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Status of Pre-Corneal Tear film by Conjunctival Impression Cytology in Diabetic and Non-Diabetic Patients Attending BIMS, Belagavi

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Abstract

Background: Ocular surface involvement has been found in diabetic patients, dry eye in particular leading to corneal complications. Thus, early detection of dry eye in diabetic patients is necessary. As Conjunctival impression cytology is easy to perform, minimally invasive, and yields reliable information about the area sampled with minimal discomfort to the patient, this study was undertaken to compare pre-corneal tear film by conjunctival impression cytology in diabetic and non-diabetic. **Methodology:** This was a hospital based, comparison study between 47 diabetics and 47 nondiabetics between February 2021 to August 2022, where patients satisfying the inclusion criteria were recruited for the study and detailed history was taken. Patients underwent Conjunctival impression cytology, Random blood sugar, HbA1c. Association between each was determined. **Results:** Prevalence of dry eye among diabetics was 74%. Dry eye severity increased with increased duration or poor control of DM. There was a statistically significant difference ($p<0.001$) between goblet cell density between diabetics and non-diabetics. **Interpretation & conclusion:** Long-term Diabetics and/or with poor glycemic control have a higher chance of dry eye and CIC is a valuable tool to pick up early ocular surface abnormality, assess the severity of the disease and to monitor the prognosis after treatment.

Key Words: Conjunctival impression cytology; Random blood sugar; HbA1c; goblet cell density

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Introduction

Diabetes is one of the leading causes of blindness in 20-74 years old. [1] The prevalence of diabetes worldwide is reported to be 2.8% for all age-groups in 2000. The prevalence increases with age and is reported as being 13% amongst people above 65 yrs. of age. [2] In India, the number of people with diabetes was estimated to be 31 million in 2000. [3]

Diabetic mellitus is associated with numerous ocular complications which include Diabetic retinopathy, Dry eye, Neovascular glaucoma, Cataract, chronic inflammation of lids, acute orbital infection and also blindness. [4, 5] However, diabetic patients have also been found to have foreign body sensation, itching, blurred vision, and photophobia indicative of ocular surface involvement, dry eye in particular. [1] These patients suffer from a variety of corneal complications including superficial punctate keratopathy, trophic ulceration and persistent epithelial defects.[6] The higher the glycosylated hemoglobin values, the higher the rate of dry eye.[7]

Thus, there is an implication of a correlation between diabetes mellitus and tear film abnormalities. Therefore, by doing early detection of dry eye in diabetic patient decreases the corneal complications secondary to dry eye.

Conjunctival impression cytology is non-invasive, easy to perform, and yields reliable information about the area sampled with minimal discomfort to the patient. This makes it a valuable tool in the understanding of ocular surface disorders. [8]

In present study we aimed to determine the status of pre-corneal tear film in eyes of diabetic individuals by conjunctival impression cytology and compare the goblet cell density in relation to glycemic index among diabetics; to compare status of pre-corneal tear film in eyes of diabetic and non-diabetic individuals.

Inclusion criteria

All patients willing to give informed consent, known case of diabetes mellitus (aged above 20 years) and nondiabetic patients as control group (aged above 20 years).

Exclusion criteria

1. Patients with any ocular disorder known to produce dry eye.

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2. Patients suffering from any systemic diseases, (other than diabetes mellitus), associated with dry eye such as connective tissue disorders (Sjogren's syndrome, Rheumatoid arthritis, Lupus erythromatosis).
3. Patients on any drug treatment which produces dry eye (such as MAO inhibitors, Alpha agonists, Beta blockers, Thiazides, NSAIDs etc.).
4. Patients having undergone any ocular surgery in the past 2 years.
5. Wearers of contact lenses.

Methods

A total of 47 diabetics (94 eyes) and 47 nondiabetic patients (94 eyes) who attended Out-Patient Department of Ophthalmology in Belagavi Institute of Medical Sciences, Belagavi from to August 2022 were recruited for the study. This was a hospital based, comparison study.

Detailed history, general physical examination, detailed ophthalmologic examination and relevant investigations were recorded for each patient enrolled in the study.

