Conjunctival surface changes in diabetics: an unusual cytological study

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Abstract

Background: To study the conjunctival surface changes in diabetics with and without retinopathy, its relation to systemic factors and comparison to control.

Methods: In this study, 123 eyes of 74 subjects were divided into two study groups (Diabetes with and without retinopathy) and control. Diabetes mellitus (DM) without retinopathy comprised of 34 cases with 50 eyes, diabetic retinopathy (DR) of 19 cases with 33 eyes and control of 21 cases with 40 eyes. Conjunctival impression cytology (CIC) was compared in the three groups. We also noted relationship of CIC to the sign and symptoms of dry eye, duration of diabetes, the status of retinopathy and metabolic control in diabetes.

Results: CIC analysis showed that goblet cell density (GCD) was significantly lower in diabetics as compared to controls which was related to worsening retinopathy and dry eye symptoms (p<0.05) but not with duration of diabetes and poor metabolic control. Highly significant conjunctival squamous metaplasia (CSM) was seen in diabetics as compared to control group. (p<0.0001) The median CSM grade was poorer with long duration diabetes and worsening retinopathy (p<0.015), with no relation to dry eye symptoms and metabolic control.

Conclusion: Conjunctival surface changes in diabetics include goblet cell loss and CSM. Interestingly, diabetics with retinopathy had significantly worse changes on CIC analysis than those without it, and both groups showed worse changes than controls.

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Introduction
Diabetes is one of the common causes of blindness in persons aged 20-70 years with cataract and retinopathy being well known ocular complications. Recently problems involving the ocular surface, especially dry eye, have been reported in diabetics.\(^1\) Diabetic keratoepitheliopathy is sometimes hard to cure and can induce quantitative and qualitative abnormalities in tear secretion, decreased corneal sensitivity and poor adhesion of regenerating epithelial cells.\(^2\)\(^3\)\(^4\) Researches show that most cases of dry eye associated with diabetes are caused by insufficient production of tears due to “autonomic neuropathy” affecting the nerves that control the lacrimal gland.\(^4\)

Most studies have shown an intimate relationship between dry eye and diabetes mellitus but some studies have given controversial results.\(^5\)\(^6\)\(^7\) In addition, there has been a lack of data in India about changes in conjunctival surface in diabetic patients and its relation to clinical parameters. Therefore, this study was undertaken to find the changes in conjunctival surface in patients of type-2 diabetes mellitus with or without retinopathy and compare them to controls. We also investigated the relationship of systemic factors to diabetic keratoepitheliopathy.

Materials and Methods
Fifty three cases of diabetes mellitus and 21 controls were selected from the Outpatients Department of Ophthalmology after ‘Institutional Ethical Committee’ clearance and informed consent. They were divided into two study groups and one control group. The study groups of Diabetes without retinopathy (DM) had 34 cases with 50 eyes, Diabtes with retinopathy (DR) had 19 cases with 33 eyes and control group had 21 cases. Number of eyes in cases and controls were unequal because either eye of patient was not examined if it followed exclusion criteria like dense cataract so that fundus examination could not be possible. There were no inadequate samples in the study.

Inclusion Criteria: 1. Study group: Patients were diagnosed as diabetic if the fasting blood glucose (FBG) was ≥ 126 mg/dl on two separate days or Random blood glucose (RBG) was ≥ 200 mg/dl with symptoms or two-hour plasma glucose was ≥ 200 mg/dl after 75 g oral glucose tolerance test (GTT).\(^8\)

2. Control group: Age and gender matched control subjects were selected who came for routine eye examination with FBG ≤ 126 mg/dl.

Exclusion criteria: Individuals with a history of chronic or recurrent ocular surgery, dry eye, contact lens wear, chronic ocular drug abuse, systemic diseases with dry eyes as a manifestation were excluded from the study.

Age, gender, clinical history and subjective complaints including burning, itching, foreign body sensation and photophobia were recorded. ≥ 2 of these complaints were taken as positive symptoms. The patients were classified into diabetics of short duration of < 10 yrs and long duration of ≥ 10 years. Patients with FBG / RBG < 140 mg/dl had a good metabolic control. Diabetic retinopathy was evaluated with indirect ophthalmoscopy and slit lamp biomicroscopy with 78 dioptre lens. In both patients and controls, both eyes CIC were performed at the same sitting. Inflammation was noted in 4 diabetic cases, which were excluded from statistical calculations. CIC was compared in the three groups. Relation of CIC was also noted to the sign and symptoms of dry eye, duration of diabetes, the status of retinopathy and metabolic control in diabetics.

