Original Article

Comparative Evaluation of Buccal Exfoliated Cells in Individuals with Diabetes Mellitus and Healthy Controls: A Cytomorphometric Analysis

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Abstract

Background: Diabetes mellitus is the third most frequent cause of mortality and morbidity worldwide. Patients with diabetes exhibit a variety of oral symptoms, and hence the early detection of this condition can be addressed by a dentist. **Aim:** The current study aimed to study the cytomorphometric alterations of buccal exfoliated cells in individuals with type II diabetes mellitus. **Methodology:** The study included thirty diabetics and thirty healthy controls. The smears were obtained from the buccal mucosa and stained with Papanicolaou stain and hematoxylin and eosin stain. The presence of inflammatory cells, microbial carriage, nuclear enlargement, and perinuclear halo and binucleation were examined on the slides. Cellular and nuclear parameters were quantitatively measured using Image J software. Statistical analysis was done using SPSS software, and the Student's *t*-test was employed. **Results:** No inflammatory cells or microbes were observed in Group I individuals; however, the perinuclear halo was observed in 16.6% and binucleated cells in 3.3% of the controls. Inflammatory cells, consisting mainly of neutrophils and lymphocytes were seen in 40%, microbial carriage in 26.6%, perinuclear halo in 73.3%, and binucleated cells in 36.6% of the diabetic patients. The mean nuclear diameter, area, and nuclear-cytoplasmic ratio were significantly high in diabetic patients when compared to healthy controls. **Conclusion:** Oral exfoliated mucosal cells of patients with diabetes mellitus exhibit distinct cytomorphometric alterations such as increased nuclear diameter, nuclear area, and nuclear-cytoplasmic ratio.

Keywords: Cytology, cytomorphometry, diabetes mellitus, nuclear size, oral exfoliated cells, Papanicolaou stain

Introduction

Diabetes mellitus is a noncommunicable metabolic disorder with the potential to impede the health services of both developed and developing countries. It is defined by the presence of chronic hyperglycemic state with altered carbohydrate, lipid, and protein metabolism due to inadequate insulin production or insulin action.[1] The type 2 diabetes mellitus is more prevalent in urban population (11.6%) when compared to rural population (2.4%).[2] Diabetes mellitus can serve as a predisposing factor for impaired oral health. Patients with diabetes mellitus are proven to have increased frequency of periodontitis, dental caries, xerostomia, taste alteration, periapical abscess, traumatic ulcers, lichen planus, glossodynia, lichenoid mucositis, and irritational fibromas.[3,4] They also have altered oral microflora, which increases their susceptibility to *Candida albicans*.[5]

Exfoliative cytology can be used as a safe, simple, painless, and a noninvasive procedure to assess changes in the oral mucosa when compared to the conventional pathological examinations.[6] Uncontrolled diabetes mellitus is associated with several oral changes due to increased salivary glucose levels, decreased salivary secretion, microvascular changes, impaired chemotaxis and phagocytosis, and delayed healing. Several researchers have

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shown that the changes in oral mucosal cells can be revealed by morphometric analysis of cytological smears.[3,7,8] Thus, this study aimed to determine the morphological alterations observed in the exfoliated cells of individuals with diabetic mellitus by comparing it with that of healthy controls.

Methodology

This cross-sectional study was conducted after obtaining approval from the Institutional Ethical Committee (SRMU/MandHS/SRMDC/2021/PG/009). All procedures performed in the study were conducted in accordance with the ethical standards given in the 1964 Declaration of Helsinki, as revised in 2013.

Study participants

Thirty healthy individuals (controls) and thirty known diabetic individuals (cases) were included in the study by convenience sampling method after obtaining written, informed consent from them. Individuals who were apparently healthy with random blood glucose levels of 80–120 mg/dL and HbA1c <5.7% were included under Group I and type 2 diabetic individuals with random blood glucose levels >120 mg/dL and HbA1c >6.5% were included under Group II. The participants from both the groups were between the age group of 30–50 years. The patient's complete case history was recorded before enrolling them in the study. The participants from both control and case groups with habits such as smoking, tobacco chewing, or alcohol consumption were excluded from the study. Individuals with oral lesions or any other systemic disease, and participants who were under medication for any other systemic diseases apart from diabetes, were not included in the study.

