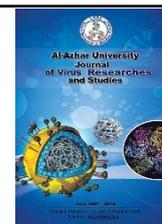




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Conjunctival Impression Cytology for Diagnosis and Grading of Dry Eye

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Abstract

Dry eye or tear film dysfunction is a common ophthalmic syndrome. Goblet cell density is significantly reduced in dry eye syndrome. The impression cytology is a useful test for the diagnosis of dry eye syndrome. The aim of the paper evaluates the conjunctival impression cytology as a diagnostic tool for diagnosis of dry eye and for grading the disease. This study is a prospective, and a non-randomized study that was held at Al-Zahraa University Hospital. It was conducted on sixty eyes of thirty patients. They were diagnosed clinically and by investigations as dry eye. Regarding impression Cytology \Nelson Grade, 14 eyes (23.3%) were grade 1 (mild), 23 eyes (38.3%) were grade 2 (moderate) and 23 eyes (38.3%) were grade 3 (severe). As the severity of dry eye increased, the cytology showed marked cell separation and lowering of N:C ratio with a decrease in goblet cell count. Conjunctival impression cytology provides detailed information about morphological alterations of the bulbar conjunctiva. It has proven to be useful in differentiating the degrees of dry eye disease.

Keywords: Dry eye, Ocular surface, Impression cytology, squamous metaplasia.

1. Introduction

Dry eye is multifactorial diseases of the tears and ocular surface that results in discomfort, visual disturbance and tear film instability. Environmental factors are often present in dry eye including exposure to pollutants, ultraviolet (UV) radiation and ozone as well as the chronic use of preserved eye drops, such as glaucoma medications [1,2]. Impression cytology is a minimally invasive test is performed under topical anesthesia to obtain the superficial cells by application of small membrane against the conjunctival surface [3]. It is a useful test to confirm the diagnosis of dry eye syndrome and to evaluate its severity. Other methods are the Schirmer tests, tear

break-up time, and functional visual acuity [4,5].

2. Patients and Methods

This study was a prospective, and a non-randomized study. It was held at Al-Zahraa University Hospital. Sixty eyes in thirty patients of both males and females aging between 20 to 60 years old were included in this study. They were diagnosed clinically and by investigations as dry eye. Each patient was subjected to the following: Uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA), slit-lamp examination for the

anterior segment, Schirmer's I test, a Schirmer's II test, tear breakup time (TBUT) test and conjunctival impression cytology.

2.1 Inclusion Criteria

Patients with different degrees of dry eye aging between 20 and 60 years old.

2.2 Exclusion Criteria

Patients with history of refractive eye surgery or lacrimal surgery. Subjects underwent the TBUT and the Schirmer's test.

2.3 Breakup Time Test

Tear film break up time is the time interval between complete blink and appearance of first randomly distributed dry spot on the cornea. Tear film was observed under the cobalt blue filtered light of the slit lamp bio-microscope. The mean TBUT scores of the right and left eyes were used for statistical analysis.

2.4 Conjunctival Impression Cytology

Cellulose acetate filter paper (Millipore filter paper) was cut into small strips of 5 mm × 5 mm. The conjunctiva was anesthetized with topical anaesthesia. The filter paper was applied to the temporal and nasal parts of conjunctiva by pressing on it by the tip of a forceps for 10 seconds. The filter paper was grasped gently with the forceps with peeling motion. The filter paper was then pressed cell side down onto a clear glass slide. Specimens were fixed in alcohol 90%, stained with Haematoxylin and Eosin slides examined under light microscope under the low and the high powers. Nelson's grading system was followed for the staging: 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, Grade II - Large polygonal and occasionally multinucleate epithelial cells with variable staining cytoplasm N:C ratio of 1:4–5. Grade III - Large polygonal epithelial cells with basophilic cytoplasm, N: C ratio of >1:6 with very few or absent goblet cells

2.5 Statistical Analysis of the Data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using range (minimum and maximum), mean, and standard deviation and Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Area under the ROC curve denotes the diagnostic performance of the test.

