Impression cytological study for ocular surface disorders of late stage eye burns

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Abstract. – OBJECTIVE: This study aims to explore the ocular surface of late stage eye burns by impression cytology (IC) and analyze the cytological changes and their relationship to ocular surface abnormalities.

PATIENTS AND METHODS: 68 eyes with late stage eye burns (thermal burn: 28 eyes; alkali burn: 26 eyes; acid burn: 14 eyes), procured from 68 patients (aged ranges from 17 to 70 years old). Ocular surface abnormalities were assessed under slit lamp and graded. These were broadly classified as eyelid, corneal, conjunctival, and tear film abnormalities. Impression cytological examination was taken by cellulose acetate filter paper for all eyes. Samples were analyzed and scored under light microscope, including the status of epithelial cells, goblet cells, mucus and inflammatory cells. All the results and data were compared and analyzed by SPSS software (version 16.0).

RESULTS: According to the IC results, loosed cell-to-cell density and nuclear abnormality, keratinization, reduced goblet cell amount, disorder of mucus, and existing of inflammatory cells were observed in almost all the cases. The IC results were significantly correlated to the ocular surface injury severity (r=0.458, p<0.01). The ocular surface injury severity mostly contains three aspects: the corneal neovascularization scales, the present or absent of recurrent epithelial erosion and the tear film break-up time. Eyes with the foreword three symptoms were inclined to have higher IC scores. The epithelial cell-to-cell density, goblet cell and mucus amount were all correlated to tear film break-up time. However, inflammatory cell density showed no significant correlation to the conjunctival hyperemia grade. But inflammatory cell density correlated to the corneal opacity grade and epithelial stability status.

CONCLUSIONS: IC examinations could reflect the cytological disorders and relative injury severity of the ocular surface in late stage eye burns. It provides further information which will be useful in surgery and therapy.

Key Words: Eye burn, Impression cytology, Ocular surface disorder, Limbal stem cell deficiency, Goblet cell, Mucus.

Introduction

Ocular traumatic events commonly occur in the condition of thermal or chemical burns. It may produce devastating injuries to the eye ball. Moderate and severe burns always result in permanent damages and cicatricial changes even though after the emergency treatment and therapy. That could be a different extent of symblepharon, lid abnormalities, corneal neovascularization and conjunctivalization. The etiology concluded limbal stem cell deficiency (LSCD), or recurrent epithelial erosion, keratinization, and so on1-3.

As for the late stage of burns, ocular surface reconstruction would be the main causes. Surgery skills and technologies were closely related to the successful of ocular surface reconstruction. The general prognosis of late stage of ocular burns was mostly decided by the presurgery status: the severity of the injury eye. Eyes with limbal stem cell deficiency would be designed to take an allo-limbal transplantation4,5, while autograft might be used in partial deficiency eye6-7. Santos et al8 think the autograft therapy would have a better outcome. In short, a complete and accurate evaluation of the trauma ocular surface and status is important and necessary for patients who need undertake tissue-engineering and stem cell technologies treatment9-11.

Usually, the assessment of ocular surface was conducted by slit lamp according to some grading systems12-14. Because of limited by clinical in vitro studies, there are few available methods to study the ocular surface in cellular levels. Im-
Impression Cytology (IC) was commonly used in various aspects as a convenient and noninvasive approach. It had many grading systems to evaluate the cytological condition of the ocular surface. But there were seldom studies about the cytological changes of ocular surface in late stage eye burns. In this research, we used IC to examine the ocular surface features at a cellular level and to find out their relationship with clinical.

**Patients and Methods**

**Patients**

All the patients were planned to take an ocular surface reconstruction surgery at the Ophthalmology Department of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, from October 2011 to March 2014. A total of 68 patients with an average of 42.06 ± 13.10 years old (17-70 years), were recruited in the study. There were 60 males, 8 females. And, a total of 68 eyes with lately ocular burns were studied: 28 eyes were thermal burns, 26 eyes were alkali burns and 14 eyes acid burns. All the eyes with chronic inflammatory and cicatricial changes were diagnosed as a late stage of ocular surface burns. The chronic inflammatory and cicatricial changes include symblepharon and eyelid abnormalities. Patients diagnosed acute eye burns and occurrent eye infections were excluded from this study. The average history was 5.80 years, ranged from 0.5 to 36 years.