Before sample collection, participants were instructed to rinse off their oral cavity using normal saline to flush off the debris and was dried with a gauze. A commercially available sterile wooden spatula was used to collect smear sample for exfoliative cytology. A unidirectional and gentle scraping stroke was given over the buccal mucosa to obtain surface cells. Two scrapes were taken from each of the participants and smeared on a glass slide, fixed, and then stained with Papanicolaou (PAP) and hematoxylin and eosin (H and E). The slides were looked in for qualitative changes such as the presence of inflammatory cells, microbial carriage, perinuclear halo, and binucleation. Fifty H- and E-stained cells with well-defined borders were selected by systematic sampling,[3] and their photomicrographs were subjected to cytomorphometric analysis Image J version 1.8 (National Institute of Health Bethesda, Maryland, United States). The photomicrographs were standardized as described by Ramadoss *et al*.,[8] and the quantitative changes such as diameter of the nucleus [Figure 1], cell diameter, nuclear area, and nuclear–cytoplasmic ratio were evaluated. The cellular and nuclear diameter and area were taken in three high-power fields, and their mean was calculated.

Statistics

Data analysis was done using SPSS for Windows (SPSS Inc., Chicago, IL, USA) version 16. Unpaired Student's *t*-test was used to compare various cytomorphometric parameters, and a $P < 0.05$ was the level of significance considered for the study. Pearson's correlation coefficient was used to correlate HbA1c values and nuclear area and diameter between the groups.

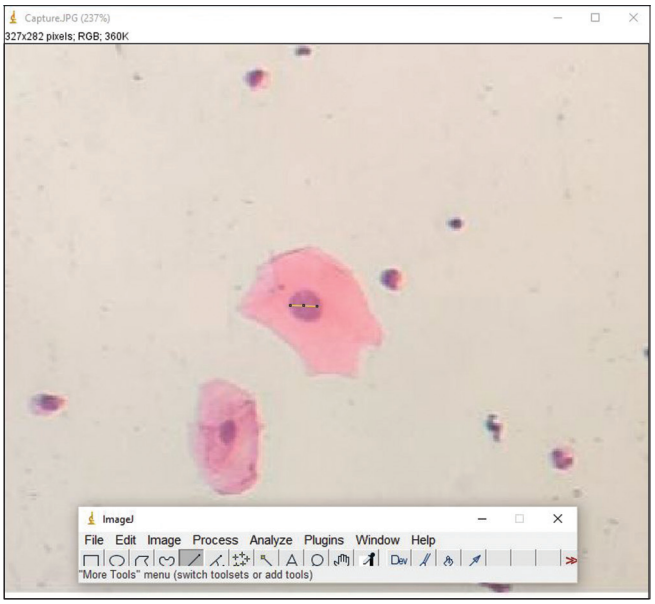

Figure 1: Increased nuclear diameter observed in a diabetic patient measured using image j software

Results

In our study, each group comprised 15 men and 15 women. The average age of the participants in Group I was 48.56 ± 15.8 years and in Group II was 42.37 ± 32.57. The mean random blood glucose and HbA1c levels were 93.98 ± 8.65 mg/dl and 4.25 ± 0.73% for Group I and 188.70 ± 17.22 mg/dl and 8.14 ± 1.01% for Group II, respectively. Inflammatory cells, consisting mainly of neutrophils and lymphocytes, were seen in 40%, microbial carriage in 26.6%, perinuclear halo in 73.3%, and binucleated cells in 36.6% of the Group II patients. Perinuclear halo was clearly evident in PAP-stained smears. Most of the cells were from the surface layer (orange color) and subsurface layer (blue–green). Inflammatory cells [Figure 2a] and microorganisms [Figure 2b] were better evident in H- and E-stained smears. Candidal hyphae [Figure 2c] were observed in one of the samples. No inflammatory cells or microbes were observed in Group I individuals; however, perinuclear halo [Figure 3] was observed in 16.6% and binucleated cells in 3.3% of the controls [Figure 4]. The mean nuclear diameter (11.59 ± 3.39 μm) was more in Group II with $P = 0.0004$, whereas the cytoplasmic diameter (45 ± 4.86 μm) was more in Group I [Table 1] with $P = 0.4891$. The mean nuclear area and nuclear-cytoplasmic ratio [Table 2] were more in Group II (90.43 ± 3.85 μm^2 and 0.03 ± 0.003) when compared to Group I (75.17 ± 2.62 μm^2 and 0.02 ± 0.002). When gender-wise comparison was done, it was found that only small statistically nonsignificant differences were observed between males and females for all the parameters [Figure 5].

