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To Evaluate Ocular Surface Abnormalities in Eyes with Pterygium: Role of Conjunctival Impressioncytology

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Abstract: ***Aim: To study ocular surface abnormalities in eyes with primary and recurrent pterygium Conjunctivalimpression cytology was explored as a tool for the assessment of the cytological changes. Materials and methods: A comparative case control study was undertaken to evaluate ocular surface disorders in pterygium. 29 eyes with primary pterygium and 9 eyes with recurrent pterygium were enrolled. Normal eye of the patients was taken as control. Patients were evaluated for the tear film break up time (TBUT), schirrmer's test and their normal control values. Pre and post op values were also compared. The spectrum of changes in cytomorphology was studied using conjunctival impression cytology. Results: Tear film breakup time and schirmer's strip wetting were found to be statistically significant in pterygium eyesas compared to their normal control eye in both the pterygium groups as all the values were found to be highly statistically significant.In the post op period TBUT and schirmer's showed significant difference in both primary and recurrent pterygium.On cytomorphology squamous metaplasia and pyknotic changes in nuclei of different grades were observed more frequently in eyes with pterygium as compared to control. Comparison between primary and recurrent pterygium showed statistical significant value of TBUT (p value<.0001) and schrimers value (p value<.0002) Conclusion: We conclude that aqueous tear film deficiency leading to ocular surface disorders brings about a change at the cytological level too.***

Keywords: Primary pterygium, Recurrent pterygium, Impression cytology

1. Introduction

The term "Pterygium" is a latinized version of Greek term "pterygion" meaning small wing. [1,2] Pterygium is a wing shaped growth of fibrovascular tissue on cornea and conjunctiva. It occurs in the palpebral fissure, much more often nasally than temporally, although either or both ("double pterygium") occurs. Pterygium is a common external ocular disease and its prevalence increases with age and higher in people living in tropical areas near the equator. Outdoor work and both blue and ultraviolet light have been implicated in its causation. Ocular surface inflammation at the site of the pterygium is thought to contribute to the symptoms of irritation, mucoid discharge, and dryness often experienced by patients with pterygium.Evidence of clinical correlation of dry eye conditions with pterygium has been accumulating in the recent years.[3,4]Dry eye disease can decrease the performance of activities of daily living and is associated with an overall decrease in quality of life.

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. The objective of the present study was to compare the tear film functions in patients with primary and recurrent pterygium in diseased and control eye and to study changes in tear film functions before and after pterygium excision to determine the association between ocular surface disorder and pterygium.

2. Materials and Method

The present study was conducted on 29 patients with primary and 9 patients with recurrent pterygium presented at outpatient department of Upgraded department of ophthalmology of Sarojini Naidu Medical College Agra. Patients other eye was taken as control. Patients already diagnosed as having Sjorgens syndrome and other conditions which may lead to dry eye were excluded. Assessment of precorneal tear film stability was done on the basis of tear film break up time (TBUT) , Schirmer' s test was performed using no. 41 Whatman filter paper(35 x 5) without topical anesthesia (Schirmers I)

Early surgical intervention can therefore reduce effects of corneal morbidity due to pterygium induced corneal distortion and visual disturbance arising from the encroachment of the pterygium into the visual axis.

Conjunctival impression cytology was taken using cellulose acetate filter paper (millipore filter paper, 0.22micron pore size). The filter paper was cut into assymetric shapes and aligned on the bulbar conjunctiva in a specific orientation .A longer piece of cellulose acetate paper was used in the pterygium to straddle the area above and below it , in case of control the medial site of other eye was selected. The strips were placed in fixative solution , they were stained using GIEMSA stain . Grading of cytology done according to Nelson's grading system (Nelson et al 1983)

All surgeries were performed under peribulbar block by conjunctival auto grafting technique. Topical antibiotic eye ointment application with pad and bandage was done to the eye for 24 hours.