Filter paper technique was used for CIC. Millipore cellulose acetate filter paper of 1 cm diameter and 0.45µ pore size was used. After instilling 0.5% paracaine, lids were retracted and excess tears dried. A D-shaped strip of filter paper of size 15x10 mm, with straight side towards limbus was applied dull side down to the lower nasal bulbar conjunctiva adjacent to the corneal limbus with a blunt, smooth tipped forceps, pressed for 2-3 seconds and gently removed in a peeling motion, avoiding shearing and applied face up on a glass slide. The slide was immediately placed in a petridish for 20 minutes in a freshly prepared solution of 2% formaldehyde, formalin and ethyl alcohol in ratio of 1:1:20 and then fixed in absolute alcohol. Two slides were prepared from each patient and stained with Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) stain. The smear was scanned at 4X, 10X and 40X and goblet cells counted in four HPF (x40X) and GCD was calculated in 1 HPF. CIC was compared in patients and controls, both eyes CIC were performed at the same sitting. Inflammation was noted in 4 diabetic cases, which were excluded from statistical calculations. CIC was compared in the three groups. Relation of CIC was also noted to the sign and symptoms of dry eye, duration of diabetes, the status of retinopathy and metabolic control in diabetics.
small irregular goblet cells were noted. The area of squamous cells and morphology showing normal small, round cells with high N:C ratio or metaplastic large, polyhedral cells with pyknotic nuclei were also noted. Inflammatory cells and micro-organisms were identified.

In our study, we modified the Nelson’s grading scheme, 1988. [9]

Grade 0: >500 goblet cells/ mm²; small, round epithelial cells with large nuclei.

Grade 1: 200 to 500 goblet cells/ mm²

Grade 2: 100 to 199 goblet cells/ mm²

Grade 3: < 100 goblet cells/ mm²; large, polygonal epithelial cells with small nuclei.

Goblet cell density <200 cells/ mm² and CSM Grades 2 and 3 were taken as abnormal.

Statistical Analysis: Independent t test, analysis of variance (ANOVA) followed by Post HOC Tukey HSD test, Kruskal Wallis test were used. Statistical significance was set at p value < 0.05. 95% CI was quoted.

Result
Mean age was 52.6±5.2 years in DR group, 51.5±8.5 years in DM group and 48.4±5.8 years in control group. Long duration of diabetes ≥ 10 years was seen more in DR group, 6 cases (31.6%) than in DM group, 5 cases (14.7%). Poor metabolic control was seen more in DR group, 9 cases (47.4%) than in DM group, (26.5%).

On comparing the CIC grades, abnormal grades were seen in 32 (97.0%) DR and 45 (90.0%) in DM groups as compared to control group of 20 cases (50.0%) (Table 1). Grade 0 showed monolayered sheets of small round squamous epithelial cells with large central nuclei and cylindrical columnar goblet cells (>500) with basal nuclei (Figure 1). Grade 2 showed sheets of squamous cells with marked loss of polarity, irregular shapes and smaller nuclei with anisonucleosis. Goblet cells are small and irregular in shape (<200) (H&E, x40) (Figure 2). Grade 3 showed anucleate, large polyhedral squamous epithelial cells, irregular shapes, overlapping, curled up and very few goblet cells (<100) (H&E, x40) (Figure 3).

The comparison of GCD and CSM in DR & DM with control showed significantly poor GCD and CSM grades in DR and DM groups as compared to control group (p < 0.0001) (Table 2). Intergroup comparisons revealed significant differences in GCD and CSM grades between DR and DM groups (p < 0.0001) as compared to control but not between the two study groups: (p values of 0.575 in GCD vs 0.192 in CSM) (Table 3).

Relation of ocular surface parameters in diabetics with and without retinopathy to systemic factor showed that GCD was significantly lower in diabetics as compared to controls in relation to increasing age (p=0.036), worsening DR (p
and Dry eye symptoms (p = 0.016). The median CSM grade was poorer with long duration diabetes (p = 0.015) and worsening DR (p < 0.0001) (Table 4).

Table 1: A comparison of conjunctival impression cytology grading in Diabetic retinopathy (DR), Diabetes without retinopathy (DM) and control group.

<table>
<thead>
<tr>
<th>Grading on no. of goblet cells and squamous metaplasia</th>
<th>Diabetic Retinopathy (n = 33)</th>
<th>Diabetes Mellitus (n = 50)</th>
<th>Control group (n =40)</th>
<th>Total (n = 123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal† (grades 0 &amp;1) Goblet cells ≥200/mm²</td>
<td>1 (3%)</td>
<td>5 (10%)</td>
<td>20 (50%)</td>
<td>26 (21.14%)</td>
</tr>
<tr>
<td>Abnormal‡ (grades 2 &amp;3) Goblet cells &lt;200/mm²</td>
<td>32 (97%)</td>
<td>45 (90%)</td>
<td>20 (50%)</td>
<td>97 (78.86%)</td>
</tr>
</tbody>
</table>

Table 2: A comparison of goblet cell density and conjunctival squamous metaplasia in DR, DM and control groups (n = 123 eyes), (p Value on ANOVA).

