

# Exfoliative cytology of buccal squames: A quantitative cytomorphometric analysis of patients with diabetes

Bharat Sankhla, Abhishek Sharma<sup>1</sup>, Raju Singam Shetty<sup>2</sup>, Sheetal Chowdary Bolla<sup>3</sup>, Naga Sribala Gantha<sup>4</sup>, Prasun Reddy<sup>3</sup>

Departments of Oral Pathology and <sup>1</sup>Public Health Dentistry, Government Dental College and Hospital, Jaipur, Rajasthan, <sup>2</sup>Department of Orthodontics, Awadh Dental College, Jamshedpur, Jharkhand, <sup>3</sup>Department of Prosthodontics, Mallareddy Institute of Dental Sciences, Suraram, <sup>4</sup>Department of Pedodontics, Panineeya Institute of Dental Sciences, Dilsukhnagar, Hyderabad, Telangana, India

**Corresponding author** (email: <drbhrt@gmail.com>)

Dr. Bharat Sankhla, Assistant Professor, Department of Oral Pathology, Government Dental College and Hospital, Jaipur, Rajasthan, India

## Abstract

**Background:** Diabetes is a third leading cause of mortality and morbidity in the world. Diabetes is one of the most common endocrine metabolic disorders and its prevalence has been increasing worldwide. Oral exfoliative cytology may be a more appropriate adjunctive diagnostic tool in conditions like diabetes mellitus, where the invasive techniques lose viability. **Aims:** The purpose of this study is to analyze the cytomorphometric changes in the exfoliated cells of the oral mucosa, as an adjunct to the diagnosis of diabetes. **Materials and Methods:** Smears were taken from the buccal mucosa of 30 diabetes patients (study group) and 30 healthy individuals (control group). All the smears were stained with rapid Papanicolaou stain (PAP). In the PAP smears, the nuclear area (NA), cytoplasmic area (CA), and cytoplasmic-to-nuclear ratio (CNR) were evaluated for 50 cells in each smear, using the Image Analysis Software (Magnus Pro™) and research microscope (Lawrence and Mayo™). **Results:** The results showed that the mean NA was significantly higher ( $P < 0.001$ ) in the study group, whereas, the mean CA did not exhibit a statistically significant difference ( $P > 0.001$ ). The mean CNR was significantly lower in the study group ( $P < 0.001$ ). **Interpretation and Conclusion:** The results associated with the clinical observations suggest that diabetes can produce morphological and functional alterations in the oral epithelial cells, detectable by microscopic and cytomorphometric analysis using exfoliative cytology, which can be used in the diagnosis of the disease.

**Key words:** Cytomorphometry, diabetes mellitus, image analysis

## INTRODUCTION

Diabetes mellitus describes a metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia, with disturbances in carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both.<sup>[1]</sup>

Diabetes has become a major health issue in South-East Asia. It has been estimated by the International Diabetes

Federation (IDF) that 382 million people currently have diabetes all over the world, and by 2035, this will rise to 592 million. The number of people with type 2 diabetes is increasing in every country and 80% of the people with diabetes live in low- and middle-income countries. The greatest number of people with diabetes are between 40 and 59 years of age and still 175 million people with diabetes are undiagnosed.<sup>[2]</sup>

The major classical findings of diabetes are polyuria, polydipsia, polyphagia, weight loss and fatigue.<sup>[3]</sup> The oral complications of uncontrolled diabetes mellitus can include xerostomia, candidiasis, increased incidence of dental caries, gingivitis, periodontitis, periapical abscess, and parotid enlargement, burning mouth syndrome, lichen planus, lichenoid reactions, traumatic ulcers, irritational fibromas, glossodynia, median rhomboid glossitis, neurosensory dysesthesias, and taste dysfunctions.<sup>[4-6]</sup> In

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general, diabetes has a shorter life span and reduced quality of life, as compared to a healthy general population.<sup>[7]</sup>

