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Correlation of Conjunctival Impression Cytology and Clinical Examination in University Students at Lahore during the COVID-19

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ABSTRACT: *During COVID 19 pandemic every home-bound school, college, and university student was using computers and electronic gadgets for social networking and online education. To assess the effects of these gadgets on ocular and vision-related problems, very little research had been done on the Pakistani population, especially among university students. An analytical cross-sectional study was carried out on 355 students of University of the Punjab. Questionnaire-based data were collected by measurement of tear-film breakup time(TBUT), the number of corneal dry spots, Schirmer's test, and conjunctival impression cytology(CIC). Pearson correlation analysis was done on CIC score versus TBUT, Schirmer's test and Goblet Cell Density (GCD). Analysis of variance (ANOVA) was used for mean comparison of visual acuity, TBUT, cornea spots, and Schirmer's test to see the significance of the results. P-value≤0.05 was considered statistically significant. Out of the 355 students, 65% were females. The mean age of the students was 22.30 ± 6.8 years. At each level of visual acuity, there was no statistically significant difference between the means of the different levels of visual acuity (F=0.488, p=0.614). A statistically significant mean difference was found between CIC score and TBUT (F=208.45p=<0.001), CIC score and cornea spot (F=39.31, p=<0.001), CIC score and Schirmer's test (F=5.83, p=0.001). For the early diagnosis and management of CVS, in addition to applying the validated questionnaire and clinical dry eye tests, the Conjunctival impression cytology can be tested on a larger population to be proven as a gold slandered test.*

Keyword: Video Device Terminal, Computer Vision Syndrome, Conjunctival Impression Cytology, Tear Function Tests

INTRODUCTION

In March 2020 World Health Organization (WHO) declared the COVID19 disease a global pandemic (Nguyen et al., 2020). During the lock-down people had been restrained from crowding in closed places which resulted in the closure of public places like shopping malls, mass traveling in buses, and airplanes, and gathering in mosques, temples, schools, colleges, and universities (Ganne et al., 2021). Consequently, the new concept of work from home, online shopping, online health consultancy services, and online education were introduced. All these measures resulted in a rapid increase in digitalization among individuals from all walks of life. In the educational system, the normal face-to-face interaction of students in the classroom was replaced with online education (Zhao et al., 2020). This situation had deleterious effects on the overall health of the students including ocular health due to long working hours while employing VDT with inappropriate posture (Mocci et al., 2001).

In literature, computers, smart tablets, smartphones, and Liquid Crystal Display (LCD) televisions are collectively assigned the term video display terminal (VDT) (Knave et al., 1985). The ocular condition associated with excessive use of the VDT has been named computer vision syndrome(CVS) (Tsubota and Nakamori, 1993). American Optometric Association has defined computer vision syndrome as "a complex of eye and vision problems related to activities, which stress the near vision and which are experienced with or during the use of the computer" (Alemayehu and Alemayehu, 2019). Symptoms related to CVS have been divided into four categories: (i) ocular-surface symptoms as in dry eye disease, excessive watering, itching, burning, and irritation (ii) symptoms of ocular asthenia or eye fatigue, strain, and pain in the eyes and around the orbital region (iii) visual symptoms, like blurring of vision, diplopia, and inability to focus at near and (iv) extraocular symptoms, the most common of which are headache, backache, and pain in the neck (Al Tawil et al., 2020).

CVS as the name suggests is a diagnosis of a clinical entity present in all age groups but its implications and precision of diagnosis by the ophthalmologists and ocular allied health professionals are not very well appreciated. The long working hours with VDT lead to a reduced rate and quality of blinking which results in tear film instability and hence dryness of the eyes (Antona et al., 2018). The tear film assessment tests are mostly subjective and their sensitivity and specificity have not been standardized. Further, the correlation between tear film profile and intensity of computer vision syndrome is not well established. It is commonly observed that most of the patients may be symptomatic with normal tear functions and not all asymptotic patients have abnormal tear function tests (Nakaishi and Yamada, 1999). Due to the lack of standardization of these clinical tests, the need for a more reliable and objective diagnostic tool cannot be overemphasized. As CVS is an ocular surface disease hence to diagnose precisely the CVS-related dry eye disease, in addition to the clinical dry eye tests, the role of Conjunctival Impression Cytology (CIC) needs to be thoroughly assessed (Singh et al., 2005). Accordingly, in the present study, the diagnosis of CVS-related dry eye disease was assessed simultaneously by tear film function tests, and CIC. To our best knowledge, this is the first study in Pakistan that used face-to-face questionnaires along with clinical dry eye tests and cellular histological examination of the external eye surface to ascertain the frequency and severity of dry eye symptoms related to the excessive use of computer and its correlation with dry eye tests like tear breakup time(TBUT), the number of dry spots on the cornea, Schirmer's test and conjunctival impression cytology(CIC) in university students during COVID 19.

