A Phase Contrast Cytomorphometric Study of Oral Exfoliated Cells in Diabetes Mellitus Patients - An Original Study

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Abstract

Background of Study: Diabetes is the most common endocrine-metabolic disease affecting worldwide. Diagnosis of type 2 diabetes with the help of oral exfoliative cytology is a simple, noninvasive technique. Evaluation of cytomorphometric changes in exfoliated buccal cells in diabetes can help in the early detection and examination of diabetes. Aim: The aim is to study and compare the cytomorphometric features of buccal mucosal cells of normal individuals and type 2 diabetes patients and to evaluate the role of phase-contrast microscopy for visualizing cytomorphological changes in unstained smears. Materials and Methods: Fifty known type 2 diabetic patients and fifty healthy individuals were taken as study group and control group, respectively. Smears were prepared from the buccal mucosa of both study and control groups. Fixed, unstained smears were viewed under phase-contrast microscope, and cytomorphometric analysis was done using image analysis software for evaluating nuclear area (NA), cytoplasmic area (CyA), and nuclear/cytoplasmic ratio. Results: A statistically significant increase in NA ($P = 0.000$), CyA ($P = 0.000$), and N/Cy ratio ($P = 0.000$) was obtained from the smears of the study group when compared with the control group. Conclusions: Cytomorphometric analysis of exfoliated cells can aid in predicting systemic disease such as type 2 diabetes. With the use of phase-contrast microscope, the cellular details can be easily and quickly evaluated and it is a cost-effective method in cytomorphometric studies.

Keywords: Cytomorphometry, diabetes mellitus, oral exfoliative cytology, phase-contrast microscopy

INTRODUCTION

Diabetes mellitus (DM) designates a group of metabolic diseases characterized by chronic hyperglycemia as a result of defects in insulin secretion, insulin action, or both. Along with hyperglycemia, insulin deficiency also leads to abnormalities in carbohydrates, lipids, and protein metabolism.[1] Diabetes is classified as type 1 diabetes, type 2 diabetes, and gestational DM, and specific types of diabetes are due to other causes such as monogenic diabetes syndromes, diseases of the exocrine pancreas, and drug- or chemical-induced diabetes.

In recent years, it was estimated that there are more than 500 million prevalent cases of type 2 diabetes worldwide. The number was expected to increase to 693 million by 2045. Approximately 5 million deaths worldwide were ascribed to diabetes in 20–99 years’ age range. It was found that the prevalence will increase in all countries covered over the next coming years, but the greatest growth will be experienced in low-income countries.[2,3]

DM can manifest many oral complications such as xerostomia, candidiasis, gingivitis, periodontitis, increased incidence of dental caries, periapical abscess, and traumatic ulcers.[4]

Diagnosis of diabetes is usually done by blood examination, which is an invasive and stressful situation for the patients and is more time-consuming. Therefore, there is a need to find a new diagnostic method that can take the place or can be comparable to the results obtained by the routine invasive method and thus providing better patient comfort and calm. In such circumstances, exfoliative cytology of oral mucosal cells, which is a straightforward, painless, and noninvasive method, became an attractive option for early diagnosis and screening of diabetes. It is a simple, noninvasive technique that is easy to perform and has the advantage of being less invasive than the routine blood tests.

Aims of the Study: The aims of the study are to compare the cytomorphometric features of buccal mucosal cells of normal individuals and type 2 diabetes patients and to evaluate the role of phase-contrast microscopy for visualizing cytomorphological changes in unstained smears.

Materials and Methods:

Fifty known type 2 diabetic patients and fifty healthy individuals were taken as study group and control group, respectively. Smears were prepared from the buccal mucosa of both study and control groups. Fixed, unstained smears were viewed under phase-contrast microscope, and cytomorphometric analysis was done using image analysis software for evaluating nuclear area (NA), cytoplasmic area (CyA), and nuclear/cytoplasmic ratio.

Results:

A statistically significant increase in NA ($P = 0.000$), CyA ($P = 0.000$), and N/Cy ratio ($P = 0.000$) was obtained from the smears of the study group when compared with the control group.

Conclusions:

Cytomorphometric analysis of exfoliated cells can aid in predicting systemic disease such as type 2 diabetes. With the use of phase-contrast microscope, the cellular details can be easily and quickly evaluated and it is a cost-effective method in cytomorphometric studies.

