

Conjunctiva Impression Cytology as a Diagnostic Tool for Assessment of Dry Eye in Presence of Normal Tear Film Function Tests

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Abstract

Aims: To explore the conjunctival impression cytology as a tool for the assessment of the cytological changes in type 2 DM & pterygium patients with normal tear film function tests. **Materials & methods:** This case control study was conducted in Navodaya Medical College Hospital & Research Centre in dept of Ophthalmology. Total 80 cases were examined i.e. 20 patients with pterygium, 20 patients with type 2DM & 40 control group and conjunctival imprint smear cytology results were analysed. The presence of local tear film abnormalities was assessed by TBUT & Schirmer test. The changes in cytomorphology was studied using conjunctival imprint cytology. The data is entered on excel sheet & analysis is carried out by using SPSS 19.0 statistical software. **Results:** In our study inspite of normal tear film function tests, abnormal conjunctival impression cytology reports were found in 10 type 2DM cases & 11 cases of pterygium. Statistically significant difference was found between study & control group ($p < 0.001$). **Conclusion:** Thus conjunctival impression cytology is a useful tool to assess the dry eye in pterygium & type 2 DM patients in presence of normal tear film function tests.

Keywords: Conjunctival impression cytology; Dry eye; Tear film; Pterygium; Type 2 DM.

Introduction

Dry eye

Disorder of tear film due to tear deficiency or excessive tear evaporation, which causes damage to the interpalpebral ocular surface & is associated with symptoms of ocular discomfort.

Conjunctival impression cytology refers to application of cellulose acetate filter paper to the ocular surface to remove superficial layers of ocular surface epithelium therefore allows analysis of the cells having reached their final differentiation. Cells then further processed for histological, immunohistological or molecular analysis. It is non invasive, painless, easy to perform, yields the minimum. 3 main populations of conjunctival cells can be found in impression cytology specimens - epithelial cells, goblet cells & inflammatory cells.

AIMS: To explore the conjunctival impression

cytology as a tool for assessment of the cytological changes in type II DM & pterygium patients with normal tear film function tests.

Materials & Methods

This case control study was conducted in Navodaya Medical College Hospital & Research Centre, Raichur in Department of Ophthalmology. Total 80 cases were examined i.e. 20 patients with type II DM, 20 patients with pterygium & 40 control group. Complete history was taken. Thorough slit

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lamp examination, Tear film break up time, RBS , Basal Schirmer test was done & then Impression Cytology was done. Patients with abnormal tear film function tests were excluded from the study TBUT was done using 2% fluorescein strip. Mean of three readings was taken. Reading of more than 10 seconds was taken as normal. RBS was performed before Schirmer's test to avoid false positive staining of conjunctiva obtained at a place where strip is placed. Basal Schirmer test was done using Whatman no. 41 filter paper strips (5x35mm) wetting of atleast 15mm was taken as normal.

Conjunctival impression cytology was done using; Acetate cellulose filter paper strips of pore size 0.20µm size, 13 mm in diameter. The filter paper was divided into two 'D'- shaped halves & smooth surface was marked. After anaesthetizing eye with 4% xylocaine drops & drying the lacrimal lake at inner canthus, filter paper strip was applied on the inferonasal bulbar conjunctiva with a blunt smooth edged forceps. The paper was allowed to remain in contact with the eye for approximately 5-10 seconds, gentle pressure was applied over strip with blunt end of the forcep & then the strip was removed in a peeling off motion. The paper was fixed for 10 minutes in a solution containing glacial cetic acid, formaldehyde, & ethyl alcohol in a 1:1:20 volume ratio.

Steps for Staining the Strip were

1. Fresh tap water - 2 min
2. 0.05% periodic acid - 2 min
3. Tap water - 2 min
4. Schiff's reagent diluted 1:1 - distilled water - 8 min
5. Tap water - 2 min

6. 0.05% sodium metabisulfite - 2 min
7. Tap water - 2min
8. Harri's haematoxylin - 30 sec
9. Tap water - 2 min
10. 95% ethyl alcohol - 2 min
11. Absolute alcohol - 3 min
12. Xylene - 20 min

Xylene makes filter paper transparent . Before mounting, the filter paper is placed with the epithelial cells facing up. The completed slides are examined by light microscopy for squamous metaplasia & goblet cell density. The grading was done according to Nelson's classification .

