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Research Article

# ASSESSMENT OF CONJUNCTIVAL IMPRESSION CYTOLOGY AND ITS DIFFERING PATTERNS IN CONTROLLED AND UNCONTROLLED DIABETIC PATIENTS

**Dr. Aashish Saluja[1], Dr. Vandana Mahaur[2], Dr. T.S. Ahluwalia[3], Balvir Singh Tomar[4], Dr. Yelda Vyas[5]**

[1]Department of Ophthalmology, National Institute of Medical Sciences and Research, NIMS University, Jaipur, Rajasthan, India.
[2,3]Professor & HOD, Department of Ophthalmology, NIMS University, Jaipur, Rajasthan, India.
[4]Institute of Medical Sciences and Research.
[5]Associate Professor, Department of Pathology, NIMS University, Jaipur, Rajasthan, India.

**Corresponding Author*:** Dr. Vandana Mahaur, Professor & HOD, Department of Ophthalmology, NIMS University, Jaipur, Rajasthan, India.

**Email ID:** vandanarai10@gmail.com

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## ABSTRACT

**Background:** Diabetes mellitus has a wide spectrum of effects on the ocular surface. Conjunctival impression cytology (CIC) is a relatively easy and non-invasive technique that allows for the evaluation of the morphology of the conjunctival epithelium and changes in the conjunctival goblet cells.

**Objective:** To assess conjunctival impression cytology patterns in controlled and uncontrolled diabetic patients and correlate cytological findings with HbA1c levels.

**Materials and Methods:** A comparative observational cross sectional study conducted in the Department of Ophthalmology, National Institute of Medical Sciences and Research, Jaipur, for 18 month. The total 216 patients with type 2 diabetes mellitus were divided into two groups: controlled (HbA1c level was ≤6.5%) and uncontrolled (HbA1c level was >6.5%). Ophthalmological detailed examination and impression cytology of the conjunctiva were carried out. The nasal bulbar conjunctiva was sampled under topical anaesthetic, stained for cytological examination and classified using Nelson's classification. Descriptive statistics and Student's t-test were used in SPSS and Microsoft software for data analysis.

**Results:** 216 subjects participated in the study, with a mean age of 59.3 ± 10.2 years. The goblet cells were more common in the controlled group (80.0%) than in the uncontrolled group (59.4%), while the metaplastic cells were more common in the uncontrolled group (24.5% vs 10.0%). Nelson Grade 0 was mainly seen in controlled patients and Grades 2 and 3 were more common in uncontrolled patients ($p=0.001$). There was a significant association among fundus findings and HbA1c status ($p=0.001$) with the uncontrolled group having more cases of proliferative DR and clinically significant ME. Nelson grade ($p=0.008$) was significantly associated with the duration of diabetes. Patients with HbA1c >6.5% were more likely to have reduced corneal sensation (10.18%) compared with patients with HbA1c <6.5% ($p=0.001$) who had no reduced corneal sensation.

**Conclusion:** There were positive associations between glycaemic control and duration of diabetic disease and increasing severity of abnormalities in the conjunctival epithelium. CIC may be a helpful non-invasive tool for early detection of changes in the ocular surface in diabetic patients.

**KEYWORDS:** Diabetes Mellitus, Conjunctival Impression Cytology, HbA1c, Goblet Cells, Squamous Metaplasia, Nelson Grading, Diabetic Retinopathy.

## Introduction

Diabetes mellitus refers to a chronic metabolic disease in which blood sugar levels are consistently elevated, and which is linked with a wide variety of microvascular and macrovascular complications. Diabetic retinopathy is the most widely known complication of diabetes, but diabetes can also impact the ocular surface such as the conjunctival epithelium, goblet cells, corneal nerves and tear film[2,7]. Prolonged hyperglycaemia, microangiopathy, neuropathy, oxidative stress and inflammation adversely affect the function of the epithelium. The study documents these changes at the surface of the eye as being associated with, and perhaps even a natural consequence of, clinically observed retinal changes[6,11,12].