Table 1: Comparison of nuclear diameter/cytoplasmic diameter between groups

Group	Nuclear diameter (μm)		C hba1c hba1c ell diameter (μm)		Cytoplasmic diameter (μm)		Nuclear diameter/ cytoplasmic diameter	
	Mean±SD	*P*	Mean±SD	*P*	Mean±SD	*P*	Mean±SD	*P*
Group I	9.07±1.86	0.0004	54.07±4.03	0.0039	45±4.86	0.4891	0.21±0.06	0.0044
Group II	11.59±3.39		56.57±2.77		44.97±4.36		0.27±0.10	

SD: Standard deviation

Table 2: Comparison of nuclear area/cytoplasmic area between groups

Group	Nuclear area (μm²)		Cytoplasmic area (μm²)		Nuclear cytoplasmic ratio	
	Mean±SD	*P*	Mean±SD	*P*	Mean±SD	*P*
Group I	75.17±2.62	0.00001	3200±402.82	0.0185	0.02±0.002	0.00001
Group II	90.43±3.85		3003.33±369.62		0.03±0.003	

SD: Standard deviation

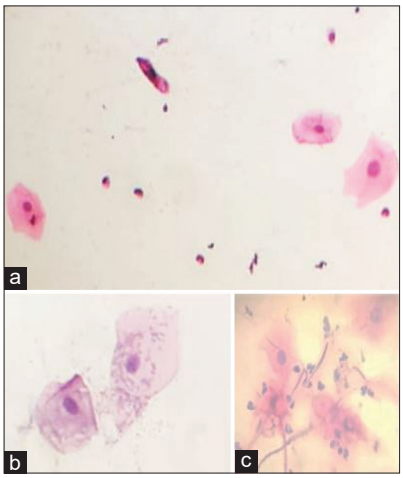

Figure 2: Exfoliated cells from diabetic patient exhibiting inflammatory cells (a), microbial carriage (b), candidal hyphae (c)

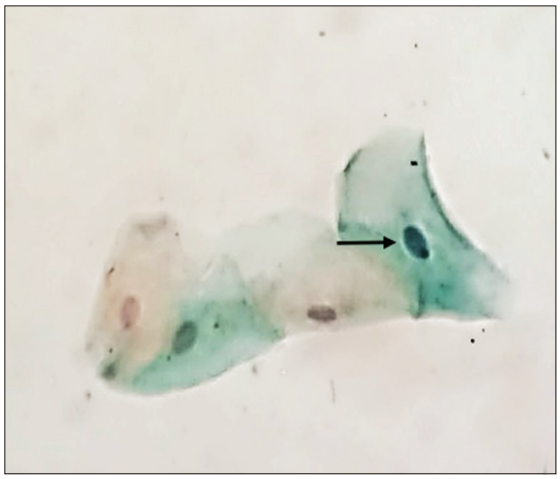

Figure 3: Perinuclear halo

HbA1c values positively correlated with the nuclear area and diameter between the groups; however, it was weak and not significant [Table 3].

Discussion

It is reported that dentists come across treating more diabetic patients when compared to the other specialties.[9,10] Hence, it becomes an important responsibility of the dentist to detect and treat these patients with an utmost care. Early and prompt identification of the oral changes caused by diabetes can help to interfere from progressing into advanced pathology. Since these patients are more prone to infections and have a poor healing capacity, it becomes a challenge for the dentist to perform invasive procedures to diagnose the lesions. The exfoliative cytology in such cases acts as a boon in diagnosing. However, its use in the assessment of cellular alterations is restricted since interpretations are subjective and chance of getting false-negative result is more. These limitations can be overcome using image analysis. This makes it easier to use and more reliable.[9]

Pap stain is a polychromatic differential stain, commonly employed for staining cytological smears. It provides good nuclear details and cytoplasmic transparency. In a study by Panneerselvam *et al*., it was found that cytological details were well appreciated with conventional PAP stain.[11] H and E stain is a gold standard technique used for staining tissues. In the current study, both PAP and H and E stains were used, and it was found that nuclear changes and inflammatory cells were better appreciated with H- and E-stained slides. Inflammatory cells were more commonly observed in diabetes patients which could be due to a positive feedback mechanisms to compensate for the impaired immune response. Prolonged hyperglycemia observed in diabetes can result in increased oxidative stress and exaggerated inflammatory response due to the production of advanced glycated end products and alterations in oral microbial flora. In our study, microbes were most commonly seen in diabetics which may be attributed to the reduced efficiency of neutrophils, monocytes, and macrophages to kill the microbes.[12] Age, the use of denture, increased glucose levels in saliva, low salivary pH, and xerostomia facilitate colonization of Candida in individuals with diabetes