MATERIAL and METHODS

An analytical cross-sectional study was performed on students from July 2020 to August 2021 at the eye clinic of the attached University Teaching hospital university of Punjab, Lahore. All students were included with non-stop working for greater than or equal to 4 hours per day on VDT by consecutive sampling. Prior approval of the study

was obtained from the institutional ethical review committee of the University of the Punjab, Lahore, Pakistan. A total of 425 students were enrolled for the study but 90 subjects didn't return the proforma and sample size ended at 355 with 95% level of confidence, level of precision 5% and a prevalence of CVS at 64% (Zenbaba et al., 2021). Subjects with previous eye diseases, eye surgery, using topical drops and contact lenses were excluded from the study. After thorough literature search, an indigenous questionnaire and clinical examination proforma was developed (Finis et al., 2014; Han and Kim, 2016). After observing the COVID 19 protocol and protective gears, the students were selected from the batches visiting the eye unit of the attached hospital. All the subjects were briefly explained about the CVS and questionnaire. The questionnaire was distributed among the students. After filling up the consent form, the questionnaire was briefly explained and the subjects were given the proforma to be returned within a week. Out of 425 subjects, 355 students returned the proforma. Rests of the 90 subjects were non-responders. It was followed by a clinical examination, and at the end, samples were secured for the conjunctival impression cytology.

The Snellen chart was used to record the best-corrected vision. The tear film breakup time was first assessed by putting one drop of proparacaine hydrochloride 0.5% eye drop (Alcaine Eye drop, Alcon Couvreur purrs; Belgium) on the sterilized fluorescein sodium ophthalmic strip. The time between the last blink and the appearance of a first dry spot on the cornea was noted. A tear break-up time (TBUT) of less than 10 seconds was suggestive of clinical indication of CVS. The number of dry spots appearing on the cornea was counted and the average of the three readings was made on the examination chart. More than four corneal spots were taken as abnormal and a sign of dryness related to CVS.

After few minutes Alcaine eye drops were repeated to perform the conjunctival impression cytology. A circular cellulose acetate filter paper of 0.22 micron thick with 13 mm diameter (Sartorius, Gottingen, Germany) was cut into strips of 5x5 mm. The strip was applied over the superior non-exposed bulbar conjunctiva by asking the subjects to look downgaze and after drying the inferior conjunctival sac with a cotton tip applicator. The samples were taken from both the superior nasal and temporal non-exposed bulbar conjunctiva of the right eye of each subject. The filter paper was gently

pressed over the conjunctiva with a cotton tip applicator instead of a glass rod as conventionally used. The cellulose acetate filter paper was removed by gentle peeling motion after 4-5 seconds and it was carefully applied to a glass slide thinly coated with albumen and glycerin (1:1). The filter paper was then removed from the slide and the slide was labeled and marked with a serial number. The slide was stained with periodic acid – Schiff reagent followed by counterstaining with hematoxylin and eosin. The mounted slide was first examined at 10x low power field (LPF) magnification followed by 40x high power field (HPF) magnification. At least five areas at HPF were examined for recording goblet cell density and epithelial cell morphology. For the scoring and grading of conjunctival impression cytology specimens, the Nelson scoring system was followed (Nelson et al., 1983).

STATISTICAL ANALYSIS

All the data obtained from the questionnaire, clinical examination tests, visual acuity, tear break-up time, cornea dry spots, Schirmer's test score, and conjunctival impression cytology score were recorded and entered on SPSS vs.25. Pearson correlation analysis was done on CIC score versus TBUT, Schirmer's test and GCD. Analysis of variance (ANOVA) was used for comparison of means of visual acuity, TBUT, cornea spots, and Schirmer's test to see the significance of the results. A multiple comparison test (LSD) was used to compare the significant differences. P-value ≤ 0.05 was considered significant.