Goblet cells are found in the conjunctiva which produces mucin and stabilizes the tear film. Diabetic changes may include decreased goblet-cell numbers, enlargement of the epithelium, changes in the nuclear/cytoplasmic ratio, keratinization and squamous metaplasia[10,13-16]. The changes can be identified by using a minimally invasive technique, called conjunctival impression cytology, whereby very superficial conjunctival cells are collected onto a membrane and examined under the microscope[17]. The study highlights that CIC is able to identify subclinical changes of the ocular surface and that the severity of the cytological abnormalities may be linked to glycaemic control[3,4,8,9].

Conjunctival epithelial changes are graded according to Nelson's classification, which corresponds to the progressive squamous metaplasia and goblet-cell loss. The present study thus aimed to compare the CIC pattern in controlled diabetic patients with that of uncontrolled, and study the relationship between these patterns and HbA1C, duration of diabetes, DR and CS[1,5].

## Materials and Methods

It was an observational cross-sectional study done in the department of ophthalmology, National Institute of Medical Sciences and Research, Rajasthan, Jaipur. Study was approved by ethical committee of our institute and adhered to the tenets of declaration of Helsinki. The patients with diabetes mellitus were stratified into two groups: Group 1 (HbA1c ≤6.5%) and Group 2 (HbA1c >6.5%). The stated study period was 1 July 2024 to 31 December 2025 (18 months).

Patients were included if they had known type 2 diabetes mellitus and either HbA1c ≤6.5% or HbA1c >6.5%, were able to give informed consent and were age >40, regardless of sex. The exclusion criteria were: use of contact lenses, smoking, vitamin A deficiency, previous eye trauma or surgery, lid/adnexal disorders or corneal pathology, topical/systemic eye medication, and anaemia.

### Conjunctival Impression Cytology

The cellulose acetate filter paper disc (pore size 0.2 µm) was applied to the nasal bulbar conjunctiva, adjacent to the limbus, after topical anaesthesia with 0.5% proparacaine, and was gently pressed for about 10–15 seconds. Specimen placed on albumin coated slide, fixed prior cytopathological processing. Study describes staining with Papanicolaou stain in the processing protocol and also it reports that the samples were stained with PAS and H&E for cytological examination[18-20]. Microscopic examination was undertaken by a pathologist who had no knowledge of the clinical information. Cytological assessment was made on the basis of epithelial morphology and goblet-cell density, and classified as per Nelson's classification.

| Nelson grading of goblet cells | |
|---|---|
| **Grade** | **Features** |
| 0 | >500 goblet cells / mm<br>Small ,round epithelial cells with large nuclei |
| 1-2 | 100-500 goblet cells/mm |
| 3 | <100 goblet cells/mm<br>Large , polygonal epithelial cells with small nuclei |

Statistical analysis was performed using IBM SPSS Statistics and Microsoft Excel.

Ethical approval for the study was obtained from the Institutional Ethics Committee of NIMS University/National Institute of Medical Sciences & Research, Jaipur. Written informed consent was obtained from all participants prior to their enrolment in the study.

## Results

A total of 216 participants were included. The overall mean age was 59.3 ± 10.2 years. There were 150 males (69.4%) and 66 females (30.6%). The controlled group (group 1) had a mean age of 58.9 ± 9.8 years and the uncontrolled group (group 2) 59.7 ± 10.6 years.

**Table 1: Demographic characteristics of study participants**

| Characteristic | HbA1c ≤6.5% | HbA1c >6.5% | Overall |
|---|---|---|---|
| Sample size | 108 | 108 | 216 |
| Male | 77 (70.0%) | 73 (68.9%) | 150 (69.4%) |
| Female | 33 (30.0%) | 33 (31.1%) | 66 (30.6%) |
| Mean age, years | 58.9 ± 9.8 | 59.7 ± 10.6 | 59.3 ± 10.2 |