Each patient underwent the following clinical assessments:

- In Diabetic patients, duration since onset of diabetes mellitus was noted.
- Evaluation of anterior segment was done under Slit lamp biomicroscopy.
- Conjunctival impression Cytology:
  - It was done to all subjects in 3 stages:
  - Stage 1: Sample collection-
  - Materials required-
    - Cellulose acetate filter paper (0.45µ pore size, 13mm diameter)
    - Glass slides
    - Cover slip
    - Iso propyl alcohol
    - Distilled water
    - Xylene
    - Staining dishes
    - Slide tray
    - PAS stain

  - Procedure
    - All subjects were informed about the procedure.
    - One drop of proparacaine 0.5% was instilled to both eyes and excess tears wiped out.
    - Under aseptic precaution, 0.45µ pore size, 13mm diameter cellulose acetate filter paper was placed over the temporal bulbar conjunctiva.
    - Goldmann applanation tonometer head was used to apply uniform pressure for 3-5 seconds.
    - Filter paper was taken out in peeling fashion and placed over sterile glass slide.
    - Slide was marked and placed in Coplin jar containing isopropyl alcohol as fixative.

  - Stage 2: Staining-
  - All the slides were stained with Periodic Acid- Schiff (PAS) to demonstrate goblet cells.
  - Steps of PAS staining-

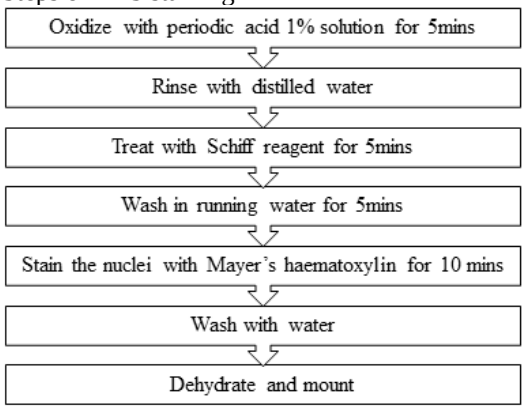

Figure 1

  - Stage 3: Grading-
  - Nelson's grading system was used to grade the conjunctival impression cytology as listed below:

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Table

Grade	Findings
Grade 0	• The epithelial cells are small and round. • The nuclei are large. • The goblet cells are abundant, plump, and oval.
Grade 1	• The epithelial cells are slightly larger and more polygonal. • The nuclei are smaller. • The goblet cells are decreased in number; however they still maintain their plump and oval shape.
Grade 2	• The epithelial cells are larger, more polygonal and occasionally multinucleated. • The nuclei are small. • The goblet cells are markedly decreased in number and are smaller with well-defined cellular borders.
Grade 3	• The epithelial cells are large and more polygonal. • The nuclei are small and pyknotic. • The goblet cells are completely absent.

- Grade 0 and 1 were considered normal cytology and grade 2 and 3 were considered abnormal cytology

Following this clinical assessment, patients were investigated for RBS and HbA1c levels.
Cytological grading was correlated with Duration of Diabetes, Random blood sugar levels and Glycaemic control. The Cytological grading between diabetics and non-diabetics were also correlated.
Data was collected, entered in MS Excel and analysed by descriptive statistics. Association between different attributes will be seen using chi square test. P <0.05 were considered as significant.

Results

Among the number of eyes with diabetics, maximum patients were between 51-60 years (31.9%), whereas maximum patients were of the age group 61-70 years (31.9%) in number of eyes with non-diabetics. (Table 1) Mean age of the diabetic patients was 57.3 ± 13.5 years whereas it was 63.4 ± 11.5 years in non-diabetics.

Table 1: Age distribution among patients

Age distribution (in years)	21-30	31-40	41-50	51-60	61-70	71-80	81-90	Total
No. of eyes with diabetes mellitus (%)	4 (4.2)	10 (10.6)	12 (12.8)	30 (31.9)	22 (23.4)	14 (14.9)	2 (2.1)	94
No. of eyes without diabetes mellitus (%)	0 (0)	0 (0)	20 (21.3)	20 (21.3)	30 (31.9)	18 (19.1)	6 (6.4)	94

