Original Article

# Reliability and Utility of Impression Cytology in the Diagnosis of Dry Eye

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## Abstract

**Introduction:** Dry eye disease is a multifactorial disease of the tears and ocular surface that can result in ocular discomfort and visual impairment. There are numerous tests to evaluate the ocular status in dry eye disease. Among the different tests for diagnosing dry eyes, Impression Cytology is considered a practical and minimally invasive investigation. **Objective:** The aim of this study was to characterize ocular surface symptoms typical of dry eyes, study pattern of dry eye in various systemic diseases, assess whether impression cytology samples could be used to define conjunctival surface changes and describe changes in conjunctival cells. We also aimed to compare the sensitivity of impression cytology with other diagnostic tests. **Methods:** A cross sectional study was conducted among 100 patients with symptoms of dry eye. After eliciting a complete history which included any systemic disease, occupation and drug intake these patients were subjected to various tests like Schirmers test, Tear Break Up Time (TBUT), Lissamine green staining and Conjunctival impression cytology. **Results:** All clinical tests have limited diagnostic value if performed individually, but impression cytology showed the highest sensitivity. **Conclusion:** It is recommended that impression cytology is an ideal method of investigating ocular surface disorders when diagnosis is not clinically obvious or when clinical diagnosis needs substantiation. It is recommended that major ophthalmic centers should develop and introduce this technique into routine clinical practice.

**Key words:** Dry eye, impression cytology, lissamine green, tear break up time

## Introduction

Dry eye disease is a major healthcare problem due to its prevalence and impact on patients' quality of life. Dry eye disease is a multifactorial disease of the tears and ocular surface that can result in ocular discomfort and visual impairment. The prevalence of dry eye is considered to be more common in Saudi Arabia due to dry environmental conditions.[1]

The National Eye Institute Industry Workshop on Clinical Trials in Dry Eyes defines "dry eye" as "a disorder of the tear film due to tear deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface."[2] Despite significant advances, our understanding of the pathogenesis of dry eye is still in evolution. There is no single test to completely evaluate ocular tear film as each test examines part of a process, and results of various tests taken together can get a more complex look at this dynamic process.[3]

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Impression cytology is a practical and minimally invasive method performed under topical anesthesia to obtain superficial cells by application of small membrane against conjunctival surface.[4] It is considered to be a useful test for dry eye syndrome.[5]

Conjunctival impression cytology has a wide range of applications in ophthalmology in the realm of diagnosis, therapy, and prognosis of ocular surface disorders. It facilitates the diagnosis of ocular surface disorders such as keratoconjunctivitis sicca, ocular surface squamous neoplasia, and ocular surface infections.[6] It is a useful test in the

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diagnosis of dry eye, in which there is a significant reduction in goblet cell density[5] and a higher Nelson's grade in severe dry eye.[7] It has been found to correlate well with the duration of computer use[8] and used to evaluate the efficacy of topical cyclosporine in different grades of dry eye.[9] Impression cytology is being increasingly used at present to assist in the diagnosis of ocular surface disease, improve the understanding of the pathophysiology of ocular surface disease, and provide biomarkers to be used as outcome measures.[6,10] Impression cytology with scanning electron microscopy has also been used to detect reduction in microvilli, which could detect dry eye damage even before epithelial damage occurs.[11]

The objective of this study was to characterize conjunctival surface cellular changes typical of dry eyes, study conjunctival cell patterns of dry eye in various systemic diseases, assess whether impression cytology samples could be used to define conjunctival surface changes, and describe changes in conjunctival cells. We also aimed to compare the sensitivity of impression cytology with other diagnostic tests.

## Materials and Methods

In this cross-sectional study conducted in the Ophthalmology Department of the medical college from September 2012 to August 2013, a total of 100 eligible patients above 20 years of age with symptoms suggestive of dry eye (based on ocular surface disease index [OSDI]) were studied. It consists of three sub-scales including a vision-related function scale, ocular surface symptoms scale, and environmental trigger scale.[12] All patients were taken into the study only after fully informed consent was obtained.

After eliciting a complete history which included any systemic disease, occupation, and drug intake, these patients were subjected to various tests such as Schirmer's test, tear break up time (TBUT), lissamine green staining, and conjunctival impression cytology.