Keywords: Cytomorphometry, diabetes mellitus, oral exfoliative cytology, phase-contrast microscopy

Access this article online

Quick Response Code:  
Website:  www.ijds.in  
DOI: 10.4103/IJDS.IJDS_10_19

How to cite this article: Mridula RG, Akhil S, Kumar RB, Thomas J, Saji AM, Iype AK. A phase contrast cytomorphometric study of oral exfoliated cells in Diabetes mellitus patients - An original study. Indian J Dent Sci 2019;11:103-7.
Diagnostic method, can be well thought-out as a more practical technique to diagnose the disease.\textsuperscript{[5]}

Quantitative cytomorphometric assessment of exfoliated cells has become a valuable diagnostic tool during these years. Parameters such as nuclear area (NA), cellular area, nuclear/cytoplasmic ratio, nuclear shape, nuclear discontinuity, optical density, and nuclear texture can be evaluated for the diagnosis of diabetes.\textsuperscript{[6]}

Even though these cellular features of exfoliated cells can be analyzed with the help of light microscopy, the visualization of viable cellular specimens is complex and it can be enhanced by the use of special microscopy such as phase-contrast microscopy. It provides improved observation of very transparent objects which are almost invisible by ordinary transmitted light, in clear detail and in good contrast to their surroundings, and to see very small differences in thickness and density within the cell by depicting the differences in refractive indices between regions of the specimen visible in the form of differences in intensity resulting in high-contrast images.\textsuperscript{[7]}

Therefore, to study the morphologic changes in the exfoliated cells, phase-contrast microscopy can be a useful technique.

The present study attempted to determine the cytomorphometric changes taking place in the cells of the buccal mucosa in type 2 diabetic patients and assess the role of phase-contrast microscopy as a substitute and straightforward method of cytological evaluation of wet and unstained smears.

**Materials and Methods**

This study was conducted in the Department of Oral Pathology and Microbiology, Malabar Dental College and Research Centre, on a total of fifty known type 2 DM patients and healthy individuals each.

**Inclusion criteria**

Fifty each of known type 2 diabetic patients and healthy individuals were included after obtaining informed consent.

Type 2 DM patients aged between and above 40 years with a minimum duration of 1 year, irrespective of whether they were under any medication for diabetes or not, and participants with clinically healthy oral mucosa were included.

Patients were considered as diabetic when the fasting blood sugar (FBS) level was $\geq 126$ mg/dl (as per the American Diabetes Association criteria for the diagnosis of DM), whereas patients with FBS levels $<110$ mg/dl were considered normal and random serum glucose concentration $>200$ mg/dl (11.1 mM).

For the control group, participants with clinically healthy oral mucosa without clinical signs of systemic diseases and positive laboratory findings (absence of diabetes) were included.

**Smear preparation**

The smears were taken from the buccal mucosa of the study and control groups and spread it over a large area to prevent clumping of cells on a clean, fresh, and dry glass slides. The smears were fixed immediately using biofix spray.

**Cytomorphometric analysis**

Exfoliated cells were evaluated using a phase-contrast microscope with camera attachment.

In each slide, areas with uniformly spread exfoliated cells were viewed through $\times 10$ and $\times 20$ magnifications and images were captured. Cytomorphometric analysis was done using IS capture image analysis software for evaluating NA, cellular area, and nuclear/cytoplasmic ratio.

Cell area was measured in square microns (µm$^2$). For measurements, the cell boundary was traced with digitalized cursor using interactive measurement tool, and the software automatically calculated the cell area [Figure 1]. NA was measured in square micron (µm$^2$) automatically by tracing the nuclear boundary [Figure 2]. Cytoplasmic area (CyA) was calculated using the formula: $\text{CyA} = \text{cell area} - \text{NA}$. Nuclear/cytoplasmic ratio (N/Cy ratio) was another parameter calculated using the formula: $\text{NCR} = \text{NA}/\text{CyA}$.

**Results**

The exfoliated cells showed a considerable change in the NA, CyA, and nuclear/cytoplasmic (N/Cy) ratio in the diabetic group on comparison with the control group.

The mean NA obtained was $4.68$ [Graph 1], CyA $146.82$ [Graph 2], and nuclear/cytoplasmic ratio was $0.038$ [Graph 3] in the DM group [Table 1].

The cytomorphometric data were statistically assessed between DM and control groups by independent sample test. The NA and CyA of the exfoliated cells were more in the study group compared to the control group with $P = 0.000$ ($P < 0.05$), which was highly significant. The nuclear/cytoplasmic ratio was found to be increased in the study group than the control group with a statistically significant result with $P = 0.000$ ($P < 0.05$) [Table 2]. The mean NA, CyA, and N/Cy

**Figure 1:** Phase contrast ($\times 100$) of exfoliated buccal cells showing morphometric measurement of cell area.
Deleterious effects of DM on the oral mucosa, which have shown the morphological alterations on the oral mucosa resulting in retarded tissue function and promotion of oral infection and oral neoplasia. In diabetes, changes in the biological structures occur at the cellular level which can be found by simple studies on the exfoliated cells. The changes are caused due to the loss of oxidation equilibrium which causes the depression of the activities of the antioxidant scavengers and enzymes because of the high concentration of glucose, excessive formation of free radicals, and protein glycation.

We conducted a study to evaluate the cytomorphometric changes in exfoliated cells of the buccal mucosal cells of known type 2 DM patients. The study revealed a significant increase in the NA, CyA, and N/Cy ratio in the study group when compared to the control group.

