Conjunctival impression cytology versus routine tear function tests for dry eye evaluation in contact lens wearers

ABSTRACT
Aims: Prolonged contact lens wear is often accompanied by dryness of the eyes. The aim of this study was to compare conjunctival impression cytology (CIC) and tear film tests such as tear film break up time (TBUT) and Schirmer test for dry eye evaluation in contact lens wearers and measure their correlation with dry eye symptoms.

Setting: A case control study was done at three referral eye centers.

Materials and Methods: The eyes of 230 contact lens users were compared to 250 eyes of age- and sex-matched controls. Participants were recruited based on their response to a questionnaire of dry eye symptoms, (Dry Eye Scoring System, DESS) and measurements of TBUT, Schirmer test, and CIC was done. A correlation analysis between symptom severity and tear film tests was performed. Pearson's coefficient, R² > 0.5 was considered significant.

Results: As compared to controls (r² = 0.010), Nelson grade correlated significantly with dry eye symptoms (r² = 0.765), among cases. However, there was moderate correlation between dry eye symptoms, Schirmer test, and TBUT (r² = 0.557 and 0.530, respectively) among cases and a weak correlation among controls (r² = 0.130 and 0.054, respectively). The sensitivity of TBUT was 86.4%, specificity was 82.4%, positive likelihood ratio (LR) was 4.50 [95% confidence interval (CI) 3.46-5.85], and negative LR was 0.09. The sensitivity of the Schirmer test was 48.2%, specificity 88%, LR 2.12 (95% CI 1.48-2.96), and negative LR 0.83.

Conclusion: CIC correlates better than Schirmer and TBUT with dry eye symptoms. It may be the most appropriate test for dry-eye evaluation in contact lens wearers.

Key words: Conjunctival impression cytology (CIC); contact lens; dry eye; lens wear time

Introduction
Contact lenses are commonly used to correct refractive error and are preferred by females due to cosmetic reasons. Although generally claimed to be safe, prolonged lens wear may cause corneal hypoxia, microtrauma, and decreased turnover of corneal epithelial cells.[1] Secondly, lens wear not only disrupts the tear film and increases evaporative tear loss, it may also cause corneal dehydration leading to dryness of eyes.

It is estimated that approximately 20-30% of soft contact lens wearers and 80% of rigid contact lens wearers suffer from dryness of eyes. Cytologically, dry eye is associated

Access this article online
Website: www.jcytol.org
DOI: 10.4103/0970-9371.171242

How to cite this article: Kumar P, Bhargava R, Arora YC, Kaushal S, Kumar M. Conjunctival impression cytology versus routine tear function tests for dry eye evaluation in contact lens wearers. J Cytol 2015;32:261-7.
with alteration in the morphology of epithelial cells of conjunctiva and reduction in conjunctival goblet cell density (GCD).<sup>2</sup> Various factors such as lens material, lens water content, concurrent use of visual display terminals (VDTs), ambient humidity conditions, and lens wear time may influence cytological changes on the ocular surface.<sup>3,4</sup> Ocular surface health may thus be a reasonable indicator of contact lens-induced problems.

Conventionally, dry eye in contact lens wearers is evaluated by routine tear function tests such as Schirmer, tear film break up time (TBUT), and Rose Bengal staining (RBS). However, most of these tests are poorly standardized, inaccurate, and unrepeatable, making comparison between studies tenuous at best.<sup>5,6</sup>

In addition, there is lack of correlation between ocular symptoms and signs observed on routine tear film tests.<sup>7</sup> Consequently, symptom-based assessments (dry eye questionnaire) have become a key component of clinical diagnosis for dry eye conditions.<sup>8</sup> For example, a long-term contact lens wearer may have normal Schirmer and TBUT, but may show early cytological changes in the conjunctiva and cornea on impression cytology. Conjunctival impression cytology (CIC) may be useful under such circumstances and may detect early dry-eye changes (undetected by routine tear function tests) so that appropriate measures may be instituted and development of squamous metaplasia prevented.<sup>9,10</sup>

Although a few studies have evaluated cytological changes in conjunctiva using CIC, a search of major databases (including Medline) did not reveal any study comparing the sensitivity, specificity, and diagnostic accuracy of CIC and routine tear film tests in contact lens wearers.