Early diagnosis of diabetes mellitus is an important aspect of healthcare.<sup>[8]</sup> Exfoliative cytology is considered a moderate, straight forward, painless, and noninvasive technique compared to the conventional anatomopathological examination.<sup>[3]</sup>

Oral exfoliative cytology is a powerful diagnostic tool. Cytomorphometric analysis of the exfoliated cells can be a noninvasive diagnostic marker for Diabetes Mellitus. Exfoliative cytology has the potential to be developed as a routine investigation for the screening of diabetes. Thus, the purpose of this study is to investigate the cytological alteration of the exfoliated oral mucosal cells of diabetic patients, to establish its role as a diagnostic criterion.

## MATERIALS AND METHODS

The study was conducted on 30 diabetic patients and 30 non-diabetic subjects, as the control group, who were visiting the Clinical Laboratory of the Department of Oral Pathology, Darshan Dental College and Hospital, Udaipur. The patients' detailed case histories were recorded in the Department of Oral Pathology. All the subjects were clinically, hematologically, and biochemically examined, to exclude the possibility of any other oral or systemic disease.

### Inclusion criteria

The 30 patients who were diagnosed with diabetes mellitus were in the age group of 30 to 60 years, visiting the Clinical Laboratory of the Department. All the cases from the study group and control group underwent venipuncture under aseptic conditions for estimation of fasting blood sugar (FBS) levels, using the Glucose Oxidase Dehydrogenase–Pyruvate Oxidase Dehydrogenase (GOD–POD) method, with the GOD–POD kit and a digital photo colorimeter. The 30 in the control group, of age 30-60 years, were randomly selected from patients who were free of any signs or symptoms of diabetes mellitus, visiting the Clinical Laboratory of the our Department.

### Exclusion criteria

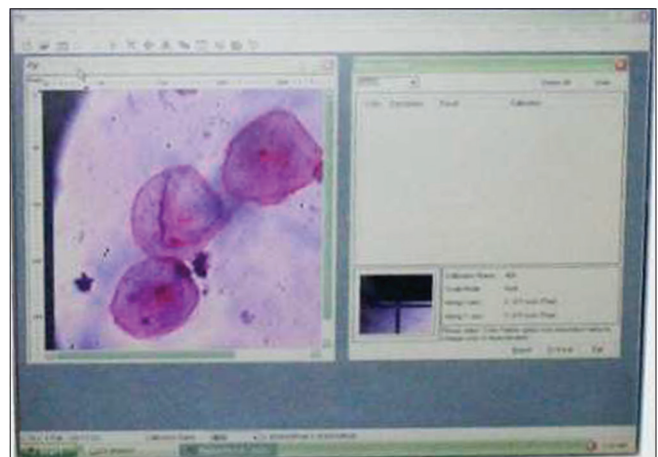
The control and diabetic group patients with habits, such as, tobacco smoking/chewing, betel quid chewing, alcoholism, with other oral and systemic diseases or taking medications other than the diabetic medications were excluded from this study.

## Exfoliative cytology and preparation of smears

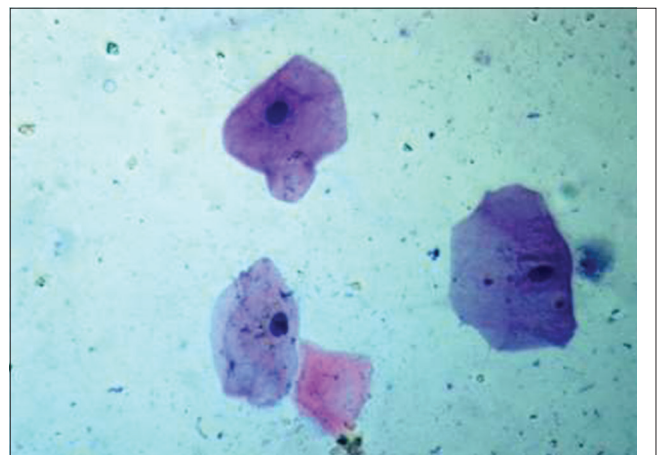
A commercially available wooden spatula was used for cytology. The spatula was used in only one direction over the buccal mucosa, with moderate pressure, thus obtaining clear epithelial cells. For the preparation of smears, clean, fresh, dry glass slides were used. The scraps were smeared onto the center of the glass slides and spread over a large area, preventing the clumping of cells. The slides were immediately sprayed with Biofix™ spray fixative to ensure proper fixation. The smears were stained by using the Papanicolaou (PAP) staining method.

