ABSTRACT

The objective of the present study was to assess the effect of diabetes on buccal mucosa by using cytomorphometry and to emphasize its relevance as an additional tool to aid in the evaluation of oral mucosal changes in diabetes mellitus. The study Group consisted of 100 subjects divided into 50 control subjects (Group C) and 50 Type II diabetic subjects (Group D). We analyzed and compared between the groups the nuclear and cell morphometric features i.e. mean nuclear area (MNA), mean nuclear perimeter (MNP), mean of maximum nuclear diameter (Max-ND), mean of minimum nuclear diameter (Min-ND), mean cell area (MCA), mean cell perimeter (MCP), mean of maximum cell diameter (Max-CD), mean of minimum cell diameter (Min-CD) and nuclear to cell parameter ratio. Buccal epithelial cells of these individuals were collected with a brush and fixed smears were stained with Papanicolaou (PAP) stain and cytomorphometric analysis performed using Image J image analysis software. In Group D, the nuclear parameters i.e. MNA, MNP, Max-ND, Min-ND were higher, while cell parameters i.e. MCA, MCP, Max-CD, Min-CD were lower as compared to Group C. The nuclear to cell parameter ratio were higher in Group D. Univariate analysis of variance (ANOVA) showed a significant group effect for nuclear parameters, cell parameters and ratio of nuclear to cell parameters. (p value <0.001).

There is increase in nuclear parameters, decrease in cellular parameters and increase in ratio of nuclear to cellular parameters in smears from diabetics as compared to normal subjects. This study might contribute to the general understanding of the alterations in the cellular pattern of buccal mucosa cells in diabetic patients and can be used as an additional tool to aid in the evaluation of oral mucosal alterations in diabetes mellitus.

Key Words: Cytomorphometry, diabetes, oral mucosa

INTRODUCTION:

Diabetes mellitus is one of the most common, chronic endocrine metabolic disorders, and its prevalence has been increasing worldwide.[1] The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in year 2000 and will increase to 4.4% in year 2030. The total number of people with diabetes is projected to rise from 171 million in year 2000 to 366 million in year 2030.[2] Diabetes mellitus (DM) is characterized by hyperglycemia, associated with irregularities in the metabolism of carbohydrates, lipids, and proteins.[3] Chronic hyperglycemia causes damage to the eyes, kidneys, nerves, heart and blood vessels.[4] Diagnosis of diabetes is achieved by evaluating the blood glucose levels. Either a random blood sugar estimation or analysis of fasting/post-prandial blood sugar levels is the commonest diagnostic test for diabetes. Recently, however, monitoring of glycosylated hemoglobin levels has become much commoner.[5]

It has been shown that diabetes may also cause various changes in the cells of the oral mucosa, which can be easily obtained by exfoliative cytology.[6] Application of quantitative techniques has largely improved the potential accuracy of exfoliative cytology.[7] The purpose of this study was to analyze the cytomorphometric change of buccal mucosa cells of diabetic subjects using computerized image analysis based on quantitative parameters such as nuclear and cell area, perimeter, minimum diameter, maximum diameter and their ratios, as well as to evaluate potential dysplastic transformation.
MATERIALS & METHODS:

The study was done on OPD patients of a general hospital in Bhopal for the period of 6 months on 50 Type II diabetics (Group D) and 50 control subjects (Group C). The criteria for selection of diabetic group was Type II diabetes of more than 5 years duration. The criteria for control group included healthy subjects with FBS less than 110 mg/dl. The sex ratio for both control and diabetic subjects were approximately (M:F) 1:1. The age group for both control and diabetic group was 20 to 70 years. Patients with any other systemic disease such as anemia, clinically apparent oral mucosal lesions and previous benign or malignant lesions were excluded from this study. Both control and diabetic subjects were non alcoholics and non-smokers. The smears were taken from clinically normal buccal mucosa. The subject was asked to rinse the mouth with drinking water. Taking all the aseptic precautions, a wooden spatula was then used to scrape the sample area (inner side of the cheek) three to four times with firm pressure. The scrapings were smeared on to the center of glass slide. The slides were immediately sprayed with commercially available spray fixative to ensure proper fixation. All cytological smears were stained by PAP staining technique.

