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Mast cells and angiogenesis in primary and recurrent pterygia

Primer ve nüks piterijiyumlarda mast hücreleri ve anjiyogenezis

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ABSTRACT

Pterygium is a common benign lesion of limbus but the pathogenesis are not completely understood. Pterygia have a chronic inflammatory cellular infiltrate and a rich vasculature. Mast cells are a heterogeneous group of multifunctional tissue-resident cells. It has been suggested that mast cells and their products may be responsible for the formation of new blood vessels. We investigated the number and phenotype of mast cells and neovascularization in pterygia specimens and compared with those in normal conjunctival specimens

Pterygia tissues were obtained during excisional surgery from 32 eyes of 32 consecutive patients. Seventeen of all cases were recurrent pterygia. Superior bulbar conjunctival tissue from the same eye was also sampled as control tissues. The tissue sections were stained with routine hematoxyline-eosin and toluidine blue stain for mast cells. For immunohistochemical studies anti-factor VIII-related antigen, monoclonal anti human mast cell tryptase and chymase were used as an endothelial and mast cell marker.

The mean number of mast cells in pterygia was significantly higher than that in the normal conjunctival tissue and microvessel counts was significantly higher than the counts of the controls in both primary and recurrent pterygia. There was no correlation between microvessel numbers and mast cell numbers. There was no phenotypic difference between the mast cells in the ptergyia and those in the normal conjunctival tissues.

This study confirms that mast cells are prominent in pterygia and our results suggest that mast cells and angiogenesis are independent factors in the genesis and progress of ptergyium.

Key words: Angiogenesis, chymase, tryptase, mast cell, pterygium

ÖZET

Piterijiyum limbusun en yaygın, iyi huylu lezyonudur ama patogenezi tam olarak bilinmemektedir. Piterijiyumlar kronik iltihabi infiltrasyona sahip ve damardan zengindir. Mast hücreleri dokularda bulunan, bir çok görevi olan heterojen bir grup hücredir. Mast hücreleri ve ürünlerinin yeni damar oluşumuna neden olabileceği öne sürülmüştür. Bu çalışmada piterijiyum örneklerinde mast hücre sayısı ve fenotipleri ile yeni damar oluşu incelendi ve normal konjonktival doku ile karşılaştırıldı.

Piterijiyum dokuları 32 hastadan eksizyonel biyopsi olarak elde edildi. Olguların 17 tanesi nüks piterijiyum idi. Kontrol olarak aynı göz üst bulbar konjonktiva dokusu örneklendi. Doku kesitleri rutin hematoksilen-eozin, mast hücreleri için toluidin mavisi ile boyandı. İmmunhistokimyasal çalışmalarda endotel ve mast hücre belirleyicisi olarak anti-faktörVIII ilişkili antijen, anti mast hücre triptazı ve kimazı kullanıldı.

Primer veya nüks piterijiyumlarda ortalama mast hücre ve mikrodamar sayısı normal konjonktiva dokusuna göre anlamlı olarak yüksekti. Mikrodamar sayısı ve mast hücre sayısı arasında bir ilişki yoktu. Normal konjonktiva dokusu ile piterijiyumlarda bulunan mast hücreleri arasında fenotipik farklılık yoktu.

Bu çalışma mast hücrelerinin piterijiyumlarda baskın olarak bulunduğunu desteklemektedir. Sonuçlarımız damar oluşumu ve mast hücrelerinin piterijiyumların oluşma ve gelişmesinde bağımsız faktörler olduğunu düşündürmektedir.

Anahtar sözcükler: Anjiogenez, kimaz, triptaz, mast hücresi, piterijiyum

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INTRODUCTION

Pterygium is a common lesion of limbus encountered in Turkey. Environmental factors, such as ultraviolet irradiation have been suggested as the main causative factor in the development of the disease. However, the aetiopathogenesis of pterygium remains obscure (1).

Pterygium is an active, invasive, inflammatory process. In a two-stage process, 'conjunctivalization' of the cornea occurs with tissue characterized by extensive chronic inflammation, cellular proliferation, connective tissue remodeling and angiogenesis. Mast cells (MCs) are known to have important roles not only in allergic-type reactions, but also in chronic inflammatory and collagen-vascular diseases (1,2). It has also been suggested that secreting mast cells are able to induce and enhance angiogenesis via multiple, and partly interacting pathways (3,4).

Mast cells are composed of groups of cells that are heterogeneous with respect to structure and function. On the basis of their content of neutral proteases, human mast cells have been divided into two phenotypes. One is the tryptase positive containing tryptase but not chymase: This is the predominant type observed in alveoli of the lung and in the small intestinal mucosa. The other is the tryptase positive, chymase positive mast cell, which is the predominant type observed in the skin and in the small intestinal submucosa. The phenotypic characteristics of a mast cell population can be changed by alterations of pathological conditions. Distribution of mast cell subset may have important pathogenetic and therapeutic implications (5-7).