Post-Operative Care: Bandage was removed after 24hrs, topical instillation of antibiotic & steroid combination (moxifloxacin& betamethasone) eye drops were advised. For initial two weeks the frequency of drops were 4 times a day, tapered over the next 4 weeks & then stopped. Artificial

tear drops were not prescribed to patients post-operatively. All patients were followed post-operatively 24hrs, 1 week, 2 week, 1 month & 2 months. TBUT, schirmer's tests were performed 2 months post-operatively.

3. Statistical Analysis

Results were analysed using statistical software MSexcel /SPSS VERSION 17.0 for windows. Statistical significance between the groups was evaluated using student 't ' test . A p value <0.05 was considered as statistically significant and p value of < 0.01 was considered statistically highly significant.

4. Result

In the present study 29 eyes of primary and 9 eyes of recurrent pterygium was studied and their other eye was taken as controls. Of the total cases analyzed of primary pterygium,19 (65.51%) were males and 10(34.48%) were females and in recurrent pterygium cases 7 (77.77%) were males and 2 (22.22%) were females .Following observation was made

Table -1 depicts the difference between mean TBUT of diseased eye pre operatively and control eye in cases of primary pterygium was 3.586 (p <.02) and difference between mean schirmers value was2.11 (p<.03) , all the values were found to be significant .

Table 2 shows the mean difference between TBUT andschirmers value in cases of recurrent pterygium is 4.56(p<.0003) and 6.66 (p<.0001) respectively,all values were found to be highly significant.

Table 3 and table 4 shows the difference between mean TBUT and schirmers value in cases of primary and recurrent pterygium pre and post operatively respectively.

Table 4: Pre and Post op value of TBUT and schirmersin Recurrent Pterygium

Parameters	Recurrent Pterygium			
	Pre op	Post op	Mean diff	P value
TBUT	6.11	10.83	-4.72	.0023
Schrimmer's	5.56	13.17	-7.16	.0034

Table 5: Comparison between primary and recurrent pterygium

Parameter	Primary Pterygium	Recurrent Pterygium	P value
TBUT	8.72	6.17	.002
Schirmer	17.76	5.17	.004

Table 5 shows the comparison between primary and recurrent pterygium .Studying the result showed that mean difference between TBUT and schirmers value is 2.55 (p<.0001) and 12.59 (p<.0002) respectively which were found to be highly statistically significant.

The cytomorphology of the conjunctival imprint smears were studied in detail.In the present study group, cellularity was significantly lower in the pterygium group compared to control .The metaplastic cells were enlarged with pyknotic nuclei and abundant cytoplasm. The number of goblet cells was reduced.Squamous metaplasia of different grades was observed more frequently in 24/29(82.75%) compared to 14/29 (48.3%) in normal control. While some degree of squamous metaplasia was observed in all patients of recurrent pterygium as compared to 66 .66% of their normal control.

Table 1: TBUT and schirmers comparison in Primary Pterygium

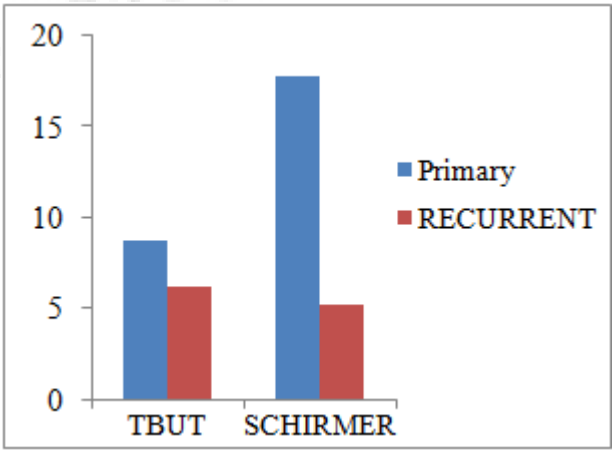
Parameter	Primary Pterygium			
	Cases	Control	Mean diff	P value
TBUT	8.72	12.31	-3.59	0.001
Schrimer's	17.76	19.87	-2.11	0.032

