Evaluation of Buccal Mucosal Cell in Type 2 Diabetic Patients: (Cytomorphometrical Study)

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Abstract: Background: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, associated with disturbances in the metabolism of carbohydrates, lipids, and protein. Diabetes can cause considerable cellular changes. Objectives: The Objective of this study was to detect the cytomorphometric measurements (the cytoplasmic diameter, nuclear diameter, and nucleus: cytoplasm ratio) for exfoliated buccal mucosal cells of type 2 diabetic patients and healthy control subjects by use the cytoplasmic smears, and to determine those measurements in different study groups. Methods: The total sample composed of 75 adult, aged 30-60 years. The study sample was divided into three groups. Group 1 was Control healthy, Group 2 was controlled diabetics with HbA1C ≤ 7.0% and Group 3 was uncontrolled diabetics with HbA1C ≥ 7.0%. Smears were obtained from normal buccal mucosa from each subject. The freshy obtained specimens stained with Papanicolaou technique for cytomorphometric analysis. An eyepiece micrometer was used to take mean values of ND, CyD, and N: C ratio. Twenty (20) clearly defined cells were measured in each case in a step wise manner, to evade quantifying cells once more. Comparison of Nuclear Diameter (ND), Cytoplasm Diameter (CyD) and ratio of Two Diameters (N: C) among three groups was performed by using ANOVA. Results: The results showed that statistically significant increase in ND (P=0.001) with statistically significant decrease in CyD (P=0.001) was found in diabetic patients compared to controls. Degree of glycemic control significantly affected ND (P=0.001) and N: C ratio (P=0.001). In general, as the severity of diabetes increases, ND and N: C ratio rise gradually. Conclusion: Diabetes produces definite cytomorphometric changes in the buccal mucosal patients. The results suggested that nuclear diameter of buccal mucosal cells increased while cytoplasmic diameter was decrease in type 2 diabetic patients.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by inappropriate blood hyperglycemia, resulting from the failure of the pancreatic beta cells to produce insulin and/or inability of the body to employ the insulin produced because of insulin deficiency in the body cells. In the body, insulin is the only hormone that reduces blood glucose levels while other hormones such as thyroid hormone, glucagon, growth hormone, catecholamine (epinephrine and norepinephrine) and glucocorticoids all elevate the blood glucose levels (7). The broad categories of DM are designated as Type1 known as Insulin Dependent diabetes mellitus (IDDM) and Type 2 known as Non-Insulin Dependent diabetes mellitus (NIDDM). Type 2 DM is a heterogeneous group of disorder usually characterized by insulin resistance, impaired insulin secretion and increase glucose production. Distinct genetic and metabolic defect in insulin action and secretion give rise to common phenotype of hyperglycemia in type 2 DM (8,9). The main complications associated with DM are retinopathy, nephropathy and/or micro/macropathy. It damages tissue repair processes and cause stochastic problems of dental interest. Several studies suggest a higher prevalence and severity of oral pathologies like gingivitis, periodontitis, candidiasis and other manifestations such as alteration of salivary flow and burning sensation (4). A significant indicator of common condition and possible diabetes complications is metabolic control, which is monitored through the blood glucose level (BGL) and glycosylated hemoglobin level (HbA1c). In 2009, an international committee for the diagnosis of diabetes recommended use of glycated haemoglobin (HbA1c), an index of average plasma glucose over several weeks, as a marker for the disease (5). This recommendation was also made by the American Diabetes Association (ADA) in 2010, which suggested that HbA1c > 6.5% (48 mmol/mol) be considered diagnostic of diabetes (6). HbA1c is considered equal to fasting plasma glucose (FPG) as a predictor of diabetes (7). Several studies have examined the deleterious effects of diabetes on oral mucosa. It was reported that diabetes adversely affects the morphology of cheek mucosa, which may compromise tissue function to favour the occurrence of oral infections and neoplasia (6,9). Oral exfoliative cytology may be more appropriate in condition like DM where invasive techniques lose viability. The morphologic and functional changes in oral mucosa can be studied at cellular level by using exfoliative cytology which can help in diagnosis with better patient acceptability. Exfoliative cytology is the study of superficial cells which have been exfoliated from mucous membrane or which have been scraped or pulled from surface. The rationale of exfoliative cytology lies in epithelial physiology. Normal epithelium undergoes continuous exfoliation or shedding of superficial cells, and it is replenished by a new crop of cells from the basal layer. These exfoliated cells are stained by various stains for example Papanicolaou (PAP) according to need. With the advancement in the field of quantitative exfoliative cytology there has been a reemergence of oral exfoliative cytology as a powerful diagnostic tool. By using cytomorphometric analysis various parameters such as cytoplasm diameter, nuclear diameter and nuclear to cytoplasmic ratio can be evaluated. CD, ND, and N: C ratio have shown to be significant in diagnosis of oral and systemic diseases (10).

