



# CORRELATION OF TEAR FUNCTION TESTS AND CONJUNCTIVAL IMPRESSION CYTOLOGY IN DRY

### **EYES PATIENTS**

Dr. Sagarika Rao<sup>1</sup>, Dr. Sarita Aggarwal<sup>2</sup>, Dr. Somesh Ranjan<sup>3</sup>, Dr. Shivang Patel<sup>4</sup>, Dr. Nupur Bhatnagar<sup>5</sup>, Dr. Mishika Bhasin Kandhari<sup>6</sup>

Article History: Received: 12.12.2022 Revised: 29.01.2023 Accepted: 15.03.2023

### **Abstract**

The presence of dry eye disease and its effects on patient's quality of life makes it a significant healthcare issue. According to estimates, dry eye syndrome will affect approximately 75% of adults over 65 years. This was a hospital based cross sectional analytical study that was conducted in 80 patients divided equally, with and without dry eyes in of the age group of 10-70 years during the time period of 18 months. An evaluated history and ocular examination was done with tear function tests like schirmer's test, tear break up time test, rose bengal staining and conjunctival impression cytology. The goal of the study was to determine the correlation between cytology grading and clinical tests for dry eyes in order to determine whether the cytological test can be alternative in the diagnosis and prognosis of dry eye disease. This study found that among 40 cases with dry eyes, maximum patients were in the age group of 51-60 years (32.5%) and minimum in the age group of 11-20 years (7.5%) and 31-40 years (7.5%). Cases affected with dry eye were more males (52.5%) than females (47.5%). There is statistically significant correlation between the Schirmer's test grading and tear break up time grading with that of conjunctival impression cytology grading. This research concludes that there is strong correlation between conjunctival impression cytology and tear function tests. Thus this non-invasive, rapid and easy investigation is valuable in assessing dry eye disease and in evaluating the prognosis of dry eye disease.

**Keywords:** Dry eye, Dry eye syndrome, tear function tests, conjunctival impression cytology, ocular surface disease index, .

DOI: 10.31838/ecb/2023.12.s3.071

<sup>&</sup>lt;sup>1,4,5,6</sup>Santosh Deemed To Be University No.1, Santosh Nagar, Ghaziabad-201009,Ncr Delhi, India

<sup>&</sup>lt;sup>2,3</sup>Department of Ophthalmology Santosh Medical College & Hospital Ghaziabad, Ncr Delhi

#### 1. Introduction

The International Dry Eye Workshop defines dry eye as a multifactorial disease of the tears and ocular surface that causes symptoms such as discomfort, visual disruption, and tear film instability, as well as potential ocular surface damage. Along with it, the tear film's osmolarity increases, and the ocular surface becomes inflamed.(International Dry Eye WorkShop 2007)

The presence of dry eye disease and its effects on patient's quality of life makes it a significant healthcare issue. Dry eye prevalence rises with advancing age. According to estimates, dry eye syndrome will affect approximately 75% of adults over 65 years. (Hashemi H et al 2014)

Dry eye prevalence ranged from 18.4 to 40.8% in India. (Basak SK et al 2012, Gupta N trauma al 2014, Brewitt H et al 2001)

The absence of a widely accepted gold standard test has made it more challenging for ophthalmologists to diagnose and treat dry eye clinically.(Bhargava R et al 2014)

Slit lamp examination, Schirmer's test, tear film break up time, and Rose Bengal staining are all used to establish a clinical diagnosis of dry eye. Conjunctival biopsy is the traditional method for examining the histopathological abnormalities.

Since it is invasive, this technique is not regularly performed. Conjunctival impression cytology, a more contemporary test, has been created to acquire conjunctival impressions and examine cellular morphology. It uses a filter paper approach with millipore cellulose acetate paper.

The Schirmer's test is the most commonly used method of measuring tear production. Due to reflex tearing, it cannot be repeated and ignores the evaporative features of dry eye. (Nichols KK et al.)

2004) On the other hand, tear film stability can be evaluated using the tear film break up time (TBUT). Considering that it can be somewhat repeated, this test appears to be more accurate than Schirmer's.(Patel S et al 1985, Cho P et al 1996)

The level of ocular surface damage is measured by staining with essential dyes like Rose Bengal, but this method might miss milder cases. In dry eye states, it can also be used in conjunction with other tests like the TBUT.( Argueso P et al 2006)

A qualitative approach to assess the alterations in conjunctival morphology and goblet cell density in dry eyes is conjunctival impression cytology. (Guazzi A et al 1988)

In 1954, Larmande and Timsit originally characterised it as a diagnostic tool for squamous neoplasia of the ocular surface. (Larmande A et al 1954) In 1977, Egbert et al. published a report on the application of impression cytology for assessing goblet cell densities and ocular surface diseases. (Egbert PR et al 1977)

In conjunctival impression cytology, the ocular surface's morphology is preserved while the outer layers of the conjunctival epithelium are collected for morphological analysis. It is repeatable, minimally invasive, offers the opportunity to track the progression of morphological changes, and provides accurate information on the area sampled with little discomfort to the patient.( Natadisastra G et al 1987, Puangsricharern V et al 1995, Lee GA et al 1995)

This technique can be used to analyse conjunctival goblet cells, which are decreased in people with severe dry eye, as well as to identify early abnormalities in the process of squamous metaplasia before they become clinically obvious and permanent. (Murube J et al 2003)

It is a commonly used approach for studying the ocular surface in diseases such as viral conjunctivitis,

keratoconjunctivitis sicca, (Marner K et al 1980, Nelson JD et al 1984) vitamin A insufficiency, (Wittpenn JR et al 1986) chronic renal failure, psoriasis, ocular surface tumors. (Dry Eye Workshop, 2007) However, its greatest benefit has been in the treatment of dry eye condition, when goblet cells are depleted.