The aim of the present study was to compare the diagnostic accuracy of CIC and routine tear function tests such as Schirmer and TBUT for dry eye in contact lens wearers and to measure the extent to which these tests correlate with dry eye symptoms. The sensitivity, specificity, and likelihood ratio (LR) of these tests, taking CIC as the reference standard, were also calculated.

**Materials and Methods**

**Patients**

A case control study was done at three referral eye centers. The trial was approved by the institutional review boards and the local ethics committee. Written informed consent was obtained from all patients willing to participate in the study based on the Helsinki protocol.

**Inclusion criteria**

A survey was conducted in regional universities, medical schools, and information technology parks. Symptomatic female contact wearers experiencing dry eye symptoms and lens wear discomfort were identified and invited to take part in the trial. Age- and sex-matched subjects working under similar conditions but who did not wear contact lens served as controls.

Subjects were recruited based on their response to the Dry Eye Scoring System (DESS)<sup>5</sup> a questionnaire of dry eye-related symptoms [Table 1] and a specially designed scale for lens wear comfort; all participants were using monthly- or daily-wear soft contact lens. The average daily lens wear time was calculated as hours per day. The total lens wear time was calculated in months. Lens types worn were separated according to the U.S. Food and Drug Administration (FDA) categories.

**Exclusion criteria**

Patients with active ocular infection and patients on medications likely to influence tear film tests (tetracycline, corticosteroids, or antiglaucoma drugs) were excluded; those with past history of laser in situ keratomileusis (LASIK), herpetic eye disease, diabetes, and/or liver diseases were also excluded. Other exclusion criteria included pregnant or lactating mothers, patients of human immunodeficiency virus (HIV) infection, patients of hepatitis B and C, those on aspirin or anticoagulant therapy, and those allergic to fluorescein. Topical medications (other than artificial tear supplements) that could affect tear film or meibomian gland functions were also discontinued prior to intervention. However, patients were instructed not to use artificial tear preparations, 2 h prior to testing. Computer usage was not allowed during the course of the study as concurrent use of VDTs may independently influence the results.

**Outcome measures**

Changes in epithelial cell morphology, cytology, and GCD were the primary outcome measures. Correlation between

**Table 1: Dry eye questionnaire and scoring system (DESS<sup>5</sup>)**

| Symptom                  | Score (maximum 18) | Absent (0) | Sometimes (1) | Frequently (2) | Always present (3) |
|--------------------------|--------------------|------------|---------------|----------------|--------------------|
| Itching or burning       |                    |            |               |                |                    |
| Sandy or gritty sensation|                    |            |               |                |                    |
| Redness                  |                    |            |               |                |                    |
| Blurring of vision       |                    |            |               |                |                    |
| Ocular fatigue           |                    |            |               |                |                    |
| Excessive blinking       |                    |            |               |                |                    |

Scores of 0-6 were mild, 6.1-12 were moderate, and 12.1-18 indicated severely symptomatic dry eye.<sup>11</sup> *Bhargava R. Laser Eye Clinic, Noida, India*
dry eye symptom severity, tear film tests and Nelson grade were secondary outcome measures.

**Assessment of dry eye symptoms and lens wear comfort**
The DESS© was administered to all participants (cases and controls) prior to ophthalmic examination and tests. A score was assigned to common symptoms of dry eye [Table 1]. Dry eye symptom severity is assessed on a scale of 0-18, with higher scores representing dry eye severity. A symptom score of 0-6 represents mild, 6.1-12 moderate, and 12.1-18 severe dry eye.[12-16] Contact lens comfort was graded on a specially designed scale from 0 to 6 (0 = no discomfort to 6 = severe discomfort).

**Ophthalmic examination and measurements**
The participants were instructed to visit the dry eye clinic in the morning, and all tests were performed at the same time of the day (between 10 AM and 12 PM) in a dimly lit room. Patients were instructed not to use artificial tear preparations 2 h prior to testing. At each visit, the subjects underwent a detailed ocular examination by an independent investigator (not a study surgeon, KS). This included recording of corrected distance visual acuity (CDVA), and slit lamp examination; this included assessment of lid margins, eyelashes, and meibomian gland orifice for any blockage or occlusion. One eye was selected at random for evaluation.