### Conjunctival impression cytology

Method: Cellulose acetate filter paper (millipore filter paper) is cut into small strips of 5 mm × 5 mm. The conjunctiva is anesthetized with topical anesthesia. With a blunt, smooth-edged forceps, the filter paper is applied with dull side down to the bulbar conjunctiva. Gentle pressure is applied with a smooth glass rod for 3–5 s and then removed with a peeling motion. The filter paper is then immediately pressed cell side down onto a clear glass slide. The slides are placed horizontally in a Petri dish for 3–4 h in a solution containing freshly prepared three parts acetone and one part of a mixture of ¼ 95% methanol and ¾ 95% ethanol. The slides are then fixed in absolute alcohol and stained with Papanicolaou stain and mounted in DPX. These slides are then examined under light microscope under low power and high power. Nelson's grading system was followed for the staging.[13]

### Nelson's grading system

Grade 0 - Small round, epithelial cells with scanty eosinophilic cytoplasm, large basophilic nuclei with N:C ratio of 1:2 and plump, and oval intensity passive abundant goblet cells.

Grade I - Larger polygonal epithelial cells with eosinophilic cytoplasm N:C ratio of 1:3, and plump oval shapes periodic acid-Schiff (PAS) +ve goblet cells which were decreased in number.

Grade II - Large polygonal and occasionally multinucleate epithelial cells with variables staining cytoplasm N:C ratio of 1:4–1:5, and smaller, less intensity PAS-staining goblet cells, markedly decreased in number with poorly defined cellular borders.

Grade III - Large polygonal epithelial cells with basophilic cytoplasm, N:C ratio of >1:6 with very few or absent goblet cells.

### The inclusion criteria

- Patients older than 20 years
- No history of ocular surgeries
- No history of chronic ocular diseases
- No history of contact lens wear.

### The exclusion criteria

- Patients <20 years of age
- Acute ocular infections such as conjunctivitis and keratitis
- Patients who have undergone intraocular surgery or extraocular surgeries such as cataract or refractive surgeries
- Diagnosed cases of dry eye already on treatment
- Contact lens users.

### Statistical analysis

Statistical analysis was done on the SPSS version 17 (SPSS Inc Chicago). The association between two diagnostic tests was done using the Pearson Chi-square test and agreement between 2 tests was analyzed. $P < 0.05\%$ was considered statistically significant.

## Results

A total of 100 cases were studied over a 1 year span. The youngest patient in our study was 21 years and the oldest 79 years [Table 1]. The mean age was 45.18 (standard deviation was 13.8). A majority of the patients were in the age group of 40–60 years and involvement was more in females, especially those in the perimenopausal/menopausal group.

The most frequent symptom was difficulty to read at a distance seen in 76% patients. The next most frequent symptoms were blurring of vision and painful eyes seen in 72% patients [Table 2].

**Table 1: Age and Sex Distribution of Cases**

| Age group (in years) | Males | Females | Total |
|---|---|---|---|
| 20-30 | 7 | 11 | 18 |
| 31-40 | 9 | 5 | 14 |
| 41-50 | 11 | 21 | 32 |
| 51-60 | 10 | 12 | 22 |
| 61-70 | 6 | 4 | 10 |
| >70 | 3 | 1 | 4 |
| Total | 46 | 54 | 100 |

Of the 100 patients with positive symptomatology, the sensitivity of various diagnostic tests were positive Schirmer's in 83%, positive TBUT in 82%, positive lissamine green in 26%, and positive impression cytology in 94%.

The impression cytology was graded in accordance to the Nelson grading system and the most common grade was Grade I (65%) [Table 3].

The agreement between the different tests was analyzed. Both the tests – Schirmer's and TBUT – were positive in 66 patients (79.5%) ($P$ = 0.153 and agreement was 67%). Impression cytology and Schirmer's test were positive in 77 patients (98.7%) ($P$ = 0.639 and agreement was 77%) along with TBUT and impression cytology were positive in 77 patients (98.7%) ($P$ = 0.639 and agreement was 77%) that had the best of the agreements [Tables 4a-c].

We identified many systemic diseases associated with dry eye, prominent in our study were diabetes (11 cases) and thyroid (10 cases) [Table 5]. The pattern of distribution in the impression cytology showed that all cases of diabetes had squamous metaplasia and only 6 cases had a reduced goblet cell index [Table 6].