**Clinical Examination**

In order to analyze the relationship between the clinical abnormalities and IC results, a clinical grading system was performed. All the clinical examinations were conducted under the slit lamp by the same experienced ophthalmologist.

The grading system is referred to assessment of the four ocular surface components: the eyelid, the cornea, the conjunctiva and the tear film. (1) Evaluation for the eyelid: ectropion or entropion, trichiasis, defects, lid margin or/and palpebral conjunctiva keratinization. Each complication accounts for 1 score. So the eyelid abnormality is graded on a scale from 0 to 4. (2) Evaluation for the cornea: include 3 parts: the corneal neovascularization scales, the corneal opacity degrees and the corneal epithelial stability. The scale of corneal neovascularization is graded from 0 through 3, where 0 = no neovascularization, 1 = neovascularization involving less than half of the limbus, 2 = neovascularization involving half or more than half of the limbus, but not full circle, and 3 = neovascularization involving the whole circle of the limbus area. Neovascularization also contains extensive symblepharon formation which reached to the cornea. The corneal opacification is graded from 0 through 3. 0 = clear cornea with iris details clearly visualized, 1 = partial obscuration of the iris details, 2 = iris details poorly seen with pupil margin just visible, and 3 = completely obscuration of iris and pupil details. The corneal epithelial stability is score for 0 to 1. 0 = complete and stable epithelium, and 1 = recurrent epithelial erosion. (3) Evaluation for the conjunctiva. The assessment for conjunctiva include the symblepharon scales and conjunctiva hyperemia degrees. According to Kheirkhah et al, the assessment for conjunctiva include the symblepharon length and width, also the hyperemia degrees which gave information of inflammatory activity. (4) Tear film break-up time: This was judged by fluorescein staining under slit lamp and scored from 0 to 2. 0 = longer than 10 seconds, 1 = 10 or less than 10 seconds, but more than 5 seconds, 2 = less than 5 seconds. The total clinical evaluation grading system was count from 0 to 22. A high clinical evaluation score indicated a wide ranged damage of the ocular surface.

Impression Cytology

The evaluation for IC results were composed of two parts: the analysis for the bulb conjunctival epithelial cells and the corneal surface epithelial cell types. A cellulose acetate filter paper (milipore filter paper, 0.22 µm pore size) is used in IC. The filter papers were pre-cut into pieces (4×8 mm) with a pointed tip on one corner. The patients were administered topical anesthetics (0.4% oxybuprocaine eye drops). The cellulose acetate filter paper pieces were then applied to four sites: upper, lower, nasal, and temporal limbus including both parts of cornea and conjunctiva areas (Figure 1). The pieces were stained periodic acid Schiff (PAS) and hematoxylin eosin. All the examinations under a light microscope were performed by the same researcher, who was unaware of the clinical status.

According to the grading system by Haller-Schober et al, the scores incorporate biology characteristic of epithelial cells (including the
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Results

Clinical Results

The average clinical examination score for the clinical evaluation was 12.31±3.84 (ranged from 2 to 20). The evaluation results had no correlation to disease history and age. 65 eyes existed eyelid abnormality. There were 42 eyes have full scale of neovascularization of cornea, the rest 26 eyes contained partial neovascularization. Different extent of corneal opacity occurred in 65 eyes, corneal epithelial defects were found in 8 eyes, symblepharon existed in 51 eyes, and all eyes had a limited Breakup time (BUT) (Table I).

Impression Cytological Results

A total of 232 pieces of IC samples were obtained from 68 eyes. Some eyes presented severe symblepharon, due to which the sample size was affected by only 1 to 3 pieces of samples could be procured. Almost all the eyes were turned out to have a declined conjunctival epithelial healthy status. There were 67 eyes had cell-to-cell adhesion abnormality, 66 eyes incorporated epithelial cell keratinization, 67 eyes encompassed reduced goblet cells density, 64 eyes involved mucus amount disorder and 66 eyes existed inflammatory cells. Besides, 36 eyes had declined nucleo/cytoplasmic ratio (N/C ratio) and 59 eyes had pathological nuclear changes (Table II). The average IC score was 15.84±4.32 (ranged from 8 to 26). Data analysis revealed that IC score had no significant correlation with disease course or age. It had no statistical difference between gender and etiologies. Besides, corneal surface cell type analysis showed that conjunctiva cells were detected in all neovascular invasion eyes. Among the 42 full-scale neovascularized eyes, 40 eyes without corneal epithelial cells existence, and the rest 2 eyes had both corneal and conjunctival epithelial cell (Figure 3).

