Cytological and Cytomorphometric Study of Exfoliated Cells of the Oral Mucosa in Diabetic Patients

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Background: Systemic disorder like diabetes mellitus is on the rise in developing countries like India and in many parts of the world. Early diagnosis of this disease will help to prevent the complications due to uncontrolled diabetes mellitus. Dentist helps in the early diagnosis of this disease as patients manifest several oral manifestations. Aims and Objectives: The aim of this study was to study the cytological and cytomorphometric changes of exfoliated cells of oral mucosa in diabetic patients. Materials and Methods: Thirty diabetic patients and 15 healthy controls were included in the study. From the buccal mucosa, smears were collected and Papanicolaou stain was used for staining. Cytological and cytomorphometric study was carried out. The cell diameter and nuclear diameter were measured on these smears and were compared with the control group with the help of “unpaired Student t test.” Results: Buccal mucosa exfoliated cells’ mean nuclear diameter of diabetic group and control group was 11.198 and 9.494 µm, respectively and the difference was highly significant. Conclusion: Our study suggests significant cellular changes in the diabetic patients, which may aid us in early detection of diabetes.

Keywords: Cytology, cytomorphometry, diabetes mellitus, exfoliated cells, oral mucosa

Received : 23-01-2020.
Revised : 24-02-2020.
Accepted : 13-03-2020.
Published : 28-08-2020.

INTRODUCTION

Diabetes mellitus is characterized by abnormal carbohydrate, fat, and protein metabolism that results in acute and chronic complications due to the absolute or relative lack of insulin.[1] It is a slowly evolving symptomless disease, described in 1500 BC by Egyptians. This is the disease of interest in modern society due to its increased incidence and prevalence. It has been reported that about 40 millions in India are diabetic and the alarming thing is more than half of the population are unaware of the presence of this disease.[2-4]

Sometimes they are diagnosed at late stages with patients having severe hyperglycemia and landing in diabetic coma. This syndrome will bring to bear its effect on various organs of the body such as eye, kidney, heart, nerves, blood vessels, and oral cavity. Thus, it is imperative that dentists must have a fundamental knowledge of the disease. Reports suggest that dentist will treat more of diabetic patients than any other health professionals. Dentists come across various oral manifestations of this disease. If they have proper understanding of these manifestations, then they can diagnose the disease at initial stage itself. Improvement is seen in diabetic control after treatment for periodontal disease. Reduction in inflammation associated with oral infection is believed to reduce harmful effects that lead to poor metabolic control. Hence dentists have major opportunities for health promotion, disease prevention, diagnosis, and therapy related to diabetes.[1-6]
Oral pathologists also play a significant role in identifying this disease. Exfoliative cytology is the microscopic examination of shed cells from an epithelial surface. It is a rapid and noninvasive procedure. Its principal use has been in screening for dysplasia and carcinoma of the uterine cervix.[3] This technique uses the rationale of exfoliation of superficial epithelial cells as a part of physiologic turnover and exfoliation of deeper cells in case of neoplasia as cells lose their cohesiveness. The patients with diabetes mellitus are susceptible to various mucosal epithelial changes due to weaker immunity of oral mucosa and various manifestations of the disease in the oral cavity because of altered metabolism. Such epithelial changes are studied by exfoliative cytology. Hence exfoliative cytology can be applied in diabetic patients to study the morphological and morphometric aspects of the cells.[5-10] This study was carried out to find the quantitative and qualitative alterations in oral epithelial cells of diabetic patients.

**Materials and Methods**

This study was carried out in Yenepoya Medical and Dental College campus, Derlakatte, Mangalore, Karnataka, India. Thirty diabetic patients above the age of 30 years were included for diabetic group after obtaining institutional ethical committee clearance and subjects’ consent. Fifteen age-matched healthy nondiabetic people formed the control group.