For determining the severity of dry eye and making a diagnosis, it is just as reliable as a biopsy. It aids in determining the level of dry eye severity.

However, it is still not the foremost investigation in dry eye syndrome. Currently, these tests are frequently used in conjunction to diagnose and grade the severity of dry eye.

Conjunctival impression cytology applications in dry eye syndromes include:

- 1-Dry eye diagnosis
- 2. To determine the severity level.
- 3. To understand how dry eyes affect the ocular surface
- 4-To evaluate how a treatment will affect the prognosis and the course of the disease. 5-To find another ocular surface pathology that is related.

Considering the advantages of this minimally invasive technique over other methods, the goal of the current study is to determine the correlation between cytology grading and clinical tests for dry eyes caused by various etiologies and in healthy individuals, to assess the degree to which these tests correlate with dry eye symptoms in order to determine whether the cytological test can be alternative to conjunctival biopsy in the diagnosis and prognosis of dry eye syndromes.

### 2. Material And Methods

This was a cross sectional analytical study that was conducted in 80 patients divided into 40 cases (Group I ) with Dry Eyes and 40 controls (Group II) which were age and sex matched individuals without dry eyes in of the age group of 10-70 years during the time period of April 2021 to October 2022 who attended

the Department of ophthalmology, Santosh Medical College and Hospital, Ghaziabad, UP.

### **Inclusion Criteria:**

Those patients who presented to Department of Ophthalmology with symptoms of dry eyes and positive for any one of following tests were included in group I.

- 1)schirmers test(<10mm in 5min)
- 2)Tear film break up test(<10sec)
- 3)Rose Bengal staining positive.

And age and sex matched population without symptoms of dry eyes and negative for tests mentioned above were included in group II.

- 1) schirmers test(>10mm in 5min)
- 2) 2)Tear film break up test(>10sec)
- 3) Rose Bengal staining negative.

### **Exclusion Criteria:**

- 1) Acute conjunctivitis.
- 2)Diagnosed case of dry eye already on treatment.
- 3) History of any past ocular surgery.

During the above said period ,individuals satisfying the inclusion criteria were selected among the patients presented to Santosh Medical College and Hospital,Ghaziabad,UP.

### **Ethical Approval**

Ethical approval was taken by the ethical committee of the medical college and hospital.

### History

All individuals provided written consent after being fully informed. The principal indicators of this condition are subjective dry eye symptoms. Burning, foreign body sensation, stinging, dryness, itching, pain, photophobia, heaviness of the lids, and ocular strain are only a few possible presenting methods. Patients who suffer from dry eyes often experience similar symptoms.

### **Schirmer Test**

A popular, affordable, and simple clinical test called the Schirmer test is used to qualitatively assess tear production. Whatman number 41 strip, 5 mm wide by 35 mm long, was inserted into lower eyelid for this test. A well-standardized test called the ST without anaesthesia or basal schirmers test assesses the basal tear secretion caused by conjunctival-lacrimal trigeminal reflex. ( Hida RY et al 2006, Cho P et al 1993)

Schirmer's test was performed with out topical anaesthesia. The strip was placed between medial 2/3rd and lateral 1/3rd of lower lid margin and asked the patient to keep the eye closed. Results were noted after 5 minutes. The strip's rate of wetting from the tears was measured in millimetres per five seconds. The test was evaluated based on grading- 4 grades (1-variable, 2-</= 10, 3-</=5,4-</=2).

### Tear Film Break Up Time( Tbut)

Tear break-up time (TBUT) is common, cheap and easy to perform. Tear breakup time test is the standard clinical procedure that was introduced by Norn and its high sensitivity suggests a strong connection to the dry eye. (Begley CG et al 2006)

Fluorescein 1 mg strips were used to stain the subjects' eyes, and they were instructed not to blink. TBUT is the time interval between a complete blink and the first appearance of a dry spot in the precorneal tear film after the installation of fluorescein viewed with a cobalt blue filter. ( Cho and Brown et al 1936, Savini G et al 2008) The grading of TBUT is as follows-(1-variable, 2-</=10, 3-</=5, 4-immediately).