2. Materials and Methods

Patients attending at the Diabetic Clinic in AL- Mawani General Hospital (Diabetic –Endocrinology Center ) in Basra city during the period from (February-2017 to June

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significant decrease in buccal mucosa for both uncontrolled and controlled diabetes groups (45.33±0.026,49.20±0.027) respectively, when compared with control healthy group (50.93±0.060). In addition, there was significant decrease in uncontrolled DM when compared with controlled DM.

Table 3 showed Cytomorphometric Comparison of N:C ratio between control and two groups of diabetes (controlled and uncontrolled) of buccal smear. The present study showed statistical significant increase in N:C ratio in buccal mucosa for uncontrolled DM (0.210±0.026) and controlled DM groups (0.180±0.014) when compared with control healthy group (0.151±0.013). Also there’s significant increase for uncontrolled DM when compare with controlled DM. The N:C ratio again showed similar trends as the ND. It is raised significantly in the UCD group when it is compared with the other groups (P value=0.001)

### Table 1: Cytomorphometric Comparison of ND between control and two groups of diabetes of buccal smear (Mean± SD)

<table>
<thead>
<tr>
<th>Cell parameter</th>
<th>Nuclear Diameter (ND)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Buccal mucosa</td>
</tr>
<tr>
<td>Control healthy</td>
<td>7.72 ±0.58</td>
</tr>
<tr>
<td>Controlled DM</td>
<td>8.88 ±0.36</td>
</tr>
<tr>
<td>Uncontrolled DM</td>
<td>9.89 ±0.52</td>
</tr>
</tbody>
</table>

* A significant if p-value less than 0.05 (p<0.05)

### Table 2: Cytomorphometric Comparison of CyD between control and two groups of diabetes of buccal smear (Mean± SD)

<table>
<thead>
<tr>
<th>Cell parameter</th>
<th>Cytoplasm Diameter (CyD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>groups</td>
<td>Buccal mucosa to multiple comparison</td>
</tr>
<tr>
<td>Control healthy</td>
<td>50.93±0.060</td>
</tr>
<tr>
<td>Controlled DM</td>
<td>49.20±0.027</td>
</tr>
<tr>
<td>Uncontrolled DM</td>
<td>45.33±0.026</td>
</tr>
</tbody>
</table>

* A significant if p-value less than 0.05 (p<0.05)

### Table 3: Cytomorphometric Comparison of Nuclear/Cytoplasmic ratio between control and two groups of diabetes of buccal smear (Mean± SD)

<table>
<thead>
<tr>
<th>Cell parameter</th>
<th>Nuclear/Cytoplasmic ratio (N/CyD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Buccal mucosa to multiple comparison</td>
</tr>
<tr>
<td>Control healthy</td>
<td>0.151±0.013</td>
</tr>
<tr>
<td>Controlled DM</td>
<td>0.180±0.014</td>
</tr>
<tr>
<td>Uncontrolled DM</td>
<td>0.210±0.026</td>
</tr>
</tbody>
</table>

* A significant if p-value less than 0.05 (p<0.05)

3. Results

Out of the total 75 participants who were included in the study (25 patients were with uncontrolled diabetes, 25 were controlled diabetes patients which came to the diabetic clinic randomly and 25 were healthy control patients).

Table 1 showed Cytomorphometric Comparison of ND between control and two groups of diabetes (controlled and uncontrolled) of buccal smear. It was noted that the mean nuclear diameter ND rises steadily from the control group (7.72±0.58) to the uncontrolled diabetic group (9.89±0.52). ND in the UCD group is raised significantly when it is compared with the other groups.

