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Original Article

Evaluation of transforming growth factor-beta1 gene expression in pterygium tissue of atopic patients

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Abstract

Background: The exact pathogenesis of pterygium is still not fully understood. Growth factors are considered to play an important role in the formation of pterygium. Transforming growth factor (TGF)-β1 is considered to be one of the main mediators of fibroblast stimulation and tissue remodeling in allergic conditions. The objective of the present study was to investigate the association between TGF-β1 gene expression and pterygium in atopic and nonatopic participants.

Methods: We used questionnaires to record demographic and clinical information from patients who underwent pterygium excision surgery. Skin prick examination was done to confirm or rule out atopy in 30 patients with atopy (Case Group) and 30 individuals without atopy (Control Group). Additionally, measurement of serum immunoglobulin E, cytokines, including interleukin-4 and interferon-γ, and peripheral blood eosinophil count was performed to confirm atopy in 30 consecutive patients (Case Group). A semiquantitative reverse transcription polymerase chain reaction was performed to determine TGF-β1 gene expression in all individuals.

Results: TGF-β1 mRNA gene expression was significantly higher ($p = 0.0001$) in atopic patients 2.50 ± 1.11 compared to nonatopic individuals 1.40 ± 0.46. Eosinophil count and serum immunoglobulin E were significantly higher ($p = 0.031$ and $p = 0.001$, respectively) in atopic patients compared to the Control Group. Serum interleukin-4 was also significantly higher ($p = 0.01$) in atopic patients compared with nonatopic individuals.

Conclusion: Excess expression of TGF-β1 gene in pterygium tissue of atopic individuals suggests that growth factors play a role in the pathogenesis of pterygium.

Keywords: atopy; gene expression; pterygium; transforming growth factor-β1

1. Introduction

Pterygium is a benign condition, characterized by invasive fibrovascular growth of the conjunctiva to the cornea, which is generally linked with overexposure to UV radiation.[1] Although some environmental factors (e.g., wind, dust, heat, infection, smoke, chemicals, dry eye, and pollens) are suggested to play a role in the pathogenesis of pterygium,[1–3] the exact etiology of this condition is still not fully understood.

Conflict of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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Some investigators have proposed an allergic and immunological basis for the pathogenesis of pterygium.[4,5] Several growth factors, cytokines, and metalloproteinase enzymes are identified in the cornea during the recovery time after photorefractive keratectomy. These biological factors, which are produced by migratory leukocytes, are considered to play an important role in the formation of pterygium.[3] In addition, it has been demonstrated that UV may activate signaling pathways in the epithelial cells of pterygium, resulting in production of cytokines and growth factors.[6]

Both acute and chronic complications have been observed in atopic patients due to the release of inflammatory mediators. Transforming growth factor (TGF)-β1 is considered to be one of the main mediators of tissue remodeling in patients with asthma.[7] It stimulates fibroblasts to produce extracellular matrix proteins and cell-adhesion molecules such as collagen, fibronectin, and integrins; decreases production of collagenase, heparinase, and stromelysin; and results in extracellular matrix deposition. Additionally, it directly induces angiogenesis *in vivo*. Therefore, TGF-β1 is considered an effective regulator of tissue invasion and metastasis.[8] Stroma of pterygium cells and fibroblasts plays a fundamental role in the remodeling process of pterygium tissue. Matrix metalloproteinases have been found in these cells.[9] Furthermore, TGF-β1 enhances matrix metalloproteinase expression and fibroblast activity.[10]

To the best of our knowledge, no previous investigation has considered the association between pterygium and TGF-β1 levels in atopic patients. In the present study, TGF-β1 mRNA gene expression in pterygium tissue of atopic patients was evaluated to investigate its probable association with increased susceptibility for pterygium formation. In addition, the present study aimed to determine if TGF-β1 overexpression in atopic individuals promotes tissue remodeling in pterygium formation. The histopathological differences in pterygium between atopic and nonatopic patients have also been investigated.