**Inclusion criteria**

Diabetic patients for at least 1 year (fasting whole venous blood sugar level >110 mg/dL) were included in the study.[9]

**Exclusion criteria**

(1) Patients with known local and systemic disease (other than diabetes mellitus)
(2) Medically compromised patients
(3) Patients with habits (pan chewing, alcohol abuse, and smoking)
(4) Frank local lesions other than gingivitis.

**Methodology**

The examiner was unaware of the diabetic group and the control group, and took the smears from right buccal mucosa adjacent to the first and second maxillary molars, above the line of occlusion. The site was wiped with a gauze swab to remove surface debris and excess saliva. Oral B (Procter & Gamble Ltd., Los Angeles, CA, USA) interdental brush was placed firmly against the buccal mucosa and rotated for 5–10 times on the mucosa [Figure 1]. Two smears of each individual were taken on a coded glass slide and immediately fixed in absolute alcohol for 30 min, and then stained with Papanicolaou stain. Stained smears were observed under a binocular light microscope “Labomed” (Noida, India). The microscope stage was moved from left to right then down and across to avoid measuring the same cells repeatedly. Clumped, folded, and distorted cells were not considered. The cytological features of the smear were observed and noted...
[Figures 2 and 3]. Measurement of 50 cells was taken for the nuclear diameter and cell diameter along the longest axis of the cell using 10× eye piece graticule and 40× objective. The readings of the graticule were calculated using stage micrometer under 40× objective. The obtained nuclear diameter and cell diameter were recorded and the mean of 50 cells were calculated.

Another observer recorded the findings of the same slide to eliminate the interobserver bias. The same observer recorded the findings at different intervals of time to eliminate the intra-observer bias. After the findings were recorded, the code of each smear was decoded. The calculated mean nuclear diameter and cell diameter of the diabetic group was equated with that of the group of control category using “unpaired Student t test.” Reliability of intra- and interobserver bias was also calculated. Photomicrographs were taken using photo microscope “Olympus CX41” (Chennai, India) under 40× magnification.

**RESULTS**

The demographic details were recorded and it revealed that most of the patients (25) were between 31 and 50 years, and females were slightly more in number (24) than males (21) [Table 1]. The nuclear and cell diameters of exfoliated cells of the buccal mucosa of diabetic group and control group were compared and there was a significant difference ($P < 0.01$): [Table 2].

We found that observations obtained by first observer at different intervals of time were reliable (because agreement scale is more than 0.6, it is reliable [Table 3]). The interobserver values were recorded [Table 4]. We found that observations obtained by two observers at different intervals of time were reliable (because agreement scale was more than 0.6, it is reliable [Table 5]). The cytological features of both the diabetic and control groups were compared [Table 6].

**DISCUSSION**

In our present study, we have applied cytology to study the cellular and morphometric changes in the exfoliated cells of the buccal mucosa of diabetic patients. We found that in smears of diabetic patients, unusual changes at cellular level such as karyorrhexis, binucleation, and micronucleus were observed. Our findings are similar to those by Alberti et al. [9]

We observed a significant difference between the exfoliated cells’ mean nuclear diameter of diabetic group and group belonging to control category, which is in agreement with that of Alberti et al. [9]

This increase in the nuclear diameter may be seen in several other conditions such as smokers and nutritional deficiency such as hypochromic anemia, megaloblastic anemia, and vitamin B12 and folic acid deficiency. Hence we excluded such patients from our study.

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**Table 1: Age and sex distribution of the individuals of both diabetic group and control group**

| Age (years) | Number | Male | Female | Diabetic group | Control group |
|------------|--------|------|--------|----------------|---------------|
| 31–40      | 13     | 4    | 9      | 4              | 9             |
| 41–50      | 12     | 5    | 7      | 8              | 4             |
| 51–60      | 11     | 6    | 5      | 9              | 2             |
| 61–70      | 8      | 6    | 2      | 8              | —             |
| 71–80      | 1      | —    | 1      | 1              | —             |
| Total      | 45     | 21   | 24     | 30             | 15            |