At each examination, subjects underwent tests of tear film characteristics such as Schirmer, TBUT, and CIC. Furthermore, the subjects were given dry eye questionnaire at each visit. The independent investigator (KS) was masked to the information obtained from the questionnaire.

TBUT was performed 30 min after the removal of contact lens. Excessive eyelid manipulation was avoided at this stage as it might have adversely influenced the results. A sterile fluorescein strip containing 1 mg fluorescein sodium (Madhu Instruments, Delhi, India) was applied over the inferior bulbar conjunctiva. The strip was moistened with normal saline solution prior to application. The patient was instructed to blink naturally, without squeezing, several times to distribute the fluorescein. The tear film was observed on slit lamp using cobalt blue filter. The interval between the last complete blink and the first appearance of a dry spot on the cornea was recorded with a timer. A TBUT of less than 10 s was considered consistent with dry eye. Three readings were taken in succession and averaged. The subject then waited for another 30 min and the Schirmer test with anesthesia (0.4% oxbupracaicone hydrochloride) was done with the eyes closed. A length of wetting less than 6 mm was considered consistent with dry eye.

To ensure uniformity and eliminate bias, CIC was performed by a single examiner who was masked to the information obtained from the questionnaire. One eye of each patient was selected at random for examination.

**Technique**
CIC was performed by transfer method after anesthetizing the eye with one drop of 0.4% oxbupracaicone hydrochloride.[17] The lacrimal lake at the inner canthus was dried with a cotton tip applicator. A circular 0.22-micron filter paper measuring 13 mm in diameter (Sartorius, Gottingen, Germany) was grasped with a blunt-tipped forceps and applied over the inferior bulbar conjunctiva. CIC samples were obtained from the nonexposed conjunctiva to eliminate the influence of environment-related factors on the ocular surface in the exposed part.

The paper strip was gently pressed with a glass rod held in the other hand. The filter paper was removed in a peeling fashion after 4-10 s and the specimen transferred to the lab for fixation (ethyl alcohol, formaldehyde, and glacial acetic acid in 20:1:1 volume ratio) and staining. Due to the relative ease of handling, the filter paper was first placed on a glass slide with albumin paste to transfer the specimen to the slide, instead of working directly. However, loss of adhered material to the filter may be a potential disadvantage. The filter paper was then removed from the slide and the slide labelled and numbered. The slide was kept at room temperature and stained with periodic acid Schiff (PAS) and counterstained with hematoxylin and eosin (H&E). The mounted slide was first examined under the light microscope with 100× low power field (10× objective lens). After localization, cells were then analyzed with 400× final magnification (40× objective). At least 10 high power fields (HPF) were examined for goblet cells and epithelial cells. The number of goblet cells per HPF was marked and counted.

The mean goblet cell count per HPF and the standard deviation (SD) were calculated. The coefficient of variation (COV) (%) = SD × 100/Mean. Estimated GCD = number of goblet cells counted per HPF divided by sampling area covered in mm². Grading and scoring were carried out by the criteria suggested by Nelson.[11] Nelson grades 0 and 1 were regarded as normal, whereas grades 2 and 3 were considered to represent abnormal cytology.

**Statistical analysis**
Statistical analysis was performed using SPSS software for Windows (version 22, IBM Corp.). Means of groups were compared using t-tests. Chi-square tests were used for proportions. A $P$ value < 0.05 was considered statistically significant. Analysis of variance (ANOVA) was used when there was a significant difference in proportions. A $P$ value < 0.05 was considered statistically significant. Analysis of variance (ANOVA) was used when there was a significant difference in proportions.
were more than two variables (between type of contact lens and symptom severity). Correlation analysis was done to study the relationship between symptoms and the outcome variables (Schirmer TBUT and Nelson grade) in cases and controls. Pearson’s correlation coefficient $R^2 > 0.5$ was significant. Sensitivity, specificity, and LR were calculated with CIC as the reference standard.