Grade 0: Small round epithelial cells with a eosinophilic staining cytoplasm, large basophilic nuclei with an n/c ratio of 1:2, abundened goblet cells(>500 cells/mm²)

Grade 1: Slightly larger andmore polygonal cells ,smaller nuclei,with n/c ratio of 1:3 , goblet cells are reduced in number (350-500 cells/mm²), preserved plump , with an intensely oval shape PAS positive cytoplasm.

Grade 2: Even larger& polygonal epithelial cells, occassionally multinucleated with variable staining cytoplasm, small nuclei with n/c ratio 1:4- 1:5, goblet cells markedly decreased in number (100-150 cells/mm²) , smaller and less intensely PAS positive.

Grade 3: Large & polygonal with basophilic staining cytoplasm small pyknotic nuclei , occassionly completely absent, n/c ratio >1:6, very few goblet cells(100 cells/mm²).

The data is entered on excel sheet & analysis is carried out by using SPSS 19.0 statistical software.

Table 1: Result of impression cytology in cases & controls

Impression	Grade 0	Grade 1	Grade 2	Grade 3	Total
Cases	31	5	2	2	40
Controls	40	0	0	0	40

Table 2: Result of impression cytology in Type II DM & pterygium (Compared with control)

Disease	Grade 0	Grade 1	Grade 2	Grade 3
DM II (n=20)	10	4	3	3
Pterygium (n=20)	9	9	2	0

Table 3: Comparison between cases & controls

Cases	Mean	S.D.	t- value	'P'	Remarks
DM II	0.95	1.14	3.7	<0.001	H.S.
Pterygium	0.65	0.67	4.3	<0.001	H.S.

Results

Total 80 cases (pterygium – 20, type II DM – 20 & control group – 40) were studied & no significant difference in terms of results of TBUT & Schirmer test between study & control group population. However statistically significant difference ($p < 0.01$) was found between two population in terms of impression cytology results.

In our study inspite of normal tear film function tests, abnormal conjunctival impression cytology reports were found in 10 type 2DM cases & 11 cases of pterygium. Statistically significant difference was found between study & control group ($p < 0.001$).

Discussion

- Conjunctival impression cytology is a valuable tool in investigating ocular surface disorders [2].
- Can calculate goblet cell density & staging of squamous metaplasia, especially in dry eye, monitoring effects of treatment [1].
- Dr. Pushpa (2008) studied 20 eyes of patients with pterygium, DM II & allergic conjunctivitis for TBUT, Schirmer's & impression cytology. They found statistically significant difference between cases & control groups in terms of CIC [3].
- Ranjana Bandyopadhyay, Dipanwita Nag, studied ocular surface abnormalities in 50 patients with pterygium by using CIC. They found, in case of Schirmer's test (2%) had borderline results while in case of CIC significant difference was found between cases & control groups [5].
- Chan et al. have evaluated CIC technique to study ocular surface changes in pterygium. They have found significant squamous metaplasia with altered goblet cell density in these patients [6].
- Martin goebbel (2000) studied TBUT, Schirmer's test & CIC in 86 patients with DM II. They found significant difference in terms of CIC in controls & cases groups but no difference in terms of TBUT [7].
- In our study, we found there is no significant difference in terms of results of TBUT & Schirmer's DM II can be diagnosed early by using CIC

compared with commonly used tear film function tests.

- It is helpful in early establishment of treatment ocular surface disorders found in pterygium & DM II can be diagnosed early by using CIC compared with commonly used tear film function tests.
- It is helpful in early establishment of treatment .

Conclusion

Thus conjunctival impression cytology is a useful tool to assess the dry eye in pterygium & type 2 DM patients in presence of normal tear film function tests.

Abbreviations

- DM II – Diabetes Mellitus type 2
- CIC – Conjunctival Impression Cytology
- TBUT – Tear Break Up Time

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