3. Results

Our Age of patients ranged between 20 and 60 years. (Mean: 45.23 ± 10.97 years). 56.7% of patients were in the age group between 45 and 60 years while 43.3% were at age group between 20 and 44 years. As regards to sex, 56.7% of patients were females, while 43.3% were males. As regard comorbidities, it was observed that none of the participants in the study were diabetics nor hypertensive Table (1). There were statistically non-significant differences between OS and OD regarding visual acuity, best corrected visual acuity, TBUT and Shirmer I & II tests. Also, there was statistically non-significant difference between OS and OD regarding Impression Cytology \Nelson Grade Table (2). According to the impression Cytology \Nelson Grade, it was significantly increased with the increase in age. Burning sensation, blurring of vision, photophobia, redness and gritty sensation were significantly higher in the severe group. On the other hand, there were statistically non-

significant differences between Impression Cytology \Nelson Grade and sex as well as lacrimation Table (3). There was statistically non-significant difference between the mild and moderate groups, and also there was statistically non-significant difference between the moderate and severe groups. There were statistically significant differences between Impression Cytology \Nelson Grade and BCVA, Shirmer I as well as Shirmer II Table (4). Comparison regarding TBUT showed that there was statistically non-significant difference between mild and moderate groups, and also between moderate and

severe groups. There was an important change between mild and severe groups. So, a ROC curve was applied between mild and severe groups to assess the cutoff point in-between; the best cut off point was found ≤ 5 to differentiate between the mild and severe groups (sensitivity and specificity was 78.26% and 78.57% respectively). Figure (1). As the severity of dry eye increased, the cytology showed marked cell separation and lowering of N:C ratio with a decrease in goblet cell count. Figures 2 (a, b, c)

Table (1): Demographic and clinical characteristics among the studied cases.

		No. = 30 pts
Age (years)	Mean \pm SD	45.23 \pm 10.97
	Range	20 – 60
	20 – 44 yrs.	13 (43.3%)
	45 – 60 yrs.	17 (56.7%)
Sex	Females	17 (56.7%)
	Males	13 (43.3%)
DM	Negative	30 (100.0%)
	Positive	0 (0.0%)
HTN	Negative	30 (100.0%)
	Positive	0 (0.0%)

Table (2): Comparison between the laterality of eye as regards clinical examination and impression Cytology \Nelson Grade.

		OD	OS	Test value	P-value	Sig.
		No. = 30	No. = 30			
VA	Mean \pm SD	0.30 \pm 0.28	0.29 \pm 0.29	-0.181•	0.856	NS
	Range	0 – 0.778	0 – 1.301			
BCVA	Mean \pm SD	0.08 \pm 0.09	0.13 \pm 0.24	-0.352•	0.725	NS
	Range	0 – 0.176	0 – 1.301			
TBUT	Mean \pm SD	6.47 \pm 3.27	6.63 \pm 3.48	-0.135•	0.893	NS
	Range	3 – 16	3 – 17			
Shirmer I	Mean \pm SD	6.17 \pm 2.41	6.23 \pm 3.02	-0.174•	0.862	NS
	Range	3 – 12	2 – 15			
Shirmer II	Mean \pm SD	9.37 \pm 2.77	9.53 \pm 3.01	-0.134•	0.893	NS
	Range	4 – 14	4 – 18			
Impression Cytology \Nelson Grade	Grade 1 (Mild)	10 (33.3%)	4 (13.3%)	3.354*	0.187	NS
	Grade 2 (Moderate)	10 (33.3%)	13 (43.3%)			
	Grade 3 (Sever)	10 (33.3%)	13 (43.3%)			
		10 (33.3%)	13 (43.3%)			

Table (3): Relation between impression Cytology \Nelson Grade and demographic and clinical data.

