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ARAŞTIRMA / RESEARCH

The role of mast cell in pterygium recurrence

Pterjium nüksünde mast hücresinin rolü

Meydan Turan[1], Gülay Turan[2]

[1]Balıkesir Ataturk City Hospital, Ophthalmology Clinic, Balıkesir, Turkey
[2]Balıkesir University Faculty of Medicine, Department of Pathology, Balıkesir, Turkey

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Abstract

Purpose: To investigate the role in pterygium recurrence of mast cell, which is known to play a role in inflammatory and immunological events, we aimed to determine the mast cell number in primary and recurrent pterygium tissue samples and to determine its relationship with angiogenesis.

Materials and Methods: The study included 61 patients with pterygium who underwent primary pterygium excision with a bare sclera technique, but recurrent after a while. Primary and recurrent pterygium tissue samples of the same patient were examined histopathologically. The mean mast cell count was calculated by counting from 3 different areas under light microscope at 400 x magnification. In addition, the number of vessels was scored at 200x magnification.

Results: 34 of the patients were male and 27 were female. The mean age was 57.02 ± 7.96. The mean mast cell count was 8.46±2.52 in the primary pterygium tissue and 14.07±3.05 in the recurrent pterygium tissue. The mean vascular count was 12.90 ± 3.17 in primary pterygium tissue and 16.21 ± 2.24 in recurrent pterygium. Statistical analysis revealed a significant increase in the number of mast cells and vascular number in the recurrent pterygium. Mast cell number and vascularity were correlated in primary pterygium and recurrent pterygium tissue samples.

Conclusion: Increased mast cell in pterygium tissue may play an important role in the recurrence of pterygium. Determining mast cell count in preventing pterygium recurrence may be important.

Keywords: Mast cell, pterygium, recurrence, angiogenesis

Öz

Amaç: İnflamatuar ve immünolojik olaylarda rol oynadığı bilinen mast hücresinin pterygium nüksündeki rolünü araştırmak için primer ve tekrarlayan pterygium doku örneklerinde mast hücre sayısını belirlemeyi ve anjiyogenez ile ilişkisini saptamayı amaçladık.

Gereç ve Yöntem: Çalışmaya çıplak sklera tekniği ile primer pterjiyum eksizyonu yapılan, ancak bir süre sonra tekrarlayan pterjiyumlu 61 hasta dahil edildi. Aynı hastanın primer ve tekrarlayan pterygium doku örnekleri histopatolojik olarak incelendi. Ortalama mast hücre sayısı, 400x büyütmede ışık mikroskobu altında 3 farklı alandan sayılarak hesaplandı. Ek olarak, damarların sayısı 200x büyütmede skorlandı.

Bulgular: Hastaların 34'ü erkek, 27'si kadındı. Yaş ortalaması 57.02 ± 7.96 yıl idi. Ortalama mast hücre sayısı, primer pterjiyum dokusunda 8.46 ± 2.52, tekrarlayan pterjiyum dokusunda 14.07 ± 3.05 idi. Ortalama vasküler sayı primer pterjiyum dokusunda 12.90 ± 3.17 ve tekrarlayan pterjiyumda 16.21 ± 2.24 idi. İstatistiksel analiz, tekrarlayan pterjiyumda mast hücre sayısında ve vasküler sayıda anlamlı bir artış olduğunu gösterdi. Primer pterjiyum ve tekrarlayan pterjiyum doku örneklerinde mast hücre sayısı ve vaskülerite korelasyon göstermekteydi.

Sonuç: Pterjium dokusunda artmış mast hücresi, pterjiumun nüksünde önemli bir rol oynayabilir. Pterjium nüksünün önlenmesinde mast hücre sayısının belirlenmesi önemli olabilir.

Anahtar kelimeler: Mast hücresi, pterjiyum, nüks, anjiyogenez.

Yazışma Adresi/Address for Correspondence: Dr. Meydan Turan, Balikesir Ataturk City Hospital, Ophthalmology Clinic, Balıkesir, Turkey E-mail: meydanturan@gmail.com
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INTRODUCTION

Pterygium, one of the common lesions of the ocular surface, is characterized by the triangular or wing-like abnormal conjunctiva growing on the cornea. Pterygium exhibits proliferative and inflammatory properties as well as degenerative and hyperplastic changes[1]. Although the pathogenesis of pterygium is still unclear, many different factors have been blamed. For example, proinflammatory cytokines such as interleukin (IL) -1, IL-6, IL-8 and tumor necrosis factor (TNF) -α, which are secreted after exposure to chronic ultraviolet (UV) radiation[2,3]. Inflammation induces angiogenic pathways resulting in neovascularization contributing to the development and growth of the pterygium[4]. Morphological changes in the pterygium as well as immunological changes are seen. The presence of IgG and IgE detected by immunofluorescent methods in the pterygium tissue was evidence of this[5]. Mast cells (MC) were first described by Ehrlich as connective tissue cells with large granules in the sense of "mastzellen-eating cell"[6]. The most effective factor in maintaining the development, differentiation, proliferation, adhesion, tissue migration, activation and viability of MC is the chemotherapeutic factor (Stem cell factor = c kit ligand)[7].