Computerized Cytomorphometry:

PAP stained smears were examined under a light microscope. Only cells that were fully included in the field of vision and with clearly defined cellular and nuclear outlines were selected. Cells that were clumped or folded and cells with unusually distorted outline or nuclei were not considered for the analysis. A 640 X 400 pixel digital image was taken by a camera on the microscope with 10X eyepiece and 40X objective. Using the Image J 1.47 image analysis software, morphometric analysis of 50 cells/case was done. The following nuclear and cell morphometric features were analyzed i.e. mean nuclear area (MNA), mean nuclear perimeter (MNP), mean of maximum nuclear diameter (Max-ND), mean of minimum nuclear diameter (Min-ND), mean cell area (MCA), mean cell perimeter (MCP), mean of maximum cell diameter (Max-CD) and mean of minimum cell diameter (Min-CD). Based on the analyzed values, the nuclear to cell parameter ratios were calculated for each case.

The mean values were obtained in square micrometers for area and in micrometers for perimeter and diameter. The data obtained was subjected to Univariate analysis of variance (ANOVA) showing a significant group effect for nuclear parameters, cell parameters and ratio of nuclear to cell parameters (p value <0.001) for the two groups.

RESULTS:

This study was conducted on 100 individuals, which included 50 diabetics (Group D) and 50 controls (Group C).

In the present study, most of the diabetics were in the age group of 51-60 years (40%), followed by 41-50 years (26%), 31-40 years (22%), 61-70 years (8%) and 21-30 (4%): Table I.

The mean Fasting blood sugar (FBS) levels in Group C and Group D were 80.67 mg/dl and 196.37 mg/dl respectively.
The contents of Table 2 show results of nuclear and cellular parameters i.e. MNA, MNP, Max-ND, Min-ND, MCA, MCP, Max-CD and Min-CD respectively.

The MNA (µm²) for Group D and Group C were 77.32 ± 9.31 and 55.40 ± 10.01. The MNP (µm) of nucleus was found to be 31.87 ± 1.97 and 27.41 ± 2.59 for Group D and Group C respectively. Max-ND and Min-ND (µm) for Group D were 11.43 ± 0.704 and 8.660 ± 0.645, whereas for Group C they were 10.093 ± 0.853 and 7.010 ± 0.749 respectively. (p<0.001)

The MCA (µm²) for Group D and Group C were 2573.65 ± 368.36 and 2929.75 ± 379.20. The MCP (µm) of cell was found to be 194.32± 14.29 and 210.59 ± 18.21 for Group D and Group C respectively. Max-CD and Min-CD (µm) for Group D were 68.24 ± 4.97 and 50.13 ± 3.87, whereas for Group C those were 75.76 ± 5.13 and 52.94 ± 3.74 respectively.

The nuclear versus cell parameter ratios of both the groups were calculated and are shown herein: Table III. In Group D, the ratio of nuclear to cell area was 0.0304 ± 0.0043 compared to 0.0193 ± 0.0044 in Group C. Similarly in Group D, the N/C ratio of perimeter, maximum diameter, and minimum diameter were 0.1645 ± 0.0116, 0.1681 ± 0.0125, and 0.1734 ± 0.0151 respectively. The corresponding values in Group C were 0.1308 ± 0.0136, 0.1338 ± 0.0141 and 0.1358 ± 0.0198 respectively. (p<0.001)

The data obtained was statistically analyzed and compared for the two groups using one way ANOVA, post hoc, for nuclear parameters, cell parameters and ratio of nuclear to cell parameters and was found to be statistically significant (p value <0.001)

Table 1: Age wise distribution of Diabetics and Control subjects.

