Cytomorphology of Conjunctival Epithelium in Ocular Surface Disorders

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Abstract

Introduction: To study cytomorphological changes in various ocular surface disorders and find out the sensitivity, specificity and the diagnostic accuracy of impression cytology.

Materials and Methods: One hundred and ten patients (84 cases and 26 controls) were included in this study. Out of these 84 cases, 25 cases having vitamin A deficiency (VAD), 19 cases are having vernal keratoconjunctivitis (VKC), 30 of dry eye and 10 of chemical burn. Impression cytology was performed with each patient and the results were noted. Biopsy was done in 2 patients from each group. Patients were examined for goblet cell density in the form of clumping of goblet cells and degree of squamous metaplasia in terms of loss of cell cohesion, altered cell morphology and keratinisation.

Results: On impression cytology, all cases of vitamin A deficiency showed loss of goblet cells and 92% showed squamous metaplasia. In case of vernal conjunctivitis showed clumping (37%), loss of goblet cells (63%) and squamous metaplasia (64%). In dry eye group, severe reduction of goblet cells (73%) with moderate to severe grade of squamous metaplasia (88%) was seen. All cases of chemical burn showed marked reduction of goblet cells and squamous metaplasia. Control group, showed mild reduction in goblet cells (23%) and mild grade of squamous metaplasia (15%). Sensitivity, specificity and diagnostic accuracy of impression cytology was 89.28%, 76.92% and 86.36% respectively. Cytological findings were well correlated with histological findings.

Conclusion: Impression cytology was found to be good technique for diagnosing metaplastic changes in various ocular surface disorders.

Keywords

Impression cytology; Vitamin-A deficiency; Vernal keratoconjunctivitis; Dry eye; Chemical injury of eye

Introduction

The term “ocular surface” describes the entire mucosal epithelial lining bordered by the skin at the superior and inferior eyelid margins [1]. This includes the epithelium from the muco-cutaneous junction of the eyelid margin on to the back of the lids, into its reflection back over the globe, including that which covers the cornea [2]. Ocular surface disorder is a term that includes the disease of various types of aetiology which disturbs the delicate balance between the ocular surface, the adnexa and the tear film. Clinical diagnosis of ocular surface disorder based on slit-lamp examination, Schirmer’s test, tear film break up time and Rose Bengal staining do not show changes at the cellular level. The histopathological features are conventionally studied by conjunctival biopsy. This procedure being invasive is not done routinely and repeated biopsies are avoided to prevent scarring and loss of limbal stem cells. Recently a filter paper technique utilising millipore cellulose acetate paper has been developed, to obtain the conjunctival impressions and study the cellular morphology [3]. It ensures good adhesion of surface epithelial cells to the biopore membrane with the result; adequate specimens are obtained in a very high percentage of cases [3]. Sensitivity of this method is high (78-87%); the main disadvantages of impression cytology are some loss of morphological details and poor cell yield in cases of keratinizing lesions [5]. The objective of this study was to assess the various changes of ocular surface disorder with the evaluation of alterations at the cellular level in the conjunctiva, by using impression cytology and conjunctival biopsy.

Materials and Methods

In a period from August 2014 to September 2016, 84 patients of both the genders reporting the symptoms of discomfort and ocular irritation such as redness, pain, discharge, blurred vision, itching, watering or tearing of eye were included. Informed consent has been taken from all the patients who are willing to take part in the case control study based on Helsinki protocol. The trial was approved by the ethics committee at our institution. All the selected patients were subjected to detailed history taking, physical examination and clinical tests. Dry eye, vernal keratoconjunctivitis, vitamin A deficiency and chemical burn, were the most common ocular surface disorder amongst subjects included in the study. Those patients who had conjunctivitis have various types of aetiologies, except allergic, and were excluded. Impression was taken from the inferotemporal bulbar conjunctiva. The patients lay down in supine position. Topical anaesthesia (Proparacaine 0.5%) was instilled into the conjunctival sac. After applying lid speculum, the impression was taken from the inferotemporal quadrant of conjunctiva. Nitro cellulose acetate membrane filter paper (Whatman 0.2 μm) was prepared into 5x6mm pieces. A blunt, smooth ended forceps grasped one corner of filter paper; while a smooth glass rod held in other hand, with the help of glass rod we gently press the paper on to the conjunctiva. Then the filter paper was kept on to the surface for 3-5 sec and it was removed with the peeling motion. This was then applied to a clean glass slide, at room temperature and the impression was transferred by uniform, gentle pressure. Fixing was done by 90% ethanol for 10 min. The slides were stained with PAS and PAP stains. The slides studied under low and high power with a light microscope. The cytology was graded according to scheme suggested by Nelson [6].

