Conjunctival impression cytology used as a gold standard test in evaluating the cases of Computer Vision Syndrome among young university students

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ABSTRACT

Purpose: To find the correlation between clinical dry eye tests and conjunctival impression cytology examination for the assessment of computer vision syndrome in university students in Lahore.

Methodology: At Punjab University, 225 students participated in this cross-sectional study. Conjunctival impression cytology under topical anaesthesia, tear-film breakup time measurement, the number of corneal dry spots, and Schirmer's test were among the questionnaire-based data gathered. The association between the CIC score and Schirmer's test, TBUT, and Goblet Cell Density (GCD) was examined using Pearson correlation analysis. Schirmer's test, TBUT, cornea spots, and visual acuity were all compared between groups using analysis of variance (ANOVA). P-value≤0.05 was considered statistically significant.

Results: Sixty-three percent of the 225 participants were female, while 37 percent were male. The pupils were 19 ± 3.8 years old on average. The mean difference between the CIC score and Schirmer's test ($p=0.001$), cornea spot ($p<0.001$), and TBUT ($p<0.001$) was statistically significant.

Conclusion: The results of the study showed that on statistical analysis, there was a strong coefficient of correlation between dry eye tests usually practiced by ophthalmologists and laboratory examination by the CIC methodology. It was suggested that studies with large strata of population may be planned to find the pathophysiologic basis and diagnostic association with gold standard tests like CIC in cases of CVS using visual device terminals for longer periods

Key Words: Video Device Terminal, Computer Vision Syndrome, Dry Eye Disease Questionnaire, Conjunctival Impression Cytology, Tear Function Tests.

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INTRODUCTION:

Computer Vision Syndrome (CVS), also known as digital eye strain, has emerged as a significant public health concern due to the widespread use of digital devices and increased screen time. University students, in particular, are at high risk of developing CVS due to prolonged exposure to

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screens for academic, research, and social networking. Symptoms of CVS include foreign body sensations, eye strain, dryness, itching, irritation, blurred vision, and headaches, which can negatively impact productivity and quality of life.[1,2] One of the most frequently reported symptoms in CVS is ocular surface discomfort, which mostly results from tear film instability and ocular surface alterations caused by reduced blinking and increased tear evaporation during prolonged screen use.[3] Commonly employed diagnostic techniques for CVS were primarily based on subjective symptom-based questionnaires and clinical assessments such as tear breakup time (TBUT), Schirmer's test, and fluorescein staining.[4] However, these methods have certain limitations in accurately assessing ocular surface health at a cellular level. Recently, Conjunctival Impression Cytology (CIC) has emerged as a gold standard, minimally-invasive test for evaluating ocular surface changes in conditions affecting the tear film and conjunctival epithelium, including dry eye disease and CVS.[5,6]

CIC involves the collection of superficial conjunctival epithelial cells using a cellulose acetate filter or similar microporous membrane, followed by cytological and histopathological analysis of the retrieved specimen. This technique enables early detection of squamous metaplasia, goblet cell loss, and epithelial cell abnormalities, which are key indicators of chronic ocular surface stress due to prolonged digital device exposure.[7] Several studies have demonstrated that CIC provides objective, reproducible, and quantitative evidence of ocular surface alterations, making it a valuable tool in assessing CVS-related ocular changes.[8] Despite its diagnostic accuracy, the role of CIC in evaluating CVS-related ocular surface pathology among young university students has not been frequently addressed. This study aims to establish CIC as the gold standard test for assessing ocular surface changes in CVS, comparing it with conventional clinical tests and subjective symptom scores obtained by using the validated questionnaire. The findings will provide deeper insights into CVS-induced ocular surface alterations, facilitating early diagnosis, preventive measures, and targeted interventions to improve eye health among university students.

MATERIALS & METHODS:

This cross-sectional study was conducted at the Department of Ophthalmology at the Punjab University Teaching Hospital. A total of 225 students were enrolled, study was conducted from mid-July 2022 to the end of August 2023. Participants who had continuously worked on visual display terminals for more than four hours per day were included. Before the study commenced, IRB ethical approval was sought from the Punjab University, Lahore. However, students with a prior history of eye disease, previous eye and laser surgery for removing glasses, or who were using topical eye drops were excluded from the study. After consulting with two surface eye disease experts, a questionnaire and clinical examination proforma were developed.[9,10] Additionally, informed consent was obtained, and the procedure and methodology were clearly explained to the study participants.