**Table 2: Distribution of Symptomology among the Cases**

| Symptom | Constant | Frequent | Infrequent |
|---|---|---|---|
| Sensitive to light | 26 | 22 | 12 |
| Feeling of grittiness | 28 | 32 | 6 |
| Painful eyes | 20 | 40 | 12 |
| Blurred vision | 8 | 52 | 12 |
| Poor vision | 4 | 40 | 12 |
| Unease in reading | 18 | 40 | 18 |
| Driving at night | 14 | 18 | 8 |
| Working with computers | 10 | 4 | 2 |
| Watching TV | 20 | 44 | 0 |
| Windy climate | 12 | 28 | 10 |
| Low humidity | 8 | 28 | 18 |
| Working in AC | 29 | 8 | 4 |

**Table 3: Distribution of Impression Cytology Score**

| Nelson's grading | Number of cases |
|---|---|
| Nonrepresentative cases | 5 |
| Grade 0 | 1 |
| Grade 1 | 65 |
| Grade 2 | 27 |
| Grade 3 | 2 |

**Table 4a: Correlation among Schirmer's, Tear Break Up Time, Lissamine Green, and Impression Cytology**

| | Schirmer's$^+$ (%) | Schirmer's$^-$ (50) | Agreement (%) | *P* |
|---|---|---|---|---|
| TBUT$^+$ | 66 (79.5) | 16 (94.1) | 67 | 0.153 |
| TBUT$^-$ | 17 (20.5) | 1 (5.9) | | |
| Lissamine$^+$ | 20 (24.1) | 6 (35.3) | 31 | 0.338 |
| Lissamine$^-$ | 63 (75.9) | 11 (64.7) | | |
| Impression$^+$ | 77 (98.7) | 17 (100) | 77 | 0.639 |
| Impression$^-$ | 1 (13) | 0 | | |

TBUT: Tear break up time; + : positive; – : negative

**Table 4b: Correlation among Tear Break Up Time, Lissamine Green, and Impression Cytology**

| | TBUT$^+$ (%) | TBUT$^-$ (%) | Agreement (%) | *P* |
|---|---|---|---|---|
| Lissamine$^+$ | 24 (29.3) | 2 (11.1) | 40 | 0.112 |
| Lissamine$^-$ | 58 (70.7) | 16 (88.9) | | |
| Impression$^+$ | 77 (98.7) | 17 (100) | 77 | 0.639 |
| Impression$^-$ | 1 (13) | 0 | | |

TBUT: Tear break up time; + : positive; – : negative

**Table 4c: Correlation Between Impression Cytology and Lissamine Stain**

| | Impression cytology$^+$ (%) | Impression cytology$^-$ (%) | Agreement (%) | *P* |
|---|---|---|---|---|
| Lissamine$^+$ | 25 (100) | 0 | 26 | 0.548 |
| Lissamine$^-$ | 69 (98.6) | 1 (1.4) | | |

+ : positive; – : negative

## Discussion

Patients with symptoms suggestive of dry eye were a common presentation during our study period. Although the problem may appear trivial, it does impact the quality of life of the patients. We, therefore, felt that it is imperative and diagnosed all cases.

### Age and sex distribution

The patients in the age group of 41–60 years had maximum number of dry eyes and this correlates well with other studies.[14] We assume that this could be due to the age factor, onset of chronic diseases as well as the increasing use of devices such as computers, mobiles, and laptops. Other studies had a relative peak in the age group of 31–40 years.[15] The association between older age and increase in dry eye symptoms is consistent with a Melbourne study.[16] This is likely as a result of normal changes in tear production associated with advancing age. Reduction of tear volume and flow and increase in evaporation have been noted in older people.

The sex distribution in our study is consistent with various other studies conducted by Moss and Klein and Schaumberg *et al*., which showed females had higher prevalence of dry eyes than males.[15,17] We also noted women in perimenopausal age group having dry eyes were 16%. Menopause causes estrogen deficiency and consequent change in local hormonal milieu of lacrimal gland.

### Presenting symptomatology

The most common symptom in most studies was ocular discomfort, while others showed dryness, soreness, and light sensitivity as frequent symptoms.[18,19] The common symptoms among computer users (more than 6 h/day) were eye strain, foreign body sensation, and blurred vision. This is probably due to decrease in blinking reflex among computer users and also use of air conditioners leads to increased tear vaporization. Normal blinking frequency is about 12/min which is reduced

**Table 5: Distribution of Systemic Conditions Associated with Dry Eyes**

| Disease | Number |
|---|---|
| DM | 11 |
| Thyroid disease | 10 |
| SLE | 1 |
| RA | 8 |
| Vitamin A | 3 |
| Postradiation | 2 |

DM: Diabetes mellitus, SLE: Systemic lupus erythematosus, RA: Rheumatoid arthritis

**Table 6: Pattern of Distribution of Impression Cytology among Various Systemic Associations of Dry Eyes**

| Systemic conditions | Squamous metaplasia (%) | Reduced goblet cell index (%) |
|---|---|---|
| DM | 11 (100) | 6 (54.5) |
| Hypothyroid | 10 (100) | 4 (40) |
| RA | 5 (71.4) | 6 (85.7) |
| Vitamin A deficiency | 1 (33.3) | 3 (100) |
| Postradiation | 3 (100) | 3 (100) |

DM: Diabetes mellitus, RA: Rheumatoid arthritis

by up to 50% during computer use. Most of the patients included in this study having dry eyes were computer operators, drivers, salesmen, field workers, and mechanics exposed to environmental factors which comprised 30%. Home makers and students comprised 29% whereas office workers were 13%. Thus, various environmental factors have a propensity to dry eyes which include wind, sunlight, high temperature, and air pollution.