### **Rose Bengal Stain**

The superficial ocular surface can be evaluated using dyes to find damage that may be present in dry eye disease. 1% liquid rose bengal is instilled into the eye. The examiner uses green filter to assess the amount of staining on slit lamp biomicroscopy. Intensity scored in 2 exposed conjunctival zones ( nasal and temporal conjunctiva) and cornea Score. The staining pattern was documented, and the samples were assessed using Van Bijsterveld's scale 0–3, a maximum of 9 score. Scores under 4 are considered normal, and those over 4 are considered abnormal (American-Europe criteria). ( van Bijsterveld O et al 1969, Vitali C et al 2002 )

### **Conjunctival Impression Cytology**

Impression cytology is the process of removing the outermost layers of the ocular surface epithelium using a cellulose acetate filter applied to the eye. The histological, immunohistological, or molecular examination of these cells is then possible.

This minimally invasive technique for examining conjunctival goblet cells was first introduced by Egbert et al. (Egbert PR et al. 1977)

It is non-invasive, simple to use, and produces accurate results regarding the area tested while causing the patient the least amount of discomfort.

Implementations of impression cytology include the etiological diagnostic test of numerous ocular surface disorders, describing sequential variations in the conjunctival and corneal outer layer over time, surveilling clinical outcomes and staging conjunctival squamous metaplasia, as well as as an investigational tool for analysing ocular surface disease with immunostaining and DNA analysis.

### **History**

Conjunctival samples were collected by Egbert et al using Millipore filters, and they were afterwards air dried and stained with periodic acid Schiff (PAS) and hematoxylin.(Egbert PR et al 1977)

Tseng changed the procedure for collecting specimens and stained them using PAS and Papanicolaou stains. (Tseng SCG et al 1985)

Maskin and Bode have described a method for using impression cytology to collect conjunctival epithelial cells for electron microscopy research. (Maskin SL et al 1986)

To enable immunocytochemical staining, pure nitrocellulose membranes and Biopore membrane apparatus have been utilised. (Krenzer KL et al 1987, Thiel MA et al 1997)

### Sample Collection-

The majority of authors utilise surfactant-free filter paper with pores ranging from 0.22 mm to 0.44 mm.(Vadrevu VLD et al 1994) Millipore's cellulose acetate filter paper, which is trimmed into a 5 mm strip with one square end and one tapered end, is used in Tseng's modified method of collecting specimens.(Tseng SCG et al 1985 )The pointed tip and asymmetrical form make it easier to hold and move the paper to the right location while using forceps with blunt, smooth edges.

All subjects underwent cytology in three phases.

A-Sample collection

**B-Staining** 

C-Grading of Cytology

#### Steps.

- -A 13 mm-diameter piece of Millipore paper cut in half in a "D" form.
- For alignment, the end of the paper that will be adhered to the nasal side is trimmed. -Excessive tear fluid and medication are removed, and one drop of local anaesthetic is injected into the eye.
- -The paper is placed over the cornea, bulbar conjunctiva or both with aseptic precautions while straddling the limbus. The underlying pathology determines the area that needs to be sampled.
- -After about 5 to 10 seconds of staying in contact with the eye while putting gentle pressure, the paper is removed using forceps.
- -It is essential that the lids are kept apart from the paper during the period of contact and that it is not permitted to be wetted by tear fluid, which may occasionally arise as a result of stimulation of lacrimation.
- Cell yield will be low if the paper becomes very saturated
- -The tissue is placed on a glass slide before being placed in a fixative solution-filled Koplin jar.

The generally utilised stains for routine histological staining of impression cytology specimens are papanicolaou or haematoxylin and PAS stains.

### **Grading:**

Nelson's Grading was used to grade the conjunctival impression cytology.26

### Grade 0 -

The epithelial cells are tiny and rounded. Large nuclei are present. The goblet cells are thick, rounded, and numerous.

#### Grade 1-

The epithelial cells are slightly bigger and more oblong. The nuclei are more compact. Despite having fewer of them, the goblet cells continue to have an oval shape and are still plump.

### Grade 2 -

Larger, more polygonal, and occasionally multinucleated epithelial cells are present. The nuclei are very relatively small. The goblet cells are far fewer, smaller, and have distinct cellular boundaries.

#### Grade 3 -

The nuclei are tiny and pyknotic, and the epithelial cells are big and more polygonal. There are no goblet cells at all.

Grades 0 and 1 were considered normal whereas grades 2 and 3 were considered to have aberrant cytology.

### **Statistical Analysis:**

For statistical analysis, all data were entered into a computer data base using latest version of Microsoft excel,data analysed using appropriate tests. To examine statistical significance, chi square tests were used, (p value of 0.05 or higher is deemed significant) to measure the association for categorical dataset, analysed using appropriate tests.

### 3. Results

### Table-1 ( Cases Of Dry Eyes According To Age )

Among 40 cases with dry eyes, maximum patients were in the age group of 51-60 years (32.5%) and minimum in the age group of 11-20 years (7.5%) and 31-40 years (7.5%). 30% cases were below the age of 40 years and 70% cases were above the age group of 40 years.

### **Table-2** ( Gender Distribution According To Age ) Amongst 40 cases with dry eyes, maximum males

were from the age group of 51-60 years (33.3 %) and minimum from 31-40 years (9.5%) while maximum females were from the age group of 51-60 years (31.57%) and minimum were equally from 11-20 years (5.2%) and 31-40 years (5.2%). And cases affected with dry eye were more males (52.5%) than females (47.5%).