**Table 2: Comparison of nuclear diameter and cell diameter of exfoliated cells of the buccal mucosa of diabetic group and control group**

|                | Mean (µm) | Standard deviation |
|----------------|-----------|--------------------|
| Diabetic group |           |                    |
| Nuclear diameter | 11.198   | 0.62370            |
| Cell diameter   | 56.52     | 3.73675            |
| Control group   |           |                    |
| Nuclear diameter | 9.49440  | 0.48246            |
| Cell diameter   | 53.14853  | 3.97918            |

**Table 3: Reliability test for the observations obtained by first observer at different intervals of time**

| Group           | Comparison of first and second time recording | Intra-class correlation coefficient values |
|-----------------|-----------------------------------------------|------------------------------------------|
| Diabetic group  | ND 1 vs. ND 1¹                               | 0.8997                                   |
| Diabetic group  | CD 1 vs. CD 1¹                               | 0.8849                                   |
| Control group   | ND 1 vs. ND 1¹                               | 0.8603                                   |
| Control group   | CD 1 vs. CD 1¹                               | 0.9042                                   |

ND 1 = nuclear diameter of first time recording, ND 1¹ = nuclear diameter of second time recording, CD 1 = cell diameter of first time recording, CD 1¹ = cell diameter of second time recording.
Instruments such as metal spatula, cotton sticks, wooden spatula, and brush are used for adequate sample collection. Jones et al. [11] suggested that with cytobrush, one can obtain sufficient number of cells, and these cells were found to be dispersed in a thin uniform layer. We used interproximal brush for sample collection.

Several morphometric methods have been used by different authors for cytometric studies. Cowpe et al. [12] measured nuclear area and cytoplasmic area in normal mucosa and in premalignant lesions using two methods namely planimeter and image analysis. Both techniques exhibited an enhancement in nuclear area and decline in cytoplasmic area in premalignant lesions. According to them, image analysis is more appropriate than planimetry.

In our study, we have manually measured nuclear diameter and cell diameter using eyepiece graticule under binocular microscope. Similar method was used by Lavanya et al. [13] and Ramaesh et al. [14] in their study performed in smokers, where they measured nuclear diameter and cell diameter using micrometer and compound microscope.

Caldeira et al. [15] compared nuclear and cytoplasmic volume of 50 basal epithelial cells in the buccal mucosa of the autoimmune nonobese diabetic mice with Balb/C mice (control). The results showed marked decrease in nuclear volume and cytoplasmic volume in diabetic mice compared to nuclear volume and cytoplasmic volume of the control mice. They used stereological parameters for their measurement.

Femiano and Scully [16] used DNA cytometry in oral leukoplakia and oral lichen planus. This technique was evaluated using densitometry in the Feulgen-stained section. This system is found to be more sensitive in the evaluation of oral potentially malignant lesions into low risk and high risk. Pektas et al. [17] evaluated nuclear morphometry and DNA ploidy status. They used Feulgen stain.

According to Dandona et al. [18] poor diabetic control is related with an increased cancer risk, owing to oxidative damage to DNA. Seppälä et al. [19] reported lowered immunity in diabetic patients and microangiopathy, which may together play a role in oral cancer development.

Dikshit et al. [20] explained insulin-like growth factor-I might be related to p53 mutations, which is quite common in head and neck tumor. Whereas according to Tsuji et al. [21] and Seril et al. [22] alterations occurred in the oxidative equilibrium of free radicals in diabetic patients. They suggested that elevated blood glucose levels may result in excessive formation of free radicals. The activity of antioxidant scavengers and enzymes will be reduced due to protein breakdown. Both these factors might promote carcinogenesis.

Further studies are necessary to exactly determine the role of morphometric changes in the early detection of diabetes and also the association with several neoplastic and inflammatory diseases.
CONCLUSION

Our study suggests that there are significant morphometric alterations in the exfoliated cells of buccal mucosa in the diabetic group in comparison to the control group. This knowledge can be helpful in early detection of cellular changes in diabetes mellitus.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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