

# Mast cell density in pterygium, and its association with ultraviolet exposure in different climatic conditions: A series of 140 cases

## Piterijiyumda mast hücreleri ve farklı iklim şartlarındaki ultraviyole etkisi ilişkisi: 140 olguluk seri çalışma

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### ABSTRACT

This study compared the number of mast cells and histopathologic findings in 140 randomly selected pterygium and 30 normal conjunctival specimens. The pterygium specimens came from medical centers in two regions of Turkey with different climatic conditions (Adana with high sunlight exposure and Ankara with relatively low UV ray exposure). Slides were stained with routine H&E and toluidine blue to detect mast cells. The mean mast cell counts for 140 pterygium and control specimens were  $17.9 \pm 8.39$  and  $9.23 \pm 6.86$  and the difference was statistically significant ( $p < 0.05$ ). The mean counts estimated in Adana and Ankara specimens were  $18.44 \pm 8.77$  and  $16.51 \pm 7.23$  respectively without any statistically significant difference. The pterygium specimens from Adana ( $n=101$ ) had demonstrated a significantly higher frequency of inflammation (six times higher) than those from Ankara ( $n=39$ ). This finding is related to increase of mast cell degranulation via ultraviolet rays. The results suggest that mast cells are actively involved in the pathogenesis of pterygium and ongoing changes in these lesions and many factors other than UV rays might directly influence or induce mast cell-related effects that are involved in the pathogenesis of pterygium.

**Key words:** Pterygium, mast cell, ultraviolet

### ÖZET

Bu çalışmada rasgele seçilmiş 140 piterijiyum olgusundaki ve 30 normal konjunktiva dokusundaki histopatolojik bulgular ve mast hücre sayıları karşılaştırılmıştır. Piterijiyum örnekleri Türkiye'deki farklı iklim özelliklerine sahip iki bölgedeki merkezlerden toplanmıştır (Yüksek oranda güneş ışığı alan Adana bölgesi ve nispeten daha az güneş ışığı alan Ankara bölgesi). Kesitler rutin H&E ile ve mast hücrelerini saptamak için toluidin mavisi ile boyandı. Yüz kırk piterijiyum olgusundaki toplam mast hücre sayısının ortalaması  $17.9 \pm 8.39$  iken kontrol örneklerindeki ortalama mast hücre sayısı  $9.23 \pm 6.86$  idi ve bu fark istatistiksel olarak anlamlı bulundu. Adana ve Ankara örneklerindeki toplam mast hücre sayısı ortalaması sırasıyla  $18.44 \pm 8.77$  ve  $16.51 \pm 7.23$  olup istatistiksel olarak anlamlı bir fark yoktu. Adana bölgesi örneklerinde (101 olgu) inflamasyon Ankara bölgesi örneklerine (39 olgu) oranla 6 kat daha fazla bulundu ve bu istatistiksel olarak anlamlıydı. Bu bulgu, ultraviyole ışınları etkisinin mast hücre degranülasyonunu arttırması ile ilişkilendirildi. Bu sonuçlar, mast hücrelerinin piterijiyum patogenezi aktif olarak etkilediğine ve meydana gelen değişikliklerin ultraviyoleten başka çok sayıda faktörün direkt veya mast hücreleri aracılığı ile gelişen dolaylı etkilerine bağlı olduğuna ve bunların piterijiyum patogenezinde rol oynadığına işaret etmektedir.

**Anahtar sözcükler:** Piterijiyum, mast hücresi, ultraviyole

### INTRODUCTION

A pterygium is a triangular fold of membrane in the interpalpebral fissure that is formed

from nasal or temporal bulbar conjunctivas, extending toward the center of the cornea, with its attachments to the sclera and cornea along its base (1-6). These histologically benign wing-like lesions are covered by squamous epithelium rich in columnar or goblet cells. The stroma is

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composed of collagen and degenerated elastic fibrils, and frequently consists of congested, dilated vessels (1-10). In rare cases, the epithelium overlying a pterygium undergoes morphological changes. These range from mild dysplasia to in situ carcinoma, and even to invasive squamous cell carcinoma (1-6). Acute or chronic inflammation, mast cells and melanophages are frequent findings in the stroma of a pterygium (2). Although pterygia have been studied extensively, the etiopathogenesis of this abnormality is not known. Ultraviolet rays (UV), human papilloma virus, herpes simplex virus, long-term exposure to petrochemicals, thermal damage, smoking and chronic irritants have been implicated (1-16).

