Abstract: Growing evidence indicates that TNF-alpha is involved in the pathogenesis of POAG in several ways, primarily by induction of retinal ganglion cells apoptosis and therefore optic nerve degeneration. TNF-alpha and POAG relationship has been studied at the genetic level with variable results in different populations. The transcription rate and the release of the TNF-alpha cytokine have been reported to be affected by polymorphisms in the promoter of the TNF-α gene. Polymorphisms at positions -238 and -308 are the most frequent studied. Another polymorphism, at the position -863 in the promoter region, has been less studied, but a homozygous AA allele appears protective in a Chinese population. Our aim was to assess the potential association of -863C/A TNF-alpha gene promoter polymorphisms with POAG in an Egyptian group of subjects. Genotyping of the TNF-alpha (-863) polymorphism was done for 228 POAG patients and 230 control subjects using the PCR-based, Restriction Fragment Length Polymorphism (RFLP) assay. TNF-alpha (-863) A/A genotype was absent in both groups. There was no significant difference between both groups as regards to TNF-α (-863) A allele carriage (6.14 versus 10.43%; p = 0.099). Also the genotype TNF-α (-863) C/C and the frequency of the tumor necrosis factor-alpha (-863) C allele did not significantly differ between both groups (93.86 versus 89.57%; p = 0.099) and 96.93 versus 94.78%; p = 0.107) respectively. Our data indicated that the TNF-alpha (-863) allele is not linked with primary open angle glaucoma protection among Egyptian patients.

Keywords: Polymorphism, PCR-RFLP, POAG, TNF-α, Glaucoma

Introduction

Glaucoma is a progressive optic neuropathy associated with Retinal Ganglion Cell (RGC) degeneration-optic disc cupping and defects of the visual field. A crucial and common risk factor is Intraocular Pressure (IOP) elevation and about seventy million people have glaucoma throughout the world (Quigley and Broman, 2006). Primary Open Angle Glaucoma (POAG) and Primary Angle Closure Glaucoma (PACG) are subdivisions of Primary glaucoma (Shields, 2005), with POAG being more common in most populations, (AAOGP, 2010). The mechanism is multi factorial and complex neurodegeneration results from interactions between genetic, epigenetic, aging-related and other factors (Yang et al., 2011). Tumor necrosis factor-alpha is implicated in various inflammatory and immune reactions. It is mainly secreted by monocytes, but significant amounts are secreted also by other cell types (Beutler and Cerami, 1989). The clinical and investigational studies about the role of tumor necrosis factor-alpha in the etiology of glaucoma denote that ischemic glial cells release tumor necrosis factor-alpha, to prime retinal ganglion cells apoptosis and the degree of optic nerve degeneration is matched with the tumor necrosis factor-alpha expression from the retina and optic nerve head (Tezel and Wax, 2000).

The upstream region of the TNF-alpha locus at the Major Histocompatibility Complex (MHC) class III region on chromosome 6 p 21.3 is polymorphic and related to modifications in TNF-alpha production
(Hajeer and Hutchinson, 2001). While there may be some control on the tumor necrosis factor-alpha gene transcription, promoter region polymorphisms have been related to TNF-alpha gene expression or protein synthesis (Skoog et al., 1999) although with inconsistent results in various populations worldwide.

The most repeatedly investigated Single Nucleotide Polymorphisms (SNPs) in the upstream region are those at positions -238 and -308. A third SNP at position -863 has been studied less extensively (Lee and Song, 2015; Simionescu et al., 2015; Al-Dabbagh et al., 2011). The aim of this study was to determine whether this SNP affected the susceptibility of Egyptian patients to primary open angle glaucoma.

**Methods**

**Study Subjects**

This study included 228 primary open angle glaucoma patients and 230 healthy individuals as a control group. All participants were unrelated Egyptians matched for gender and age. The inclusion criteria were distinguishing markers in the monocular field of according to Anderson's criteria for minimal glaucoma abnormality, optic nerve heads cupping, open Anterior Chamber (AC) angles on gonioscopy, IOP of ≥21 mmHg at a point in the path of the disease and the absence of secondary causes of glaucoma (Suzumura et al., 2011). Subjects with a previous history of ocular traumatic injury or surgery or other ocular or systemic diseases were excluded. A written informed agreement was obtained as per the Declaration of Helsinki.