## Cytomorphometric analysis

The PAP-stained smears were subjected to cytomorphometric analysis, using the Magnus Pro 3.0 [Figure 1] image analysis software with a Research microscope (Lawrence and Mayo). The images [Figure 2] were captured using a closed circuit device



**Figure 1:** Buccal mucosal cells measured by image analysis software. (Magnus Pro™)

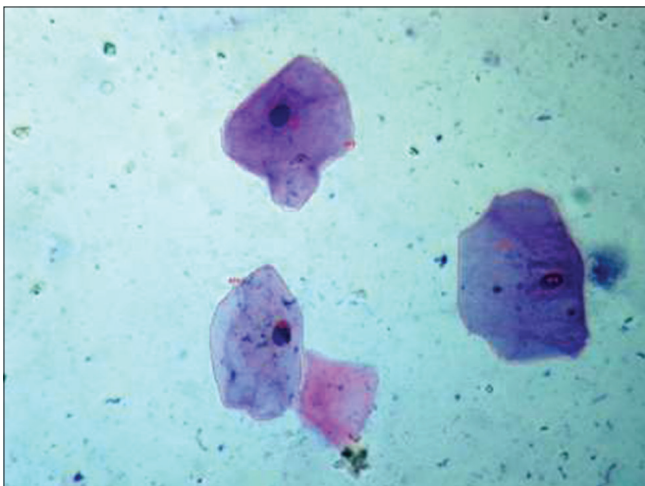


**Figure 2:** Pre-analyzed photomicrograph of buccal mucosal cells (x400)

(CCD) camera attached to the research microscope. The final images captured, had a magnification of x400 on the monitor. Fifty clearly defined cells, with good staining, were selected by systematic sampling in a stepwise manner, moving the microscope stage from left to right, and then down and across, in order to avoid measuring the same cells again. The nuclear area (NA) [Figure 3] and cytoplasmic area (CA) [Figure 3] were obtained by drawing around the nuclear and cell boundaries using the digitizer cursor. The cytoplasmic ratio and nuclear ratio (CNR) were calculated.

## RESULTS

The cytormorphometric data were compared between the diabetic and control groups by the student's *t*-test. Each group comprised of twenty two males and eight females, ranging from 30 to 60 years, and enjoying good oral health. The time of disease was greater than two years in 85% of the diabetic patients, and at least one kind of medication was being used (oral hypoglycemic). The level of fasting blood sugar (FBS) was  $92.97 \pm 8.89$  mg/dl for the control group versus  $157.70 \pm 16.22$  mg/dl for the diabetic group. The cytormorphometric results showed that the mean nuclear area in the control group was  $67.493 \mu\text{m}^2$  and in the diabetic group was  $87.271 \mu\text{m}^2$ . The mean NA showed a statistically significant increase in the study group, as compared to the control group ( $P < 0.001$ ). The mean cytoplasmic area of the diabetic group was  $2642.892 \mu\text{m}^2$  and that of the control group was  $2731.431 \mu\text{m}^2$ . No significant difference was found in the mean CA of the study group as compared to the control group ( $P > 0.001$ ). The mean CNR showed a statistically significant decrease in the study group as compared to the control group ( $P < 0.001$ ) Table 1.