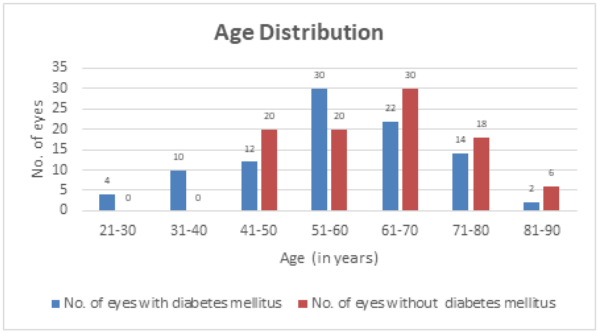

Figure 1

In this study, 70.2% (66 eyes out of 94 eyes) of diabetic's eyes were males while it was 55.4% (52 eyes out of 94 eyes) in non-diabetic's eyes. (Table 2)

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Table 2: Sex distribution

No. of eyes with	Male	Female	Total
Diabetes mellitus	66 (70.2%)	28 (29.8%)	94
Without Diabetes mellitus	52 (55.3%)	42 (44.7%)	94

CIC with Duration of DM

We found a statistically significant association between CIC and Duration of Diabetes (chi square= 28.99, p value= <0.001). Among 24 eyes with normal cytology, duration of diabetes was less than 5 years in 16 eyes (66.7%). (Table 3)

Table 3: Association of CIC grading with Duration of DM

CIC GRADE	Duration of Diabetes			Total (% out of 94)
	< 5 years	5-10 years	>10 years	
Normal Cytology	16 (66.7)	6 (25)	2 (8.3)	24 (25.5)
Abnormal Cytology	8 (11.4)	38 (54.3)	24 (34.3)	70 (74.5)
TOTAL	24 (25.5)	44 (46.8)	26 (27.7)	94 (100)
CHI SQUARE= 28.99	P < 0.001			

CIC with RBS levels

We found a statistically significant association between CIC grade and RBS levels (chi square= 13.68, p value= <0.001). Among 70 eyes with abnormal cytology 55 eyes (78.6%) had RBS more than 200mg/dL. (Table 4)

Table 4: Association of CIC grading with RBS levels

CIC GRADE	RBS Levels (mg/dL)			Total (% out of 94)
	< 140 mg/dL	140 -200 mg/dL	> 200 mg/dL	
Normal Cytology	8 (33.3)	5 (20.8)	11 (45.8)	24 (25.5)
Abnormal Cytology	4 (5.7)	11 (15.7)	55 (78.6)	70 (74.5)
TOTAL	12 (12.8)	16 (17)	66 (70.2)	94 (100)
CHI SQUARE= 13.68	P < 0.001			

CIC with HbA1C control

We found a statistically significant association between CIC grade and Glycaemic control (HbA1c) (chi square= 35.11, p value= <0.001). Among 70 eyes with abnormal cytology, none of eyes had normal control of HbA1C while majority (50 eyes) i.e., 71.4% had poor control. (Table 5)

Table 5: Association of CIC grading with Glycaemic control

CIC GRADE	HbA1c Levels				
	Normal (<6)	Good Control (6-6.7%)	Fair Control (6.8-7.65%)	Poor control (>7.65%)	Total (% out of 94)
Normal Cytology	8 (33.3)	5 (20.8)	7 (29.2)	4 (16.7)	24 (25.5)
Abnormal Cytology	0 (0)	9 (12.8)	11 (15.7)	50 (71.4)	70 (74.5)
TOTAL	8 (8.5)	14 (14.9)	18 (19.1)	54 (57.4)	94 (100)
CHI SQUARE= 35.11	P < 0.001				

CIC between DM and NON-DM

We found a statistically significant association between CIC Grading in Diabetics vs CIC Grading in non-diabetics (chi square= 57.77, p value= <0.001). Among the 94 eyes of diabetics' majority (74.5%) had abnormal cytology, while 24 eyes (25.5%) had normal cytology. Among the 94 eyes of non-diabetics' majority (80.9%) had normal cytology, while 18 eyes (19.1%) had abnormal cytology. (Table 6)

Table 6: Association of CIC grading between diabetic and non-diabetic groups

CIC GRADE	Diabetic vs non-Diabetic	
	Diabetic	Non-Diabetic
Normal Cytology	24 (25.5)	76 (80.9)
Abnormal Cytology	70 (74.5)	18 (19.1)
TOTAL	94 (100)	94 (100)
CHI SQUARE= 57.77	P < 0.001	

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Discussion

In our study, the prevalence of dry eye in DM patients was 74% which is agreement with a prevalence of dry eye at 70% as reported by Seifart et al. [7] Diabetic patients are more susceptible for autonomic dysfunction, leading to decreased tear secretion. This might be a potential factor for the reported high prevalence. An additional factor for high prevalence could be the presence of dry and tropical climate in the region of the study.