**Clinical Evaluation**

We took a thorough history including age, gender, duration of primary open angle glaucoma, medications and previous surgeries. We did a comprehensive ophthalmic examination for all subjects in the form of unaided and corrected visual acuity, slit lamp biomicroscopy, IOP measuring and indirect ophthalmoscopy. For the glaucoma group only, the central 30 degrees of the visual field was evaluated using Humphrey perimeter and he optic nerve head evaluation was done using Ocular Coherence Tomography (OCT).

**DNA Preparation and Genotyping**

We collected venous blood sample in 5 mL EDTA tubes then genomic DNA isolation was done using the QIAamp. DNA Blood MiniKit, (QIAGEN, Valencia, CA). The DNA concentration and purity was estimated by a Nanodrop spectrophotometer (Optima SP-3000+, Japan). Using a Nanodrop spectrophotometer (Optima SP-3000+, Japan), we estimated the concentration and the purity of DNA.

TNF-alpha (-863C/A) polymorphism was genotyped by PCR and detected by restriction fragment length polymorphism (PCR-RFLP) assay. The primers were: forward (5’-GGC TCT GAG GAA TGG GTT AC-3’) and reverse (5’ - CTA CAT GGC CCTGTCC TTC GTT ACG3’). DNA samples were amplified in a total reaction volume 25 µL aliquots containing 12.5 µL Taq PCR Master Mix (QIAGEN, Valencia, CA), 1 µL of each primer (0.4 µM final conc.), extracted DNA to 0.1 microgram final conc., RNase-free water to a total volume of 25 µL. The G- storm thermal cycler (Gene technologies. Ltd, England) was used for the amplification process according to the following program: 95°C, 1 min, (Initial denaturation) -95°C, 1 min, (denaturation) -57°C, 15 Sec, (annealing) -72 248°C, 45 Sec, (extension)- No. of cycles: 35 and final extension at 72°C for 5 min.

Products of amplification were signified by electrophoresis on 1.5% agarose gel (stained with ethidium bromide) and gave an expected PCR product size of 125 bp (Fig. 1), then digested with the Fast Digest Tail restriction endonuclease (Thermo-scientific - ThermoFisher Scientific Inc.). For digestion, the following reaction components were combined at room temperature: 17 µL nuclease-free water, 2µL 10 X Fast Digest Buffer, 10 µL PCR product and 1µL Fast Digest enzyme then mixed gently and incubated at 65°C in a heat block for 5 min. The digested products were detected using electrophoresis on a 1.5% agarose gel (stained with ethidium bromide). The bands were visualized using a uv Transilluminator (254 nm, Alpha Inno ztechcoporation, USA), then photographed and analyzed using a digital camera (Olympus. ED lens. 6.3 megapixel, USA). Photos were transferred to computerized analysis using Gel Documentation System (Alpha Innotech, USA).

The Tail restriction enzyme cleaved the mutated (-863 A) 125 bp allele (restriction sequence: ACGT), giving rise to fragments of 104 and 21 bp, of which only the 104 bp fragment was detected on the gel. Thus, in subjects homozygous for the wild-type (-863C) allele, a single band of 125 bp is observed; in heterozygous subjects (-863 C/A), two bands of 104 and 125 bp; and in subjects homozygous for the mutated (-863A) allele, a band of 104 bp is observed (Fig. 2). Selected samples underwent sequencing to ensure accuracy.

**Statistical Analysis**

All the analyses were done using SPSS, 20.0 (SPSS Inc., IBM, USA) and results were stated as mean ± Standard Deviation (SD). To assess the statistical differences between the categorical data, we employed the chi-squared test. The independent sample t test was used to compare variables. Any P value ≤ 0.05 reflected a statistically significant value.
**Results**

Our study included 228 Egyptian glaucoma patients (97 males and 131 females) and 230 healthy individuals (101 males and 129 females). Mean age was 67.4±5.9 years in the primary open angle glaucoma group and 66.9±4.3 years in the control group.