**Figure 3:** Post-analyzed photomicrograph of buccal mucosal cells (x400)

## DISCUSSION

Diabetes is an ancient disease. Its symptoms, which include excessive drinking of water and frequent urination (to wash away the excess sugar in the blood) were noted on a scrap of Egyptian papyrus more than 3,500 years ago.<sup>[9]</sup>

Hyperglycemia is a hallmark of diabetes mellitus. Diabetes mellitus, a complex metabolic disorder, is a syndrome characterized by abnormalities in carbohydrate, lipid, and protein metabolism that results either from a profound or an absolute deficiency of insulin (IDDM) or from target tissue resistance to its cellular metabolic effect (NIDDM).<sup>[8]</sup>

It has been reported that diabetes mellitus adversely affects the morphology of the oral mucosa, which may compromise the tissue repair, growth, and regeneration functions.<sup>[10]</sup>

The exfoliative cytology technique is a noninvasive method for initial and early diagnosis of cancers, as an adjunct to clinical examination. A review of literature shows that this technique has been in use since the 1960s and 1970s, as a cancer evaluation and diagnostic technique, with acceptable sensitivity and specificity.<sup>[11]</sup> Cytology can be used as a diagnostic technique in detecting early changes of diseases even in the absence of clinical manifestations. This technique has some advantages, including easy and fast implementation, adequate diagnostic value, noninvasiveness, it is painless, with a low-cost, and reproducibility.<sup>[12,13]</sup>

Planimeters have been replaced by semiautomatic image analysis techniques, which are faster, more accurate, and more reproducible. The modern image analysis software encompasses morphometry, densitometry, neural network, and expert system.<sup>[14]</sup>

In our present study, an attempt has been made to study the morphometric and cytological changes in

**Table 1: Mean of CA, NA and ratio of NA: CA (after end of the result paragraph in text)**

| Parameters   | Control                | Diabetic               | <i>P</i> value |
|--|------------------------|------------------------|----------------|
| Mean nuclear area ( $\mu\text{m}^2$ )                  | $67.493 \pm 6.780$     | $87.271 \pm 8.921$     | $< 0.001$      |
| Mean cytoplasmic area ( $\mu\text{m}^2$ )              | $2731.431 \pm 239.186$ | $2642.892 \pm 243.186$ | $> 0.001$      |
| Mean cytoplasmic and nuclear ratio ( $\mu\text{m}^2$ ) | $40.855 \pm 5.415$     | $30.449 \pm 2.929$     | $< 0.001$      |

CA=Cytoplasmic area, NA=Nuclear area

the exfoliated cells of the buccal mucosa of the normal controls and diabetic patients.

In the diabetic group, the maximum value of a fasting blood sugar level was 165.33 mg/dl in the 51 – 60 year age group. However, in the control group, the maximum value of FBS was 97.00 mg/dl in the 31 – 40 year age group. The mean of the FBS showed a statistically significant increase in the diabetic group as compared to the control group ( $P < 0.001$ ) in the above-mentioned age groups [Graph 1].

In this present study, the NA of the exfoliated cells from the buccal mucosa of the study group ranged from 86.288  $\mu\text{m}^2$  to 89.525  $\mu\text{m}^2$ , with a mean value of 87.271  $\mu\text{m}^2$ . Although, in the control group the NA ranged from 66.337  $\mu\text{m}^2$  to 70.800  $\mu\text{m}^2$ , with a mean value of 67.493  $\mu\text{m}^2$ . On statistical analysis, a significant difference was found in the mean values between the diabetic and control groups ( $P < 0.001$ ) [Graph 2].