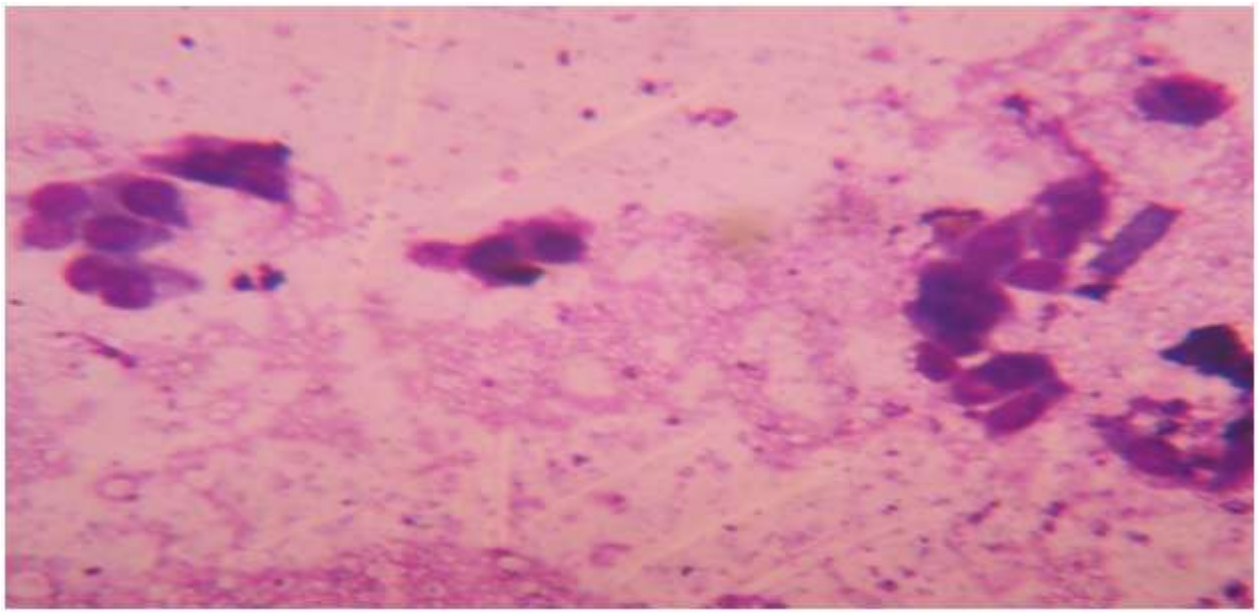
Table 2: Comparison in recurrent pterygium

Parameter	Recurrent Pterygium			
	Cases	Control	Mean diff	P value
TBUT	6.11	10.67	-4.56	0.003
Schirmers	5.56	12.22	-6.66	0.004

Table 3: Pre and Post comparison in Primary Pterygium

Parameter	Primary Pterygium			
	Pre -op	Post op	Mean diff	P - value
TBUT	8.72	11.45	- 2.73	.0023
Schirmer's	17.76	19.69	-1.93	.0034





Impression cytology depicting pyknotic changes in recurrent pterygium cases

5. Discussion

Pterygium is an ocular degenerative condition that has been attributed to environmental factors. It is found in areas of bright sunlight and may be linked to ultraviolet (UV) light. It is thought that UV light causes destruction of the tear film due to rapid evaporation. But even today there is no consensus regarding the exact pathophysiological mechanism underlying pterygium formation although environmental factors, limbal stem cell deficiency, abnormal proliferation of conjunctival epithelium have all been suggested. Tear film dysfunction has been found in subjects with pterygium. The present study was a comparative one aimed to find out if there is any ocular surface disorder and corneal astigmatism associated with pterygium

In. the present study, TBUT in diseased eye was lower significant pre-operatively than in control eye (P <0.0001) Bekibele et al. also reported that TBUT was lower among cases than among their corresponding control eyes.{5} Wang S et al found that TBUT in the eyes with pterygium when compared with the opposite i healthy eyes where significantly low (p <.05) {6} El-Sersy TH found that the mean TBUT was 11.70 ± 2.16 s in normal healthy eyes wheras in eyes with pterygium this value was markedly reduced to 5.91 ± 1.95 s.12. {8}

Mittal *et al.* reported in 1991 that both BUT and Schirmer's test values were significantly reduced in cases of pterygium indicating the inadequacy of tear film in these patients {7} Bandyopadhyay et al. reported significantly different Schirmer strip wetting length & TBUT test results in study & control groups.{8 } Roka N, et al. (2013) had also found statistically significant difference between mean Schirmer's test value of pterygium patients and control group.. Our study results also corroborated with these findings.