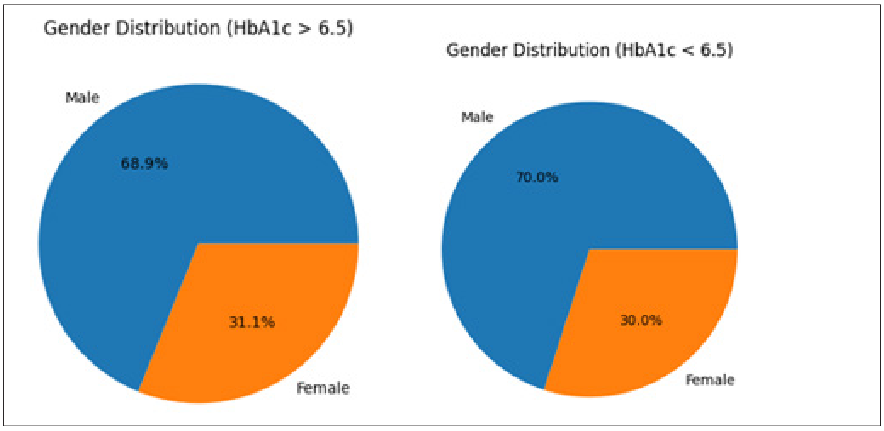


**Figure 1. Gender distribution according to HbA1c level**

**Table 2: Distribution of conjunctival cell types according to HbA1c level**

| Cytological finding | HbA1c ≤6.5% | HbA1c >6.5% | Total |
|---|---|---|---|
| Goblet | 88 (80.0%) | 63 (59.4%) | 151 |
| Anucleate | 33 (30.0%) | 21 (19.8%) | 54 |
| Squamous | 25 (22.7%) | 24 (22.6%) | 49 |
| Dysplastic | 25 (22.7%) | 24 (22.6%) | 49 |
| Degenerative | 14 (12.7%) | 19 (17.9%) | 33 |
| Keratinized | 16 (14.5%) | 5 (4.7%) | 21 |
| Pigmented | 11 (10.0%) | 5 (4.7%) | 16 |
| Columnar | 8 (7.3%) | 12 (11.3%) | 20 |
| Metaplastic | 11 (10.0%) | 26 (24.5%) | 37 |

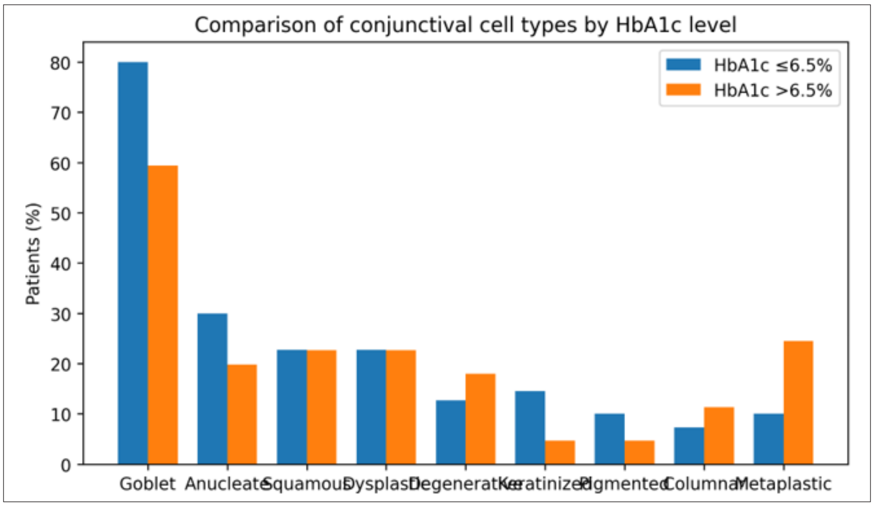


**Figure 2.Comparison of conjunctival cell types by HbA1c level**

Cytological examination demonstrated multiple epithelial cell types. Goblet cells were the most frequent finding, occurring in 88 (80.0%) patients in the group1 and 63 (59.4%) in group 2. Metaplastic cells were more frequent in group 2 (26; 24.5%) than in group 1(11; 10.0%). Degenerative cells were also somewhat more frequent in group 2.

Nelson grading showed a significant association with glycaemic status (p=0.001). Grade 0 was predominantly seen in the HbA1c ≤6.5% group (37; 88.1% of Grade 0 cases). In contrast, Grades 2 and 3 were more frequent in the HbA1c >6.5% group, accounting for 62.1% and 66.7% of cases within those grades, respectively.