In the present study, the number and phenotype of mast cells and their possible role in blood vessel formation in primary and recurrent pterygia specimens were examined and compared with those in normal conjunctival specimens.

MATERIALS and METHODS

Pterygia tissues were obtained during excisional surgery from 32 eyes of 32 consecutive patients with primary pterygium and recurrent pterygia tissues were obtained from 17 patients. Duration of the pterygia was not considered. Superior bulbar conjunctival tissue from the same eye was also sampled as control in 49 eyes. Pterygia which invaded less than 1mm into the cornea were excluded. Patients who had ocular pathology other than pterygium or a history of any systemic immune disease and atrophic pterygia were excluded.

The tissues were fixed in buffered formalin and processed for paraffin embedding. Sections were prepared with HE and stained for 10 minutes with 1% toluidine blue (pH 4.1) for mast cells. Metachromatic cells were counted as mast cells.

The number of mast cells was counted at three high power (x400) consecutive or nonoverlapping fields by using a light microscope (Olympus BX-50). The mean mast cell number was recorded. Neovascularization was determined in 24 cases of 32 primary pterygium and 17 cases of recurrent pyterygium. Sections of eight samples were inadequate for determination of neovascularization and they were excluded. An immunohistochemical study was carried out using a labeled streptavidin-biotin peroxidase technique. The primary antibodies used in this study were monoclonal mouse anti human mast cell tryptase (Neomarkers, LabVision Corp, CA, USA), monoclonal mouse anti human mast cell chymase (Neomarkers, LabVision Coop, CA, USA), and polyclonal FVIII related protein (Neomarkers, LabVision Corp, CA, USA). Incubation with each of the primary antibodies was performed for 30 minutes at room temperature. Sections were deparaffinized and then enzymatically treated with pepsin for 10 minutes at room temperature, and before incubating with anti FVIII antibody. The slides were immersed in antigen retrieval solution and were heated in

a microwave oven for 10 minutes at 650 W for anti-mast cell tryptase or chymase antibodies. The antigen-antibody immunoreaction was visualized using aminoethylcarbazol (AEC) as chromogen.

Microvessel density was counted using Olympus BX-50 microscope. Areas of the tissue containing the highest density of capillaries and small venules were identified. Large caliber vessels were omitted and even single cells with positive staining were counted as a microvessel. Three different fields were counted with x400 magnification in the most intensely stained area. Mean numbers were based on these three counts for each pterygia and control cases.

Statistical analysis was performed with SPSS version 9.05 for windows using Wilcoxon signed ranks test. A p value of less than 0.05 was considered significant. Correlation between parameters was studied using the Pearson correlation test.

RESULTS

The sex distribution of these 49 patients was 28 males, 21 females and the mean age was 55.0 years (range 38 to 73). The mean duration of the lesion was 2.5±1.0 mm and 3.6±1.0 mm for primary and recurrent pterygia respectively. All the pterygia were in nasal localization.

Histopathologically, all mast cells were identified as mononuclear cells with metachromatic granules in substantia propria. Many of mast cells in pterygia and normal conjunctival tissues were observed beneath the epithelium and around the blood vessels (Figure 1a,b). The mean mast cell counts in primary pterygia was 17.39±8.74 and 8.36±3.0 in control specimens (Table 1).

In the pterygia and the normal conjunctival tissues, all tryptase-positive cells and all chyma-

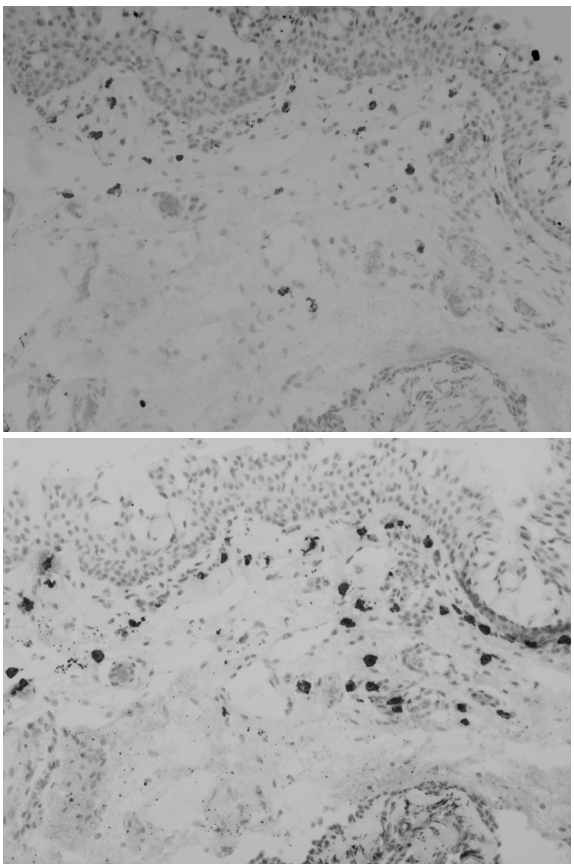

Figure 1. Immunohistochemical staining with monoclonal antibodies directed to chymase (a), and tryptase (b) performed on adjacent sequential sections in a recurrent pterygium (x400).