Mast cells, which contain primary and secondary mediators of inflammation, are the main cells that characterize acute allergy. These cells are produced by bone marrow and they also play a role in chronic inflammation via the mediators they contain. These mediators include biogenic amines (histamine, adenosine), chemotactic factors for eosinophils and leukocytes, enzymes (protease and acid hydrolase), primary mediators such as leukotrienes (ie: proteoglycans heparin and chondroitin sulfate), prostaglandins, and platelet-activating factors, and secondary mediators such as tumor necrosis factor, interleukin (IL)-1, IL-4 and IL-5 (7-10, 17-20). Accumulation of mast cells and release of their mediators affects immunity, tissue repair and angiogenesis (19). Several groups of researchers have investigated the probable role of mast cells in the pathogenesis of pterygium (7-10). These studies have revealed that pterygium lesions contain considerably higher numbers of mast cells than normal conjunctiva. Some investigators showed that UV rays increase the speed and rate of mast cell degranulation which results in the increase of secretion of vasoactive amines leading to a higher inflammation rate (21).

The aim of our investigation was to assess the effects of exposure to UV light on mast cell numbers in pterygium lesions. We tested these

effects in patients from the Turkish cities of Adana and Ankara with different climatic conditions. Adana, and its surroundings situated in southeast Mediterranean region has a very sunny climate between April and October, so the local population are exposed to higher levels of UV. In contrast, central Anatolian city of Ankara receives much less sunshine and people are exposed to significantly lower UV levels. We compared mast cell counts in pterygium and normal conjunctival tissue specimens coming from these two regions, and also tried to assess whether mast cell counts correlated with histopathological findings.

## **MATERIALS and METHODS**

The study involved 140 randomly selected pterygium specimens and 30 control specimens of healthy conjunctival tissue. Thirty-nine pterygium specimens came from Başkent University Hospital in Ankara while 101 were from Başkent University Hospital in Adana. Control conjunctival specimens were collected from patients which were operated for cataract.

Archived hematoxylin-eosin (H&E) stained slides from each case were re-examined microscopically. The control specimens of conjunctiva were fixed in 10% buffered formalin and embedded in paraffin. Five micrometer-thick sections were cut from each block, stained with H&E, and then examined with light microscopy. Any findings of koilocytosis, epithelial hyperplasia, epithelial dysplasia, melanin accumulation in the basal layer of the overlying epithelium, stromal degeneration, calcification, congestion or inflammation were recorded for each control and pterygium specimen. Sections of each pterygium and control specimens were also evaluated for perivascular eosinophil leukocyte accumulation in the stroma. In all sections, presence of inflammation was classified in three categories according to the density of inflammatory cells: no inflammation, mild inflammation, or severe inflammation with lymphoid

follicles. Presence and severity of epithelial dysplasia was also categorized as none, mild, moderate, or severe. All other histopathological parameters were recorded as “present” or “absent”.

In addition to H&E preparations, five micrometer-thick sections were cut from each archived and also recently prepared control specimens embedded in paraffin, and these sections were stained with toluidine blue (0.5% solution at pH 3.0 for 10 minutes) to detect mast cells. In each specimen we counted the numbers of mast cells in five high-power fields (x400) and recorded this as the total number of mast cells for each control and pterygium cases.

We statistically compared total mast cell counts in the pterygia and the control specimens, coming from Adana and Ankara regions. At the same time we correlated mast cell numbers with presence and/or absence of eosinophil leukocytes, inflammation, epithelial hyperplasia and/or dysplasia, stromal congestion, or stromal degeneration, and melanin accumulation on

the basal layer of epithelium. All calculations were done using software SPSS for Windows version 9.05 with chi-square nonparametric test.

## RESULTS

Seventy three (140/73; 52.1%) pterygium specimens were from male and 67 (140/67; 47.9%) were from female patients. The mean ages of the males and females were  $53.69 \pm 15.01$  and  $55.89 \pm 12.63$  years, respectively. The histopathological findings in the two groups of pterygia (Adana and Ankara) and in control cases are summarized in Tables 1 and 2.

There were no significant differences between the two pterygium groups with respect to the presence of stromal calcification, epithelial dysplasia and hyperplasia, eosinophil leukocytes, melanin accumulation and koilocytosis. The pterygium specimens from Adana (n=101) had demonstrated a significantly higher frequency of inflammation (six times higher) than those from Ankara (n=39) ( $p < 0.001$ ). Forty eight