Table 2 showed Cytomorphometric Comparison of CyD between control and two groups of diabetes (controlled and uncontrolled) of buccal smear. The results of CyD showed

The smear was taken from buccal mucosa. Before sample was taken the patients was asked to wash their mouth with tap water to remove any debris, then cells from right and left buccal mucosa were collected by using a cytobrush of Pap smear (disposable kit of pap smear). Unttaminated, new, dry glass slides were used to fix the smears. Scrapings were placed on the middle of glass slide and spread over a large area to avoid clumping of cells. The smears were fixed in 95% ethanol and stained by the Papanicolaou method. An eyepiece micrometer was used to obtain the nuclear/cytoplasmic diameter (CyD) and nuclear diameter (ND), Cytoplasmic diameter (CyD) and nucleus to cytoplasm ratio (N:C). Continuous variables were presented as Mean ± standard deviation (SD). Analysis of variance was applied to associate means of quantitative data in 3 groups. Data were analyzed using SPSS version 22 for Windows and p values less than 0.05 were considered statistically significant.

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close results when compared to this study for the variable of ND and found the nuclear diameter showed gradual increase in size from control to uncontrolled diabetic group (13). This finding are also in consistent with several studies (14,15). Where found the nuclear change was significantly higher in diabetic group.

The reason for ND increase among the study group might related to sustained hyperglycemia which could be explained by delay in keratinization of oral epithelium, effects of ageing, dehydration/atrophy, and inflammatory process. Delay in the keratinization is attributed to glycation changes. Sustained hyperglycemia causes greater accumulation of advanced glycation end products by abnormal glycation of proteins, lipids, and nucleic acids in the walls of large blood vessels as well as in the basement membrane of the microvasculature. The progressive narrowing of the vessel lumen leads to decreased perfusion of the affected tissue and consequently decreases cell turnover, thereby explaining the delay in the keratinization process of the epithelium. This delay in the process of epithelial differentiation leads to an increase in the number of mature cells, which present a large nucleus as a primary characteristic (18,20).

Another parameter assessed was cytoplasm diameter, the results of the present study showed the difference in mean of CyD between study groups was statistically significant decrease in buccal mucosa cells from patients with uncontrolled and controlled diabetes when compared to control patients. This is similar to the findings of Prasad et al. noted a clear and definite decrease in cytoplasmic diameter in uncontrolled diabetes (12). Also agree with Shareef et al. in a similar study found a statistically significant decrease in CyA, which could be due to the cell shrinkage caused by dehydration (16). But contradictory to the findings of Jajarm et al. who reported a significant increase in cytoplasmic area the in diabetic patients (15).

In the present study, a significant increase was found in the mean values of N:C ratio of the exfoliated cells from the buccal smear with uncontrolled and controlled diabetes when compared to control patients. A gradual increase in N:C ratio is noticed as progress from control healthy group to uncontrolled diabetics which is highly significant. The NCR values are coincident with the findings of Prasad et al. (12). This finding inconsistent with the findings by Jajarm et al. according to him the mean N:CR was significantly lower in diabetic group as compared to controls (15).

4. Discussion

The present study was conducted to assess cytomorphometric changes in the mucosal cells which were taken from buccal site in type 2 diabetic patients. The analysis tool used for these exfoliative cells was the exfoliative cytology followed by cytomorphometric analysis of these cells, which is already accepted as a very simple, low cost, rapid and painless tool for the diagnosis of oral pathologies (16). In this study measured the ND, CD, CyD and N:C ratio of exfoliated buccal mucosa cells using a micrometer in the diabetic patients (n=50) and control subjects (n=25). The diabetic patients were grouped under grades of glycemic control, based on their HbA1c levels. Analysis of the three study parameters between these groups showed that glycemic control definitely influenced ND, CyD and N:C ratio, each parameter was individually compared across control group and various grades of diabetes.

The main parameters for the cytomorphometric analysis are the diameter of nucleus and cytoplasm.

The first parameter assessed was nuclear diameter, which was increased in diabetics. The ND showed a consistent and uniform increase in diameter from control to uncontrolled diabetic group. This increase in ND is highly significant in uncontrolled diabetic patients when compared to controlled DM2 patients and to control healthy group. This finding concurs with the study by Prasad et al. where he found that diabetes severity (or in other words, the amount of glycemic control), measured with Hb1Ac, definitely influenced ND (12). Also agree with other study by Suidia et al. that gave the

5. Conclusion

- There is a clear and definite increase in ND as we progress from normal individuals to patients with uncontrolled diabetes.
- In contrast to the increase in ND, there is a decrease in CyD in uncontrolled diabetes.
- The N:C ratio gradually and steadily increases from normal individuals to uncontrolled diabetics.

Figure 1: Pap stained cytological smear of normal healthy group (×40).

Figure 2: Pap stained cytological smear of diabetic group (×40).
From the present study, we conclude that type 2 diabetes produces definite morphometric changes in the exfoliated buccal mucosal cells.

References