A complete eye examination was conducted, which included assessing best corrected visual acuity. After instilling one drop of local anesthetic using a

sterile fluorescein sodium ophthalmic strip, the tear break-up (TBU) time was measured. That was the time interval between the final blink and the onset of the cornea's first dry patch. A tear break-up time of <10 seconds was considered a critical sign for diagnosing computer vision-related dry eye disease, as recommended by the dry eye specialist. Additionally, because of the instability of the tear film, dry spots on the cornea were counted. For data collection, at least three readings were recorded. More than four dry spots on the cornea were deemed abnormal, indicating dry eye disease related to excessive screen time by the subjects. The same topical anesthetic drop was reapplied for conjunctival impression cytology specimen retrieval. A 0.22-micron-thick, 13 mm diameter cellulose acetate filter paper (Sartorius, Göttingen, Germany) was cut into 5+5 mm strips. Samples were taken from the upper nasal cavity and the right eye's temporally non-exposed bulbar conjunctiva. Participants were instructed to look downward, and the inferior conjunctival sac was dried with a cotton bud before gently pressing the filter paper onto the conjunctiva. Unlike the conventional method using a glass rod, a cotton-tip applicator was used to apply gentle pressure. After a few seconds, the filter paper was carefully peeled off. The specimen was then transferred onto a glass slide, which had been fixed with a mixture of albumin and glycerin at a 1:1 ratio. The impression was stained with a special stain called periodic acid-Schiff reagent and subsequently counterstained first with hematoxylin and then with eosin. Each slide was initially examined under a 10X low-power field (LPF), followed by 40X high-power field (HPF) magnification. Goblet cell density and epithelial cell morphology were assessed in at least five areas. The Nelson scoring system was utilized for grading conjunctival impression cytology specimens.

The data collected from various clinical examination parameters and the questionnaire were entered and analyzed using the Statistical Package for Social Sciences version 24. Pearson correlation analysis was performed to establish the relationship between the CIC score, TBT, Schirmer's test, and GCD. Analysis of variance (ANOVA) was conducted to statistically compare the means of visual acuity, Schirmer's test score, dry spots on the cornea, and TBUT. A multiple comparison test (LSD) was used to identify significant differences among the variables, with a significance level of $p < 0.05$.

RESULTS:

This study was conducted on 225 subjects. The female students were 131 (52.8%), and the male students were 94 (41.8%). They were categorized into three groups. Most of them (82.7%) were in the 18-25 age group, followed by the 26-30 age group (15.6%). However, less than 2% belonged to the 31-40 age group, as depicted in Table 1.

On statistical analysis, it was revealed that there was no significant difference between different scores of visual acuities ($p=0.359$). Moreover, when the values of the CIC score were statistically compared with TBT ($p < 0.001$), dry cornea spots ($p < 0.001$), and Schimer's test readings($p=0.015$) there was a statistically significant difference (Table 2).

For grading of the impression cytology specimen, a well-established Nelson grading system was followed. The results showed grades 0 and 1in 52.6% of cases, who used the computer for a longer

duration. The morphology and number of goblet cells were normal (GCD >500 cells/mm2). Meanwhile, in grade 2, histopathological changes were documented in 32% of the samples (100-500 GCD /mm) with keratinization of the conjunctiva. In addition, grade 3 changes with abnormal conjunctival epithelial cell morphology were documented in 16% of the students, who used a computer for more than 4 hours a day.

In Table 3, it was shown that there was a statistically significant negative correlation between CIC score and tear film function tests. Moreover, CIC grading had a weak correlation with Schirmer's test, whereas GCD had a strong negative relationship with CIC.

Table No. 1: Demographic data of subjects

		Frequency	Percentage
Gender	Male	94	41.8
	Female	131	52.8
	Total	225	100.0
Age groups	18-25	186	82.7
	26-30	35	15.6
	31-35	04	1.8
	Total	225	100.0

Table No. 2: Comparison between Clinical test and CIC score.

		CIC score		Kruskal Wallis –test	p-value
		Mean	S.D		
Visual Acuity	6/6	1.8	0.912	2.047	0.359
	6/9	3.00	0.00		
	6/12	2.00	0.00		
TBUT	<5 sec	2.80	0.451	121.752	<0.001
	5-10 sec	1.79	0.622		
	10 sec	0.88	0.824		
Cornea spot	1-2	1.24	.894	44.758	<0.001
	2-4	1.76	.840		
	4-5	2.10	.718		
	5-6	2.50	.745		
	>7	1.86	1.345		
Schirmer's test (mm)	Less than 5	2.57	.535	10.492	0.015
	5-10	2.10	.960		
	10-15	1.84	0.729		
	Greater than 15	1.68	1.019		