**Results**

A total of 480 subjects participated in the study. After discarding the poorly stained slides ($n = 12$), the eyes of 230 contact lens wearers and of 250 controls were evaluated. The mean age in cases ($22.4 \pm 4.8$ years, range 14-36 years) was comparable to controls ($22.3 \pm 3.9$ years), $P = 0.525$. The mean total lens wear time was $38 \pm 12$ months and the mean daily lens wear time was $8.2 \pm 1.7$ h.

The mean symptom score in contact lens wearers was $7.9 \pm 2.3$ as compared to $2.1 \pm 2.5$ in controls ($P < 0.0001$). Table 2 shows the mean baseline values in the respective groups.

Among contact lens wearers, 49 (21.3%) patients were mildly symptomatic, 167 (72.6%) moderately, and 14 (6%) severely symptomatic at baseline. In the control group, 185 (74%) were symptom-free, 62 (24.8%) were mildly symptomatic, and 3 (1.2%) moderately symptomatic.

The mean lens wear comfort was $3.1 \pm 1.0$ (graded on a scale of 0-6).

There was a moderate positive correlation between dry eye symptoms, Schirmer, and TBUT (Pearson’s coefficient, $r^2 = 0.557$ and $r^2 = 0.530$, respectively). The correlation was not significant among controls ($r^2 = 0.130$ and $r^2 = 0.054$, respectively) [Figures 1 and 2].

There was a significant correlation [Figure 3] between Nelson grade and symptom severity ($r^2 = 0.765$) in contact lens users as compared to controls ($r^2 = 0.010$). On regression analysis, 76% of the variability in Nelson grade (cytological alteration) could be explained by symptom severity. On ANOVA, the probability corresponding to the $F$ value <0.0001 suggests that there was less than 0.01% risk in assuming that the null hypothesis (no effect of symptoms) was wrong.

Impression cytology was abnormal (Nelson grades 2 and 3) in 87 (37.8%) contact lens wearers [Figure 5]. However, in the control group, 230 (92%) had Nelson grade 0 [Figure 4] and 20 (8%) Nelson grade 1 changes, respectively.

In eyes with Nelson grade 3, mean GCD was $76 \pm 9.6$ with a COV of 12.6%. Eyes with Nelson grade 2 had a GCD of $280 \pm 68$ and COV of 24.3%. Eyes with Nelson grade 1 had a GCD of

Table 2: Mean baseline characteristics in cases and controls

| Parameter               | Cases        | Controls     | $P$ value (t-test) |
|-------------------------|--------------|--------------|-------------------|
| Symptom score           | 7.9±2.3      | 2.1±2.5      | $<0.0001$         |
| TBUT (s)                | 8.7±2.7      | 13.1±1.6     | $<0.0001$         |
| Schirmer (mm)           | 9.6±4.5      | 16.3±4.3     | $<0.0001$         |
| Nelson grade            | 1.6±0.8      | 0.6±0.6      | $<0.0001$         |
| GCD (cells/mm$^2$)      | 369±210      | 820±117      | $<0.0001$         |
| Lens wear time (daily)  | 8.2±1.7 h   | NA           | NA                |
| Lens wear time (total)  | 38±12 months | NA           | NA                |

GCD: Goblet cell density, NA: Not applicable, TBUT (Tear film break up time)
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440 ± 65 with COV of 14.8%, and in eyes with Nelson grade 0, GCD was 680±145 with COV of 21.3% [Table 3].

Variability in GCD estimates
Based on 100 µm scale marker, the average image area was 440 × 320 µm² and the average image size was 0.048 ± 0.003 mm². The coefficient of variation for baseline GCD across all samples ranged 12-25%. The overall mean was 18.2 ± 5.5.

The sensitivity of TBUT in diagnosing dry eye in computer users was 86.4% (95% CI 80.2-90.4%), specificity was 82.4% (95% CI 76.5-88.4%), positive LR was 4.50 (95% CI 3.46-5.85), and negative LR was 0.09 (95% CI 0.05-0.18).

The sensitivity of the Schirmer test in diagnosing dry eye in computer users was 48.2% (95% CI 22.8-54.2%), specificity 88% (95% CI 82.8-92.3%), positive LR was 2.12 (95% CI 1.48-2.96), and negative LR 0.83 (95% CI 0.76-0.92).