Table 1. Histopathological findings

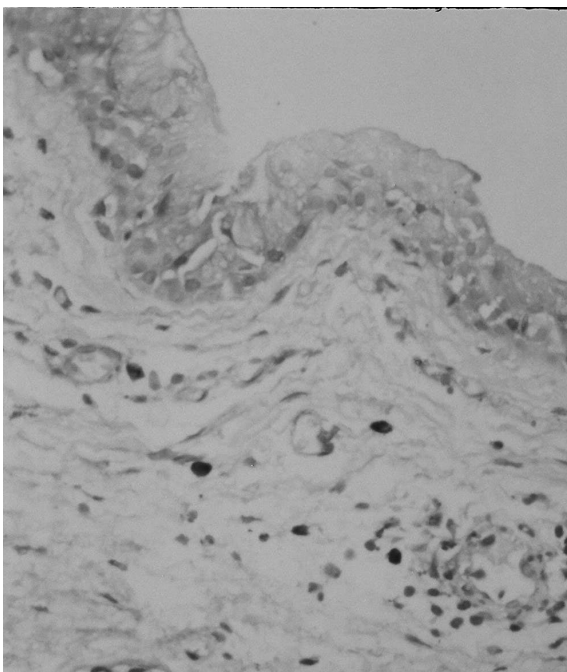
	Adana n=101 %	Ankara n=39 %	Control n=30 %	Total n=170 %
Pterygium	101 (100)	39 (100)	30 (100)	170 (100)
Epithelial hyperplasia	98 (97.02)	37 (94.8)	10 (33.3)	145 (85.2)
Koilocytosis	98 (97)	37 (94.9)	10 (33.3)	145 (85.2)
Melanin accumulation	50 (49.5)	15 (38.4)	5 (16.6)	70 (41.1)
Stromal degeneration	101 (100)	39 (100)	1 (3.3)	141 (82.9)
Stromal calcification	10 (9.9)	0 (0)	0 (0)	10 (5.9)
Stromal congestion	96 (95.04)	39 (100)	7 (23.3 %)	142 (83.5)
Mast cells	18.44±8.77	16.51±7.23	9.23±6.86	16.37±8.77

Table 2. Dysplasia and inflammation in Adana, Ankara and control cases

	Dysplasia				Inflammation $p < 0.001$		
	Absent	Mild	Moderate	Severe	Absent	Mild	Severe
Adana n=101	83	15	2	1	18	77	6
%	(83,1)	(14,9)	(1,9)	(0,9)	(17,8)	(76,3)	(5,9)
Ankara n=39	34	5	0	0	24	11	4
%	(87,2)	(12,8)	(0)	(0)	(61,5)	(28,2)	(10,3)
Control n=30	30	0	0	0	20	9	1
%	(0)	(0)	(0)	(0)	(66,7)	(30)	(3,3)
Total n=170	147	20	2	1	62	97	11
%	(86,4)	(11,7)	(1,1)	(0,8)	(36,4)	(57,1)	(6,5)

(34.3%) pterygium specimens had large numbers of eosinophils surrounding the stromal vessels.

We observed that most of the mast cells were clustered around vessels and around the basophilic degenerated collagen and elastic fibrils (Figure 1). The mean ( $\pm$ SD) mast cell count for 140 pterygium specimens was  $17.90 \pm 8.39$ , and the mean count for 30 control specimens of conjunctiva was  $9.23 \pm 6.86$ . This difference was statistically significant ( $p < 0.05$ ). The mean mast cell counts in Adana and Ankara pterygium specimens were  $18.44 \pm 8.77$  and  $16.51 \pm 7.23$ , respectively ( $p > 0.05$ ). Mast cell counts were positively correlated with the presence of inflammation ( $p = 0.048$ ), and melanin deposits within the basal layer of the overlying epithelium ( $p = 0.041$ ). However, mast cell counts were not significantly correlated with presence of eosinophil leukocytes, epithelial dysplasia and hyperplasia, stromal congestion, or stromal degeneration.



**Figure 1.** Mast cells in pterygium stroma (Toluidine blue x400)

## DISCUSSION

Although etiopathogenesis of pterygium is not completely understood, recent studies have focused on UV rays and viruses as etiologic factors (1,4-6,11-16). Some other investigations have demonstrated increased mast cell density in pterygia (7-10). Similarly we found that the mean mast cell counts in our 140 pterygium specimens were twice the mean counts observed in the specimens of normal conjunctivas. This supports the theory that mast cells play a role in the pathogenesis of pterygium. However, we found no significant difference in mast cell counts between the specimens obtained from the two locations with different climatic conditions, ie: Adana and Ankara. There was a trend towards higher mast cell counts in the specimens from Adana, and long-term exposure to UV rays might have induced this change. However mast cell numbers were nearly the same. Adana group had a significantly higher frequency of inflammation (six times higher) when compared with Ankara group. Walsh showed that UV rays induce mast cell degranulation which results in the increase of secretion of vasoactive amines and leads to a higher inflammation rate (21). With that finding in mind, we can explain the reason for higher inflammation rate and our data supports that hypothesis as well.

