α*) gene polymorphisms as risk factors of migraine, and whether they influence the TNF-α level.
Materials and methods: Two hundred patients with migraine and 200 controls were enrolled in this study. Polymorphisms of *TNF-α* gene were detected using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Serum TNF-α level was measured using enzyme-linked immunosorbent assay (ELISA).
Results: *TNF-α*-308 GA, AA genotypes and A allele, *TNF-α*-857 CT genotype and T allele were associated with increased risk of migraine, while the *TNF-α*-238 polymorphism was not. *TNF-α*-308 GA, AA genotypes and A allele or AA genotype were associated with increased risk of migraine with aura (MA) and migraine without aura (MO) respectively; this was more significant in female patients with MA than in males. *TNF-α*-857 CT genotype was associated with increased risk of MO, or MA in females or males. While -857T allele was significantly associated with MO or MA in males and with MA only in females. On the other hand, we didn't find any significant associations of *TNF-α*-238 polymorphism with MO, or MA in males or females. TNF-α levels were higher in patients with migraine, MA, or MO than in controls ($P < 0.001$).
Conclusion: *TNF-α* polymorphisms were associated with migraine, MA, or MO in Egyptians.

1. Introduction

Migraine is a common neurovascular disorder characterized by severe, often unilateral, pulsatile headache that lasts 4–72 h and can be accompanied by nausea, vomiting, photo- and/or phonophobia [1]. Globally, about 11% of the population experience migraine and it is three times more common in females (15–18%) than in males (6%); this thought to be due to changes in ovarian hormones [2,3]. Migraine sub-classified into migraine with aura (MA) and migraine without aura (MO) [4]. It has a complex etiology affected by genetic and environmental factors [1]. Approximately 50% of patients with migraine have an affected first degree relative. Family or twin studies have provided conflicting results with respect to the mode of inheritance of migraine. Notably, there are no clinical differences between sporadic and familial cases [5].

Although the pathophysiology of migraine remains unknown, it is thought that cytokines play an important role in the modulation of pain threshold and may be involved in its pathogenesis [6]. Inflammatory activation plays a vital role in the pathophysiological mechanisms of migraine, exerts deleterious effects on the progression of tissue damage and may be involved in migrainous infarction [7]. Therefore, a thorough understanding of how inflammatory activation occurs under migraine conditions is important.

Recently, there is an increasing interest on the possible relationship between genetic polymorphisms and the risk for migraine, and many possible candidate genes have been the matter of studies. Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine involved in brain, immune, and inflammatory activities, and also appears to play a role in migraine [8]. When administered centrally or peripherally, TNF-α can cause hyperalgesia and initiate ectopic activity in nociceptive primary afferent neurons [9]; it can also strongly increase hyperalgesia by eliciting prostanoid release and increasing the expression of nerve growth factor and bradykinin receptors [10].

TNF-α gene cluster is located within the class III region of the highly polymorphic major histocompatibility complex (MHC) on human chromosome 6p21. Recently, a large number of single nucleotide polymorphisms (SNPs) have been identified in the *TNF-α* gene promoter [11]. Notably, TNF-α production is affected by SNPs in gene promoter and regulated at the transcriptional and posttranscriptional levels; and have also been found to be associated with altered levels of circulating TNF-α [12,13]. A moderate TNF-α level is necessary to maintain cell

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insetting and to prevent certain diseases through regulating immune response [14]. However, excessive TNF-α due to pathological conditions is associated with autoimmune diseases, infectious diseases, and malignant tumors [15].

Because migraine is considered a multi-factorial disease with various genetic etiologies and few studies concerning the association of *TNF-α* gene polymorphisms with migraine susceptibility have presented conflicting results in different populations. To the best of our knowledge, there are no studies elucidating the role of *TNF-α* SNPs and/or TNF-α serum levels in the susceptibility to migraine in Egypt. Therefore, the aim of our study was to investigate possible associations the *TNF-α* gene promoter polymorphisms (-308G/A, -238G/A, -857C/T), and whether they influence TNF-α level in our population.

2. Materials and methods

This case–control study included 200 unrelated patients with migraine who had made their first visit or a follow-up visit to the outpatient clinics of Internal Medicine and Neurology Departments, Zagazig University Hospitals between May 2012 and December 2013. Migraine was diagnosed based on the criteria of the International Classification of Headache Disorders from the International Headache Society [16]. Two hundred unrelated, migraine-free, control participants were selected from a community. They were of the same ethnic origin (Egyptian) and matched to cases by age, sex, and as far as possible for smoking habits. Migraine, positive family history of migraine, and any type of severe or recurrent headache in first-degree relatives were excluded in all controls through personal interview. All subjects included in this study underwent complete history taking and a thorough medical examination.

2.1. Ethical consideration

A written informed consent was taken from all of the participants after explaining details, benefits as well as risks to them. The study design was approved by the Ethical Committee of Faculty of Medicine, Zagazig University.