2. Methods

2.1. Study population

This study was conducted in accordance with the Helsinki Declaration of 1975 (as revised in 1983) and approved by the Research Ethics Committee of Islamic Azad University of Mashhad, Mashhad, Iran. Informed consent was obtained from all participants after the nature of the study was explained. Predesigned questionnaires were used to record demographic information and past medical history of individuals who were diagnosed with pterygium and referred to the Eye Hospital of Mashhad University of Medical Sciences from June 2010 to May 2011. Thereafter, 30 pterygium patients without any history of allergic reactions were enrolled in the Control Group. Those with a history of at least one allergic condition (e.g., asthma, allergic rhinitis, atopic dermatitis, hives or angioedema, or food allergies) underwent skin prick testing and measurement of total serum immunoglobulin (Ig)E level. Correspondingly, 30 pterygium patients with positive skin prick test and IgE level > 100 IU/mL were included in the Case Group. According to ophthalmological examination, surgical excision of pterygium was performed on all 60 patients who were enrolled in the study. The exclusion criteria were previous treatment with corticosteroids during the past 2 months, immunodeficiency, and absence of indication for excisional surgery. Participants aged ≥ 60 years were also excluded from the study population due to decreased wheal and flare in skin prick tests.[11] In addition, patients were advised to withdraw from the use of drugs and medications at least 48 hours before the skin test because some topical corticosteroids or antihistamines may affect the validity of skin prick tests.[12] Additionally, we certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

2.2. Immunological assessment

All patients were evaluated by skin prick test on the inner forearm, using 19 common standard allergen extracts (Stallergenes, Antony, France; Hollister−Stier Laboratories, Spokane, WA, USA). Blood samples were obtained by

Table 1
Sequence of primers used in this study.

mRNA	*Primer*
TGF-β1 mRNA (137 bp, RT-PCR)	Sense primer: 5′-AAGGACCTCGGCTGGAAGTG-3′ Anti-sense primer: 5′-CCCGGGTTATGCTGGTTGTA-3′
GAPDH mRNA (266 bp, RT-PCR)	Sense primer: 5′-GGAAGGTGAAGGTCGGAGTCA-3′ Anti-sense primer: 5′-GTCATTGATGGCAACAA TATCCACT-3′

GAPDH = glyceraldehyde-3-phosphate dehydrogenase; RT-PCR = reverse transcription polymerase chain reaction; TGF-β1 = transforming growth factor-β1.

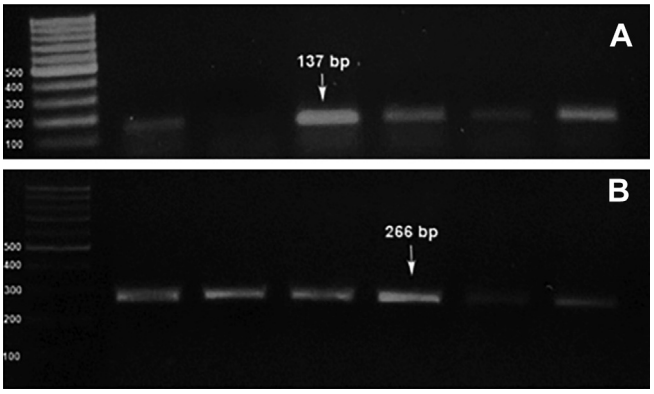

Fig. 1. Transforming growth factor (TGF)-β1 gene expression compared with the ladder was measured using reverse transcription polymerase chain reaction (RT-PCR). RT-PCR products from TGF-β1 mRNA with 137-bp fragment (A) and glyceraldehyde-3-phosphate dehydrogenase mRNA with 266-bp fragment (B) were separated on agarose gels and stained with green viewer. According to the Mann−Whitney *U* test, mean relative expression level of TGF-β1 mRNA was significantly higher ($p = 0.0001$) in atopic patients (2.50 ± 1.11) compared with that in nonatopic individuals (1.40 ± 0.46).