Evaluation of morphologic and cytomorphometric changes in oral mucosa in Type II diabetes mellitus – An exfoliative cytology study

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Abstract

Background: It is well known that disease process of diabetes mellitus has effects on various tissues of the body. Diabetes mellitus adversely affect the morphology of oral mucosa, which may compromise tissue functions to favor the occurrence of oral infections and oral neoplasia. These effects can be studied at cellular level using oral, buccal exfoliative cytology.

Aims and Objectives: To evaluate the alteration in the morphology and cytomorphometric parameters of exfoliated buccal mucosal cells of Type II diabetes using exfoliative cytology.

Materials and Methods: Oral cytological smears were made from 20 Type II diabetes patients and 20 healthy individuals. The smears were stained with papanicolaou (PAP) stain. Evaluation of cells for morphologic and cytomorphometric changes were done using research microscope.

Results: Specific qualitative and quantitative changes were observed in the study group. There was a strong relation between diabetes and cytomorphometric parameters.

Conclusion: Cellular alterations (cytomorphometrical and morphological) observed in diabetic patients were related to glycosylated hemoglobin levels. Cellular alterations in oral exfoliated cells can be used as one of the investigative tool which can assist in the diagnosis of diabetes mellitus.

Keywords

Cytomorphometry, diabetes mellitus, exfoliative cytology, oral exfoliative buccal cells

Introduction

Diabetes mellitus is one of the most common endocrine disorders. It is the fifth most chronic condition. It is genetically heterogeneous metabolic disease characterized by abnormally elevated blood glucose level and dysregulation of carbohydrate, protein, and lipid metabolism. Since its prevalence is increasing worldwide, hence, calls for an appropriate action. Several studies have demonstrated the deleterious effects of diabetes mellitus on oral mucosa, which may compromise tissue functions, to favor the occurrence of oral infections and neoplasia. These effects can be studied at cellular level using oral exfoliative cytology.

Currently, monitoring of glycosylated hemoglobin (HbA1c) levels has become much commoner. However, in specific conditions such as diabetes, many invasive techniques lose viability as a result of variations in blood glucose and disease itself.

These days interest has been increasing in non-invasive diagnostic testing. With the advancement in the field of quantitative exfoliative cytology, there is a reemergence of oral cytology as a powerful diagnostic tool. Morphological and functional alteration in oral epithelial cells are detectable by microscopic and cytomorphometric analysis. It is proved that techniques such as computer-aided morphometry has been used to investigate the cellular changes which are more reliable, objective, and reproducible.

The early diagnosis of diabetes is an important aspect of health care. Conventional cytopathology has improved in the early detection of changes in individual exfoliated cells. The detection of qualitative and quantitative cellular changes may assist in the diagnosis of diabetes mellitus.
Aims and objectives

- To evaluate the alterations in the morphology and cytomorphometry of exfoliated buccal mucosal cells of Type II diabetes mellitus using exfoliative cytology
- To correlate the cellular changes between control and study group
- To ascertain the reliability of cellular changes as a diagnostic tool in Type II diabetes mellitus.

Materials and Methods

The study was conducted in the Department of Oral Medicine and Radiology, M.R. Ambedkar Dental College and Hospital, Bengaluru.

Method of collection of data

This study consisted of a total sample size of 40 participants with age ranging from 30 to 60 years inclusive of both the genders. The participants were divided into two groups which include 20 cases of normal healthy individuals as controls and 20 cases of diagnosed Type II diabetes mellitus.

The control group participants were advised for fasting and postprandial blood glucose levels, and the study participants were confirmed by HbA1c values.

Based on the HbA1c values, the study samples were divided into the following subgroups:

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Level of HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Well controlled (HbA1c ≤8%)</td>
</tr>
<tr>
<td>Group B</td>
<td>Moderately controlled (HbA1c &gt;8% ≤10%)</td>
</tr>
<tr>
<td>Group C</td>
<td>Poorly controlled (HbA1c &gt;10% and ≤12%)</td>
</tr>
<tr>
<td>Group D</td>
<td>Uncontrolled (HbA1c &gt;12%)</td>
</tr>
</tbody>
</table>

Written informed consent from the selected patients was taken for the procedure to be carried out on them subsequently.

Inclusion criteria

Control group - healthy controls with no history/risk factors of diabetes.

Study group - participants diagnosed with Type II diabetes mellitus with or without treatment.

Exclusion criteria

Patients with deleterious habits, systemic disease, pregnant women, medications other than diabetes, any active lesions on the buccal mucosa, radiation exposure, and viral infections.