Genotype and allele frequencies of TNF-alpha-863C/A are shown in Table 1. In both groups none of the studied subjects were homozygous for the A/A allele and the A allele carriage in the primary open angle glaucoma group and the control group was 6.14 and 10.43% respectively (No statistically significant difference was found; p = 0.099) and so the frequency of the tumor necrosis factor-alpha (-863) A allele did not significantly vary between the two groups (3.07 versus 5.22%; p = 0.107). The C/C genotype in the primary open angle glaucoma group and the control group was 93.86 and 89.57% respectively (p = 0.099) and the frequency of the tumor necrosis factor-alpha (-863) C allele did not significantly vary between both groups (96.93 versus 94.78%; p = 0.107) respectively.

**Discussion**

This work is the first to address the role of the tumor necrosis factor-alpha (-863) gene polymorphisms in Egyptian patients with primary open angle glaucoma. Others have studied the G-380 SNP (Hamid et al., 2016) and found that it was related significantly with primary open angle glaucoma in another Egyptian population.
In our work, we determined the genotypes of the TNF-alpha (-863) polymorphisms in 228 patients with primary open angle glaucoma and 230 control subjects, matched for age and gender. Allelic frequencies and genotype distributions did not significantly differ between the two groups. This is in line with Funayama and associates (Funayama et al., 2004) who investigated the association between sequence variation in the Optineurin gene and the polymorphisms in the TNF-alpha promoter region, at positions -308, -857 and -863 in Japanese POAG patients. Optineurin expression is induced by tumor necrosis factor-alpha and interferes with adenoviral E3-14.7K protein which safeguards cells from the neurodegenerative effect of TNF-α. Funayama and associates found that there was no significant difference in genotype or allelic frequency was observed between POAG patients and Japanese control for the 3 SNPs of TNF-alpha, they also observed that POAG Patients who were TNF-alpha -863 A and optineurin/603A carriers had significantly worse (p = 0.026) visual field scores than those who were TNF-alpha-863A and non-optineurin/603A carriers. These contradictory outcomes may be owing to variable genotype distributions among different populations.

On the other side, our results are opposite to those of (Wang et al., 2012) who stated that the TNF-alpha -863 A allele is linked with primary open angle glaucoma protection in the Chinese as the AA genotype was less common in primary open angle glaucoma patients (7%) than in controls (11%), (p = 0.037) and the TNF-alpha -863A allele frequency was significantly decreased in the primary open angle glaucoma group (22 versus 30%, p = 0.007) while our results showed absence of the homozygous AA allele in the two groups and no statistically significant difference was found as regard to the A allele carriage (p = 0.099) and A allele frequency (p = 0.107). These contradictory outcomes may be owing to variable genotype distributions among different populations.

Our results suggest that the TNF-alpha -863A allele is not linked with primary open angle glaucoma protection, but we do not exclude the role of TNF-alpha -863 polymorphism in the glaucoma pathogenesis as neurodegeneration results from interactions between genetic and epigenetic factors and so further studies that correlate the association of TNF-alpha -863 polymorphism with polymorphisms in other genes impacted in POAG like Optineurin gene should be followed. Our work was restricted by the relatively small number of subjects and so, population-based studies are needed.

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Author’s Contributions

Walaa Bayoumie Abdulwahhab: Conceived and designed the research, molecular biology technique, analyzed and interpreted the data, critical revision of the manuscript and corresponding author.

Shaymaa Mohamed Abd El Rahman: Molecular biology technique, drafted the manuscript, revision of the manuscript.

Mohamed Nagy Elmohamady: Collecting samples and clinical data, writing and revision of the manuscript.

Ahmed Mohamed Saed: Collecting samples and clinical data.

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Disclosure of Interest

There is no conflict of interest that I should disclose.

References


