



ORIGINAL RESEARCH PAPER

Ophthalmology

STUDY OF PATHOLOGICAL CHANGES IN THE CONJUNCTIVA AND CORNEA USING IMPRESSION CYTOLOGY IN CASES OF PTERYGIUM: A CROSS-SECTIONAL STUDY CONDUCTED AT ONE OF THE TERTIARY CARE CENTERS OF NORTHERN INDIA.

KEY WORDS:

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ABSTRACT

Background: Pterygium is a benign, proliferative, degenerative condition of conjunctiva and sub conjunctival tissue that has the potential to impair vision. Impression cytology is the process of removing the outermost layers of ocular surface epithelium using cellulose acetate filter paper. There are various methods to investigate Pterygium, we carried out this study through impression cytology. **Materials and Methodology:** This was a cross-sectional study carried out in Career Institute of Medical Sciences, Lucknow including 100 patients with pterygium. From each eye, 4 impressions were collected through impression cytology, which included 2 conjunctival and 2 corneal impressions to study the cytological changes occurring throughout inter-palpebral ocular surface in the eye with pterygium. **Results:** The cytology of the surface cells overlying the pterygium exhibits squamous metaplasia, goblet cell hyperplasia, and keratinization of the epithelial cells. These cytological changes are not only seen over pterygium conjunctiva and cornea around the pterygium but they are also seen over the unaffected sites of the interpalpebral conjunctiva and cornea in the same eye. **Conclusion:** Pterygium is a graded series of the ocular surface variations occurring through out the inter-palpebral bulbar conjunctiva and cornea, with most advanced variation shappingening directly over pterygium surface, confirming that pterygium is certainly anocular surface disorder. This study suggests that impression cytology represents a fast, cost-effective, and non-invasive tool for understanding the pathology of pterygium.

INTRODUCTION:

Pterygium is a common external ocular disease in the tropics. An ocular surface condition called pterygium is also referred to as surfer's eye. It is described as a benign, proliferative, degenerative condition of conjunctiva and sub-conjunctival tissue that has the potential to impair vision.

Ocular surface inflammation at the site of the pterygium is thought to contribute to the symptoms of irritation, and dryness often experienced by patients with pterygium.^{1,2}

Although the epithelium of pterygium has been histologically evaluated with the electron microscope, including evaluation of the epithelial ultrastructure and observation of the intraepithelial capillaries in pterygium,^{3,4,5} Impression cytology has the benefit of analyzing the surface morphology and topographic cell-to-cell relationship of the epithelial cells in pterygium that are not discernible in conventional histologic sections.

Such studies are very scarcely found in India and especially in Northern Province; So, this study was aimed to study the pathological changes in the conjunctiva and cornea using impression cytology in cases of pterygium. We performed impression cytology in patients with pterygium to assess the morphology of their surface epithelium.

We also compared the cytology of the epithelium over the pterygium with that of the other sites of the bulbar conjunctiva and cornea in the same eye to determine whether similar ocular surface changes are seen in these clinically unaffected sites.

MATERIALS AND METHODOLOGY:

After getting approval from Institutional Ethical committee, A Cross-Sectional study was conducted along with specified interventions at Ophthalmology Department, at Career Institute of Medical Sciences, Lucknow, for the duration of 2 years (from January 2021 to December 2022). All cases of pterygium, taking treatment from our department and willing to participate in this study were included as study participants after taking prior consents from the individual study participants. Patients having Vernal keratoconjunctivitis, Trachoma, Uveitis, Pseudopterygium and

such allergic and inflammatory conditions of the eyes were excluded from the study to avoid misinterpretation of the study.

The sample size of 100 patients was calculated by taking 7% prevalence of pterygium in India based on a previous study conducted by Ang et al., 2012⁶, and putting this value in the formula of quantitative sampling method formula⁷

- $n = Z^2 P(1-P)/d^2$,
- (at 95% level of confidence and error rate, usually set at 0.05 level is 4) Where,
- n = sample size,
- Z = Z statistic for a level of confidence, for the level of confidence of 95%, which is conventional, Z value is 1.96.
- P = expected prevalence or proportion (in proportion of one; if 13%, P = 0.13),
- d = precision (in proportion of one; if 5%, d = 0.05).