CIC with Duration of DM

We found a significant association between CIC grade and duration of diabetes mellitus, indicating higher rates of dry eye and abnormal cytology with longer duration of diabetes. In eyes with diabetes more than 10 years, 24 eyes had abnormal cytology and dry eye while only 2 eyes had normal cytology.

According to Khetwani *et al.*[9], the duration of DM was strongly linked with dry eyes (P=0.01). In a study carried out in Tamil Nadu, India, Nasar et al. [10] found a similar substantial correlation between dry eyes and poor glycaemic control (P<0.001) and a longer duration of DM (P<0.05), Gannur et al. [11], noted that patients with five years of DM had a higher prevalence of dry eyes (a 2.65-fold increase) in Vijaypura Patients with uncontrolled DM observed to have dry eyes (P<0.001).

CIC with RBS levels

We found a significant association between CIC grade and RBS levels i.e., high RBS levels were associated with abnormal cytology and dry eye. 55 eyes (58.5%) of 94 eyes had RBS more than 200 mg/dL which amounted for 78.6 % of the total abnormal cytology in the study.

Chinese community-based study [12] found a connection between DED and higher blood sugar levels (OR 1.240, p<0.001) and levels of HbA1c (OR 1.108, p<0.001). Kaiserman *et al.* [13] found that good blood sugar regulation is critical for the prevention and control of DES in diabetic patients.

CIC with HbA1C control

We found a significant association between CIC grade and HbA1C levels, indicating higher rates of dry eye and abnormal cytology with poor control of diabetes. 71.4% had abnormal cytology with poor control (>7.65%) of diabetes.

This is consistent with the study by Seifart U. *et al.* [7] who found an association between HbA1C and dry eye syndrome and concluded that people with poor metabolic control are more likely to develop dry eyes. They also found, an association between HbA1C and conjunctival cytology, tear film status, and both. Tear film instability affected 86.3% of patients with poor metabolic control, while conjunctival morphological abnormalities affected 58.3% of patients. Sarkar et al. [14] found that patients with poor glycaemic control (HbA1c >8%) had a higher degree of dry eyes and diabetes management was highly statistically significant with dry eyes.

Additionally, Kaiserman *et al.* [13] discovered that diabetes participants with higher HbA1c levels used artificial tears more frequently.

CIC Between DM and NON-DM

We found a significant association between CIC grade in diabetic eyes and non-diabetic eyes. 70 eyes (74.4%) out of the 94 eyes of diabetics had abnormal cytology whereas only 18 (19.1%) eyes of non-diabetic had abnormal cytology while majority i.e., 76 eyes (80.6%) of non-diabetics has normal cytology. This shows that diabetics had a higher decrease in goblet cell density and hence have higher prevalence of dry eye when compared to controls.

Previous studies by Mehmet C et al. [15], Yoon KC et al. [16], Dogru M et al. [17], Khan AA et al.[18], and Figueroa-Ortiz LC et al. [19] also showed significantly worse CIC grading among diabetic patients compared to non-diabetic patients.

Conclusion

Dry eye negatively impacts the quality of life, and this study showed it is worse in at-risk populations like diabetics when compared to their counterparts. It showed that increase in duration of DM increases the risk of tear film alterations that result in abnormalities on the ocular surface and dry eyes with statistical significance. (p<0.001) It further confirmed that poorer is the glycaemic control in diabetics more severe is the dry eyes (p<0.001). Thus, Conjunctival impression cytology being an easy, minimally invasive, less time-consuming procedure, it is a valuable tool to pick up early ocular surface abnormality, assess the severity of the disease and to monitor the prognosis after treatment. Given that the prevalence of dry eye among diabetics in this hospital-based study with limited sample is significant then the prevalence in the community must be higher and needs attention.

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