Role of conjunctival impression cytology in various diseases of eye

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ABSTRACT
Background: Conjunctival impression cytology is a recent method to diagnose various eye diseases. Aim of this study is to observe the cellular changes in conjunctival superficial layer in various diseases of eye and establishing the diagnosis and helping in the management of the disease.
Methods: Cellulose acetate filter paper is used to take impression from the bulbar conjunctiva.
Results: 80 cases were studied for a span of 3 years.
Conclusions: Conjunctival impression cytology is a useful method of screening, diagnosing and managing eye disease.

Keywords: Conjunctiva, Impression cytology, Cellulose acetate paper, Eyes

INTRODUCTION

Conjunctival impression cytology is a recently introduced method for study and diagnosis of eye diseases affecting conjunctiva. A cellulose acetate filter paper is applied on the conjunctival surface to remove the superficial layer of the conjunctiva. The sample is then stained by PAS and Haematoxylin and cytological analysis is done.

This method was first used by Egbert PR et al to study conjunctival goblet cells.¹ This method can be used to detect dry eye syndrome, vitamin A deficiency and trachoma, metaplastic and dysplastic changes can also be diagnosed.² Early diagnosis of neoplasm of eye can be done by this method.³

This technique can also be applied for documenting sequential changes in conjunctiva over the time, monitoring effect of treatment and staging conjunctival squamous metaplasia, as investigational tool for analyzing ocular surface disease with immunostaining and DNA analysis. Aim of our study is to observe the cellular changes in conjunctival superficial layer in various diseases of eye and establishing the diagnosis and helping in management of eye diseases.

METHODS

Cellulose acetate filter paper is used to collect specimen. Cellulose acetate paper is cut in size of 3 x 10mm with diagonal end. Eye is anesthetized by one drop of paracain. Palpbral fissure is widened by retracting eye lid with finger and thumb of one hand, with other hand filter paper applied over the bulbar conjunctiva and left for 4-6 seconds.⁴ Then filter paper is removed with forceps in a peeling motion.

The specimen is then fixed by dipping in a fixative containing glacial acetic acid, formaldehyde and ethyl alcohol in ratio 1:1:20 for 10 minutes.⁵ Then it is rinsed in tap water for two minutes. The strip is dipped in 0.05% periodic Schiff reagent for 8 minutes sodium metabisulfite for 2 minutes and in Haematoxylin for 30 seconds. The strip is rinsed for 2 minutes with tap water after each step above. Now 95% ethyl alcohol is applied over strip for 2 minutes. The strip is dehydrated with
absolute alcohol. Lastly xylin is applied over filter paper to make it transparent.

The stained specimen is mounted over a slide with coverslip and examined under microscope. Guazzi A et al also did conjunctival cytology using cellulose acetate paper. 5 Meena MKB et al used nylon paper instead of cellulose acetate paper. 6

RESULTS

A total of 80 patients were examined in a span of 3 years from 2012-15 at Patna Medical College and Hospital, Patna. Age group was from 5 to 90 years. 45 were males and 35 were females.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Total no. of cases</th>
<th>Metaplasia (No. Of cases)</th>
<th>Goblet cells (No.of cases)</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Refractory error</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Corneal ulcer/keratitis</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Red eye</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blepheritis</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Itching</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dry eye</td>
<td>12</td>
<td>5</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Pterygium</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Exposed eye</td>
<td>5</td>
<td>-</td>
<td>2</td>
<td>-</td>
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<tr>
<td>Watering eye</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Episcleritis</td>
<td>2</td>
<td>-</td>
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</tr>
</tbody>
</table>

DISCUSSION

9 patients showed squamous metaplasia. Classification of squamous metaplasia according to Reddy V et al. 2

1. Mild slightly enlarged polygonal cells, N/C ratio 1:2 to 1:4. No keratinization.
3. Severe- N/C ratio 1:8 Advanced keratinization.

Inflammation was present in 39 cases. Goblet cell was absent in 2 cases; scanty in 14 cases and normal in 64 cases. Nelson JD et al have counted the goblet cell but counting of goblet cells were not done in this study. 8,9

12 cases have refractory error and 2 cases of watering eyes had a normal cytology. 18 cases of conjunctivitis had inflammation on cytology. 10 Corneal ulcer, 6 cases, 5 of which had inflammation, Red eye (5 cases), Blephitis (6 cases), Episcleritis 2 cases had inflammatory findings. Itching (8 cases) showed inflammation with scanty goblet cells in 2 cases. Dry eye 12 cases of which 5 showed mild metaplasia, 2 moderate metaplasia, exposed eye 5 cases of which 2 showed mild metaplasia. 11

Natadisastra G et al and Amedee-Manesma O et al studied conjunctival impression cytology in Vitamin A deficient patients and concluded that this was a good method to detect vitamin A deficiency and also to follow up after treatment. 12,13

Its use can be further extended by transferring the material to gelatin coated glass slides then polymerases chain reaction, immunohistochemistry and flowcytometry done. 14 It can diagnose ocular neoplasm, staging of metaplasia and monitoring effect of treatment. 15

CONCLUSION

The conjunctival impression cytology is new, reliable, non-invasive easy and comfortable to patients. This is a suitable method to obtain information from conjunctival surface. It gives good result with high rate of picking abnormalities in suspected specimen. It is cheap and easy procedure and can be incorporated as one of the routine investigation for patients with conjunctival disease. It is an outdoor procedure with a little expertise needed.

Before this method conjunctival scapping and tissue pieces were taken. This caused a lot of trauma to the patients. Egbert PR et al tried this method. They used different materials like photographic films, cellophane tape and synthetic fibres. Ultimately cellulose acetate filter paper was found to be most effective.

Higher investigations such as polymerase chain reaction, immunohistochemistry and flowcytometry can be done by transferring the material obtained on cellulose acetate filter paper to gelatin coated glass slide. These way malignant neoplasms can be diagnosed. So impression cytology can be used for screening, early diagnosis and management of various eye diseases.
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REFERENCES