In this study, Impression cytology was done by removing the outermost layers of ocular surface epithelium using cellulose acetate filter paper. These slides were stained with H&E and PAS stain. After studying the stained slides under microscope, the data regarding the presence/absence of squamous metaplasia, goblet cell density and keratinization were noted and recorded in MS excel data sheet and Statistical Analysis was done by using SPSS software version 20.0. Appropriate statistical tests were applied to find the significance result of the study (p < 0.05)

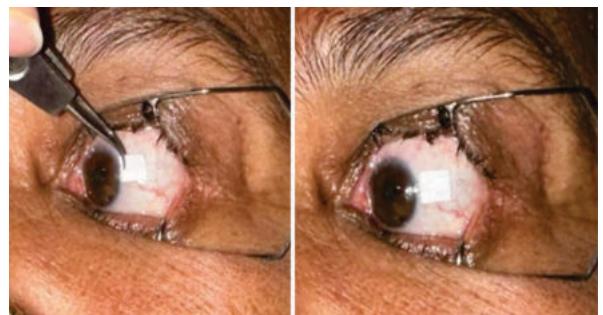


Figure 1: Collection of impressions of conjunctiva and cornea in patients with Pterygium

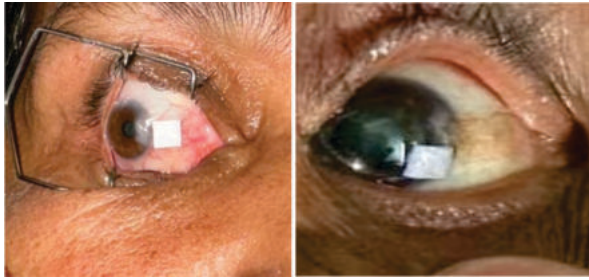
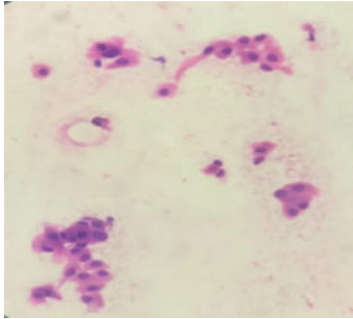


Figure 2: Squamous metaplasia on H&E stain at 40X



RESULTS:

Table 1: Distribution of Study Participants as per the selected Variables. (N = 100)

Variables	Categories	N (%)
1. Gender	Males	61
	Females	39
2. Age Group	18-30	7
	31-40	31
	41-50	19
	51-60	33
	>60	10
3. Site of Pterygium	Nasal	92
	Temporal	3
	Double-headed	5
4. Grades of Pterygium	1	16
	2	34
	3	35
	4	15
5. Sunlight Exposure	Yes	77
	No	23
6. Work in Hot and Humid Climate	Yes	63
	No	37

In the present study, a total of 400 impressions were collected (i.e 4 impressions from each eye). Which included 200 conjunctival and 200 corneal impressions. Out of 200 conjunctival impressions, 105 impressions were collected from the pterygium (92 nasal+ 3 temporal+ 5x2=10 double-headed) and 95 impressions were collected from the non- pterygium interpalpebral conjunctiva. Similarly, out of 200 corneal impressions, 105 impressions were collected from the cornea around the pterygium (92 nasal+ 3 temporal+ 5x2=10 double- headed) and 95 impressions were collected from the cornea not covered with pterygium.

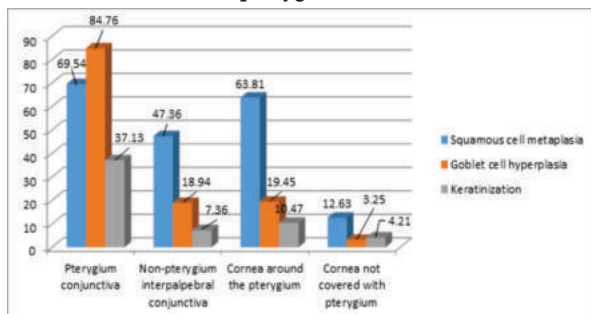


Figure 3: Distribution of Impression cytology sample results

Table 2: Comparison of study results based on Impressions

Impression results	Squamous Cell Metaplasia			Goblet cell Hyperplasia			Keratinization		
	N	OR#	p-Value	N	OR	p-Value	N	OR	p-Value
Pterygium conjunctiva	73	-	-	89	-	-	39	-	-
Non-ptyerygium interpalpebral conjunctiva	45	2.54	0.001*	18	23.8	<0.001*	7	7.4	<0.001*
Cornea around the pterygium	67	1.29	0.379	20	23.64	<0.001*	11	5.0	<0.001*
Cornea not covered with pterygium	12	15.78	<0.001*	3	170.6	<0.001*	4	13.44	<0.001*

*=Significant (p<0.05), Chi-Square test, # = 95% Confidence Interval is taken as calculation.

