Original Article

Conjunctival surface changes in diabetics: an unusual cytological study

Adeeb Alam Khan[1], Divya Kesarwani[1], Shaista Mannan Vasenwala[2], A.K. Amitava[1], Zia Siddiqui[1], Kafil Akhtar*[2]

[1]Department of Ophthalmology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India
[2]Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India

Keywords: *Diabetic retinopathy, Conjunctival impression cytology, Dry eye.*

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.

***Corresponding author:**
Dr Kafil Akhtar, Associate Professor, Department of Pathology, J N Medical College, Aligarh Muslim University, Aligarh (U.P) India. Email: drkafilakhtar@gmail.com

Date of Submission: May 27, 2014 **Date of Acceptance:** Aug 1, 2014 **Date of Publishing:** Sept 28, 2014

How to cite this paper:
Khan AA, Kisarwani D, Vasenwala SM, Amitava AK, Siddiqui Z, Akhtar K. Conjunctival surface changes in diabetics: an unusual cytological study. Annals of Pathology and Laboratory Medicine. 2014;1(2):A1-A5

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, Diabetes 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 ≤ 126mg/dl.

Exclusion criteria: Individuals with a history of chronic ocular drug abuse, contact lens wear, ocular surgery within previous 3 months; abnormalities in cornea, conjunctiva or eyelid and secondary ocular and 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 glacial acetic acid, 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.[8] The smear was scanned at 4X, 10X and 40X and goblet cells counted in four HPF (x40X) and GCD was calculated in 1 HPF. Calculation of GCD was according to Nelson's criteria using a calibrated grid at 200 or 400 over an area of 0.03 or 0.008 sq.mm respectively. The mean total of each such 10 areas was recorded for each specimen. One Low power field (LPF) = 3.0 mm^2 and one High power field (HPF) = 1.0 mm^2 was taken as the field diameter of the microscope used. Loss of granules seen as decreased PAS intensity and

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/ mm^2; small, round epithelial cells with large nuclei.

Grade 1: 200 to 500 goblet cells/mm^2

Grade 2: 100 to199 goblet cells/ mm^2

Grade 3: < 100 goblet cells / mm^2; large, polygonal epithelial cells with small nuclei.

Goblet cell density <200 cells/ mm^2 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 (Figure1). Grade 2 showed sheets of squamous cells with marked loss of polarity, irregular shapes and smaller nuclei with anisonucleosis with small and irregular goblet cells (<200) (Figure 2). Grade 3 showed anucleate, large polyhedral squamous epithelial cells, irregular shapes, overlapping, curled up and very few goblet cells (<100) (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).

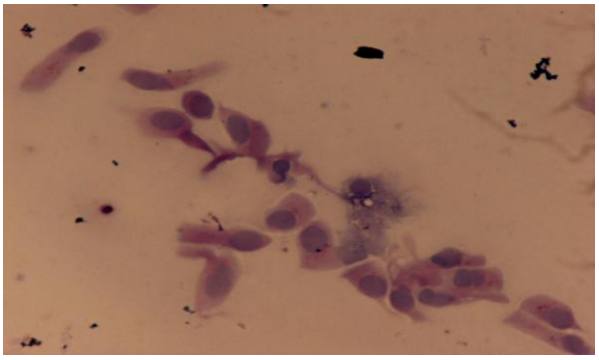

Figure 1: Grade 0 shows monolayer sheets of small round squamous epithelial cells with large central nuclei and cylindrical columnar goblet cells (>500) with basal nuclei. (H&E, x40).

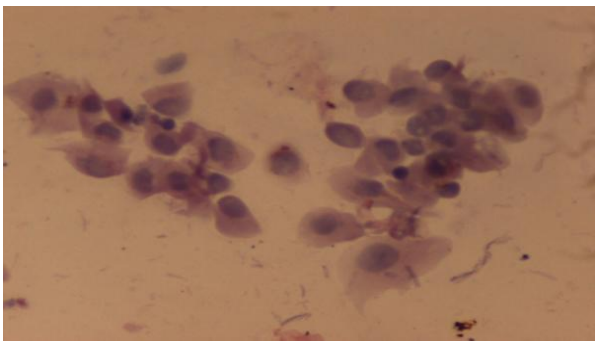

Figure 2: Grade 2 shows sheets of squamous cells with marked loss of polarity, irregular shapes and smaller nuclei with anisonucleosis. Goblet cells are small and irregular in shape (<200) (H&E, x40).