**Table 3: Intergroup comparison of ocular surface parameters between DR, DM and control groups. p values (Post-hoc Tukey on ANOVA and non parametric Kruskal wallis test ) and difference of mean (95% CI) are quoted.**

<table>
<thead>
<tr>
<th>Ocular surface parameters</th>
<th>Control - DR</th>
<th>Control - DM</th>
<th>DM – DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goblet Cell Density (cells/mm²) Mean (SD)</td>
<td>189.73 (132.12 to 247.33)</td>
<td>166.47 (114.51 to 216.43)</td>
<td>23.26 (-31.68 to 78.19)</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.575</td>
</tr>
<tr>
<td>Conjunctival Squamous Metaplasia Median</td>
<td>2 Vs 3</td>
<td>2 Vs 3</td>
<td>3 Vs 3</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.102</td>
</tr>
</tbody>
</table>

**Table 4: Relation of ocular surface parameters in all diabetics, with and without retinopathy to systemic factors**

<table>
<thead>
<tr>
<th>Parameters in all diabetics (with and without retinopathy)</th>
<th>Goblet cell density (cells/mm²) Mean (SD)</th>
<th>p value</th>
<th>Conjunctival Squamous metaplasia (grades)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt;50</td>
<td>164.07 (156.27)</td>
<td>0.036</td>
<td>2(0 to 3)</td>
<td>0.350</td>
</tr>
<tr>
<td>≥50</td>
<td>114.01 (106.26)</td>
<td>0.407</td>
<td>2.5(0 to 3)</td>
<td></td>
</tr>
<tr>
<td>Dry eye symptoms</td>
<td>Present</td>
<td>107.13 (105.19)</td>
<td>0.016</td>
<td>3(1 to 3)</td>
</tr>
<tr>
<td>Absent</td>
<td>169.65 (153.68)</td>
<td>0.015</td>
<td>0(0 to 3)</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>&lt;10</td>
<td>85.65 (70.35)</td>
<td>0.06</td>
<td>3(1 to 3)</td>
</tr>
<tr>
<td>≥10</td>
<td>47.78 (39.04)</td>
<td>0.15</td>
<td>3(2 to 3)</td>
<td></td>
</tr>
<tr>
<td>Metabolic control</td>
<td>Good</td>
<td>85.83 (70.81)</td>
<td>0.587</td>
<td>3(1 to 3)</td>
</tr>
<tr>
<td>Poor</td>
<td>69.61 (55.29)</td>
<td>0.367</td>
<td>3(2 to 3)</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

In our study, CIC analysis showed significantly lower goblet cell density and higher grade of squamous metaplasia in both DR group and DM group than in control group (p < 0.0001). Dogru reported that the average grade of CSM was significantly higher and the average GCD (goblet cells/mm²) was significantly less in diabetics as compared to controls (p<0.001). The GCD was not affected by status of retinopathy and duration of diabetes.[4]

Goebbell study evaluation of CIC according to Tseng,[10] and reported significantly more frequent and more pronounced signs of CSM in diabetics as compared to controls.[5] Re-analyzation of their data showed that significantly more IDDM patients had abnormal CSM grades (> grade 2) as compared to controls: 65.11% Vs 14.28% (p<0.05).[5]

Goblet cell density illustrates the condition of the ocular surface. The loss of goblet cells and abnormal morphology as small, irregular distorted shapes of goblet cells are related to the degree of squamous metaplasia. Epithelial cells in squamous metaplasia become larger and polygonal with high N:C ratio and in severe cases, the nuclei are pyknotic or even absent. The mechanisms producing these morphological changes in the cells of ocular surface in the course of diabetes are still not clear. These changes were present in 97% eyes of DR group and 90% eyes of DM group as compared to 50% of control group. Yoon reported similar findings that the CSM grade was directly related to the presence of retinopathy but not the duration of the diabetes.[6] It is interesting to note, that the
abnormal changes observed in CIC in diabetes were also seen in healthy control eyes. We suggest that it could possibly be due to the age related changes and external harsh surrounding environmental conditions like extremes of temperature, dry wind and dust at Aligarh.

In our study, we also assessed relationship of ocular surface changes with age, presence of dry eye symptoms, duration of diabetes, presence of diabetic retinopathy and metabolic control. In particular, we found that the decreasing goblet cell density was related to increasing age and presence of dry eye sign symptoms and squamous metaplasia was related with increasing duration of diabetes. Evidence from our study is essentially in accordance with the majority of the results in the literature, that ocular surface changes are more pronounced in diabetics than in controls and worsen with the worsening grades of retinopathy.[6,7]

**Conclusion**

We felt that conjunctival impression cytology (CIC) is a useful technique. It provides the grades of goblet cell loss and an objective analysis of the goblet cell and epithelial cell morphology. Therefore, routine examination and follow up of ocular surface parameters should form a part of the workup of all diabetic patients.

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None.

**Competing Interests**

None declared

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**References**