RESULTS

The female preponderance of about 65% was recorded out of the 325 students, who fully participated in the study. Majority of the students (84.5%) were in the age group of 18 -25 years, and 13.8% were in the 26 -30 years, age group. There were five individuals (1.4%) in the age group of 31 -40 years and only 01 (0.3%) in the group for 36 -40 years. The mean (±SD) of age was 22.30 (±6.82) years (Table 1).

Table 1: Demographic data of subjects

		Frequency	Percentage
Gender	male	140	39.4
	female	215	60.6
	Total	355	100.0
Age groups	18-25	300	84.5
	26-30	49	13.8
	31-35	5	1.4
	36-40	1	0.3
	Total	355	100.0

While studying the effects of VDTs usage by the university students during COVID 19 lockdown, it was revealed that at each level of visual acuity, there was no statistically significant difference between the means of the different levels of visual acuity (F=0.488, p=0.614). Statistically significant difference found between CIC score and TBUT (F=208.45p=<0.001), cornea spot (F=39.31, p=<0.001), and Schirmer's test (F=5.83, p=0.001) (Table 2).

Impression cytology specimens revealed normal results (Nelson grade 0 and 1) in 42.5% after prolonged use of computers. In this group goblet cell density(GCD,>500cells/mm2) and epithelial cell morphology was normal on histological examination. Whereas about 36% of the sample had grade 2 changes with decreased goblet cell density (100-500 GCD/mm2) and abnormal conjunctival cytopathology. Due to the prolonged use of VDT, 22% of CIC samples had grade 3 changes (GCD, <100/mm2) and abnormal conjunctival epithelial cells morphology.

A Pearson correlation analysis was done between conjunctival impression cytology (CIC) Nelson score with TBUT, Schirmer's test and GCD. The Correlation analysis between conjunctival impression cytology (CIC) and tear function tests yielded a significant moderate relationship (r=-0 .525, p=<0.001). Schirmer's test had a week correlation (r= -0.153, p=0.015) with CIC score. Moreover, the Goblet cell density had a strong significant relationship with CIC (r=-0.923, p=<0.001) (Table 3).

Table 2: Mean comparison between Clinical test and CIC score

		CIC score		F –test	p-value	Significant pair (if applicable)
		Mean	**S.D**			
Visual Acuity	6/6	1.84	.898	0.488	0.614	• Not applicable as F-test is insignificant
	6/9	2.00	0.00			
	6/12	2.33	.577			
TBUT	<5 sec	2.72	.530	112.099	<0.001	• <5 sec vs. 5-10 sec • < 5 sec vs. 10 sec • 5-10 sec vs. 10 sec
	5-10 sec	1.80	.642			
	10 sec	1.05	.952			
Cornea spot	1-2	1.26	.902	15.159	<0.001	• 1-2 vs. (2-4, 4-5, 5-6, >7) • 2-4 vs. (4-5, 5-6, >7)
	2-4	1.77	.838			
	4-5	2.05	.720			
	5-6	2.35	.849			
	>7	2.17	.985			
Schirmer's test	<5 mm	2.67	.500	3.762	0.011	• <5 mm vs. (5-10 mm, 10-15 mm, >15 mm) • 5-10 mm vs. (>15 mm)
	5-10 mm	2.02	.950			
	10-15 mm	1.84	.736			
	>15 mm	1.76	.975			

Table 3: Correlation between CIC score and clinical tests

	CIC score versus		
	TBUT	Schirmer's	Goblet cell density GCD
Pearson Correlation	-0.525**	-0.153**	-0.923**
Sig. (2-tailed)	<0.001	0.015	<0.001
No. of cases	355	355	355

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

DISCUSSION

During COVID 19 pandemic, following the lockdown, use of computers and smart electronic devices increased drastically among the students in particular and other individuals in general. In this study, effect of VDT use by the students during COVID 19 was measured through various clinical tests like TBUT, Schirmer's test, and dry cornea spots and the statistical correlation was worked out between these tests and CIC.