There was no significant difference in dry eye symptom between the types (FDA types) of contact lenses (ANOVA, \( P = 0.340 \) and 0.245, respectively).

Discussion

Dry eye syndrome is a multifactorial disease of tears on the ocular surface leading to ocular surface inflammation and increased tear film osmolarity; ocular surface inflammation may be associated with expression of proinflammatory cytokines and markers such as human leukocyte antigen (HLA)-DR.[18-21]

Diagnosis of dry eye is a challenging task due to the limitations of tests such as TBUT and Schirmer and their lack of correlation with dry eye symptoms. Therefore, questionnaire-based studies have found a higher prevalence rate of dry eye in contact lens wearers; in dry eye research, a questionnaire can serve as a screening instrument and help to define treatment groups according to symptoms.[22] One important limitation, however, of symptom-based screening is that it does not predict the extent of cytological changes in the ocular surface, and the resolution of symptoms alone after dry eye therapy cannot be used as an outcome measure. Having said this, CIC has not yet become the first-line investigation in dry eye only because it is a relatively time-consuming procedure.

In a study on 75 patients with dry eye, Nichols et al. evaluated the relation between dry eye symptoms and routine tear function tests by correlation analysis and multivariate logistic regression; the authors found a lack of association between dry eye symptoms and clinical signs. None of the clinical tests significantly predicted frequently reported symptoms after adjustment for age and artificial tear use.[23] In the present study, there was moderate correlation between dry eye symptoms and Schirmer test (Pearson’s correlation coefficient, \( r^2 = 0.557 \)). Likewise, there was a moderate correlation with TBUT \( (r^2 = 0.530) \). However, there was a strong correlation of dry eye symptoms with Nelson grade \( (r^2 = 0.765) \).
In a case-control study on 216 patients with dry eye of varied etiology, Kumar et al. found that CIC correlated better with dry eye symptoms as compared to Schirmer, TBUT, and RBS. Schirmer, TBUT, and RBS were less sensitive than CIC in diagnosing dry eye.\(^{[16]}\)

In the present study, there was a significant reduction of GCD in contact lens wearers (369 ± 210/mm\(^2\)) as compared to controls (820 ± 117/mm\(^2\)) (\(P < 0.001\)). However, the GCD estimates were significantly higher than in other studies considering the mean lens wear time of 3.4 ± 1.2 years; the probable explanation is that there is a wide variability in goblet cell distribution, not only in sampling area used for selection but also the location selected for sampling (exposed versus nonexposed conjunctiva).\(^{[23,24]}\)

In a case control study, Anshu et al. evaluated eyes of contact lens wearers by CIC \((N = 80)\) and found that symptomatic lens wearers had a significant increase in Nelson grades when compared with their asymptomatic counterparts. No correlation was, however, found between duration of lens wear time and cytological grades. Cytological changes were more severe in soft contact lens users.\(^{[25]}\)

In another study \((N = 100)\) to evaluate the cytological changes in conjunctiva following regular contact lens wear and to determine the correlation, if any, between severity of cytological alteration and symptoms, Simon et al. found that the severity of cytological changes increased with lens wear time \((P = 0.001)\).\(^{[26]}\) However, these studies evaluated eyes with both rigid and soft contact lenses, they had small sample sizes, and correlation analysis was not done to evaluate the results; this could account for the difference in observation.