We noted the numbers of goblet cells and change in epithelial cells i.e. squamous metaplasia in three forms 1. Loss of cohesion, 2. Change in N:C ratio, 3. Presence/absence of keratinisation in histopathological examination via Impression cytology and conjunctival biopsy. Normal cytological features were observed in the
form of tight epithelial sheets i.e. good cohesion of cell, N:C ratio 1:2, rounded small epithelial cell with complete absence of keratinisation. The grading of squamous metaplasia and goblet cell density was done according to Nelson’s (1988) classification [7].

On the basis of goblet cell density and distribution and also various parameters of squamous metaplasia we find different grades of cytological changes of conjunctiva in conjunctival impression cytology (Figure 1)

Grade 0 (Normal):
 a. Abundant goblet cell numbers.
 b. Good cell cohesion in the form of tight epithelial sheets.
 c. Nucleo-cytoplasmic ratio (N:C) 1:2 along with small rounded epithelial cells.
 d. Absence of keratinisation.

Grade 1 (Low normal):
 a. Mild reduction of goblet cell numbers.
 b. Loose cell cohesion in the form of clusters
 c. Nucleo-cytoplasmic ratio (N:C) 1:3 to 1:4 along with moderately enlarged cell size.
 d. Mild keratinisation.

Grade 2 (Borderline):
 a. Moderate reduction of goblet cell.
 b. Scattered cells with occasional clusters of loose cell cohesion.
 c. Nucleo-cytoplasmic ratio (N:C) > 1:5 along with enlarged cell in size and polygonal in shape.
 d. Moderate keratinization.

Grade 3 (Abnormal):
 a. Occasional presence or absence of goblet cell.
 b. Isolated cells with complete loss of cell cohesion.
 c. Anucleated cell or cell with pyknotic nuclei.
 d. Severe keratinisation.

Biopsy was done in 10 patients (2 patients from each group). Specimen was taken from the inferotemporal area of about 2-3 mm length. Specimen was placed on an absorbent mount and then spread the specimen on the mount until some moisture from the tissue runs into the mount (only about 10-15 seconds). Specimen was placed into a vial, which consist of fixative 10% saline for histopathology examination.

Statistics

Statistical evaluation was performed using Chi-square test with or without Yale’s correction to know the significance of Conjunctival impression cytology between cases and controls. Values for ‘p’ are less than 0.05 and they were regarded as statistically significant. Diagnostic value for each test was analysed for sensitivity, specificity and diagnostic accuracy.

Results

Out of 110 subjects included in the study, 84 patients with ocular surface disorders were placed in case group and rest 26 subjects were taken as control group. Demographic details of patients are shown in Table 1.

Control group

Out of 26 control, 8 (30.76%) were male and 18 (69.23%) were females. Mean age was 27.73 yrs. and median was 21 yrs. On Impression cytology, in control group (Table 2), 20/26 (76.92%) has abundant goblet cells which had shown the normal distribution of goblet cell in ocular surface, 4/26 (15.38%) had mild and 2/26 (7.62%) showed moderate reduction of goblet cell. None of them had total absence of goblet cells. Squamous metaplasia was absent in maximum of 21/26 controls (80.76%), only 4/26 (15.38%) controls showed mild grade changes in conjunctival impression cytology (CIC) and 1/26 (3.84%) had moderate grade changes with the loss of cohesion, change in N:C ratio and presence of keratinisation. On Conjunctival biopsy, goblet cell was present in abundance. There was no evidence of squamous metaplasia only some cell showed altered N:C ratio up to 1:3 and altered shape.