The NA of the exfoliated cells showed a consistent increase in the study group compared to the control group. This finding was in consistent with studies done by Nandita et al.,[8] and Sahu et al.,[10] where they found a significant increase in the NA in diabetic patients.

| Table 1: Group statistics for various parameters |
| Parameter | Group | n  | Mean   | SD   |
|-----------|-------|----|--------|------|
| NA        | Case  | 50 | 4.68   | 0.94 |
|           | Control | 50 | 2.67   | 0.73 |
| CyA       | Case  | 50 | 146.83 | 56.49|
|           | Control | 50 | 104.37 | 16.04|
| N/Cy      | Case  | 50 | 0.038  | 0.01 |
|           | Control | 50 | 0.025  | 0.007|

NA: Nuclear area, CyA: Cytoplasmic area, N/Cy ratio: Nuclear/cytoplasmic ratio, SD: Standard deviation

**DISCUSSION**

Type 2 DM is the fifth most common chronic condition among the elderly worldwide. It exhibits a number of common oral manifestations. Early diagnosis of DM is always helpful in control of blood sugar level at an early stage to prevent its various complications. Many studies have revealed the deleterious effects of DM on the oral mucosa, which have shown the morphological alterations on the oral mucosa resulting in retarded tissue function and promotion of oral infection and oral neoplasia. In diabetes, changes in the biological structures occur at the cellular level which can be found by simple studies on the exfoliated cells. The changes are caused due to the loss of oxidation equilibrium which causes the depression of the activities of the antioxidant scavengers and enzymes because of the high concentration of glucose, excessive formation of free radicals, and protein glycation.

We conducted a study to evaluate the cytomorphometric changes in exfoliated cells of the buccal mucosal cells of known type 2 DM patients. The study revealed a significant increase in the NA, CyA, and N/Cy ratio in the study group when compared to the control group.
The increase in the NA can be attributed to the metabolic control in diabetic state and factors such as reduction in epithelial nourishment, proliferation, and turnover, which occurs secondarily due to the microvascular and metabolic disorders that may be accompanied by the reduction in the stimulatory effect of insulin and insulin-like growth factor 1 on keratinocyte.[10] The presence of the sustained hyperglycemia causes accumulation of glycation 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. This progressive narrowing of the vessel lumen leads to decreased perfusion of the affected tissue and consequently decreases cell turnover and hence explains the cause for the delay in the keratinization process of the epithelium. This, in turn, delays the process of epithelial differentiation which leads to an increase in the number of mature cells which show a large nucleus as a primary characteristic.[9] The reduction in the salivary flow in Diabetes patients causes dryness of mouth and results in mucosal atrophy or ulceration, which causes exfoliation of basal and parabasal cells during exfoliative cytology. Thus the cells acquired are more of parabasal cells which are smaller in size but have relatively large nuclei, therefore giving an impression of nuclear enlargement.

We found that the CyA showed an increase in the study group compared to the control group. This result was in accordance with the study done by Jafarm et al,[13] and Lamichhane et al,[9] where they got a significant increase in CyA in the study group.

The increase in the CyA in the diabetic group could be because of the altered cell membrane integrity leading to excessive accumulation of lipid droplets in the cytoplasm, hence creating an increased intercellular space. Caldeira et al. reported that in a study on experimental animal, in DM, there are associated changes in the cells on microscopic examination such as nuclear-cellular pleomorphism, ill-defined cell layers, reduced number of cell organelles, cell membrane disorganization, the accumulation of lipid droplets within the cytoplasm, increased intercellular space, and slight superficial desquamation.[13]

The other parameter evaluated was the nuclear/cytoplasmic ratio (N/Cy ratio) of the exfoliated cells. We obtained a significant increase in the nuclear/cytoplasmic ratio of the diabetic mellitus patients compared to the control group. This finding was also in consistent with the study of Lamichhane et al.[9]

The determination of the severity of DM and the degree of control of glycemia is always beneficial taking into consideration of the adverse effects of DM on the oral mucosa. Exfoliative cytology being a noninvasive procedure can be used easily by a dentist as an adjunct for screening as in case of suspecting of diabetes. The advantages of using phase-contrast microscopy over light microscopy are that the exfoliated cells can be viewed more accurately without the interference of the staining procedures and are easier and rapid in slide preparation. It is also ideal for interpreting thin specimens and the internal structures of a particle and can detect even a small number of protein molecules. The most important emphasize of using this microscope is the ability to observe living particles in a living state.

## Conclusions

In the present study, there was an increase in NA, CyA, and nuclear/cytoplasmic ratio in the exfoliated cells of type 2 DM patients compared to the control group. This study, therefore, brings to notice the cellular alterations in the buccal mucosal cells in type 2 DM patients which can be easily evaluated by the simple exfoliative cytology. Hence, cytornophometric analysis of the exfoliated cells also being a noninvasive method can be used as a means for the mass screening and monitoring of DM.

## Financial support and sponsorship
Nil.

## Conflicts of interest
There are no conflicts of interest.

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