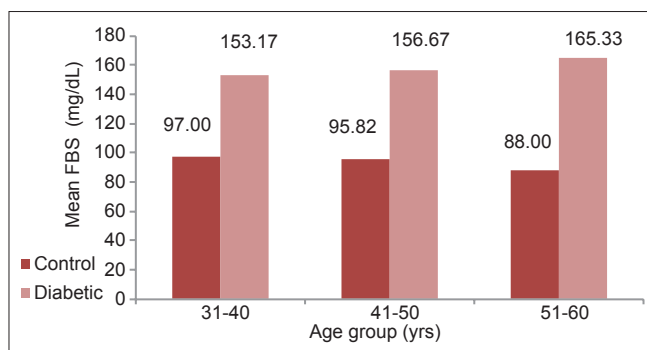
At about the turn of the century, a number of workers, notably Strasbruger (1893), Gerassimow (1902), Boveri (1905), and Hertwig (1908), suggested that the cell nucleus, and more specifically the amount of nuclear chromatin (now identified as DNA) determines the size of the cell.<sup>[15,16]</sup>

There are two theories on the cell proportions of different cellular constituents:<sup>[17]</sup>

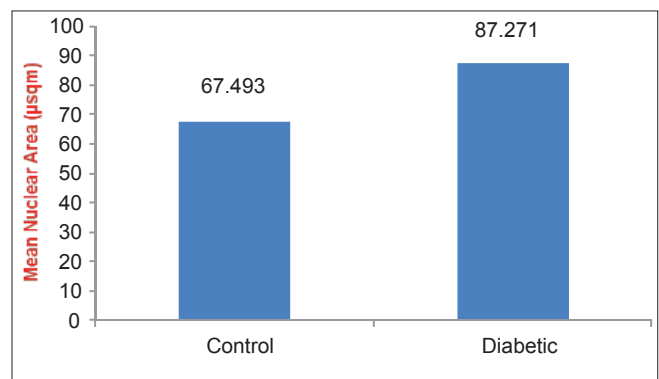
- Genetic regulation: The available experimental and clinical evidence suggests that the deleterious effect of diabetes mellitus results from metabolic defragments, mainly hyperglycemia. In addition, the coexistent hypertension, common in diabetics, contributes to atherosclerosis.<sup>[11]</sup> In diabetic patients, decreased cellular turnover might be a secondary reaction to ischemia (diminished blood flow in the tissue), caused by atherosclerosis. Ischemia causes hypoxia resulting in decreased oxidative

phosphorylation, and thus, adenosine triphosphate (ATP) generation. The resulting depletion of ATP has widespread effects on many systems within the cells. As a result the anaerobic glycolysis increases because of the decreased ATP and the associated increase in adenosine monophosphate, which stimulates the enzyme phosphofructokinase. This pathway is evolutionary, intended to maintain the cell energy by generating ATP from glycogen, and its activation leads to the rapid depletion of glycogen stores. Increased glycolysis also results in the accumulation of lactic acid and inorganic phosphates from the hydrolysis of phosphate esters, thus lowering the intracellular pH. Ribosomes lead to detachment from the rough endoplasmic reticulum. In this type of irreversible cellular injury, cellular adaptation occurs, hence, the nucleus is hypertrophied with a much higher DNA content than the normal cell<sup>[10]</sup>

- Enzyme regulation: It can be explained by the fact that in a cell the enzymes that are normally inactive can often be activated when needed. As previously mentioned, there is decreased perfusion of the affected tissues and decreased turnover and the cell may remain in a stressful situation. When most of the ATP has been depleted in the cell, a considerable amount of cyclic adenosine monophosphate (c-AMP) is found as a breakdown product of ATP. The presence of this c-AMP in turn immediately activates the glycogen splitting enzyme, phosphorylase, liberating glucose molecules that are rapidly metabolized to provide energy, which is used to replenish the ATP stores. The preservation of c-AMP in a diabetic group may be a compensatory mechanism to maintain the cell function in stress conditions like diabetes mellitus. In this manner, a constant cross-feed between the synthetic systems results in an almost equal amount of substance in the cell at all times. The enzyme system can either