2.2. Measurement of serum TNF-α concentration

Blood samples were drawn from all subjects after an overnight fast. Sera were separated immediately and stored at −20 °C. The concentration of TNF-α in serum was determined using a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Kits were provided by Biosource (Europe S.A., Belgium).

2.3. DNA extraction

Genomic DNA was extracted from EDTA whole blood using a spin column method according to the protocol (QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany) DNA was stored at −20 °C till the time of use.

2.4. Genotyping of TNF-α gene polymorphisms

Genotyping for the -238G/A (rs361525), -308G/A (rs1800629), and -857C/T (rs1799724) polymorphisms in the *TNF-α* gene promoter was performed by a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method described by Zuo et al. [13]. The PCR primers were designed on the basis of *TNF-α* gene reference sequence in GenBank accession no. NG007462), open access program Primer3 (http://www.bioinfo.ut.ee/primer3-0.4.0), and they were similar to primer sequences in a study by Zuo et al. [13]. PCR was performed in a final volume of 25 μL containing 5.5 μL of H_2O, 5 μL of genomic DNA, 1 μL of each primer (1 μM) (Promega, Madison, WI), and 1× PCR Master mix (12.5 μL) (Taq PCR Master Mix Kit, QIAGEN, GmbH, Hilden, Germany) containing (200 μmol/L of each d NTP, 5 μL of 10× reaction buffer, and 1.25 U Taq Gold Polymerase, and 4 mmol/L $MgCl_2$). The PCR protocols of *TNF-α*-238, -308, and -857 were performed with the following settings: 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 61 °C of *TNF-α*-238, 63 °C of *TNF-α*-308, 65 °C of *TNF-α*-857, for 30 s separately, and 72 °C for 45 s. A final extension step was carried out at 72 °C for 7 min. All PCR products were digested with restriction enzymes (Fermentas, Germany) according to the manufacturer's instruction. The fragments were resolved by gel electrophoresis in a 2.5% agarose (Serva, Spain) stained with ethydium bromide and visualized under a UV transilluminator. A 50 bp ladder (Fermentas, Germany) was used as a marker (Figs. 1, 2, 3). PCR primer sequences, restriction enzymes and fragment size before and after digestion were listed in Table 1. Notably, 10% of the samples were amplified twice for checking accuracy of results. Retested samples were randomly chosen and retesting was performed in order to control the test process.

2.5. Statistical analysis

Data were analyzed using SPSS software version 17 (SPSS Inc., Chicago, Illinois, USA). The appropriate sample size and power of the study were determined using PAWE-3D (Laboratory of Statistical Genetics, New York) (Gordon et al., 2005) [17]. PAWE-3D calculations showed that the sample size, together with the specified study design, allele frequencies, prevalence of disease, and allowable error rates, can give as high as 90% power and can detect the variant allele frequency of at least 0.05 and genotype relative risk of ≥1.8 at 80% power. The results for continuous variables were expressed as means ± SD. The means of genotype groups were compared with independent Student's t-test or one-way analysis of variance (ANOVA). Qualitative data were compared by the chi-squared-test (X^2) or Fisher's exact test. *TNF-α* genotype frequencies in cases and controls were tested for Hardy–Weinberg equilibrium and any deviation between the observed and expected frequencies was tested for significance using the chi-squared-test. The statistical difference in genotype distribution and allele frequencies in both control and case was assessed by using X^2 test or Fisher's exact test. Odds ratios (ORs) and confidence intervals (CIs) were calculated. A correction for multiple comparisons was applied when necessary, using the Bonferroni method. A difference was considered statistically significant if P was <0.05.

3. Results

3.1. Clinical characteristics of patients with migraine and controls

The characteristics of patients and controls are summarized in Table 2. There were no statistically significant differences between

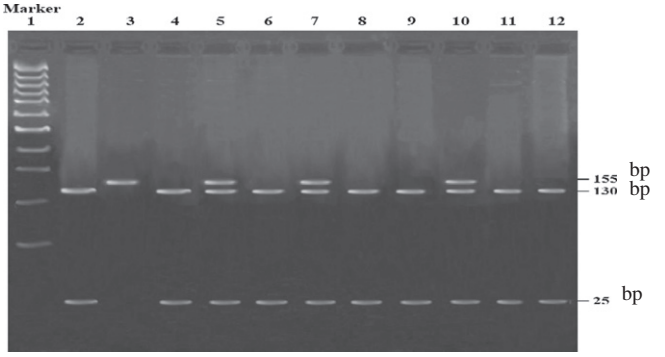

Fig. 1. *TNF-α*-238 genotypes of patients with migraine on 2.5% agarose gel. M: 50 bp marker: lane 1; *TNF-α*-238 GG genotype: lanes 2, 4, 6, 8, 9, 11, and 12; GA genotype; lanes 5, 7, and 10; and AA genotype: lane 3.