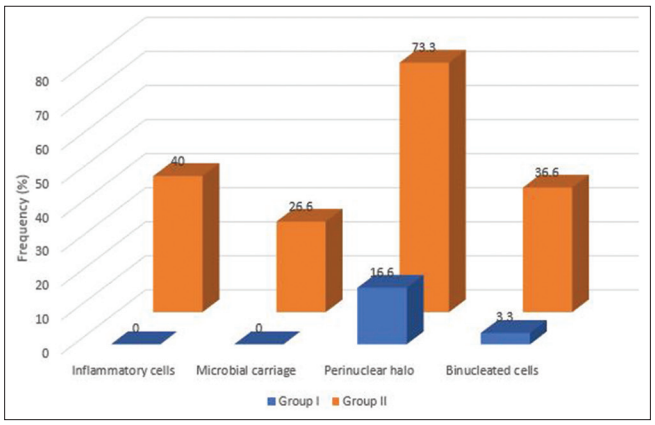

Figure 4: Qualitative alterations seen in the oral exfoliated cells

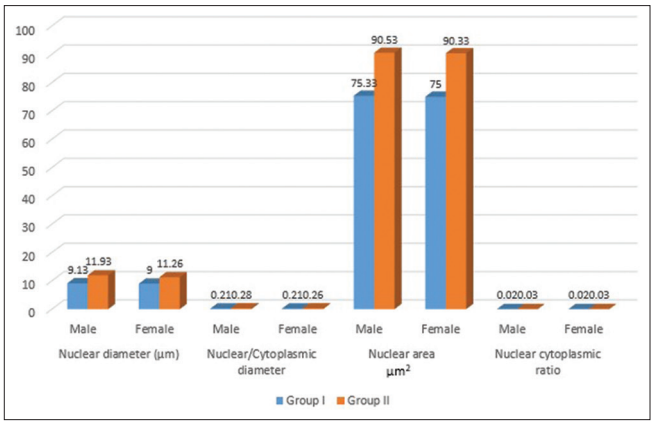

Figure 5: Gender comparison of cytomorphometric parameters

Table 3: Correltation of hemoglobin A1c levels and nuclear diameter and area

HbA1c levels	Nuclear diameter		Nuclear area	
	R	*P*	*R*	*P*
Group I	0.25	0.18	0.03	0.87
Group II	0.14	0.46	0.03	0.87

HbA1c: Hemoglobin A1c

mellitus.[13] Binucleation and perinuclear halo observed in Group II patients could be due to the accelerated cellular aging observed in diabetic state. Insulin regulates functional organization of cytoskeleton and persistent insulin deficiency causes disruption of microtubules. Since microtubules are concentrated the nucleus, its disruption in diabetes mellitus might result in perinuclear halo formation.[14]

The increase in mean nuclear diameter and nuclear area observed in the diabetic group complies with the results of few other similar studies.[3,9,15,16] In a study done by Alberti *et al*., nuclear area was larger, and the cytoplasm: nucleus was 37.4% smaller in diabetics in comparison to the healthy individuals. Ramana Reddy *et al*. similarly observed that nuclear area was significantly increased in diabetic patients, whereas cytoplasmic/nuclear ratio was significantly less in diabetic individuals. However, there was no statistically significant difference in cytoplasmic area between the two groups.[9] Increase in nuclear size could be due to the decrease in cell turnover occurring in response to ischemia following atherosclerosis, seen in diabetes.[16] Decreased salivary flow rates caused by systemic dehydration, usage of drugs, and membranopathy of salivary ducts, can result in the loss of oral mucosal cells due to trauma. To replenish the lost cells, the basal cells actively divide. In actively dividing cells, the amount of cytoplasm reduces with concomitant increase in nuclear contents.[17] This could be the reason for decreased cytoplasmic area observed in the current study. Xerostomia can also cause mucosal atrophy. When the cytology of atrophic oral mucosa is done, there are greater chances of obtaining the basal and parabasal cells with large nucleus in the smear.[16]

When intragroup gender comparison was done, the results were not statistically significant implying that gender does not have a key role in determining cellular morphology. A similar result was also observed by Sahay *et al*. and Balan *et al*.[18,19] In our study, HbA1c values positively correlated with the nuclear area and nuclear diameter. Prasad *et al*. observed a uniform increase in the nuclear diameter with different grades of glycemic control, from well-controlled to poorly controlled diabetes. They concluded that the degree of glycemic control significantly affected nuclear diameter and nuclear-cytoplasmic ratio.[16]

It is evident from the current study that qualitative as well as quantitative assessment of oral mucosal cells is essential in individuals with type II diabetes mellitus. However, there are several other confounding factors which the patient may not be aware of like nutritional deficiencies that cause similar cellular changes.

Conclusion

Oral exfoliated cells of individuals with type 2 diabetes mellitus exhibit distinct cytomorphometric alterations such as increased nuclear diameter, nuclear area, and nuclear-cytoplasmic ratio. Further studies with more sample size are essential to validate exfoliative cytology as a preliminary diagnostic tool in the clinical practice.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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