Case group

Vitamin A Deficiency (VAD) - Twenty five out of 84 patients clinically had features of Vitamin A deficiency. There were 11 (44%) females and 14 (56%) males. Mean age was 8.68 yrs. All patients of vitamin A deficiency showed (Table 2) occasional presence of goblet cell with mucin spot, patients also showed complete absence of goblet cells. But presence of adequate number of goblet cell was not seen in any patient and, along with the absence of goblet cells, all cells were scattered in clusters with few cell cohesion to isolated in distribution with complete loss of cell cohesion. Maximum patients showed moderate to severe grade change in N:C ratio. While 3/25 (12%) patients showed 1:2 to 1:3 N:C ratio and 14/25 (56%) patients had moderate to severe grade presence of keratinisation (Figure 2). On Conjunctival biopsy, all patients of this group shows absence of goblet cells, all cells were scattered in clusters with few cell cohesion to isolate in distribution with complete loss of cell cohesion. Maximum patients showed moderate to severe grade change in N:C ratio. While 3/25 (12%) patients showed 1:2 to 1:3 N:C ratio and 14/25 (56%) patients had moderate to severe grade presence of keratinisation (Figure 2).

Vernal keratoconjunctivitis (VKC) group – Out of 19 patients, five (26%) were female and fourteen (74%) were male in this group with mean age of 13.47 yrs. Goblet cell count (Table 2) was abundant in 7/19 (36.84%) patients and moderate cell count was seen in 2/19 (10.52%) patients, which were present in clump (Figure 3). Occasional presence of goblet cell was seen in 8/19 (26.31%) cases while only 2/19 (10.52%) patients showed complete absence of goblet cell. Patients in early stage of disease had clumping of goblet cells along with abundant goblet cells. 6/19 (31.57%) patients showed tight epithelial sheet without any loss of cohesion while 12/19 (63.2%) patients showed moderate to severe loss of cell cohesion. Keratinisation was completely absent in 11/19 (57.9%) patients and 6/19 (31.6%) patients showed moderate grade presence of keratinisation. Fourteen patients (73.7%) had N:C

Table 1: Demographic details of patients.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Mean Age (years)</th>
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<td>18</td>
<td>26</td>
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<tr>
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<td>19</td>
<td>13.47</td>
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<tr>
<td>3</td>
<td>Vitamin A deficiency</td>
<td>14</td>
<td>11</td>
<td>25</td>
<td>8.68</td>
</tr>
<tr>
<td>4</td>
<td>Dry eye</td>
<td>11</td>
<td>19</td>
<td>30</td>
<td>11.3</td>
</tr>
<tr>
<td>5</td>
<td>Chemical burn</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>34.3</td>
</tr>
</tbody>
</table>
Figure 1: Grading of Impression cytology.

- PAS (×400)
  - Grade 0: Abundant Goblet Cells
  - Grade 1: Mild reduction of Goblet Cells
  - Grade 2: Moderate reduction of Goblet Cells
  - Grade 3: Mucin Spot/Occasional Goblet Cell

- PAP (×400)
  - Grade 0: No Squamous Metaplasia
  - Grade 1: Loss of Cohesion in form of Cluster with N:C=1:3 to 1:4
  - Grade 2: Scattered Cells with N:C ≥ 1:5 with Moderate Keratinization
  - Grade 3: Complete loss of Cohesion with Anucleated Cells with Severe Keratinization
Conjunctival Impression Cytology of VAD Group

[Squamous metaplasia seen at the surface with Keratinization]

[PAP stain × 400]

Conjunctival Biopsy of VAD Group

[Moderate reduction of Goblet cells]

[H & E, × 100]

Squamous metaplasia with marked Keratinization

Figure 2: Vitamin A deficiency group.
Conjunctival Impression Cytology of VKC Group

[Figure 3: Vernal keratoconjunctivitis group.]

Conjunctival Biopsy of VKC Group

[Squamous metaplasia (N:C ratio 1:2 to 1:3 with mild Keratinization)]

[Cluster of Goblet cells]

[Mild reduction of Goblet cells with clusting]

[Squamous metaplasia with mild Keratinization]
On Conjunctival biopsy, we observed the early keratinization with 1:3 nucleus along with enlarged polygonal cell size. Keratinization were keratinisation. Nine patients (90%) showed 1:4 N: C ratio to pyknotic epithelial sheet clusters were present in 3/10 (30%) patients with early metaplasia, by the presence of pyknotic nuclei and anucleated cells. There was marked presence of keratinisation (Figure 4).