		Impression Cytology \Nelson Grade			Test value	P-value	Sig.
		Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe			
		No. = 14	No. = 23	No. = 23			
Age (years)	Mean ± SD	37.5 ± 12.97	45.13 ± 11.19	50.04 ± 5.53	6.954•	0.002	HS
	Range	20 – 60	21 – 60	43 - 60			
Sex	Females	10 (71.4%)	10 (43.5%)	14 (60.9%)	3.037*	0.219	NS
	Males	4 (28.6%)	13 (56.5%)	9 (39.1%)			
Burning sensation	Negative	10 (71.4%)	11 (47.8%)	3 (13.0%)	13.313*	0.001	HS
	Positive	4 (28.6%)	12 (52.2%)	20 (87.0%)			
Lacrimation	Negative	10 (71.4%)	16 (69.6%)	18 (78.3%)	0.479*	0.787	NS
	Positive	4 (28.6%)	7 (30.4%)	5 (21.7%)			
Blurring of vision	Negative	9 (64.3%)	12 (52.2%)	5 (21.7%)	7.603*	0.022	S
	Positive	5 (35.7%)	11 (47.8%)	18 (78.3%)			
Photophobia	Negative	12 (85.7%)	16 (69.6%)	8 (34.8%)	10.828*	0.004	HS
	Positive	2 (14.3%)	7 (30.4%)	15 (65.2%)			
Redness	Negative	14 (100.0%)	20 (87.0%)	12 (52.2%)	13.335*	0.001	HS
	Positive	0 (0.0%)	3 (13.0%)	11 (47.8%)			
Gritty sensation	Negative	10 (71.4%)	14 (60.9%)	6 (26.1%)	8.919*	0.012	S
	Positive	4 (28.6%)	9 (39.1%)	17 (73.9%)			

*P-value >0.05: Non-significant (NS); P-value <0.05: Significant (S); P-value < 0.01: highly significant (HS), *: Chi-square test; •: One Way ANOVA test.*

Table (4): Relation between impression Cytology \Nelson Grade and clinical examination.

		Impression Cytology \Nelson Grade			Test value•	P-value	Sig.
		Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe			
		No. = 14	No. = 23	No. = 23			
VA	Mean ± SD	0.24 ± 0.18	0.26 ± 0.31	0.36 ± 0.30	1.805	0.406	NS
	Range	0 – 0.477	0 – 1.301	0 – 0.778			
BCVA	Mean ± SD	0.04 ± 0.07	0.07 ± 0.10	0.18 ± 0.26	3.256	0.046	S
	Range	0 – 0.18	0 – 0.30	0 – 1.30			
TBUT	Mean ± SD	8.43 ± 4.16 ^a	6.52 ± 3.03 ^{a, b}	5.43 ± 2.66 ^b	8.966	0.011	S
	Range	4 – 17	3 – 12	3 – 11			
Shirmer I	Mean ± SD	5.64 ± 2.50	5.96 ± 2.80	6.78 ± 2.75	1.479	0.477	NS
	Range	3 – 10	2 – 15	3 – 13			
Shirmer II	Mean ± SD	10.00 ± 2.29	9.04 ± 2.51	9.52 ± 3.51	1.354	0.508	NS
	Range						

P-value >0.05: Non-significant (NS); P-value <0.05: Significant (S); P-value < 0.01: highly significant (HS), •: One Way ANOVA test, Different superscript letter indicate significant difference between groups.