### **Table-3 (Gender Frequency )**

The study showed males 21 in number (52.5%) having dry eyes were more than females which were 19 in number (47.5%).

### Table-4 ( Correlation Of Conjunctival Impression Cytology With Osdi Grading )

In 62 eyes with with normal cytology, 47 eyes (75.80%) had grade 0 OSDI, 10 eyes (16.1%) had grade 1 OSDI and 5 eyes (8.06%) had grade 3 OSDI and none had grade 4 OSDI.In 98 eyes with abnormal cytology, 39 eyes (39.79%) had grade 0 OSDI, 26 eyes (26.53%) had grade 1 OSDI, 25 eyes (25.5%) had grade 2 OSDI and 8 eyes (8.16%) had grade 4 OSDI. There is statistically significant correlation between OSDI AND CIC grade (chi square -21.6, p value-0.01)

### Table-5 ( Correlation Of Conjunctival Impression Cytology With Schirmers Test Grading-1 )

In 62 eyes with with normal cytology (CIC grade 0 and 1) -52 eyes (83.8%) had ST grade 1, 9 eyes (14.5%) had ST grade 2, 1 eye (1.61%) had grade ST 3 and none had ST grade 4. In 98 eyes with abnormal cytology (CIC grade 2 and 3), 56 eyes (57.14%) had ST grade 1, 24 eyes had (24.4%) ST grade 2, 18 eyes (18.36%) had ST grade 3 and none had ST grade 4.

### Table-6 ( Correlation Of Conjunctival Impression Cytology With Schirmers Test Grading-2 )

There is statistically significant correlation between the ST grading with that of CIC grading ( chi square value- 8.134 and p value 0f 0.043)

### Table-7 ( Correlation Of Conjunctival Impression Cytology With Tear Break Up Time-1)

In 62 eyes with normal cytology (CIC grade 0 and 1), 48 eyes (77.4%) had TBUT grade 1, 9 eyes (14.5%) had TBUT grade 2, 5 eyes (8.06%) had TBUT grade 3 and none had TBUT grade 4. In 98 eyes with abnormal cytology (CIC grade 2 and 3), 35 eyes (35.7%) had TBUT grade 1, 27 eyes (27.5%) had TBUT grade 2 and 3 and 9 eyes (9.18%) had TBUT grade 4.

### Table-8 ( Correlation Of Conjunctival Impression Cytology With Tear Break Up Time-2 )

There is statistically significant correlation between the TBUT grading with that of CIC grading(chi square value- 12.88 and p value 0f 0.0043)

### Table-9 ( Coorelation Of Conjunctival Impression Cytology With Van Bijsterveld Grading )

In 62 eyes with normal cytology, 59 eyes (95.16%) had normal VBS score and 3 eyes (4.8%) had abnormal VBS score and in 98 eyes with abnormal cytology, 64 eyes (65.3%) normal VBS score and 34 eyes (34.6%) had abnormal VBS score. There is statistically significant correlation between VB score and CIC grading (chi square value-22.85, p valve-0.00).

## Table-10 ( Coorelation Of Conjunctival Impression Cytology With Dry Eye Severity Grading )

The statistically significant correlation between CIC grade and DES grading with p value (0.05)

### Table-11 Comparison Of Conjunctival Impression Cytology Results Between Group 1 (Cases) And Group 2 (Controls)

From 80 eyes of cases, 63 eyes (78.75%) had abnormal CIC and 17 (21.25%) eyes had normal CIC and from 80 eyes of controls, 34 eyes (42.5%) had abnormal CIC and 46 eyes (57.5%) had normal CIC. There is statistically significant correlation in cytology grading between 2 groups. (chi sqaqre value-15.46, p value-0.001)

Table-1 ( Cases Of Dry Eyes According To Age )

| AGE (in yrs.)                    | 11-20 | 21-30 | 31-40 | 41-<br>50 | 51-60 | 61-<br>70 | TOTAL |
|----------------------------------|-------|-------|-------|-----------|-------|-----------|-------|
| CASES HAVING<br>DRY EYES         | 3     | 6     | 3     | 7         | 13    | 8         | 40    |
| % of CASES<br>HAVING DRY<br>EYES | 7.5   | 15    | 7.5   | 17.5      | 32.5  | 20        | 100   |

Table-2 ( Gender Distribution According To Age )

| AGE IN YEARS | MALE | FEMALE | TOTAL |
|--------------|------|--------|-------|
| 11-20 yrs.   | 2    | 1      | 3     |
| 21-30 yrs.   | 3    | 3      | 6     |
| 31-40 yrs.   | 2    | 1      | 3     |
| 41-50 yrs.   | 3    | 4      | 7     |
| 51-60 yrs.   | 7    | 6      | 13    |
| 61-70 yrs.   | 4    | 4      | 8     |
| TOTAL        | 21   | 19     | 40    |

Table-3 (Gender Frequency )

| 1                        | MALE | FEMALE | TOTAL |
|--------------------------|------|--------|-------|
| CASES HAVING<br>DRY EYES | 21   | 19     | 40    |
| %                        | 52.5 | 47.5   | 100   |