Dry eye group – Out of the 30 patients in this group, 11 (37%) were male and 19 (63%) female, with mean age of 34.3 years. All patients showed moderate reduction to complete absence of goblet cells. In aqueous tear deficiency group all patients showed complete absence of goblet cells while ineffective resurfacing group showed moderate to occasional presence of goblet cells. In 22/30 (73.33%) patients showed absence of goblet cells. In this group different grades of squamous metaplasia was observed, maximum patients showed moderate to severe grade changes while ineffective resurfacing group showed mild to moderate changes. In 29/30 (96.7%) showed complete loss of cell cohesion and cells appeared in isolated condition along with the presence of keratinisation in 24/30 (80%) patients. The metaplastic cells were enlarged along with the change in N:C ratio in 27/30 (90%) patients (Figure 4). On Conjunctival biopsy, we observed a moderate reduction of cells in one patient while the other patient showed occasional presence of goblet cells and severe grade of metaplasia, by the presence of pyknotic nuclei and anucleated cells. There was marked presence of keratinisation (Figure 4).

Chemical injury group - Ten patients (7 male and 3 female) were included in this group with mean age of 11.3 years. Eight patients (80%) showed complete absence of goblet cells while in 2/10 (20%) patient’s occasional presence of goblet cells was seen. Normal epithelial sheet clusters were present in 3/10 (30%) patients with early keratinisation. Nine patients (90%) showed 1:4 N:C ratio to pyknotic nucleus along with enlarged polygonal cell size. Keratinization were present in 8/10 patients (80%) of moderate to severe grade (Figure 5). On Conjunctival biopsy, we observed the early keratinization with 1:3 ratio of N:C in one patient while the other patient showed the 1:4 to 1:5 N:C ratio with polygonal in shape and enlarge in size with marked presence of keratinisation (Figure 5).

Impression cytology showed, goblet cell reduction in 91.66% cases, while 8.33% cases have normal range of goblet cell count. Abundant goblet cell found in 76.92% controls, while 23.07% controls showed reduction of goblet cell population. Squamous metaplasia was seen in 88.09% cases, while in 11.90% cases squamous metaplasia was absent. In controls, 80.76% subjects showed absent squamous metaplasia while 19.23% controls showed mild to moderate grade of squamous metaplasia.

In our study impression cytology results was positive in 75/84(89.28%) patients and negative in 9/84 (10.71%). In controls, it was positive in 6 persons (23.07%) and 20/26 (76.92%) showed normal cytological features of conjunctiva. This difference was statistically significant as ρ value < 0.001.

In our study, we found the Sensitivity of Conjunctival impression cytology 89.28%, Specificity of Conjunctival impression cytology 76.92% and Diagnostic Accuracy of Conjunctival impression cytology 86.36%.

Discussion

In ocular surface disorders, almost all the diseases lead to dry eye which affects the quality of life and may lead to sight threatening situations. Most studies report a higher prevalence of dry eye in females than males [8-11]. Our study was no exception; 36.66% males as compared to 63.33% females in dry eye patients. Impression cytology has been used to study conjunctival epithelium in several diseases of the ocular surface [12,13]. Diagnosis of dry eye is still a challenging task in many cases. Factors that are contributed to make the diagnosis difficult and required for further attention are: low performance of most conventional tests and the overlapping of dry eye symptoms with those of other conditions. Newer non-invasive tests are still confined to a few centres, because they require expensive instruments and also because of their complexity. Conjunctival biopsy is a surgical technique which is traumatic and makes the patient more apprehensive and gives the imminent complications of sub-conjunctival haemorrhage and infections. Conjunctival impression cytology is a simple, non-invasive, repeatable, easy to perform, and yields almost equal information with minimal discomfort to the patients. It allows the quantitative assessment of the number of goblet cells in the conjunctiva and qualitative analysis of metaplastic changes of epithelial cells. It not only helps in detecting those at impending risk of developing symptoms but also helps in assessing the severity of cytological alteration of symptomatic patients. Eghert first used this method to determine, the density of goblet cells in different areas of conjunctiva [3]. Impression cytology usually removes one to three cell layers and thus is ideal for studying surface epithelium rather than basal epithelium or the basement membrane [14]. The exfoliated and exudative cytological response of conjunctiva has interested investigators for many years because of the clues it offers to the diagnosis and study of the pathogenesis of many types of conjunctival disorders [15]. Impression cytology of the conjunctiva is a valuable diagnostic technique in detecting ocular surface disorders such as allergic conjunctivitis, xerophthalma, keratoconjunctivitis sicca and also in other diseases. The most widespread use of this procedure is detection and grading of squamous metaplasia. Wittpen et al reported that the conjunctival imprint cytology is abnormal in children with vitamin A levels were less than the 20 µg/dl [16]. In this study, we analysed the potential of conjunctival impression cytology for the diagnosis of ocular surface disorders, compared with