Relationship Between Clinical Results and IC Analysis

The IC score was correlated to the clinical assessment result (r=0.458, p<0.01) (Figure 3), especially to the corneal neovascularization scales, epithelial stability and BUT (r=0.367, p=0.002; r=0.312, p=0.009; r=0.491, p=0.000; respectively). The IC scores compared by Kruskal-Wallis showed significant difference between different neovascularization scale groups (p<0.01). The grade 3 group (full circle)
owned the highest IC scores which was 16.90±4.23, and the grade 2 group (half or more than half, but not full circle) which was 15.17±4.25, the grade 1 group (less than half) showed the lowest IC scores as 11.75±1.75. The more the neovascularization scale was, the higher the IC score counted. The average IC score of recurrent epithelial erosion cases was higher than the group with stable epithelial, the difference was significant ($p<0.01$). Likewise, cases with shorter tear film break-up time presented higher IC scores ($p<0.01$). In short, it was indicated that corneal neovascularization scales, epithelial stability and tear film inadequacy was responsible for the ocular surface cytological status.

![Figure 2. IC pictures under light microscope (a-e) and ocular surface photo under slit lamp (f). (a) Normal dense epithelial cells with abundant goblet cells. (b) Normal dense epithelial cells and distinctly reduced goblet cell, with pathological changes. Small block was the enlargement for goblet cell. (c) Distinct keratinization of the epithelial cells with pathological changed nuclei (arrows), the N/C ratio was 1:4-1:6. (d) Abundant mucus amount with exudate, filaments and aggregation changes. Single cells were seen among mucus. (e) IC samples for the superior quadrant of the eye in picture f. Loosened cell sheet without goblet cells, a cluster of corneal epithelium cells were seen (arrow), which was large in size, lightly stained and high nucleocytoplasmic ratio compared to conjunctival epithelium cells. Microscopic magnification for IC pictures: a-b 400×, e 200×. (f) Slit-lamp photo for the same patient of picture Figure 1 e, full circle invasion of new blood vessels into the limbus could be seen.](image-url)
The BUT showed a correlation to cell density, goblet density and mucus amount ($r=0.376$, $p=0.002$; $r=0.568$, $p=0.000$; $r=0.245$, $p=0.044$; respectively). The three parameters were considered as related to tear film stability. Notably, IC score appeared to have no significant correlation with the symblepharon scales. The keratinization grade and N/C ratio showed no significant correlation with all the assessed clinical indexes. The inflammatory cells density in IC results showed no significant correlation with the conjunctiva hyperemia grades ($p>0.05$), but had certain correlation to the corneal opacity grade ($r=0.244$, $p=0.044$) and epithelial stability status ($r=0.272$, $p=0.025$).

**Discussion**

The broadly definition for ocular surface is consisted of eyelid, conjunctiva, cornea and tear film. The assessment for the ocular surface disorder grade should include the integrity of these four parts and functions. Observation under slit lamp could offer the ocular surface as an anatomical evaluation. The IC grading system and subparameters (cell-to-cell adhesion, N/C ratio, the tendency for keratinization, nuclear changes, the morphology and density of conjunctival goblet cells and mucus, the presence or absence of inflammatory cells) used in this research gave a cellular level assessment for the ocular surface\(^1\). Besides, the type of cells on corneal surface would help to identify the total or partial LSCD\(^2,3\).
In this study, the main features of the ocular surface for late stage eye burns were found declined in the epithelial cell quality, reduced goblet cell density and existence of inflammatory for bulb conjunctival. The epithelial cells tend to be enlarged, high N/C ratio, abnormal nuclear and keratinized. These were processes to squamous metaplasia, losing the normal function of conjunctiva as a smooth and secretory stratified epithelium. Additionally, the reduced goblet cells and pathological change might be other important reasons for dry eye. Evaluation of all these aspects of ocular surface was critical for the designation of surgery.