Table-4 ( Correlation Of Conjunctival Impression Cytology With Osdi Grading )

| GRADING | P            | OS        | DI GRADIN  | G        |            |
|---------|--------------|-----------|------------|----------|------------|
| OF CIC  | 0(%)<br>TOTA |           | 2(%) 3     | 8(%)     |            |
| 0       | 19(79%)      | 5(21%)    | 0(0)       | 0(0)     | 24(15%)    |
| 1       | 28(73.6)     | 5(13.1%)  | 5(13.1%)   | 0(0)     | 38(23.75%) |
| 2       | 12(41.3)     | 11(37.9%) | 6(20.6%)   | 0(0)     | 29(18.13%) |
| 3       | 27(39.1%)    | 15(21.7%) | 19(27.5%)  | 8(11.5%) | 69(43.13%) |
| TOTAL   | 86(53.75%)   | 36(22.5%) | 30(18.75%) | 8(5%)    | 160(100%)  |

Table-5 ( Correlation Of Conjunctival Impression Cytology With Schirmers Test Grading-1 )

| GRADING |            | SCHIRM    | ER TEST G | RADING |            |
|---------|------------|-----------|-----------|--------|------------|
| OF CIC  | 1(%)       | 2(%)      | 3(%)      | 4(%)   | TOTAL      |
| 0       | 19(79.1%)  | 5(20.8%)  | 0(0)      | 0(0)   | 24(15%)    |
| 1       | 33(86.8%)  | 4(10.5%)  | 1(2.6%)   | 0(0)   | 38(23.76%) |
| 2       | 22(75.8%)  | 4(13.7%)  | 3(10.3%)  | 0(0)   | 29(18,13%) |
| 3       | 34(49.3%)  | 20(29%)   | 15(21.7%) | 0(0)   | 69(43.13%) |
| TOTAL   | 108(49.3%) | 33(20.6%) | 19(11.8%) | 0(0)   | 160(100%)  |

Table-6 ( Correlation Of Conjunctival Impression Cytology With Schirmers Test Grading-2 )

| GRADING  |             | SCHIRM     | ER TEST O  | GRADING | 3           |
|----------|-------------|------------|------------|---------|-------------|
| OF CIC   | 1(%)        | 2(%)       | 3(%)       | 4(%)    | TOTAL       |
| NORMAL   | 52(48.1%)   | 9(27.7%)   | 1(5.2%)    | 0(0)    | 62 (38.75%) |
| ABNORMAL | 56(51.85)   | 24(72.7)   | 18(14.7%)  | 0(0)    | 98 (61.25%) |
| TOTAL    | 108 (67.5%) | 33 (20.6%) | 19 (11.8%) | 0(0)    | 160 (100%)  |

Table-7 ( Correlation Of Conjunctival Impression Cytology With Tear Break Up Time-1)

| GRADING | TE        | AR BREAK  | UP TIME T | EST GRAD | DING       |  |  |  |
|---------|-----------|-----------|-----------|----------|------------|--|--|--|
| OF CIC  | 1(%)      | 2(%)      | 3(%)      | 4(%)     | TOTAL      |  |  |  |
| 0       | 19(79%)   | 4(16.6%)  | 1(4.1%)   | 0(0)     | 24(15%)    |  |  |  |
| 1       | 29(76.3%) | 5(13.1%)  | 4(10.5%)  | 0(0)     | 38(23.75%) |  |  |  |
| 2       | 8(27.5%)  | 12(41.3%) | 9(31%)    | 0(0)     | 29(18.13%) |  |  |  |
| 3       | 27(39.1%) | 15(21.7%) | 18(26%)   | 9(13%)   | 69(43.13%) |  |  |  |
| TOTAL   | 83(51.8%) | 36(22.5%) | 32(20%)   | 9(5.6%)  | 160(100%)  |  |  |  |

Table-8 ( Correlation Of Conjunctival Impression Cytology With Tear Break Up Time-2 )

| GRADING<br>OF CIC | TEAR BREAK UP TIME TEST GRADING<br>1(%) 2(%) 3(%) 4(%)<br>TOTAL |            |           |         |             |  |
|-------------------|---|------------|-----------|---------|-------------|--|
| NORMAL            | 48(57.8%)   | 9(25%)     | 5(15.6%)  | 0(0)    | 62 (38.75%) |  |
| ABNORMAL          | 35(42.1%)   | 27(75%)    | 27(84.3%) | 9(100%) | 98 (61.25%) |  |
| TOTAL             | 83(51.8%)   | 36(22.5 %) | 32(20%)   | 9(5.6%) | 160 (100%)  |  |

Table-9 (Coorelation Of Conjunctival Impression Cytology With Van Bijsterveld Grading)

| GRADING OF<br>CIC | VAN BIJSTER<br>NORMAL* | VELD SCORE<br>ABNORMAL * | TOTAL      |
|-------------------|------------------------|--------------------------|------------|
| 0                 | 24(100%)               | 0(0)                     | 24(15%)    |
| 1                 | 35(92.1%)              | 3(7.8%)                  | 38(23.76%) |
| 2                 | 23(79.3%)              | 6(20.6%)                 | 29(18.13%) |
| 3                 | 41(59.4%)              | 28(40.5%)                | 69(43.13%) |
| TOTAL             | 123(76.87%)            | 37(23.13%)               | 160(100%)  |