Tablo 1. Mast cell counts in pterygia and normal bulbar conjunctiva.

	Metachromatic cells (Toluidine blue staining) mean±SD	Chymase (+) cells mean±SD	Tryptase (+) cells mean±SD	Microvessel number mean±SD
Primary pterygia	17.39±8.7	18.09±10.7	17.53± 8.9	36.4±7.4
Normal conjunctival tissue in primary pterygia	8.36±3.0	15.4±6.2	13.29±5.3	21.37±7.8
Recurrent pterygia	25.35±8.4	19.08±3.9	25.30±6.2	13.18±2.2
Normal conjunctival tissue in recurrent pterygia	13.01±5.8	11.16±5.0	12.96±5.5	7.26±1.5

se-positive cells were observed beneath the epithelium and around the blood vessels. The mean number of tryptase-positive and chymase positive cells in primary and recurrent pterygia was significantly higher than that in the normal conjunctival tissues ($p<0.001$). Although mast cell count was different in primary and recurrent pterygia, the difference was not statistically significant ($p>0.5$).

The numbers of mast cells which stained with toluidine blue in pterygia or control conjunctival tissues correlated with the immunohistochemical staining. There was no statistically significant correlation between mast cell count and pterygia size (Pearson correlation coefficient $r=0.086$, $p=0.66$).

The average number of blood vessels both of primary and recurrent pterygia was significantly higher than that in control tissues ($p<0.05$). There was no correlation between microvessel density and mast cell counts.

DISCUSSION

The exact pathogenesis of pterygium is not yet clearly understood. The most common theory concerning the origin and pathogenesis of pterygium describes the association of the disease with environmental factors such as dust, smoke and ultraviolet radiation (1,5). Recent evidence suggests that human papilloma virus may be involved in the pathogenesis of pterygia (8). Histologically, actively growing pterygia exhibit degenerative changes. Granular-appearing material beneath the epithelium resembles degenerated collagen and elastic fibers. Pterygia have a chronic inflammatory cellular infiltrate (lymphocytes, mast cells) and a rich vasculature (9). Lymphocytes are predominantly T-cells. In addition, deposition of IgE and IgG in pterygium hasbeen reported (9,10). Mast cells play an important role in inflammation releasing stored and newly synthesized inflammatory mediators, including heparin, histamin, metallo- and serine proteases, and various growth factors, following activation. Thus, an increase in mast cells has been observed not only in allergy, but also in nonallergic chronic inflammation, angiogenesis, fibrosis, and tissue remodeling (2,3,7-10).

The number of mast cells in pterygia has been reported to be higher than in normal conjunctiva in previous studies in which mast cells were detected by metachromatic dye staining or by morphological characteristics (11-14). It has been reported that the number of the mast cells increased in vernal and allergic conjunctivitis, as compared to normal conjunctiva. Phenotype of the increased mast cells, were predominantly tryptase- positive in vernal and allergic conjunctivitis, whereas chymase-positive mast cells were the predominant type in normal conjunctiva (7,14). In the present study, although the number of mast cells was confirmed to be increased in the primary and recurrent pterygia, no significant difference was found in the phenotype of mast cells between pterygia and normal conjunctival tissues. Similar results were reported by Nakagami et al. (12).

Beden et al. (16) showed that the difference in mast cell numbers between pterygia and control groups was not significant and they suggested that cellular immunity plays an important role in pterygia formation.

There is much evidence to suggest a link between mast cells and angiogenesis. Mast cells stimulate the proliferation of microvascular endothelial cells in tissue culture and accumulate markedly in tumor angiogenesis (3,4,17-20). Many components of mast cells are angiogenic or can modulate the angiogenesis process. These include basic fibroblast growth factor, vascular endothelial growth factor, heparin, heparinase, histamine, tumor necrosis factor-α and various proteases. However, Ghosh et al. (21) demonstrated that histamine derived from other inflammatory cells plays a significant role in angiogenesis of the inflammatory granulation tissue. Also, Egozi et al. (22) suggested that mast cells modulate the recruitment of neutrophils into sites of injury, yet indicate that mast cells are

unlikely to exert a major influence on the proliferative response within healing wounds, including reepithelization, collagen synthesis, and angiogenesis. No correlation was found between the vascular density and the number of mast cells in pathological and surgical scars in another study (23).

Our results confirmed the increase in the number of mast cells in pterygia compared with normal conjunctival tissues. Mast cells may be a factor in pathogenesis of pterygia or may participate during its development. Mast cell and angiogenesis are independent factors in the genesis of ptergyium. Other inflammatory cells may play a significant role in the angiogenesis of the pterygia.

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