**Graph 1:** Comparison of FBS with different age groups of control and diabetic groups



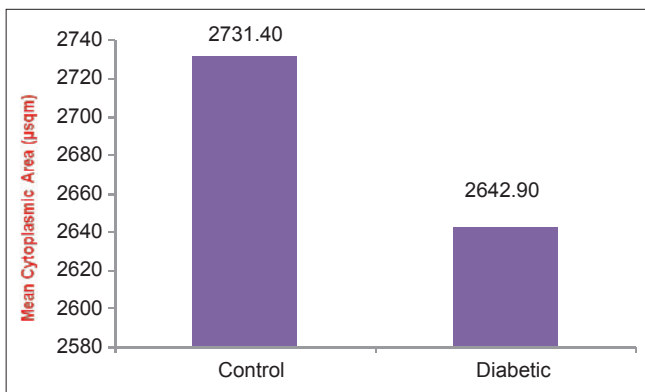
**Graph 2:** Comparison of the mean nuclear area between the control and diabetic groups

be activated or inhibited according to the need of the cell.<sup>[18]</sup>

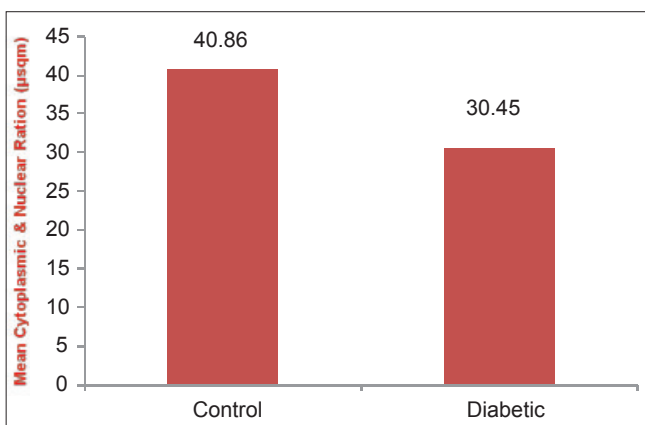
The NA values are in close relation with the study done by Alberti *et al.*,<sup>[4]</sup> Jajarm *et al.*,<sup>[5]</sup> Shareef,<sup>[8]</sup> Prasad *et al.*,<sup>[19]</sup> and Suvarna *et al.*<sup>[20]</sup>

Diabetic patients also suffer from dehydration due to the decreased salivary flow rates that may lead to mucosal atrophy. So, when smears from the atrophic oral mucosa are made, the large cells are included in the sample. Thus, the primary pattern encompasses non-keratinized cells of the parabasal layers, which are smaller in size, but have a relatively larger nucleus; thus giving an impression of nuclear enlargement.<sup>[19,21]</sup>

In this study, the cytoplasmic area (CA) of the exfoliated cells from the buccal mucosa of the study group ranged from 2553.722  $\mu\text{m}^2$  to 2708.680  $\mu\text{m}^2$ , with a mean of 2642.90  $\mu\text{m}^2$ . Although, in the control group, the CA ranged from 2663.191  $\mu\text{m}^2$  to 2797.497  $\mu\text{m}^2$ , with a mean of 2731.40  $\mu\text{m}^2$ . On statistical analysis the difference in the mean CA between the two groups was not significant ( $P > 0.001$ ). The



**Graph 3:** Comparison of the mean cytoplasmic area (CA) between the control and diabetic groups



**Graph 4:** Comparison of the mean cytoplasmic and nuclear ratio between the control and diabetic groups

CA values were in accordance with that of Alberti *et al.* (2003)<sup>[4]</sup> — according to them, the CA in the diabetic group did not exhibit a statistically significant difference ( $P > 0.05$ ) [Graph 3].

The present study showed an increase in nuclear area, but the cytoplasmic area did not present a statistically significant difference, whereas, the cytoplasmic to nuclear ratio was diminished significantly in diabetics [Graph 4].

In the background of the association of diabetes mellitus with various oral neoplastic and inflammatory diseases the early changes in oral cavity can be ascertained through cytology, more so through cytomorphometry.

## CONCLUSION

The general understanding of the alterations in the cellular pattern of oral mucosal cells in diabetic patients provide health professionals with a noninvasive tool for verification of clinical diabetes.

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