Table No. 3: Relationship between CIC score and clinical tests

	CIC score vs tests		
	Tear break up time	Schirmer's	GCD
Pearson Correlation	-0.597**	-0.192**	-0.944**
Sig. (2tailed)	<0.001	0.004	<0.001
No. of cases	225	225	225

**Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION:

In this study, a comprehensive statistical analysis was done between various clinical dry eye tests, such as tear break-up time, Schirmer's test score, total dry spots on the cornea, and laboratory histopathological evaluation of the conjunctival impression cytology specimens. A significant weak negative correlation (r= -0.525, p <0.001) was found between CIC score and TBT. Moreover, CIC had a weak negative correlation with Schirmer's test score. A study reported by Unlu et al. shows that a significant relationship exists between Ocular Surface disease(OSDI) and TBUT, whereas there was no significant correlation between OSDI score and Schirmer's test readings. However, Yaylali and Ozyurt studied a small sample of patients with Acne Rosea and showed a significant correlation between tear film break-up time and CIC score, which was in sharp contrast to the previous study. Furthermore, in another study of individuals with dry eye condition, 16.8% of cases showed grade 0 and 1 goblet cell grading after 5 minutes if their Schirmer's score was less than 10mm. Moreover, Schirmer's was abnormal in only 5.6% of controls with abnormal histology (L=0.2), while 45.6% had aberrant cytopathological alterations (p<0.001). Therefore,

it was concluded that Schirmer's score is not a reliable indicator of any changes in the cornea's and conjunctiva's morphology.

The diagnosis of CVS was based on the subject's symptoms related to the dryness and clinical tests like TBT, the number of dry spots on the cornea, and the Schirmer's test. However, there was no specific diagnostic test present to reach the final diagnosis. In the present study, we performed clinical tests along with histopathological evaluation with CIC scoring to analyze the conjunctival epithelial cell numbers and morphology. In a study, the researchers reported a positive correlation between Meibomian Gland Dysfunction (MGD) and decreased TBT. This was in sharp contrast to the previous studies because they adopted McMonigle's dry eye questionnaire (MQ). In addition, a decreased value of TBT < 7 seconds was adopted instead of the commonly used value of 10 seconds, which resulted in improving the correlation between the dry eye tests.

Parchides et al. reported that Goblet cell density did not correlate with age, gender, and TBT. They reported a negative correlation (p <0,01) of nuclear-cytoplasmic ratio with age and a positive correlation with TBT(P=0.01). This difference was because CIC samples were taken from healthy volunteers. The ophthalmic surgeon considers CIC to be a minimally invasive procedure. Although it can be easily reproduced, however, it causes discomfort and mild pain in some subjects. Sing et al. documented variability in goblet cell counts from the conjunctival and limbal specimens obtained from the exposed and non-exposed parts of the conjunctiva. Doughty et al.'s ground-breaking study discovered a range of GCD in specimens taken from the individuals' conjunctiva exposed (427+_376 cells/mm2) and non-exposed (973+_789 cells/mm2) surfaces.

It was concluded that environment-related factors predominantly affected the external eye surfaces, like the conjunctiva and cornea. which led to variable numbers and density of goblet cells in the exposed and non-exposed parts of the conjunctiva. Another aspect highlighted by Doughty et al. was to examine tissue specimens with a small sample area (high power field), which resulted in large variability in GCD count (19,20). The basic criteria in this study were to first examine the slides at 10x. later on, a minimum of 5 HPF slides were counted for the goblet cells and epithelial cells to decrease bias in calculation. After the localization of cells, the ocular of the microscope was shifted to 40x HPF magnification.[9,20]

This was the first study that included both the clinical dry tests and laboratory histopathological evaluation of the goblet cells and conjunctival and limbal tissues to study the computer-related dry eye disease in otherwise healthy university students. There were a few limitations of the study. It was a small sample and single-centered study. Only one public sector university students who used computers and related gadgets for more than 4 hours a day were recruited. A study with a large sample size and recruiting subjects from multiple public and private institutions may help establish strong links between the clinical and laboratory profiles of the students. It was suggested that studies with large population strata may be planned to find the pathophysiologic basis and diagnostic association with gold standard tests like CIC in cases of CVS using visual device terminals for longer periods.

CONCLUSION:

The results of the study showed that on statistical analysis, there was a strong correlation between dry eye tests usually practiced by ophthalmologists and laboratory examination by the CIC methodology. in cases of CVS using visual device terminals for longer periods.

Conflict of Interest: The authors declared no conflict of interest.

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