The Correlation analysis between conjunctival impression cytology (CIC) and tear breakup time yielded a significant correlation (r= -0.525, p=<0.001). Schirmer's test had a week correlation (r= -0.153, p-value=0.015) with CIC score. In a study, Ünlü et al documented a significant correlation between OSDI score and TBUT score but they found no significant correlation between Schirmer's test and OSDI score (Ünlü et al., 2012). However, this was in sharp contrast to the results of a study by Yaylali and Ozyurt. The probable explanation for this difference is that Yaylali and Ozyurt (2002) engaged a small sample size to develop a correlation between the tear film profile and CIC for Acne rosacea cases of dry eye (Yaylali and Ozyurt, 2002). Moreover, CIC was also used to examine the etiological factors responsible for the conjunctival changes in thyroid associated eye disease (Özkan et al., 1997). A cross-sectional study of one hundred and fifty patients to study the effects of computer users on conjunctiva showed that those used computers for longer period developed more cyto-pathological changes compared to those with limited use of computers (Kulkarni et al., 2017) A study reported that in dry eye patients, 16.8% of patients with Schirmer's results of less than 10 mm in 5 minutes had grade 0,1 changes in CIC and 45.6% had abnormal cytology ($p < 0.001$). Whereas only 5.6% of controls with abnormal cytology had abnormal Schirmer's ($L = 0.2$). Thus, it appears that Schirmer's does not predict any morphological changes in the conjunctiva. Likewise, in the present study Schimer's test had a week

correlation (r=-0.153, p-value=0.015) with CIC score (Kumar et al., 2014).

Due to the complex multifactorial nature of the condition, the diagnosis of CVS is not very easy but rather a bit tedious. The poor correlation had been explained by previous workers on the basis that no specific tests have been designed for the CVS. In the present study, we performed the TBUT to assess decreased tear film stability, the number of dry spots to assess severity of the condition and CIC to analyze the morphology of conjunctival epithelial cells and goblet cells density. Moore et al. by using a different questionnaire (MQ) showed a positive correlation between MGD with reduced TBUT. However, they standardized the TBUT cut-off value 0f <7s instead of the commonly used value of 10 s which resulted in improving the correlation between the dry eye tests (Moore et al., 2009). Paschides et al. reported that the Goblet cells number had a negative correlation ($p<0.01$) with age and a positive correlation with TBUT ($p=0.01$). This difference was due to the fact that CIC samples were taken from healthy volunteers. (Paschides et al., 1991)

Although, CIC is minimally-invasive, relatively easy to perform, and provides reliable information about the conjunctival area. Moreover, goblet cell counts showed gross variability in the samples obtained from exposed and non-exposed regions of the conjunctiva (Kessing, 1968). Doughty et al. have reported GCD of normal human participants from impression cytology specimens. They documented a wide range in GCD in samples obtained from exposed (427 ± 376 cells/mm2) and non-exposed (973 ± 789 cells/ mm2) surfaces of the conjunctiva of the subjects. They augmented this finding and suggested that environment-related factors predominantly influenced the ocular surface, hence there was variability in GCD in samples obtained between exposed and non-exposed quadrants of the conjunctiva (Doughty, 2012a). Moreover, in another study, Doughty, reported that with the use of a small sampling area (high power field of view) there was a high chance to get an unacceptable large variability in the

GCD estimates (Doughty, 2012b). In the present study, we initially examined prepared slides at a 10x LPF. At least 05 LPFs slides were counted for goblet and epithelial cells to reduce the bias in the calculation. After the cells were localized, they were examined at 40x HPF magnification. However, in computer vision syndrome cases it is expected to have less variability in the samples collected from different locations as compared to normal human participants.

The Secondary and tertiary eye care centers can, however, develop a team of ophthalmologists and histopathologist for the proper procurement of the samples and to facilitate this technique to be used in daily practice. In this study, clinical tear function tests were plotted against CIC to determine whether they were alongside cytological changes on the ocular surface in computer syndrome. A limitation of this study was that the young health university students using computers and related electronic gadgets confined to the home during COVID 19 were studied after some time when their routine educational activities were resumed.

CONCLUSION

The frequency and coefficient of correlation between clinical tear film tests and CIC in cases of CVS were remarkably high in university students. In the future, larger studies may be conducted to find the pathophysiologic basis and diagnostic relationship with to optimize VDTs usage and, hence lower the rate of CVS among youth pursuing higher education in colleges, and universities of the Country.

ETHICAL APPROVAL

The study was approved by the institutional ethical review committee.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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