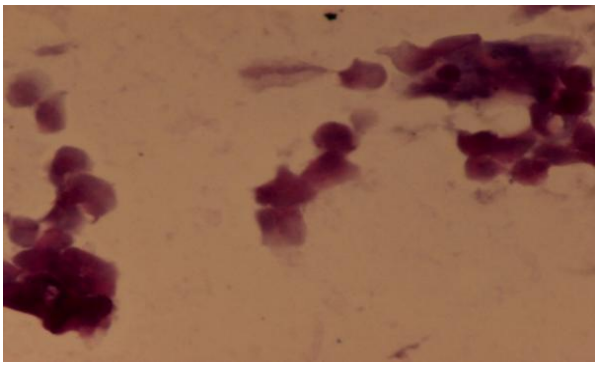

Figure 3: Grade 3 shows flat, anucleate, large polyhedral squamous epithelial cells of irregular shapes with overlapping and curling with very few goblet cells (<100) (H & E, x40).

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

<0.0001) 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.

Grading on no. of goblet cells and squamous metaplasia	Diabetic Retinopathy (n = 33) No. (%)	Diabetes Mellitus (n = 50) No. (%)	Control group (n =40) No. (%)	Total (n = 123) No. (%)
Normal* (grades 0 &1) Goblet cells ≥200mm²	1 (3%)	5 (10%)	20 (50%)	26 (21.14%)
Abnormal† (grades 2&3) Goblet cells <200/mm²	32 (97%)	45 (90%)	20 (50%)	97 (78.86%)

**: Normal (grades 0 &1): small, round squamous epithelial cells with large nuclei.*
†: Abnormal (grades 2 &3): Large, polygonal squamous epithelial cells with small or absent nuclei

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).

Ocular surface parameters	Diabetic Retinopathy (n = 33)	Diabetes Mellitus (n = 50)	Control group (n =40)	p value
Goblet Cell Density (cells/mm²) Mean (SD)	63.42 (59.2)	86.68 (69.6)	253.15 (154.1)	< 0.0001
Conjunctival Squamous Metaplasia (grade) Median (Range)	3 (1 – 3)	3 (1 – 3)	1.5 (0–3)	< 0.0001

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.

Ocular surface parameters	Control - DR	Control - DM	DM – DR
Goblet Cell Density (cells/sqmm) Mean diff (95%CI)	189.73 (132.12 to 247.33)	166.47 (114.51 to 218.43)	23.26 (-31.68 to 78.19)
p value	< 0.0001	< 0.0001	0.575
Conjunctival Squamous Metaplasia Median	2 Vs 3	2 Vs 3	3 Vs 3
p value	< 0.0001	< 0.0001	0.192

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

Parameters in all diabetics (with and without retinopathy) N= 53		Goblet cell density (cells /mm)	p value	Conjunctival Squamous metaplasia (grades)	p value
Age	<50	164.07 (156.27)	0.036	2(0 to 3)	0.350
	≥50	114.01 (106.26)		2.5(0 to 3)	
Dry eye symptoms	Present	107.13 (105.19)	0.016	3(1 to 3)	0.407
	Absent	169.65 (153.68)		0(0 to 3)	
Duration of diabetes	<10	85.65 (70.35)	0.06	3(1 to 3)	0.015
	≥10	47.78 (39.04)		3(2 to 3)	
Metabolic control	Good	85.83 (70.81)	0.587	3(1 to 3)	0.367
	Poor	69.81 (55.29)		3(2 to 3)	

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]

Goebbels studied 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.

Acknowledgements

We acknowledge the technical staff of the cytopathology lab and the departmental head for the general support.

Funding

None.

Competing Interests

None declared

References

1) Branwald E, Fauci S, Kasper D, Hauser LS, Longo DL, Jameson JL. Diabetes Mellitus. Harrison Principle of Internal Medicine. 15th Edn. 2001; pp 2121-3.
2) Saini JS, Khandalavla B. Corneal epithelial fragility in diabetes mellitus. Can J Ophthalmol 1995; 30:142-6.
3) Inoue K, Kato S, Ohara C, Numaga J, Amano S, Oshika T. Ocular and systemic factors relevant to diabetic keratoepitheliopathy. Cornea 2001; 20:798-801.
4) Dogru M, Katakami C, Inoue M. Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus. Ophthalmol 2001;108:586-92.
5) Goebbels M. Tear secretion and tear film function in insulin dependent diabetics. Br J Ophthalmol 2000; 84:19-21.
6) Yoon KC, Im SK, Sco MS. Changes of tear film and ocular surface in diabetes mellitus. Korean J Ophthal 2004; 2:168-74.
7) Braunwald E, Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL: Harrison's Principle of Internal Medicine. 17th Edn. 2008; pp 2276-9.
8) Anshu A, Munshi MM, Sathe V, Ganar A. Conjunctival impression cytology in contact lens wearers. Cytopathol 2001;12:314-20.
9) Nelson JD. Impression cytology. Cornea 1988; 7:71-81.
10) Tseng SC. Staging of conjunctival squamous metaplasia by impression cytology. Ophthalmol 1985; 9:728-33.