Malcotti and coworkers (22) and Alard and colleagues (23) found that subchronic exposure to UV rays caused edema and congestion and increased the number of mast cells in the human epidermis. In the capital city Ankara, air pollution and chronic irritation may largely explain the increase in the number of mast cells.

Mast cells are one of the main sources of basic fibroblastic growth factor (bFGF), a substance that has strong mitogenic effects on endothelial cells, fibroblasts, smooth muscle and epithelial cells (8,20). Powers and coworkers (8) showed high levels of bFGF in the basal and suprabasal cells of the epithelium of pterygia. It is also known that bFGF-2, bFGF-7, and epithe-

lial growth factor (EGF) are bound to heparin. All of these factors which are involved in the proliferation of stromal fibroblasts and thus in wound healing, are secreted by mast cells (20). The above findings suggest that epithelial hyperplasia and increased numbers of fibroblasts in pterygium may be associated with bFGF and EGF secreted by mast cells. However, our analysis did not reveal any significant relationship between mast cell counts and epithelial hyperplasia. The explanation may be that mast cells are not the only source of bFGF.

Mast cells regulate proliferation and function of endothelial cells via vasoactive mediators, such as heparin, histamine, vascular endothelial growth factor (VEGF), and bFGF, and thus play a role in angiogenesis (7-10,17-20). Research has shown that angiogenesis increases mast cell counts in many tissues, including cornea and tumor tissues, and that mast cells provoke microvascular endothelial cell proliferation in tissue cultures (7,19). Vasoactive mediators and growth factors can induce development of vascular structures and large numbers of new small capillaries in pterygia. We also observed increased congestion in our pterygium specimens. The fact that the number of mast cells in the pterygium specimens were twice as high as those found in normal conjunctiva is an evidence of likely involvement of mast cells in vasodilatation and congestion.

Proteolytic enzymes in mast cells, such as matrix metalloproteinase 9, type IV collagenase, gelatinase, triptase and chymase break down proteins in the extracellular matrix (18). These enzymes are normally inactive, but are activated by bFGF, histamine and chronic inflammation (7-9,18,19). It is thought that the degenerative changes in pterygium stroma that are attributed to UV are specifically induced by these proteolytic enzymes (7). Consistent with these findings, we also found accumulated mast cells in areas where the pterygium stroma was degenerating. However, we detected stromal degeneration both in the specimens from the geographi-

cal location where the level of UV light exposure was high and in the specimens from the location with much less sun exposure. Therefore, there must be other factors in addition to mast cells that induce stromal degeneration. Infiltration of lymphoplasmocytic inflammatory cells and the presence of eosinophils in pterygium stroma are associated with chemotactic agents released from mast cells. In turn, the resultant inflammation leads to the release of growth factors and activation of proteolytic enzymes (8,19). In this study, we found a significant correlation between mast cell counts and severity of inflammation.

Stem cell factor plays an important role in the growth and regulation of mast cells, and it is also known to increase melanocyte proliferation in human skin (9). In addition, mast cells contain mediators that increase melanogenesis in melanocytes (24). We observed melanin deposits in the basal layer of the epithelium of our pterygia specimens, and found a positive correlation between mast cell count counts and presence of melanin.

Human papilloma and herpes simplex viruses are two other factors implicated in the etiology of pterygium (14). Marshal et al. and Hosoda and colleagues reported that mediators such as histamine, protease enzymes, and leukotrienes secreted by mast cells during a viral infection have an important influence on host response (25,26).

Whatever the precise etiology of pterygium, it is likely that mast cells play an important role. These lesions contain increased numbers of mast cells, and the strong associations we observed between mast cell counts and certain histopathological features of pterygia and the mediators of mast cells support this hypothesis. However, we were especially interested in estimating mast cell counts relative to UV exposure, and our data revealed no difference in mast cell counts between the pterygium specimens in two different geographical locations. This suggests that factors other than UV rays might have



a direct influence or induce mast cell-related effects that are involved in the pathogenesis of pterygium. Further studies on mast cells and their functions will elucidate the role of mast cells in the pathogenesis of pterygia. Mast cells and their mediators trigger epithelial hyperplasia, inflammation, vascular congestion, stromal degeneration, attraction of eosinophil leukocytes and melanin accumulation at the basal cell of surface epithelium of the pterygia.

Although there were no statistical differences in mast cell counts in between Adana and Ankara groups, in Adana group inflammation was six folds greater than Ankara group. This can be explained by higher UV ray exposure in Adana. UV light causes rapid and massive degranulation of mast cells which results in increase of secretion of vasoactive amines and leads to a higher inflammation rate.

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