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**Table 3: Nelson grades according to HbA1c level**

| Nelson grade | HbA1c ≤6.5% | HbA1c >6.5% | Total | P value |
|---|---|---|---|---|
| Grade 0 | 37 (88.1%) | 5 (11.9%) | 42 (19.4%) | 0.001 |
| Grade 1 | 30 (50.0%) | 30 (50.0%) | 60 (27.8%) | |
| Grade 2 | 25 (37.9%) | 41 (62.1%) | 66 (30.0%) | |
| Grade 3 | 16 (33.3%) | 32 (66.7%) | 48 (22.2%) | |

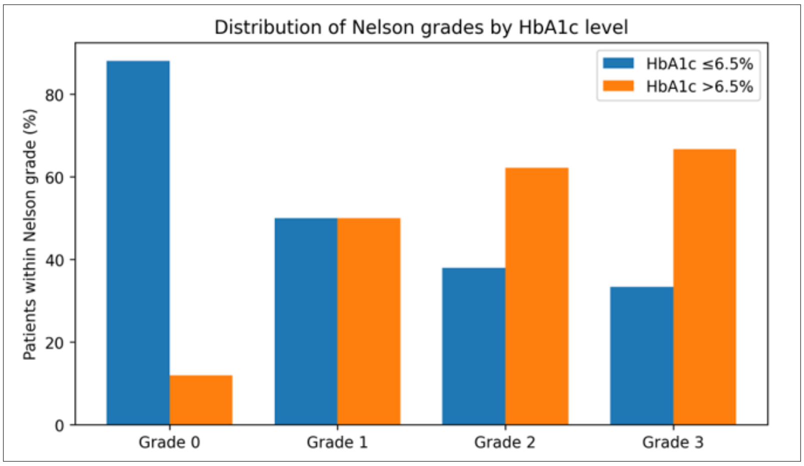


**Figure 3.Distribution of Nelson grades by HbA1c level**

**Table 4: Nelson grades correlated with fundus findings**

| Fundus finding | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Total | P |
|---|---|---|---|---|---|---|
| Normal | 46 (38.3%) | 30 (25.0%) | 29 (24.2%) | 15 (12.5%) | 120 | 0.001 |
| NPDR | 6 (15.0%) | 17 (42.5%) | 17 (42.5%) | 0 (0.0%) | 40 | |
| PDR | 0 (0.0%) | 0 (0.0%) | 11 (39.3%) | 17 (60.7%) | 28 | |
| CSME | 0 (0.0%) | 6 (21.4%) | 11 (39.3%) | 11 (39.3%) | 28 | |

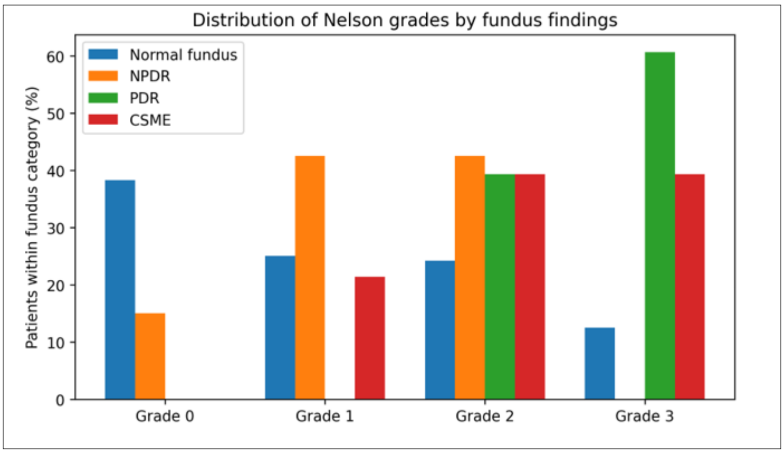


**Figure 4.Distribution of Nelson grades according to fundus findings**

Fundus findings were significantly associated with HbA1c status (p=0.001). Normal fundus findings were more frequent in group 1 (89; 82.4%) than in group 2 (28; 25.9%). Proliferative diabetic retinopathy was observed only in the HbA1c >6.5% group (25; 23.1%), while clinically significant macular edema was also substantially more frequent in group 2 (32; 29.62% versus 4; 3.7%). Nelson grades were also significantly associated with fundus findings (p=0.001). Patients with normal fundus findings showed a broader distribution with Grade 0 as the most frequent grade. In NPDR, Grades 1 and 2 were each present in 42.5% of patients. In PDR, no Grade 0 or Grade 1 cases were observed; Grade 2 occurred in 39.3% and Grade 3 in 60.7%. CSME was likewise associated with higher grades, with Grade 2 and Grade 3 each accounting for 39.3%.