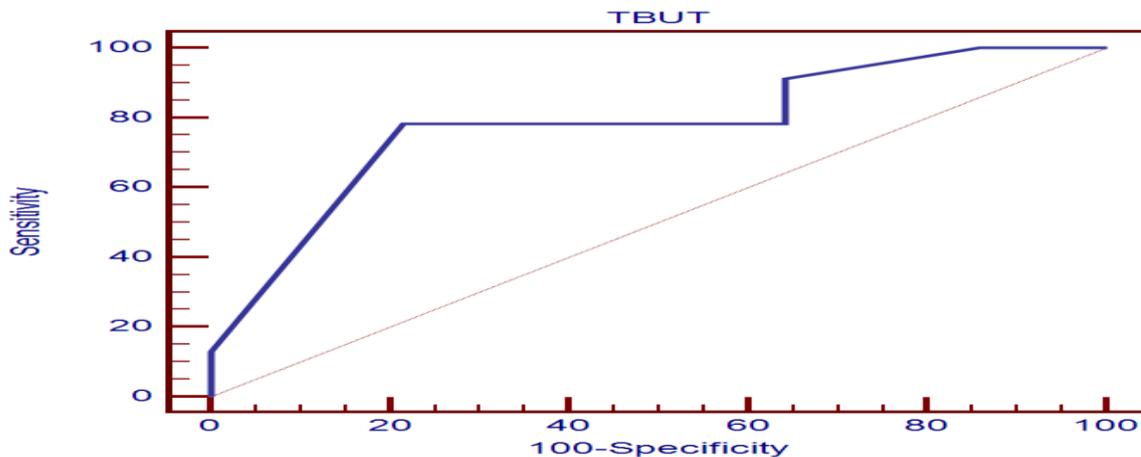
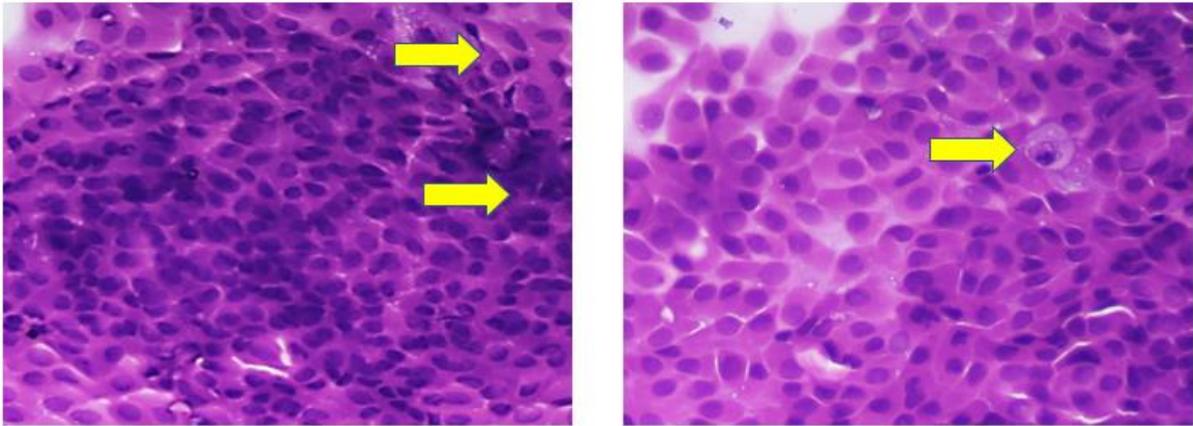