MC play an important role in angiogenesis in chronic inflammatory diseases and tumors[8]. Previous studies have shown a significant relationship between MC and microvessel numbers in malignancies such as B-cell non-Hodgkin's lymphoma, multiple myeloma, myelodysplastic syndromes, B-cell chronic lymphocytic leukemia, malignant melanoma and endometrial carcinoma[8-10]. However MC count has been reported to increase in tumor angiogenesis sites and stimulate vascular endothelial cells[11]. In addition MC include various cytokines, such as TGF-α, TNF-β, interleukin-8 (IL-8), FGF-2 and VEGF, which have a role in neovascularization[12].

The aim of this study was to determine the numbers of MC in primary pterygium and recurrent pterygium tissues and to evaluate the relationship between neovascularization and MC.

MATERIALS AND METHODS:

A total of 61 patients who underwent surgery for both primary and recurrent pterygium were included in this study between January 2011 and January 2019 at Balikesir Ataturk City Hospital. The research was conducted in accordance with the Helsinki Committee requirement. Before starting the study, the approval of the ethics committee was obtained from the Corporate Ethics Committee of Balikesir University Faculty of Medicine (Date: 10.04.2019 / Registration number: 2019/59). The exclusion criteria were as follows: (1) patients with a history of ocular surgery except pterygium, (2) patients with inflammatory or infectious disease, (3) patients with ophthalmic or systemic autoimmune disease, and (4) patients using topical or systemic drugs in last month. Excision of the pterygium was performed using the bare sclera technique. 10% formalin-fixed samples were embedded in paraffin and sections of 4 micron thickness were taken onto the glass slide. After deparaffinization and rehydration, samples stained with hematoxylin and eosin for histopathological evaluation. On histologic examination of primary and recurrent pterygium tissues of the same patient, we evaluated MC count and stromal vascularity. We scored the vascularization of ptergium by light microscopy under X200 magnification. In the stroma of tissue samples, the average MC count was obtained by counting in 3 separate regions under X400 magnification by light microscopy (The cell count is made at 400x magnification, which is equal to 0.23 mm^2).

Statistical analysis

The data obtained for statistical analysis were transferred to SPSS Statistical Software Program. Age, sex distribution, mean, standard deviation (SD) or median (min-max) values were determined by using version SPSS 20.0 (Chicago, IL, USA). Continuous variables were determined by using Kolmogorov-Smirnov test. Paired Student's t-test was used to compare the samples. Chi-square test was used to compare categorical data. P value <0.05 was considered significant.

RESULTS

The mean age of the patients was 57.02 ± 7.96 years and 34 were male and 27 were female. The mean mast cell count was 8.46±2.52 in the primary pterygium tissue and 14.07±3.05 in the recurrent pterygium tissue. The number of mast cells in the recurrent pterygium tissue was significantly higher ($p < 0.001$) (Table 1), (Figure1 A, B). The mean

vascular count was 12.90±3.17 in the primary pterygium tissue and 16.21±2.24 in the recurrent pterygium. When the mean number of vessels in the primary and recurrent pterygium tissues were compared, a significantly increased number of vessels was observed in the recurrent pterygium ($p<0.001$) (Table 1), (Figure 1 A, B). There were a positive correlation between MC count and new vessel formation both primary pterygium and recurrent pterygium groups ($p<0.001$, $r= 0.916$; $p<0.001$, $r= 0.958$, respectively).

Table 1. Comparison of mast cell count and number of vessels in primary and recurrent pterygium tissue samples.

Histopathological findings	Primary pterygium (n=61)	Recurrent pterygium (n=61)	p value*
Mast cell counts	8.46±2.52	14.07±3.05	<0.001
Microvessel counts	12.90±3.17	16.21±2.24	<0.001

*Paired Student's t-test.

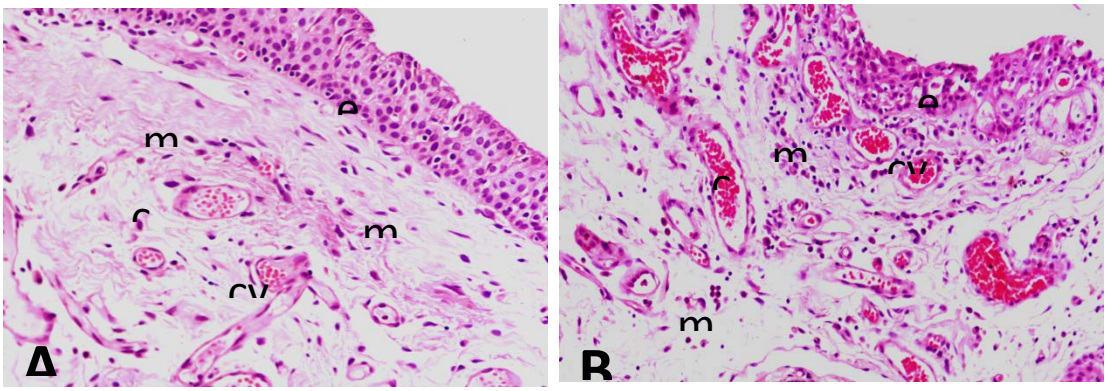

Figure 1. (A) Primary pterygium tissue sample. Increased mast cells and microvessels under the epithelium. Hematoxylin and eosin staining, x400. (ep: epithelial; mc: mast cells; cv:capillary vessel). (B) Reccurrent pterygium tissue sample. Increased mast cells and microvessels under the epithelium. Hematoxylin and eosin staining, x200. (ep: epithelial; mc: mast cells; cv:capillary vessel). MC is generally located near vascular structures in areas where intense angiogenic activity is observed.