**Table 5: Association of duration of diabetes and corneal sensation with study findings**

| Parameter | Finding 1 | Finding 2 | P value |
|---|---|---|---|
| Duration of DM vs Nelson grade | <5 years: Grade 0 most frequent | 5–10 and >10 years: higher Grade 2/3 pattern | 0.008 |
| Corneal sensation | HbA1c ≤6.5%: 108 (100%) normal | HbA1c >6.5%: 97 (89.8%) normal; 11 (10.18%) reduced | 0.001 |

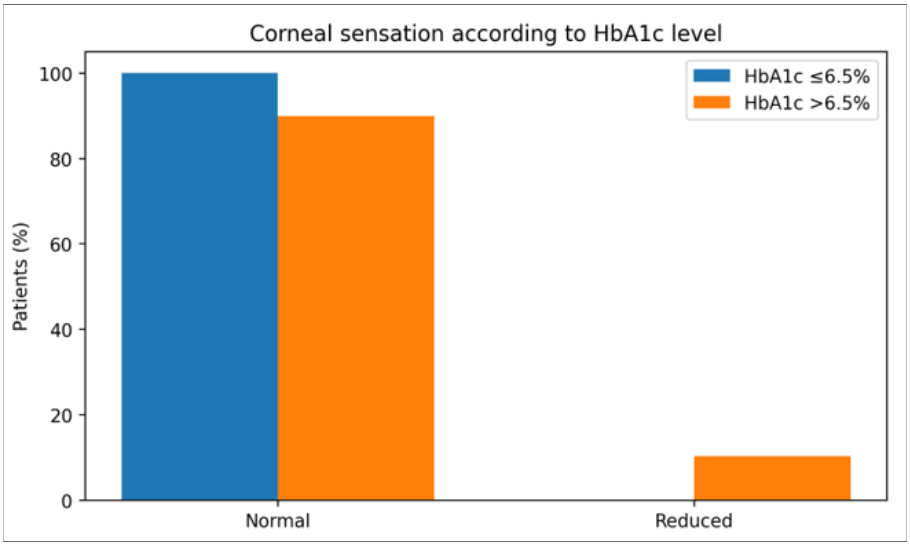


**Figure 5.Corneal sensation according to HbA1c level**

Duration of diabetes was significantly associated with Nelson grade (p=0.008). Among participants with diabetes for <5 years, Grade 0 was the most frequent category. In those with 5–10 years of diabetes, Grade 2 and Grade 3 predominated, while participants with diabetes for >10 years showed no Grade 0 cases and persistent Grade 1–3 abnormalities. Corneal sensation was assessed by the cotton-wisp test. All 108 patients in the HbA1c <6.5% group had normal corneal sensation, whereas 97 (89.8%) in the HbA1c >6.5% group had normal sensation and 11 (10.18%) had reduced sensation. The association with glycaemic status was statistically significant (p=0.001).

## Discussion

The present study evaluated conjunctival epithelial changes using conjunctival impression cytology (CIC) and examined their relationship with glycaemic control, duration of diabetes mellitus (DM), severity of diabetic retinopathy (DR), and corneal sensation. Among the 216 participants, the mean age was 59.3 ± 10.2 years, and males constituted the larger proportion of the study population. The mean age was comparable between the controlled and uncontrolled groups, making it less likely that age alone accounted for the differences observed in conjunctival cytology. A male predominance has also been observed in previous studies of ocular-surface changes in patients with diabetes[6,14]. This pattern may partly reflect differences in healthcare-seeking behaviour and access to hospital-based medical care.

One of the main findings of the present study was the significant association between poor glycaemic control and conjunctival epithelial abnormalities. Patients with higher HbA1c levels had fewer goblet cells, more metaplastic changes, and higher Nelson grades. These findings are in keeping with previous evidence showing that diabetes can affect the conjunctival epithelium and goblet-cell population, resulting in progressive ocular-surface changes[6,11-13]. The greater frequency of abnormal cytological findings in patients with poor glycaemic control suggests that prolonged hyperglycaemia may play an important role in maintaining or worsening ocular-surface damage. This relationship is clinically relevant because conjunctival changes may develop alongside other diabetic complications and may not always be apparent from symptoms alone.