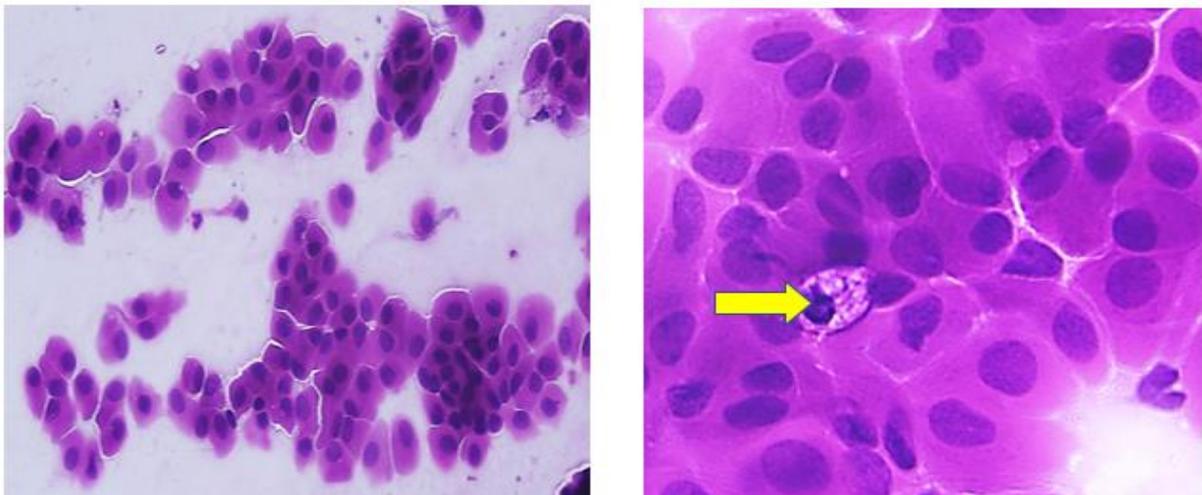
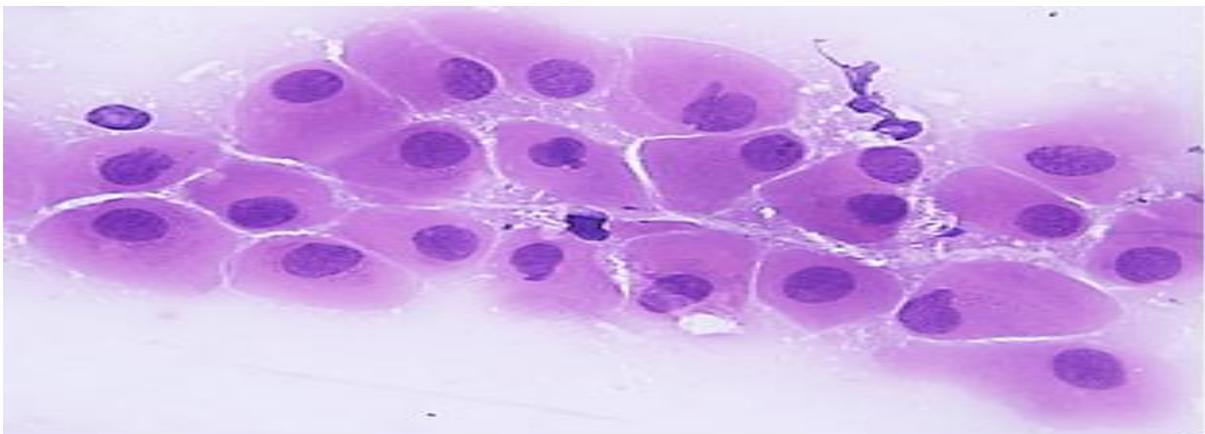