<table>
<thead>
<tr>
<th>Groups</th>
<th>Grade</th>
<th>Controls (n=26)</th>
<th>VKC (n=19)</th>
<th>Vitamin A Deficiency (n=25)</th>
<th>Chemical Burn (n=10)</th>
<th>Dry Eye (n=30)</th>
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<td></td>
<td>Absent</td>
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<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
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<td></td>
<td>Mild</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
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<td>10</td>
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<td></td>
<td></td>
<td>Severe</td>
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<td>0</td>
<td>6</td>
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</tbody>
</table>
Conjunctival Impression Cytology of Dry Eye Group

[PAP stain ×400]
All the metaplastic cells showing Keratinization

Occasional Goblet cell
Loss of cohesion with intracellular Keratinization

Conjunctival Biopsy of Dry Eye Group

[H & E × 100]
All the metaplastic cells showing keratinization

Figure 4: Dry Eye Group.
Figure 5: Chemical burns group.
the most common clinical tests used in daily practice. In this study, CIC specimens were examined and staged according to the degree of squamous metaplasia as described by Wittpen et al [17]. Wittpen et al studied the impression cytology of VKC and found two main features of goblet cells: (1) an increased number of clumped goblet cells, with mucus strands on the specimen; (2) a reduced number in specimens with signs of squamous metaplasia. We observed the same findings in the results of VKC group. Apart of this we also observed a peculiar feature in vernal keratoconjunctivitis group i.e. intraepithelial inflammatory cells, which were arranged in snake like configuration in between the epithelial cells. Nelson et al. [18] demonstrated the greater loss of goblet cell in the case of dry eye patients. As studied by Wittpen et al. [19] and Singh et al. [20] in conjunctival impression cytology of vitamin A deficiency, there was complete loss of goblet cell with the various grades of squamous metaplasia. Reddy et al. [21] reported the various grades and suggested that main features of impression cytology were squamous metaplasia and goblet cell deficiency in keratoconjunctivitis sicca. In our study, patients with vitamin A deficiency had 100% absence of goblet cell with occasional presence of mucin spots. Squamous metaplasia was seen in 24% of severe grade, 56% of moderate grade and mild grade changes was seen in 12%. However, 8% of patients have absence of squamous metaplasia. So results of our study were comparable to other studies in patients of vitamin A deficiency [6,7,19,21]. We reported different parameters to classify the CIC i.e., goblet cell count and squamous metaplasia, we did a new grading system by taken all the features of squamous metaplasia and the goblet cells on the basis of light microscope without using morphometric analysis. In our study group, we observed the goblet cell numbers, loss of cohesion between epithelial cells, nucleo-cytoplasmic ratio along with the size and shape of cells and the absence and presence of keratinisation.

According to our classification, we found various grade of ocular surface disorders. Out of 110, 84 cases 10.71% patients showed Grade 0, 9.52% had Grade 1, 30.95% came in Grade 2 and rest 48.80% showed abnormal grade i.e. Grade 3. In 26 controls, no one had abnormal grade (Grade 3), only 3.84% had Grade 2, 19.23% persons showed Grade 1 and the maximum of normal persons shown the normal cytological features of the conjunctiva. On conjunctival biopsy, we found the corresponding results with impression cytology. Although the sensitivity and specificity of biopsy is 100% with 100% diagnostic accuracy, but due to painful and traumatic procedure and to avoid unwanted complication, we did it in 10 subjects only (2 from each group) to compare the impression cytology which was carried out in all the patients. Therefore, we are not able to do statistical analysis. The limitation of our study is that distribution of the cases was not uniform in all the groups and accordingly age distribution was also different with respective to the diseases. Dry eye group was found in increased age group while the patients of VKC and VAD occurred in younger age group. In controls, due to lesser number of the persons and also the large range of age distribution, it was not comparable to a particular disease.

**Conclusion**

In conclusion, conjunctival impression cytology should be included as a screening test for the detection of ocular surface disorders, so ocular surface disease is detected at an early stage. Thus it will help in the prevention of ocular surface disease which might subsequently lead to blindness. It can be concluded that the ocular surface disorder brings a change at the cytological level, as evidenced by decreased goblet cell density and altered the epithelial cell morphology interpreted by conjunctival impression cytology, which usually remains undiagnosed by other clinical parameters.

**References**