## Sensitivity of tests

The lissamine green test had low sensitivity and this could be explained as most of the cases in this study had mild dry eyes. Impression cytology had a high sensitivity in our study as with other studies.[20] In only 5 of the 100 cases, impression cytology was nonrepresentative. We also evaluated how the grading in impression cytology (Nelson scores) compares with OSDI scores. There was only one case (1%) where the cytology was normal and the OSDI was scored as mild. This exemplifies the role of impression cytology in the diagnostic armamentarium of dry eyes. The grading in impression cytology in accordance to the Nelson grading system is relevant and reproducible index of cytological severity of dry eyes.[7] As the severity of dry eye increased, the cytology showed marked cell separation and lowering of N:C ratio with decrease in goblet cell count [Figures 1-4].

## Agreement between tests

The agreement between the different tests showed impression cytology with Schirmer's or TBUT which had the highest of 77% agreement between the two tests. All clinical tests have limited diagnostic value if performed individually, but impression cytology showed the highest sensitivity. Owing to the multifactorial nature of dry eye, there is a considerable confusion regarding the specificity of various diagnostic tests.

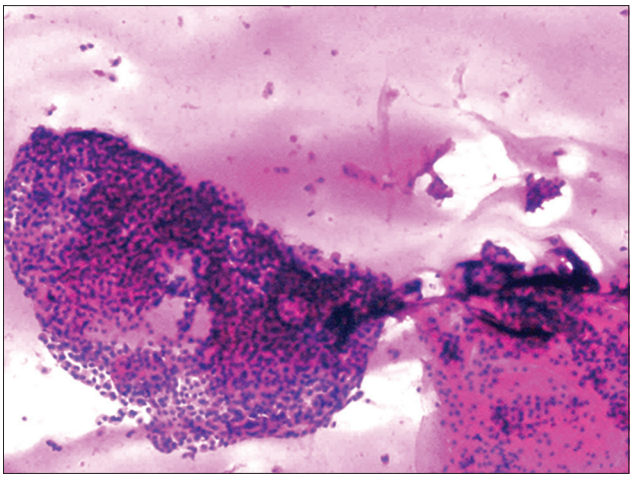

**Figure 1:** Normal impression cytology. Dense clusters of normal epithelial cells with intervening goblet cells (PAS, ×100).