DISCUSSION:

In present study, all the age groups were included, the maximum number of patients 33.0% belonged to the age group 51-60 years, and the minimum number of patients 7.0% belonged to the age group 18-30 years. Male patients 61.0% were more common than female patients 39.0%. 77.0% of patients had history of sunlight exposure and 63.0% of patients were working in a hot and humid climate. 92.0% of patients had nasal pterygium, 5.0% of patients had double-headed pterygium, and 3.0% of patients had temporal pterygium. Our demographic findings supported the findings of, Masoud Safarzadeh et al⁸, Bandyopadhyay R et al⁹, and Chan CM et al¹⁰

In present study, the cytology of the surface epithelium of pterygium conjunctiva was found 100% (105/105) abnormal. The different cytological changes seen in pterygium impressions were squamous metaplasia in 69.5% (73/105), goblet cell hyperplasia in 84.8% (89/105), and keratinization in 37.1% (39/105) epithelial cells. Reda AM et al¹¹ reported squamous hyperplasia (83%), and goblet-cell hyperplasia (31.9%) was seen in pterygium.

In the present study, it was observed that 69.5% of pterygium impressions had squamous metaplasia, followed by 63.8% of corneal impressions around the pterygium had squamous metaplasia, 47.3% non- pterygium interpalpebral conjunctival impressions had squamous metaplasia and 12.6% of corneal impressions not covered with pterygium had squamous metaplasia. Ibrahim MH et al¹² and Chan CM et al¹⁰ supported these findings, Ibrahim MH et al¹² identified squamous metaplasia in 40% pterygium patients, and Chan CM et al¹⁰ reported squamous metaplasia among 73.2% (41/56) of the pterygia studied.

As squamous metaplasia was observed in 69.5% of pterygium conjunctival impressions and was considered as the most important epithelial feature in describing epithelial changes in pterygium. On evaluating the within impression change, it was statistically significant when compared with 47.3 % in the non-ptyerygium interpalpebral bulbar conjunctival impressions (p=0.001) and 12.6% in the corneal impression not covered with pterygium (p < 0.001). Whereas, squamous metaplasia in pterygium conjunctival impressions was statistically insignificant when compared with 63.8% in corneal impressions around the pterygium (p = 0.379).

The cells in epithelium of pterygium specimens were markedly elongated, and enlarged, along with pyknotic changes in nuclei, providing nucleus-cytoplasm ratios >1:4. In contrast, other sites of bulbar conjunctiva, and the cornea exhibited an early squamous metaplasia, along with the

nucleus-cytoplasm ratios <1:4 and absence of marked cellular enlargement.

Therefore, in our study, it was observed that squamous metaplasia is not only seen over pterygium conjunctiva, and cornea around the pterygium but these metaplastic changes are also seen over the unaffected sites of the interpalpebral conjunctiva and cornea. Similarly, **Chan CM et al**¹⁰ reported Squamous metaplasia was observed in 73.2% (41/56) of the pterygia studied. This was significantly compared with 0.0% (0/42) in superior bulbar conjunctiva, 31.70% (14/44) in inferior bulbar conjunctiva (p<0.001), and 54.8% (17/31) in the clinically normal inter-palpebral conjunctiva (p<0.001).

Goblet cells of conjunctiva are the main source of mucus for the ocular surface. Epithelial cell layer which makes-up healthy conjunctival cell surface comprises mucus-secreting cells of conjunctiva, namely goblet cells. However, the mucus content is main feature of these cells and the feature by which they are likely to be identified in tissue specimens subjected to standard histological examination. **Nelson**¹³ considered the normal GCD across bulbar surface to be >500cells/mm². **Doughty MJ**¹⁴ reported the normal conjunctival goblet cell value in interpalpebral locations as 427 +/- 376 cells/mm². In our study, values greater than (427 +/- 376 cells/mm²) were considered goblet cell hyperplasia in the conjunctiva. Goblet cells don't exist on corneal epithelium in the normal condition of the ocular surface. Therefore, in our study, we considered presence of goblet cells on cornea as goblet cell hyperplasia.