Figure (1): ROC curve for TBUT with impression cytology.

Table (5): Receiver operating characteristic curve (ROC) between mild and severe impression cytology.

Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
	0.781	78.26	78.57	85.7	68.7

**Figure (2):** Normal impression cytology. Dense clusters of normal epithelial cells (Nuclear to cytoplasmic ratio is 1:2) with preserved goblet cells. Original magnification (A: 100, B: 200).**Figure (3):** (a) Nelson Grade I. Sheets of cells having a mild decrease in N:C ratio (1:3) with some preserved goblet cells (arrow), original magnification (A: 100, B: 200).**Figure (4):** Nelson Grade II. Slight dissociation with cells having a moderate decrease in N:C ratio (1:4) (original magnification; 200)

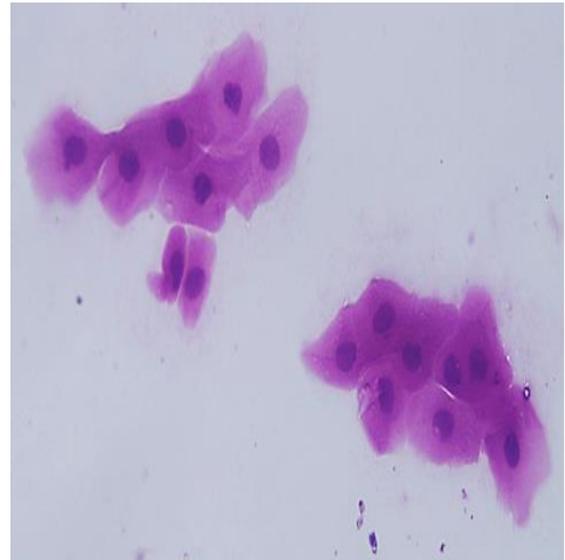
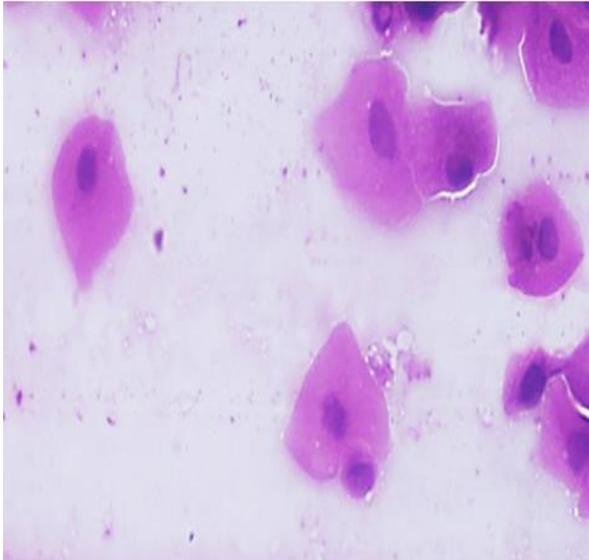


Figure (5): Nelson Grade III. Marked dissociation of cells.

4. Discussion

Conjunctival impression cytology has a wide range of applications in ophthalmology. It facilitates the diagnosis of ocular surface disorders [6,7]. Results of this study showed that, in a total of 30 patients studied, there were 18 patients with a positive burning sensation (60.0%) and 12 with were negative (40.0%), 22 (73.3%) had negative lacrimation and 8 were positive (26.7%), 17 patients (56.7%) had positive blurring of vision and 13 patients were negative (43.3%), 18 patients had negative photophobia (60.0%) and 12 Patients were positive (40.0%), 23 had negative eye redness (76.7%) and 7 patients were positive (23.3%), and 15 patients had positive Gritty sensation (50.0%). Patients who reported dry eye diagnosis presented increased symptoms of dryness, irritation, gritty eyes and light sensitivity in a study reported by Tau et al., [8], while they had less watery eyes ($p < 0.05$). Of the 100 patients with positive symptomatology in a study reported by Al Wadani et al., [4], the sensitivity of various diagnostic tests were positive Schirmer's test in 83% of cases, positive TBUT in 82% of cases, positive lissamine green in 26% of cases, and positive impression cytology in 94% of cases. There was a female preponderance in

