Impression cytology (IC) refers to the technique in which cellulose acetate filter paper is applied to the ocular surface for collection of superficial layers of the ocular surface epithelium. This technique was established in 1977\(^1\) by Egbert et al for studying goblet cells. Nelson graded the conjunctival impression cytology specimens based on the number of goblet cells (grade 0-3).

Impression cytology is also a useful technique for aetiological diagnosis of various ocular surface diseases like Keratoconjunctivitis sicca\(^2,3\), vitamin-A deficiencies, atopic\(^4\)

**Clinical photographs of the ocular lesion**


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disease, cicatrizng conjunctivitis, dry eye syndrome, stem cell deficiency and neoplasias (Figure 1). It is also useful in the monitoring effects of treatment and documenting sequential changes in conjunctiva and cornea over time. Follow up of ocular surface squamous neoplasia (OSSN) patients on Mitomycin-C (MMC) can be followed up by IC, to demonstrate recurrence of diseases.

IC is non-invasive, easy to perform and provides reliable information about the sampled area. This makes it a valuable tool in understanding of ocular surface disorders.

Cellulose acetate filter paper/millipore filter paper with pore size (0.22mm) is used. Filter paper is trimmed into 5mm strips (Figure 2) and applied to conjunctiva/ cornea or the limbus. Area to be sampled depends on the underlying pathology. Filter paper facilitates the adhesion of the cells from the surface of the lesion.

**Methodology**

One drop of local anesthesia (paracain) is instilled into the patient’s eye. Before applying filter paper on the area to be sampled, excessive tear/medication should be wiped away. Now mark the surface of the paper before applying on to the ocular surface. It is important to retract the lids from the paper (Figure 3).

At least two samples are taken from the area to be sampled depending on size of the lesion. The paper is pressed gently with a glass rod for 5-10 seconds on to the lesion and immediately transferred into a coplin jar containing 95% ethyl alcohol for fixation (Figure 4). The filter paper
Retrospective analysis at our centre over a period of 2 years revealed a total number of 855 cases which were subjected to various cytological procedures. IC was done on 662/855 (77.42%) cases. They were clinically diagnosed as ocular surface tumours 70 cases (10.5%) and limbal stem cell deficiency/dry eye deficiency 80 cases (12%) and miscellaneous 512 cases (77.34 %) including melanocytic lesions and inflammatory lesions.

Impression smears of various lesions as seen on Periodic Acid Schiff’s (PAS) stain (special stain for goblet cells) (Figure 9a) and Haematoxylin and eosin stain (Figure 9b-f).

Limitations

The ophthalmologist should be aware of the inherent draw back of false negative results which could be due to sampling error and lack of trained ophthalmologist or technical staff and non co-operation by the patient. In such cases IC should be repeated. Another disadvantage is that it cannot differentiate between severe dysplasia and invasive squamous cell carcinoma.

Being cumbersome and time consuming procedure it is not popular in all laboratories. However, it can be established in laboratories with trained laboratory personal, ophthalmologist and pathologist.

To conclude

Impression cytology is a very useful, simple, diagnostic and non invasive technique. It is helpful in the early diagnosis, staging and monitoring effects of treatment. Biopsies can be avoided in suspected malignancies and it is useful in follow up of recurrent cases.

We therefore recommend that IC may be introduced as a routine clinical practice for all ocular surface disorders.
Figure 9: Light microscopic appearance (a): Goblet cells (PAS stain). (b): Squamous metaplasia. (c): Mild dysplasia. (d): Moderate dysplasia. (e): Squamous cell carcinoma/severe dysplasia. (f): Post mitomycin-c changes
References
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