<table>
<thead>
<tr>
<th>Age group</th>
<th>control</th>
<th>%</th>
<th>diabetic</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 - 20</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21 - 30</td>
<td>7</td>
<td>14</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>31 - 40</td>
<td>17</td>
<td>34</td>
<td>11</td>
<td>22</td>
</tr>
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<td>41 - 50</td>
<td>10</td>
<td>20</td>
<td>13</td>
<td>26</td>
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<tr>
<td>51 - 60</td>
<td>12</td>
<td>24</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>61 - 70</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>TOTAL</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

DISCUSSION:

The use of oral exfoliative cytology was limited due to the subjective nature of its interpretations. This limitation in the assessment of cellular alterations can be corrected by the introduction of quantitative cytomorphometric image analysis software.
In the present study, it was found that there was a significant increase in nuclear parameters, decrease in cellular parameters and increase in ratio of nuclear to cellular parameters in smears obtained from diabetics, as compared to normal subjects. These results were comparable with the studies done by Jajarm HH et al., Alberti S et al. and Shareef B T et al., who investigated the effects of type 2 diabetes mellitus on oral epithelial cells, reported significant increase in the nuclear area (NA) with no significant difference in cellular area (CA) between type 2 diabetics and the control group. There was also a decrease in the cellular/nuclear ratio in type 2 diabetics. Opposite to our results Jajarm et al., reported CA increase in the diabetic group when compared to control groups.

Prasad H et al conducted morphologic and cytomorphometric study to analyze the effect of diabetes on oral mucosal cells. Mean values of nuclear diameter (ND), Cell diameter (CD), cytoplasmic diameter and nucleus: cytoplasm ratio (N:C ratio) were obtained for each patient. He concluded that there was a statistically significant increase in ND of diabetic patients compared to controls. Degree of glycaemia significantly affected ND (p=0.0042) and N: C ratio. In general, as the severity of diabetes increased ND and N: C ratio rise gradually.[11]

Patel V and Sheela G in their study found that the mean nuclear area and mean N/C ratio of keratinocytes was significantly higher in experimental groups (non-smoker diabetics and smoker diabetics) when compared with the control group.[12]

The cytomorphometrical changes can be attributed to the increased cellular age in patients with diabetes. A secondary reaction to ischemia caused by atherosclerosis in diabetic patients leads to decreased cellular turnover and limited production of young cells.[13]

M Suvarna et al in their study concluded that there was a statistically significant increase in average NA and significant decrease in the C/N ratio in diabetics when compared to non-diabetic healthy individuals. But the average CA did not show any statistical difference between the two groups.[14]

Zimmermann and Zimmermann suggested that the difference in cytomorphometry of oral mucosa may be related to difference in recovery rate of keratinizing cells of the oral tissue following systemic endocrine disorder like diabetes.
Ogden et al.\textsuperscript{[16]} revealed that the cytomorpho-metric changes in the buccal mucosal cells of cigarette smokers are similar to those noted in diabetics. This finding is also supported by Ramaesh et al.\textsuperscript{[17]} who reported that quantitative changes found in the buccal mucosa of smokers were attributed to the presence of larger number of non-keratinized cells of the parabasal layer. The cells were relatively smaller but having larger nuclei giving an impression of nuclear enlargement and decreased C/N ratio similar to the changes seen in type II diabetic patients. Similarly, Einstein and Sivapathasundharam\textsuperscript{[18]} also demonstrated a significant reduction in cell diameter and increase in nuclear diameter in smokers and those with a combined habit of smoking and tobacco chewing.

In the present study, we have avoided all the other possible causes that can give rise to increase in Nuclear Area (NA) by excluding subjects with any other systemic disease, clinically apparent oral mucosal lesions and previous benign or malignant lesions. Both control and diabetic subjects were non alcoholics and non-smokers.

In present study, N:C ratio appeared to be a valuable parameter, which accurately discriminated diabetic subjects from normal control group. Similar observation was also reported by Franklin and Smith.\textsuperscript{[19]} They observed that the N:C ratio has the advantage of relating nuclear volume to cellular volume and possibly represents the significant changes that occur in the cell, more accurately at a morphological level.

**CONCLUSION:**

There is increase in nuclear parameters, decrease in cellular parameters and increase in ratio of nuclear to cellular parameters in smears from diabetics as compared to normal subjects. This study might contribute to the general understanding of the alterations in the cellular pattern of buccal mucosa cells in diabetic patients and can be used as an additional tool to aid in the evaluation of oral mucosal alterations in diabetes mellitus. Further prospective studies have to be conducted with a larger sample size, and comparison with other conditions causing similar cytomorphometric changes necessary to determine the predictive value of this method.

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