DISCUSSION

Pterygium is a proliferative and invasive tissue with increased vascularity, forming a wing-like shape and entering the cornea[13]. Pterygium tissue showing histologically active growth usually exhibits degenerative and hyperplastic changes. It also contains proliferative and inflammatory findings. Pterygium may develop from a conjunctival epithelium containing normal or mild hyperplasia[14]. Effective treatment of pterygium is still surgical excision. However recurrence rates after surgery vary between 40-80%[15]. In addition, recurrent ptergium has more aggressive features than primary pterygium[16]. Mast cells have an important role in the destruction and reorganization of collagen. In the pterygium, the conjunctival stroma is damaged or destroyed[11]. Mast cells induce leukocyte infiltration by influencing vascular endothelium and leukocytes through TNF-α. This results in inflammation, angiogenesis and fibrosis[17]. The authors compared MC count in normal conjunctiva and pterygium tissue and found that there were two-fold more mast cells in pterygium tissue[11]. Furthermore Zhong et al. compared the number of MC in the normal conjunctiva, primary pterygium and recurrent pterygium and found that the number of MC in both primary pterygium and recurrent pterygium was higher[18]. There are few studies about pathophysiological changes in primary and recurrent pterygium in the literature. In our study, increased MC and microvessel number in pterygium tissue were associated with pterygium formation and recurrence. MC play an important role in chronic inflammation and immune reactions. In addition, MC synthesizes and releases many angiogenic

growth factors involved in the formation of new vessels[19].

Blair et al. reported that tryptase released by MC has an important role in neovascularization[20]. They have found that the addition of tryptase directly to vascular endothelial cells in cultures causes capillary growth. In addition, it has been found that tryptase causes dose-dependent endothelial cell proliferation. In MC granules, there are high levels of tryptase in heparin proteoglycan macromolecules. It is known that tryptase needs to interact with heparin to maintain its enzymatic activity[21]. Ribatti et al. Reported that tryptase positive MC was associated with angiogenesis which is involved in the development of pterygium. They suggested that this association is mediated by some cytokines released by epithelial, endothelial and inflammatory cells[22]. Aspiotis et al. reported significantly increased microvessel density in pterygium tissue compared to normal conjunctiva. They also stated that the most powerful angiogenetic factor was VEGF[23]. Liang et al. showed that MC in pterygium supported the formation of new vessels and there was a correlation between them[24]. In the present study, there was a positive correlation between MC number and neovascularization in both primary and recurrent pterygium. Celebi et al. compared MC number in primary and recurrent pterygium tissues, and they found that the number of MC in the recurrent pterygium was greater[25]. They suggested that MC could play a role in the recurrent of pterygium. In the study of Karahan et al. MC number in primary and recurrent pterygium tissue samples were compared, 52 primary pterygium and 12 recurrent pterygium tissues were examined. Mean mast cell count was 3.84 ± 2.45 in primary pterygium tissue and 5.08 ± 1.83 in recurrent pterygium[26]. Although the mean number of mast cells in the recurrent pterygium tissue was higher than the primary tissue, the difference was not statistically significant. In our study, the MC number of primary and recurrent pterygium tissue of the same patient was compared and the number of MCs in the recurrent pterygium was found statistically significant. We think that the reason why the results of Karahan et al. are not significant is due to the small number of patients with recurrent pterygium. The increase in vascular proliferation in recurrent pterygium has been reported to be associated with an increased number of MCs[27]. In this study, the number of MC correlated with increased neovascularization in recurrent pterygium supported this view. In our study, unlike other studies, mast cell count and angiogenesis were compared in both primary and recurrent pterygium samples of the same patient. There were some limitations in this study. As a retrospective study, the control group could not be established. Also mast cells and vascular structures were not immunohistochemically stained. Further studies are needed in this area.

There are few studies about the histopathological features of primary and recurrent pterygium. Our results showed that mast cells have an important role in the pathogenesis of pterygium. However, the number of mast cells in the recurrent pterygium was significantly higher than in the primary pterygium. Therefore, mast cell can be an important factor in recurrence of pterygium.

Yazar Katkıları: Çalışma konsepti/Tasarımı: MT, GT; Veri toplama: MT, GT; Veri analizi ve yorumlama: MT, GT; Yazı taslağı: MT, GT; İçeriğin eleştirel incelenmesi: MT, GT; Son onay ve sorumluluk: MT, GT; Teknik ve malzeme desteği: GT; Süpervizyon: GT; Fon sağlama (mevcut ise): yok.
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