Collection of exfoliated cells and method

Smears were obtained from normal buccal mucosa using a cytobrush. The smears were evenly spread over a large area so that there will be no clumping of cells.

PAP stain was used, and cells were observed under ×100 magnification, using Research microscope. Twenty clearly defined cells in each slide were evaluated. The cells were viewed in a stepwise manner to avoid measuring of the same cells again.

The cytomorphometric measurements observed were nuclear diameter (ND), cytoplasm diameter (CyD), and cell diameter (CD) [Figure 1].

The CD was determined by drawing a line with a digitizer cursor from one end of the cell to the other end, at the greatest dimension and the digital measurements were recorded. The ND was obtained by drawing a line using digitizer cursor from one side of the nucleus to other end at midlevel. The obtained values were subjected to statistical analysis.

The observed counts were expressed in numbers. The collected data were analyzed using univariate analysis, Chi-square, and Spearman rank correlation coefficient.

Discussion

Type II diabetes mellitus is the most common metabolic disorder that produces multiple systemic and oral complications. It often goes undiagnosed because many of its symptoms seem harmless. However, in certain situations, alteration in blood glucose levels and the disease itself reduce the viability of invasive techniques.

The morphological and functional changes in the oral mucosa can be studied at cellular level by using exfoliative cytology which helps in diagnosis with better patient acceptability. All the age group in the study sample showed significant relation with the disease [Table 1] and variations in the cells were also observed. Male predominance was observed.

This disparity in sex distribution could be due to the fact that men are more prone for stressful life and their lifestyle may contribute to diabetes.
This study was consistent with the study conducted by Helikkerimata and Sapra.\[^{[5]}\]

In this study, the duration of diabetes ranged from 3 to 8 years. The mean glycemic values were directly proportional to the duration of disease. The duration was found to have an influence on study parameters. However, it did not show statistically significant association with cellular changes [Table 2].

This study was consistent with the study done by Prasad et al.\[^{[1,7]}\] and Helikkerimata and Sapra.\[^{[2]}\]

There was a wide range of HbA1c levels ranging from 5.8% to 13.9%. It is interesting to report that, all levels of HbA1c had a strong effect on morphometry of the cells. This may be due to the effect of the response of the each individual to the treatment.

This study is similar to that of the study conducted by Prasad et al.\[^{[1,7]}\] and Rivera and Memdoza\[^{[14]}\] where they also found a wide range of HbA1c levels.

The maximum number of participants was observed in Group A, followed by Group C, B, and D [Table 3]. This could be due to their better cooperation, response to the treatment and healthy lifestyle.

### Table 1: Age distribution in study sample

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Control (%)</th>
<th>Study group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>30–40</td>
<td>8 (40)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Group II</td>
<td>40–50</td>
<td>5 (25)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Group III</td>
<td>50–60</td>
<td>7 (35)</td>
<td>14 (70)</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>43.3</td>
<td>49.8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

### Table 2: Duration of diabetics in the study group

<table>
<thead>
<tr>
<th>Duration (years)</th>
<th>N (%)</th>
<th>Mean±SD</th>
<th>CI-95%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-4</td>
<td>5 (25)</td>
<td>3.6±0.54</td>
<td>3.0-0.88</td>
<td>0.22</td>
</tr>
<tr>
<td>5-6</td>
<td>7 (35)</td>
<td>5.6±0.484</td>
<td>1.75-3.63</td>
<td>0.01</td>
</tr>
<tr>
<td>6-7</td>
<td>2 (10)</td>
<td>6.38±1.63</td>
<td>9.31-3.50</td>
<td>0.87</td>
</tr>
<tr>
<td>8</td>
<td>6 (30)</td>
<td>7.56±0.98</td>
<td>5.48-9.32</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>20 (100)</td>
<td>5.9±1.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard deviation, CI: Confidence interval

### Table 3: Number of participants in study subgroups according to the levels of HbA1c

<table>
<thead>
<tr>
<th>Study group</th>
<th>HbA1c level</th>
<th>Number of participants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Well-controlled (HbA1c &lt;8%)</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Group B</td>
<td>Moderately controlled (HbA1c &gt;8% and&lt;10%)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Group C</td>
<td>Poorly controlled (HbA1c &gt;10% and&lt;12%)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>Group D</td>
<td>Uncontrolled (HbA1c &gt;12%)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

The present study was not consistent with the study conducted by Prasad et al.\[^{[17]}\] where the author found maximum number of participants in poorly controlled group.