dry eye disease in a previous study on 114 dry eye cases by Shrestha et al., [5], 49.2% of eyes showed a decreased or absent goblet cell density. In 72 normal individuals 73.7% eyes showed normal goblet cell density and 26.3% of eyes showed decreased or absent goblet cells. The tear break-up time (TBUT) test was significantly more likely to be less than 10 seconds in cases as compared to the controls. Similarly, the goblet cell density was likely to be significantly reduced in cases with dry eye syndrome. Among sixty eyes of 30 patients, mean UCVA \pm SD was 0.29 ± 0.28 mean BCVA \pm SD was 0.11 ± 0.18 mean TBUT \pm SD was 6.55 ± 3.35 mean Shirmer 1 test \pm SD was 6.20 ± 2.71 (Range: 2 – 15, and mean Shirmer 2 test was 9.45 ± 2.87 . Regarding Impression Cytology \Nelson Grade, 14 cases (23.3%) were grade 1 (mild), 23 cases (38.3%) were grade 2 (moderate) and 23 cases (38.3%) were grade 3 (severe). The mean TBUT \pm SD in cases and control groups in a study reported by Bhargava et al., [9] it was 11.26 ± 1.68 and 15.68 ± 2.62 . The mean Schirmer's score in cases and controls was 24.64 ± 8.62 . Mean TBUT scores were significantly less when time spent on computers daily was more. In cases, 93 (27%) were severely symptomatic, 156 (45.3%) moderately, 35 (10.2%) were mildly symptomatic and 60 cases (17.4%)

were symptom free. DESS score was significantly higher when daily computer usage was more. In another study Schirmer test, Tear Film Break up Time (TBUT) and Rose Bengal Stain (RBS) correlate with dry eye symptoms Bhargava and Kumar, [10] found that at the baseline, 17 cases (5.4%) were severely symptomatic, 196 cases (61.2%) were moderately symptomatic, 69 cases (21.6%) were mildly symptomatic, and 38 cases (12%) had occasional symptoms. Amongst controls, 9 cases (2.6%) were moderately symptomatic, 74 cases (21.1 %) were mildly symptomatic, and 267 cases (76.3%) were symptom free. The mean symptom score in cases and controls was 8 ± 2.6 and 1.2 ± 2 , respectively ($P<0.001$). Our results showed that TBUT correlates well with conjunctival impression cytology unlike Schirmer I & II which was not correlated with it. Also, good correlation was detected between ocular symptoms and conjunctival impression cytology. In agreement with Kumar et al., [11]. Results showed that the Schirmer's test values did not correlate well with impression cytology in the experimental group, but they did somewhat amongst the controls. TBUT values correlated well with the CIC score in cases and more so among the controls. Correlation analysis was carried out for CIC and RBS scores. Contrary to our results, reported by Tau et al., [8] reported by the patients and the results of the bulbar conjunctival impression cytology. Our results cleared that age, burning sensation, photophobia and redness were significantly higher in eyes with severe impression cytology \Nelson Grade than those with moderate and mild. On the other hand, there was statistically non-significant differences between severe, moderate and mild impression cytology \Nelson Grade regarding sex, lacrimation, VA, Shirmer 1 and Shirmer 2 tests. Shrestha et al., [5] noted that, the majority of normal eyes (95.1%) had TBUT more than 10 sec, among which 72.9% had a normal cytology and only 22.2% had an abnormal cytology

for dry eyes. Using the receiver operating characteristics (ROC) curve analysis to predict patients with DED done by Ozcan et al., [12], they showed that, the highest area under the curve (AUC) was determined SII (0.761 for SII, 0.727 for NLR, and 0.653 for PLR). The diagnostic accuracy of CIC, TBUT, Schirmer and RBS in dry eye patients was evaluated and compared with age and sex matched controls in another study conducted by Bhargava and Kumar, [10] showed that, the area under the curve (AUC) was measured using ROC curve and the diagnostic accuracy was CIC (AUC=0.957) >TBUT (AUC=0.793) >Schirmer (AUC=0.765)>RBS (AUC=0.723). The sensitivity of TBUT in diagnosing dry eye severity, it was 88.6% %, specificity was 82.4 % (95 % CI-75.5 to 86.4%), positive likelihood ratio was 4.36), and negative likelihood ratio 0.14 respectively. In only 5 of the 100 cases impression cytology was no representative, also evaluated how the grading in impression cytology (Nelson scores) compares with OSDI scores in a study by Zuazoet al., [7] who reported that, there was only one case (1%) where the cytology was normal and the OSDI was scored as mild.

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