**Conclusion**

In conclusion, the results of the present study suggest that CIC correlates well with dry eye symptoms as compared to routine tear film tests; it is more sensitive for dry eye evaluation in contact lens wearers and may detect early subtle changes in the ocular surface (undetected by routine tear function tests) so that appropriate measures may be timely instituted and the development of squamous metaplasia prevented.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Lemp MA, Bielow L. Contact lenses and associated anterior segment disorders. Dry eye disease, blepharitis, and allergy. Immunol Allergy Clin North Am 2008;28:105-17, vi-vii.
2. Tomlinson A. Epidemiology of dry eye disease. In: Asbell PA, Lemp MA, editors. Dry Eye Disease. 1st ed. New York: Thieme; 2006. p. 1-15.
3. Bhargava R, Kumar P. Oral omega-3 fatty acid treatment for dry eye in contact lens wearers. Cornea 2015; 34:413-20.
4. Ramamooorthy P, Sinnott LT, Nichols JJ. Treatment, material, care, and patient-related factors in contact lens-related dry eye. Optom Vis Sci 2008;85:764-72.
5. Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. Cornea 2004;23:272-85.
6. Cho P, Brown B, Lau C. Effect of fluorescein on the tear stability of Hong Kong-Chinese. Optom Vis Sci 1996;73:1-7.
7. Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. Cornea 2004;23:762-70.
8. Nakaishi H, Yamada Y. Abnormal tear dynamics and symptoms of eyestrain in operators of visual display terminals. Occup Environ Med 1999;56:6-9.
9. Bhargava R, Kumar P. Can conjunctival impression cytology be the first line diagnostic test for evaluation of dry eye syndrome? Enliven Clin Ophthalmol Res 2015;1:004.
10. Brignole-Baudouin F, Ott AC, Warnet JM, Baudouin C. Flow cytometry in conjunctival impression cytology: A new tool for exploring ocular surface pathologies. Exp Eye Res 2004;78:473-81.
11. Kumar P, Bhargava R, Kumar M, Madaan J. Dry eye syndrome: A diagnostic enigma. Int J Contemp Surg 2013;1:72-7.
12. Bhargava R, Kumar P, Kaur A, Kumar M, Mishra A. The Diagnostic value and accuracy of conjunctival impression cytology, dry eye symptomatology, and routine tear function tests in computer users. J Lab Physicians 2014;6:102-8.
13. Bhargava R, Kumar P, Kumar M, Mehra N, Mishra A. A randomized controlled trial of omega-3 fatty acids in dry eye syndrome. Int J Ophthalmol 2013;6:811-6.
14. Bhargava R, Kumar P, Phogat H, Kaur A, Kumar M. Oral omega-3 fatty acids treatment in computer vision syndrome related dry eye. Cont Lens Anterior Eye 2015;38:206-10.
15. Kumar P, Bhargava R, Kumar M, Ranjan S, Kumar M, Verma P. The correlation of routine tear function tests and conjunctival impression cytology in dry eye syndrome. Korean J Ophthalmol 2014;28:122-9.
16. Bhargava R, Kumar P. Conjunctival impression cytology in computer users. Int J Ophthalmic Pathol 2014;3:3-4.
17. Nelson JD, Havener VR, Cameron JD. Cellulose acetate impressions of the ocular surface. Dry eye states. Arch Ophthalmol 1983;101:1869-72.
18. The definition and classification of dry eye disease: Report of the Definition and Classification Subcommittee of the International Dry Eye Workshop. Ocul Surf 2007;5:75-92.
19. Yoon KC, Jeong IV, Park YG, Yang SY. Interleukin-6 and tumor necrosis factor-alpha levels in tears of patients with dry eye syndrome. Cornea 2007;26:431-7.
20. Narayanan S, Miller WL, McDermott AM. Conjunctival cytokine expression in symptomatic moderate dry eye subjects. Invest Ophthalmol Vis Sci 2006;47:2445-50.
21. Narayanan S, Miller WL, McDermott AM. Expression of human beta-defensins in conjunctival epithelium: Relevance to dry eye disease. Invest Ophthalmol Vis Sci 2003;44:3795-801.
22. Begley CG, Caffery B, Chalmers RL, Mitchell GL; Dry Eye Investigation (DREI) Study Group. Dry Eye Investigation (DREI) Study Group. Use of the dry eye questionnaire to measure symptoms of ocular irritation in patients with aqueous tear deficient dry eye. Cornea 2002;21:664-70.
23. Doughty MJ. Sampling area selection for the assessment of goblet cell density from conjunctival impression cytology specimens. Eye Contact Lens 2012;38:122-9.
24. Kessing SV. Mucous gland system of the conjunctiva. A quantitative normal anatomical study. Acta Ophthalmol (Copenh) 1968;(Suppl 95):1+.
25. Anshu, Munshi MM, Sathe V, Ganar A. Conjunctival impression cytology in contact lens wearers. Cytopathology 2001;12:314-20.
26. Simon P, Jaison SG, Chopra SK, Jacob S. Conjunctival impression cytology in contact lens wearers. Indian J Ophthalmol 2002;50:301-6.