In control group, the exfoliated cells appeared maximum, round, and compact whereas in the study group it appeared irregular and large in size [Table 4].

The buccal exfoliated cells showed definite morphological variations with respect to hyperglycemic status. This study is in concurrence with the study conducted by Shareef et al.\[^{[12]}\]

On comparing the cytomorphometry between control and the study group, the severity of diabetes had definite effects on the study parameters.

There was definite increase in ND related to hyperglycemia, and statistically, significant difference was found in the mean values between the groups (P < 0.01) [Table 5].

This increase in ND in the study group might be an indicator of cellular aging due to delay in keratinization process caused by decreased cellular turnover. In diabetes, the glycation of proteins, lipids, and nucleic acids increase with sustained hyperglycemia causing greater accumulation of advanced glycation end products in the walls of large vessels and basement membrane of microvasculature. The effect of this is a progressive narrowing of vessel lumen, decreased perfusion of affected tissues, and decreased turnover which may cause a delay in keratinization process of epithelium thus leading to increase in cells with large nucleus as a primary characteristic.\[^{[21]}\]

In controls, the CyD appeared normal, and variations were noticed in the study group.

On statistical analysis, the observed difference in mean CyD among the two groups was not significant (P > 0.001) [Table 5]. This parameter variations in the study group could be attributed to the difference in cell turnover rate and cell maturation rate besides the effect of the existence of local inflammation.\[^{[21]}\] This is in accordance with that of Sonawane et al.\[^{[3]}\] Prasad et al.\[^{[17]}\] Baban and Garib\[^{[12]}\] who reported a similar reduction in CyD.

In controls, the CD appeared normal and was found to be increased in the study group. On comparison, it was highly statistically significant (P < 0.003) [Table 5]. However, not many studies have been done on CD to ascertain the relevant information. Studies have proved that the enzyme system can either be activated or inhibited according to the need of the cell. This regulatory mechanism most often function as feedback control system that continuously monitor the cell biochemical composition and make a correction as needed even in adverse condition.\[^{[11]}\] This study is consistent with the study done by Prasad et al.\[^{[17]}\]

We observed definite morphological changes in exfoliated buccal mucosal cells in the study group in the form of binucleation, karyorrhexis, micronuclei, perinuclear halos, and cytoplasmic inclusions. Increased frequency of morphological changes was observed in diabetes. Morphometric changes may be related to:

a. The metabolic control of the diabetic state and medication.
**Table 4:** Microscopic appearance of exfoliated cells in control and study group

<table>
<thead>
<tr>
<th>Cell appearance</th>
<th>Control group</th>
<th>Study group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>Small and compact</td>
<td>Large</td>
</tr>
<tr>
<td>Shape</td>
<td>Oval/round</td>
<td>Irregular</td>
</tr>
<tr>
<td>Cytoplasm diameter</td>
<td>187.0±1.10</td>
<td>124.56±1.11</td>
</tr>
<tr>
<td>Cell diameter</td>
<td>137.82±1.16</td>
<td>145.35±1.17</td>
</tr>
</tbody>
</table>

**Table 5:** Comparison of exfoliated cell cytomorphometry between control and study group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group</th>
<th>Study group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Nuclear diameter</td>
<td>19.14±0.21</td>
<td>0.001**</td>
</tr>
<tr>
<td>Cytoplasm diameter</td>
<td>118.70±1.00</td>
<td>0.9768</td>
</tr>
<tr>
<td>Cell diameter</td>
<td>137.82±1.16</td>
<td>0.2699</td>
</tr>
</tbody>
</table>

SE: Standard error, **denotes statistically significant

This study is concurrence with the study conducted by Alberti et al.[20] Jajaram et al.[20] Shareef et al.[12]

**Conclusion**

The present research threw light on the cytomorphometric alterations in the buccal smear of Type II diabetes mellitus suggesting that, even though the oral mucosa of diabetic patient appears clinically normal, cell changes will occur with the influence of diabetes. The early changes in the oral cavity can be ascertained through cytology, more so through morphometry. These altered changes adversely affect the oral mucosa, which may compromise tissue functions to favor the occurrence of oral infections and oral neoplasia. The application of quantitative technique has greatly improved the accuracy of exfoliative cytology. The procedure is simple and can be done on chair-side during the routine oral examination. The cellular alterations in the oral epithelium are related to diabetes and can be used as one of the investigative tool which can aid in the diagnosis of Type II diabetes mellitus apart from regular standard tests.

**References**