Table-10 ( Coorelation Of Conjunctival Impression Cytology With Dry Eye Severity Grading )

| OF CIC 1(% | <b>%</b> ) | 2(%)      | 3(%)       | 4(%) TOTA  | AT (9/3  |
|------------|------------|-----------|------------|------------|----------|
|            |            |           | -(,,,      | 1(70) 1017 | AL(70)   |
| 0          | 1          | 5         | 0          | 0          | 6        |
| 1          | 0          | 5         | 3          | 2          | 10       |
| 2          | 5          | 11        | 6          | 0          | 22       |
| 3          | 5          | 15        | 16         | 6          | 42       |
| TOTAL 110  | (13.75%)   | 36(45.0%) | 25(31.25%) | 8(10%)     | 80(100%) |

Table-11 Comparison Of Conjunctival Impression Cytology Results Between Group 1 (Cases) And Group 2 (Controls)

| CATEGORY | CONJUNCTIVAL IMPRESSION CYTOLOGY |           |           |            |          |  |
|----------|----------------------------------|-----------|-----------|------------|----------|--|
|          | 0                                | 1         | 2         | 3          | TOTAL    |  |
| CASES    | 6(7.5%)                          | 10(12.5%) | 22(27.5%) | 42(52.5%)  | 80(100%) |  |
| CONTROLS | 18(22.5%)                        | 28(35%)   | 7(8.75%)  | 27(33.75%) | 80(100%) |  |

### 4. Discussion

The study contained a total of 80 individuals divided into 40 cases ( Group I) and similar age and sex matched 40 controls ( Group II ) who met the specified inclusion and exclusion requirements of the study. Thus a total of 160 eyes were examined to determine a correlation between clinical and cytological tests used to assess the severity of dry eye and to assess the outcomes of conjunctival impression cytology in patients with dry eye and in normal individuals .

### Age Distribution-

Among 40 cases with dry eyes, maximum patients were in the age group of 51-60 years (32.5%) and

minimum in the age group of 11-20 yrs (7.5%) and 31-40 yrs (7.5%). 30% cases were below the age of 40 yrs and 70% cases were above the age group of 40 yrs. A 2017 study by Farrand K.F., discovered that the diagnosed DED group was older, with 72% of individuals being over 50 years old as opposed to 45% in the nonDED group.( Farrand K.F. Et al 2017)

### Gender Frequency-

Maximum males were from the age group of 51-60 years (33.3 %) and minimum from 31-40 years (9.5%) while maximum females were from the age group of 51-60 years (31.57%) and minimum were equally from 11-20 years (5.2%) and 31-40 years (5.2%). And cases affected with dry eye were more males (52.5%) than females (47.5%). In contrast, some research, such

as the one by Farrand K.F. in 2017 revealed that women (8.8%, 11.1 million) had a higher prevalence than men (4.5%, 5.3 million). (Farrand K.F. Et al 2017)

### Comparison Between Cic And Osdi Grading-

Higher OSDI scores (grade 2 and 3 severity) correlated better with abnormal cytology grades of CIC in the current study than lower OSDI scores (grade 0 and 1), where a large number of participants had abnormal cytology (p value -0.01).

According to Zuazo F et al., the nelsons CIC grading was substantially higher in patients with a severe grade of OSDI score (  $1.41\pm0.14$ ) compared to healthy people (  $0.86\pm0.09$ ) with p value(<0.01). (Zuazo F et al 2014)

### Comparison Between Cic And St Grading-

In this study, normal cytology was linked to lower schirmers test grades (0 and 1), whereas abnormal cytology also significantly had lower schirmer test grades. As a result, the statistical significance had a low p value (0.043).

According to a 2011 study by Shrestha E. et al., showed that there was a strong link between CIC and abnormal cytology in dry eyes with the Schirmer's value of less than 10 mm in 5 minutes. (Shrestha E et al 2011)

P Kumar et al. in 2014, found that 83.8% of normal and 52.1% of abnormal cytologies had schirmers values more than 10 mm, compared to 11.5% of normal and 27.1% of abnormal cytologies. (Kumar P et al 2014)

### Comparison Of Cic And Tbut Grading-

In this study, 77.4% of normal cytology had a TBUT of greater than 10 seconds, compared to just 35.7% of abnormal cytology. The association between the CIC and TBUT grades was therefore statistically significant. (0.006 as p value)

P Kumar et al study's from 2014 found that TBUT ranged from 5 seconds in 11.57% of normal cytology to >10 seconds in 42.14% of aberrant cytology. (Kumar P et al 2014)

### Comparison Of Cic And Vbs Of Rose Bengal

### Staining-

In this study, 95.1% of patients with normal cytology had normal VB scores, compared to 65.3% of patients with abnormal cytology who had normal VBS and 34.6% who had abnormal VBS.