Duration of diabetes was also significantly associated with CIC findings in the present study. Patients with a longer duration of DM tended to have higher Nelson grades, although the pattern was not completely linear. Similar ocular-surface abnormalities have been described in patients with longer-standing type 2 diabetes mellitus[16]. The non-linear pattern observed in our study indicates that duration of diabetes may not act independently in determining the severity of conjunctival changes. Factors such as the degree of glycaemic control, diabetic complications, neuropathy, and individual variation may also influence the condition of the conjunctival epithelium.

Another important finding was the significant association between DR severity and conjunctival cytological

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abnormalities. Higher Nelson grades were more frequently observed in patients with advanced fundus changes, particularly those with proliferative diabetic retinopathy and clinically significant macular oedema. Previous studies examining conjunctival impression cytology in diabetic retinopathy have also described ocular-surface abnormalities across different stages of retinal disease[15,17]. The parallel increase in retinal and conjunctival abnormalities may reflect the wider effects of chronic diabetes on microvascular and neural tissues. However, the cross-sectional nature of the present study does not allow a temporal or causal relationship between DR severity and conjunctival changes to be established.

The ocular-surface changes seen in diabetes are likely to result from several interacting mechanisms. Chronic hyperglycaemia can affect microvascular function and peripheral nerves and may also alter tear-film stability and ocular-surface homeostasis[6,10,11]. Changes in tear secretion and corneal sensation have previously been described in diabetic patients, suggesting that diabetic neuropathy may contribute to ocular-surface dysfunction[6,10]. In the present study, reduced corneal sensation was observed predominantly among patients with poor glycaemic control. This finding may indicate that neural involvement and epithelial changes occur together as part of the broader ocular effects of diabetes.

The relationship between cytological findings and symptoms is also noteworthy. Some participants reported dryness, burning, or foreign-body sensation, whereas others with abnormal cytological findings did not have prominent symptoms. Similar observations have been described in previous studies, where ocular-surface and conjunctival epithelial abnormalities were identified despite relatively limited clinical symptoms[6,18]. This suggests that structural changes in the conjunctival epithelium may not always correspond closely with the patient's subjective symptoms. CIC may therefore provide additional information about early or subclinical ocular-surface involvement in patients with diabetes.

Taken together, the findings of the present study show that conjunctival epithelial abnormalities were associated with glycaemic control, duration of diabetes, and severity of diabetic retinopathy. The higher frequency of abnormal Nelson grades among patients with poor glycaemic control and more advanced retinal disease highlights the potential usefulness of CIC as an additional, minimally invasive method for assessing ocular-surface involvement in diabetes. Nevertheless, these findings should be interpreted in the context of the study design. The cross-sectional nature of the study prevents conclusions regarding causality or progression over time, and the single-centre setting may limit the generalisability of the findings.

## Conclusion

A total of 216 patients with type 2 diabetes mellitus were included in this comparative cross sectional study; advanced cytological abnormalities of the conjunctiva were associated with poor glycaemic control. The goblet-cell representation was found to be lower, metaplastic changes and degenerative changes were higher and Nelson grades were higher in uncontrolled diabetic patients. HbA1c was significantly related to Nelson grading, fundus findings and corneal sensation whereas duration of diabetes showed significant association with cytological severity. CIC is an easy and non-invasive technique, which can be used as an additional tool in the work-up and early detection of changes in the ocular surface in diabetic patients.

## Limitations

The study is cross-sectional and thus cannot prove a causal or temporal relationship for cytological changes. This was done at one tertiary-care center and might not be generalizable.

## Ethics Approval and Consent

Permission was obtained from the Scientific and Ethics Committee of the National Institute of Medical Sciences & Research, Jaipur, and written informed consent was obtained from participants.

## Conflict of Interest

No specific conflict-of-interest statement was provided in the study source; this should be confirmed by the authors before submission.

## Data Availability

The underlying patient-level dataset was not provided as a public dataset in the study. Data-availability wording should be finalized according to the target journal's requirements.

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