We compared alternations of tear function before and after pterygium excision. Tear film functions were significantly different before & after pterygium excision (p<0.0001). This is in agreement with Li et al. who found that before surgery, the average TBUT was 9.74 ± 3.43 s which was significantly prolonged to 11.49 ± 3.76 s 1 month postoperatively and no significant difference was observed between preoperative & postoperative Schirmer test value (P > 0.05).They found that tear function in patients with primary pterygium improves after pterygium excision.{9}

In this study mean TBUT, Schirmer's-1 were decreased when compared to control group which indicates instability of tear film in pterygium patients. This study found a strong positive clinical correlation between dry eye and pterygium (p<0.001). Goldberg L and David R had found no correlation between dry eye and pterygium. Ishioka M et al found an association between pterygium and a shortened tear break-up time and Schirmer's test in the case-control study. They concluded that there is a correlation between pterygium formation and unstable tear film.{10} Saleem M et al had found positive correlation between dry eye and pterygium. Lekhanont K et al found that the presence of pterygium was significantly associated with positive dry eye test (11)

Impression cytology is a fast, cost-effective and non-invasive tool for the diagnosis and follow-up of ocular surface disorders. The cells collected from the conjunctiva can be subjected to both light and electron microscopic study of individual cellular characters and two-dimensional ocular surface changes can be evaluated as well. Impression cytology usually removes one to three cell layers and is thus ideal for studying surface epithelium rather than basal epithelium or the basement membrane. Egbert first used this method to determine the density of goblet cells in different areas of conjunctiva. {12}The most widespread use of this procedure is detection and grading of squamous metaplasia.

In the normal group, the predominant cells were small epithelial cells found in sheets together with presence of goblet cells and mucin spots. The goblet cells showed a tendency to aggregate into groups; those having abnormal cytology, the predominant cells were large, discrete epithelial cells with rare or no goblet cells. The borderline

abnormal showed cytology similar to abnormal, except that few goblet cells were seen. In borderline normal, only some epithelial cells showed abnormal cytology. In another study to evaluate cytological changes in dry eye states, the main feature of impression cytology was squamous metaplasia of epithelial cells and altered goblet cell density in the conjunctiva.{13}Other authors have also tried to stage the conjunctival impression cytology specimens according to squamous metaplasia as was primarily described by Wittpen. Chan *et al.* have evaluated CIC technique to study ocular surface changes in pterygium. They have found significant squamous metaplasia along with altered goblet cell density in these patients. Squamous metaplasia of conjunctival epithelium refers to pathologic transition of conjunctival epithelium involving increasing stratification of epithelial cells, together with a loss of goblet cells. Changes in the epithelial cells include enlargement, flattening and pyknotic changes in the nuclei with decreased nucleo-cytoplasmic ratio.

One limitation of our study was that there were lesser number of recurrent pterygium patients. No clear difference was apparent between primary and recurrent pterygium patients , but because of the small number of recurrent pterygium in our study , no detailed comparative analysis was made.

6. Conclusion

From the present study, we can suggest that unstable tear film is found to a greater extent in patients with pterygium than incontrol group. Pterygium excision improved tear film function . However, tear function deteriorated again with the recurrence of pterygium. Therefore, we infer that pterygium seems to cause DES and that surgical removalof pterygium alleviates pterygium-related DES. This study clearly demonstrated that tear functions improves after pterygium excision with suture less & glue free limbal conjunctival autografting which was safe & prevent recurrence of pterygium.

Conjunctival squamous metaplasia as evidenced by decreased goblet cell density and altered epithelial cell morphology is found more commonly in patients with pterygium than in control group. So it can be concluded that that aqueous tear film deficiency leading to ocular surface disorder brings about a change at the cytological level, and ocular surface disorders found in pterygium can be interpreted by conjunctival impression cytology.

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