In a 2014 study by P Kumar et al, it was found that rose Bengal staining had an abnormal VB score in 19.9% of normal and 80.09% of pathological cytology, compared to normal VB scores in 80.09% of normal and 19.21% of abnormal cytology. (Kumar P et al 2014)

### Comparison Of Cic Grading With Dry Eye Severity Grade-

In this study, 68.75 % of normal cytology had 1 and 2 DES Grade where as 43.75% of abnormal cytology had 3 and 4 DES grading. There was statistically significant correlation between CIC grade and DES grading with p value(0.05)

### Comparison Of Cic Grading Between 2 Groups-

There was a statistically significant correlation between the two groups, with aberrant cytology in those with dry eyes and considerable normal cytology in those without dry eyes. (chi squared value: 15.46, p = 0.001).

This is analogous to a research by Shrestha E. et al. in which aberrant cytology was found in the eyes of 23.6% of normal people and 49.2% of dry eye patients.(Shrestha E et al 2011). Reddy M et al. (1991) found that 76.6% of dry eyes showed aberrant cytology, and Gautam TSK et al. (2015) found that 53.7% showed abnormal cytology.( Reddy M et al 1991, Gautam TSK et al 2015)

### 5. Conclusion

Results indicated that older age groups, with a peak age group between 51 and 60 years, have a higher prevalence of dry eyes. The greater prevalence of dry eyes in men in the current study can be attributed to rural women's less frequent use of health care facilities.

Comparing dry eye patients to healthy people, it has been discovered that there is a considerable ocular surface irregularity attributed with these cases. This will help to reinforce the idea that dry eyes and abnormalities of the ocular surface cause a vicious cycle of dry eye syndromes.

Conjunctival impression cytology and tear film tests including TBUT, rose Bengal staining, and other tests have been found to significantly correlate with one another, with the exception of the schirmers test, where there was only marginally significant correlation. This may indicate that the schirmers test will identify an aqueous shortage rather than the ocular surface epithelial abnormalities that is picked up better by TBUT and rose Bengal staining.

Between the OSDI Score and the grade of dry eye severity determined by conjunctival impression cytology, there was a strong association. This will imply that the latter will be used to evaluate dry eyes. This finding indicates that conjunctival impression cytology can be effective in detecting early ocular surface abnormalities.

- 1) To identify ocular surface abnormalities linked to dry eyes in order to end the cycle. 2) To identify dry eyes
- 3) To determine the extent of dry eyes and to begin the proper course of treatment.
- 4) To keep track of the prognosis following treatment. This research concludes that there is strong correlation between conjunctival impression cytology and tear function tests. Thus this non-invasive, rapid and easy investigation is valuable in assessing dry eye disease and can serve as an excellent alternative to conjunctival biopsy in diagnosing and evaluating the prognosis of dry eye disease.

### FINANCIAL SUPPORT AND SPONSORSHIP

None

### CONFLICT OF INTEREST

There are no conflict of interest.

### **ACKNOWLEDGEMENT**

The authors acknowledge with gratitude the contribution of health workers and the patients who participated in this study.

#### 6. References

- Argueso P, Tisdale A, Spurr-Michaud S, et al. Mucin characteristics of human corneal- limbal epithelial cells %that exclude the rose bengal anionic dye. Invest Ophthalmol Vis Sci. 2006; 47:113-119.
- Brewitt H, Sistani F. Dry Eye Disease: the scale of the problem. Surv Ophthalmol 2001; 45: S199-202.
- Begley CG, Himebaugh N, Renner D, Liu H, Chalmers R, Simpson T, et al. Tear breakupdynamics: a technique for quantifying tear film instability. Optom Vis Sci 2006; 83: 15-216
- Basak SK, Pal PP, Basak S, Bandyopadhyay A, Choudhury S, Sar S. Prevalence of Dry Eye Diseases in hospital-based population in West Bengal, Eastern India. J Indian Med Assoc. 2012; 110: 789-94
- Bhargava R, Kumar P, Kaur A, Kumar M, Mishra A (2014) The diagnostic value and accuracy of conjunctival impression cytology, dry eye symptomatology, and routine tear function tests in computer users. J Lab Physicians 6: 102-108.
- Cho P, Yap M. Schirmer test I. A review. Optom Vis Sci. 1993;70(2):152-6.
- Cho P, Brown B, Lau C. Effect of fluorescein on the tear stability of Hong Kong-Chinese. Optom Vis Sci. 1996;73:1-7.
- Cho and Brown in 1993, Savini et al in 2008.
- Dry Eye Workshop. 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(2):75-92.