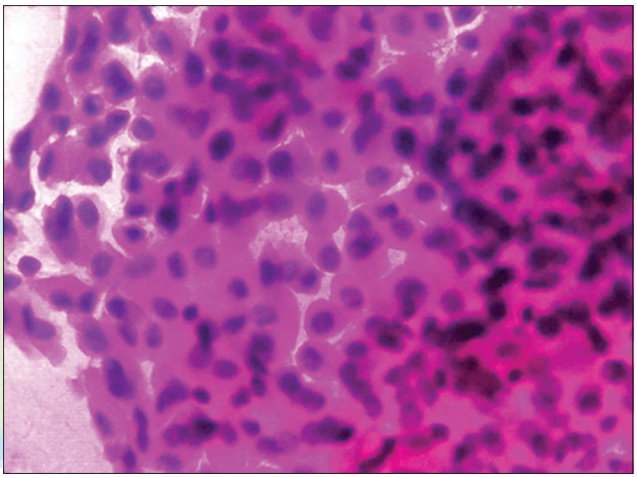

**Figure 2:** Nelson Grade I. Sheets of cells having a mild decrease in N:C ratio (1:3) (PAS, ×400).

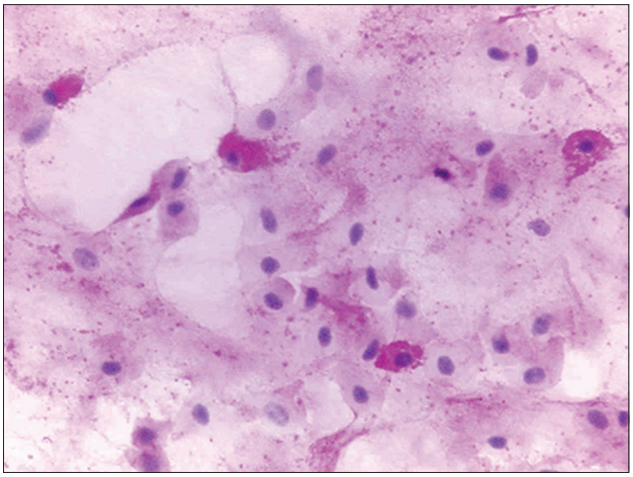

**Figure 3:** Nelson Grade II. Slight dissociation with cells having a moderate decrease in N:C ratio (1:4) with few scattered goblet cells (PAS, ×400).

The commonly used tests such as Schirmer's, TBUT, and lissamine green diagnose either aqueous or mucin-deficient

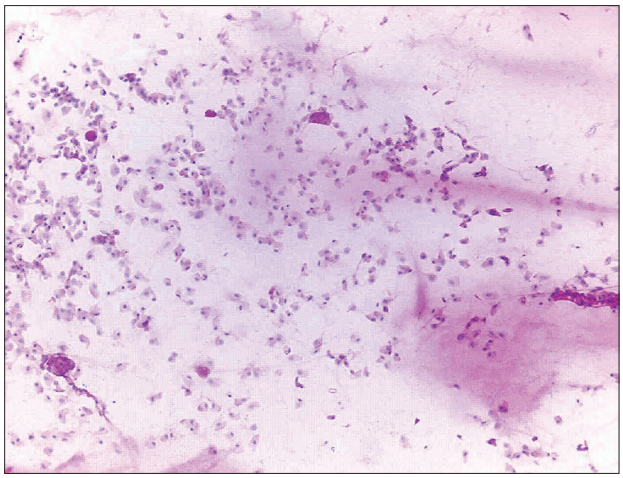

**Figure 4:** Nelson Grade III. Marked dissociation of cells with marked decrease in N:C ratio (1:5) and marked decrease in goblet cells (PAS, ×100).

states. Ocular surface keratinization, squamous metaplasia prior to keratinization, and goblet cell density can be observed only by impression cytology, which therefore provides high sensitivity and gives reproducible index of severity of dry eyes. We believe as each test examines only part of the process, it is only when these tests are reviewed collectively, a more complete picture of tear film appears.

### Association with systemic diseases

Association of dry eyes with conditions such as diabetes, arthritis, and thyroid disease is known.[15,16,21] These associations were also found in this study [Table 5]. The other associations include postradiation therapy and Vitamin A deficiency. Diabetic patients in our study have decreased Schirmer's test readings, decreased TBUT, and pathological conjunctival epithelium.[22] In the diagnostic tests employed in our diabetic patients, we had positive Schirmer's in 73%, positive TBUT in 91%, positive lissamine green in 45%, and positive impression cytology in 100%. The squamous metaplasia index was raised in all the 11 cases with 2 of the cases showing Grade III Nelson score as compared with the goblet cell index, which was reduced in 6 of the 11 cases [Table 6]. These results correlate well with other studies which showed more signs of conjunctival metaplasia among diabetic patients.[23] In diabetics, damage to the microvasculature of lacrimal gland together with autonomic neuropathy contributes to impaired function of the lacrimal gland. Diabetic sensory neuropathy of cornea also plays a role in decreased tear production. The ten patients with thyroid disease were 100% positive for Schirmer's, TBUT, and impression cytology. The eight patients with rheumatoid arthritis were 100% positive for TBUT and impression cytology, suggesting mucin-deficient state as seen in the studies by Punjabi *et al*.[24] The three patients with Vitamin A deficiency were 100% positive for TBUT and impression cytology. They showed marked reduction in goblet cell index.[25] Of the 2% patients with a history of radiation exposure, Schirmer's test, TBUT, and impression cytology were positive in both cases (100%). This correlates well with a study by Georg *et al*. where the Schirmer's test and TBUT were decreased.[26] It is well documented that there is impairment of functioning of lacrimal glands after radiation therapy.[26] All three layers of tear film are involved and there is pronounced long-term effect on tear film stability. Goblet cell index was markedly reduced in both cases (100%).

## Conclusion

We recommend that impression cytology is an ideal method of investigating ocular surface disorders when diagnosis is not clinically obvious or when clinical diagnosis needs substantiation. Equally, follow-up cases can be objectively evaluated. It is becoming a handy tool for research on pathophysiology using newer diagnostic modalities. We recommend that major ophthalmic centers should be developed and introduce this technique into routine clinical practice.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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