In this study, the majority 84.8% of pterygium impressions had increased goblet-cell density followed by 19.04% of corneal impressions around the pterygium had increased goblet-cell density, 18.9% of non-terygium interpalpebral conjunctival impressions had increased goblet-cell density and 3.2% corneal impressions not covered with pterygium had increased goblet cell density. **Ibrahim MH et al**¹² supported our findings, he identified goblet-cell hyperplasia, and squamous metaplasia along with epithelial hyperplasia. Based on results, pterygium is mostly covered by the altered conjunctival epithelium which exhibited squamous metaplasia in 40.0% cases, and the goblet-cell hyperplasia in 45.0%.

As observed, 84.8% of pterygium conjunctival impressions showed increased goblet-cell density. On evaluating the within impression change, it was statistically significant when compared to 18.9% in non-terygium interpalpebral conjunctival impressions (p<0.001), 19.04% in corneal impressions around the pterygium (p<0.001) and 3.2% in corneal impressions not covered with pterygium (p<0.001). **Chan CM et al**¹⁰ reported mucinous hyperplasia signifying the increased goblet-cell density seen over surface of pterygium in 87.5% than 2.4% in superior conjunctiva, 15.90% in inferior conjunctiva and 12.90% in clinically unaffected inter-palpebral conjunctiva (p<0.001) supporting our findings.

Squamous metaplasia of the conjunctival epithelium is typically associated with a decrease in goblet cell density as described by **Nelson**¹³. It is interesting that in this study, we found coexistence of the squamous metaplasia, and increased goblet cell density in pterygium. **Chan CM et al**¹⁰ also found the coexistence of the squamous metaplasia, and increased goblet cell density in pterygium. This unique cytologic characteristic of pterygium supports theory of the altered epithelial differentiation in pathogenesis of pterygium. Eyes with kerato-conjunctivitis sicca have been reported having relative hyperplasia of goblet-cells by **Lemp MA et al**,¹⁵ **Frost-Larsen K et al**¹⁶ and **Sjogren H et al**,¹⁷ whereas **Ralph RA et al**¹⁸ reported decreased numbers of goblet cells.

In our study, the majority 37.1% of pterygium impressions had

keratinization followed by 10.4% of corneal impressions around the pterygium had keratinization, 7.4% of non-terygium interpalpebral conjunctival impressions had keratinization & 4.2% corneal impressions not covered with pterygium had keratinization. **Moeller KL et al**¹⁹ reported conjunctival epithelial keratinization is a pathologic feature of ocular surface diseases like Sjogren's syndrome.

The present study noted, 37.1% of pterygium impressions had keratinization. On evaluating the within impression change, it was statistically significant compared to 10.4% in corneal impressions around the pterygium (p<0.001), 7.4% in non-terygium interpalpebral conjunctival impressions (p<0.001), and 4.2% in corneal impressions not covered with pterygium (p<0.001). Similarly, **Juan Peng et al**²⁰ reported keratinization was found in the superficial cells in the head of the pterygium but it was not found in the normal conjunctiva epithelium.

Therefore, in our study, it was interestingly found that, keratinization in pterygium epithelium, unaffected interpalpebral conjunctival epithelium, corneal epithelium around the pterygium, It suggests that some amount of keratinization occurs in the entire interpalpebral conjunctiva as well as in the cornea, however small, in higher grades of pterygium.

CONCLUSION:

The cytology of the surface cells overlying the pterygium was abnormal, classically exhibiting squamous metaplasia in 69.5%, goblet cell hyperplasia in 84.8%, and keratinization in 37.1% of the epithelial cells. Squamous metaplasia, goblet-cell hyperplasia, and keratinization are not only seen over pterygium conjunctiva and cornea around the pterygium but these cytological changes are also seen over the unaffected sites of the interpalpebral conjunctiva and cornea. Goblet cells are usually not present over the cornea. But presence of goblet-cells is seen over the peripheral corneal surface. This could suggest spill over/migration of the goblet cells on cornea from neighbouring conjunctiva.

This suggests graded series of the ocular surface variations occurring throughout the interpalpebral bulbar conjunctiva and cornea, with most advanced variations happening directly over pterygium surface, confirming that pterygium is certainly an ocular surface disorder. This study suggests that impression cytology represents a fast, cost-effective, and non-invasive tool for understanding the pathology of pterygium.

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