- Egbert PR, Lauber S, Maurice DM. A simple conjunctival biopsy. Am J Ophthalmol 1977;84:798–801.
- Farrand K.F., Fridman M., Stillman I.O., Schaumberg D.A.Prevalence of Diagnosed Dry Eye Disease in the United States Among Adults Aged 18 Years and Older. American Journal of Ophthalmology 2017; 182, pp. 90-98.
- Guazzi A, Nizzoli R, Tomba MC. Value of the filter imprint technique in the cytological study of ocular lesions. ActaCytol. 1988;37:601-3.
- Gupta N, Prasad I, Himashree G, D'Souza P. Prevalence of dry eye at high altitude: a case controlled comparative study. High Alt Med Biol 2008; 9: 327-34.
- Gautam TSK et al. Study of conjunctival impression cytology in assessing goblet cell density in dry eyes . Int J Res Med Sci. 2015 Dec;3(12):3819-3823
- Hida RY, Mascaro VL, Hofling-Lima AL, Goto E, Tsubota K, Gomes JA. Métodos
- Diagnósticos. In: Gomes JA, Alves MR, editores. Superfície ocular. Rio de Janeiro: Cultura Médica; 2006.
- Hashemi H, Khabazkhoob M, Kheirkhah A, Emamian MH, Mehravaran S, Shariati M. Prevalence of dry eye syndrome in an adult population. Clinical & Experimental Ophthalmology. 2014;42:242-8.
- Krenzer KL, Freddo TF. Cytokeratin expression in normal human bulbar conjunctiva obtained by impression cytology. Invest Ophthalmol Vis Sci 1997;38:142–52.
- Kumar P et al, The correlation of routine tear function tests and conjunctival impression cytology in dry eye syndrome, Korean J ophthalmol, 2014 Apr; 28(2):122-129.
- Larmande A, Timsit E . L'intérêt de cyto-diagnostiques en ophtalmologie: communication préliminaire à propos d neuf cas de tumours du limbe scléro-cornéen. Bull

- Soc Ophthalmol Fr 1954; 19: 415–419.
- Lee GA, Hirt LW. Ocular surface squamous neoplasia, Surv Ophthalmol. 1995; 39:429-450.
- Murube J, Rivas L. Impression cytology on conjunctiva and cornea in dry eye patients
- establishes a correlation between squamous metaplasia and dry eye clinical severity. Eur J Ophthalmol;2003; 13(2):115-127.
- Marner K. Snake-like appearance of nuclear chromatin in conjunctival epithelial cells from
- patients with keratoconjunctivitis sicca. Acta Ophthalmol (Copenh) 1980;58:849-53.
- Maskin SL, Bodé DD. Electron microscopy of impression-acquired conjunctival epithelial cells.Ophthalmology 1986;93:1518-23.
- Nelson JD, Wright JC. Conjunctival goblet cell densities in ocular surface disease. Arch Ophthalmol1984;102:1049-51.
- Natadisastra G, Wittpenn JR, West KP Jr, et al.

  Impression cytology for detection of vitamin A deficiency.

  Arch
  Ophthalmol.1987;105:1224-1228.
- Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. Cornea. 2004; 23:272-285.
- Patel S, Murray D, McKenzie A, et al. Effects of fluorescein on tear breakup time and on tear thinning time. Am J Optom Physiol Opt. 1985; 62:188-190.
- Puangsricharern V, Tseng SC. Cytologic evidence of corneal diseases with limbal stem cell
- deficiency. Ophthalmology.1995;102:1476-1485.
- Reddy M, Reddy P R, Reddy S C. Conjunctival impression cytology in dry eye states.
- Indian J Ophthalmol 1991;39:22-4.
- Savini, G., Prabhawasat, P., Kojima, T., Grueterich, M., Espana, E., Goto, E. (2008) The challenge of dry eye diagnosis. Clin Ophthalmol, 2(1), 31-55.
- Shrestha E et al. Conjunctival impression cytology in dry eye syndrome.Nepal J Ophthalmol 2011; 3

- (5): 39-44
- Tseng SCG. Staging of conjunctival squamous metaplasia by impression cytology.Ophthalmology 1985;92:728-33.
- Thiel MA, Bossart W, Bernauer W. Improved impression cytology techniques for the immunopathological diagnosis of superficial viral infections. Br J Ophthalmol 1997;81:984–8.
- The definition and classification of dry eye disease: report of the Definition and Classification Subcommitee of the International Dry Eye WorkShop (2007). Ocul Surf. 2007; 5:75-92.
- van Bijsterveld O, Diagnostic tests in the sicca syndrome, Arch Ophthalmol, 1969;82:10–4.
- Vadrevu VLD, Fullard RJ. Enhancements to the conjunctival impression cytology technique and examples of applications in a clinico-biochemical study of dry eye. CLAO J 1994;20:59–6
- Vitali C, Bombardieri S, et al. Classification criteria for Sjogren' s syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis ,2002;61:554-8.
- Wittpenn JR, Tseng SC, Sommer A. Detection of early xerophthalmia by impression
- cytology.Arch Ophthalmol1996;104:237-9.
- Mahapatra, S., Vickram, A. S., Sridharan, T. B., Parameswari, R., & Pathy, M. R. (2016). Screening, production, optimization and characterization of β-glucosidase using microbes from shellfish waste. 3 Biotech, 6, 1-10.
- Zuazo F, Lopez -Ponce D, Salinas- toro D et al .

  Conjunctival impression cytology in patients with normal and impaired OSDI scores. Arch Soc Esp oftalmol, 2014 Oct;89(10):391-6.