Attentions should pay to the inflammatory status, especially in those cases designed to undergo the stem cell engineering therapy. The ex vivo cultivated autologous limbal stem cells or alternative stem cells could used in cure the late stage eye burns. The alternative stem cells contained oral mucosa epithelium stem cells, mesenchymal stem cells and hair follicle stem cells. These stem cell sensitive to the survival environment and recurrent epithelial erosion. The statistical results of our research also showed that inflammatory grade was correlated to the epithelial stability condition. In those moderate or severe inflammatory eyes, anti-inflammatory drugs like glucocorticoid could be selectable administered. Postponement of the surgery was underlined to make a better microenvironment for the transplanted tissue.

Another distinct feature of this stage was the conjunctivalization of corneal surface. Conjunctival cells were detected in all neovascular invasion eyes. This was caused by the loose of normal limbal stem cells. The assessment for the exact limbal stem cell deficiency scales were crucial for the surgery strategy. The results revealed that a bigger scale of corneal neovascularization eyes would get a higher IC score, which means a worse healthy condition for the ocular surface. This was inspired that the reserve of certain areas of normal or subnormal limbus in the limbal reconstruction surgery would lead to a better surgery result. As in partial LSCD, where there had normal limbal stem cell remained, a

### Table II. Data for IC assessment results.

<table>
<thead>
<tr>
<th>IC sub-scores</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-to-cell dense</td>
<td>1(1.5%)</td>
<td>20(29.4%)</td>
<td>28(41.2%)</td>
<td>19(27.9%)</td>
<td>68(100%)</td>
</tr>
<tr>
<td>Nucleo/Cytoplasmic ratio</td>
<td>32(47.1%)</td>
<td>28(41.2%)</td>
<td>6(8.8%)</td>
<td>2(2.9%)</td>
<td>68(100%)</td>
</tr>
<tr>
<td>Keratinization</td>
<td>22(2.9%)</td>
<td>33(48.5%)</td>
<td>28(41.2%)</td>
<td>5(7.4%)</td>
<td>68(100%)</td>
</tr>
<tr>
<td>Nuclear change frequency</td>
<td>9(13.2%)</td>
<td>31(45.6%)</td>
<td>20(29.4%)</td>
<td>8(11.8%)</td>
<td>68(100%)</td>
</tr>
<tr>
<td>Nuclear change type</td>
<td>9(13.2%)</td>
<td>28(41.2%)</td>
<td>23(33.8%)</td>
<td>8(11.8%)</td>
<td>68(100%)</td>
</tr>
<tr>
<td>Goblet cell density</td>
<td>1(1.5%)</td>
<td>6(8.8%)</td>
<td>19(27.9%)</td>
<td>42(61.8%)</td>
<td>68(100%)</td>
</tr>
<tr>
<td>Goblet cell morphology</td>
<td>51(75.0%)</td>
<td>-</td>
<td>-</td>
<td>17(25.0%)</td>
<td>68(100%)</td>
</tr>
<tr>
<td>Mucus amount</td>
<td>4(5.9%)</td>
<td>6(8.8%)</td>
<td>40(58.8%)</td>
<td>18(26.5%)</td>
<td>68(100%)</td>
</tr>
<tr>
<td>Mucus morphology</td>
<td>8(11.8%)</td>
<td>10(14.7%)</td>
<td>29(42.6%)</td>
<td>21(30.9%)</td>
<td>68(100%)</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>2(2.9%)</td>
<td>37(54.4%)</td>
<td>15(22.1%)</td>
<td>14(20.6%)</td>
<td>68(100%)</td>
</tr>
</tbody>
</table>
better surgical efficiency would be expected. Although full scaled neovascularization was observed, a few of them still had detectable corneal epithelial cells. This might due to the survival of residual corneal stem cells located deep in the limbal basal layer. In this term, the IC results would give a more accurate result. Above all, it could be inferred that IC examination provided detailed and accurate cytological information of the ocular surface for late stage eye burns, which might help to conduct a better surgical design.

The major deficiency in our study is that the clinical assessment was done only by one doctor during the research and results were conducted by two or more ophthalmologists. In our further following up research works, we will study the possible relation between pre-surgery IC evaluation and surgical efficiency, as well as the post-operative complications.

Conclusions

The IC examinations reflect the cytological disorders and are related to the injury severity of the ocular surface in late stage eye burns. IC can help to give a more complete and reliable assessment of the ocular surface and guide design the surgical strategies of the late stage eye burns.

Acknowledgements

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Conflict of Interest

The authors declare that they have no conflict of interests.

References


