

Periodontal health status and implication of periodic acid–Schiff diastase - a key in exfoliative cytology among diabetics mellitus patients: A case–control study

Aesha Imran¹, Mayank Kumar Parakh¹, Satish Muthu Kumar¹,
N. Nachiammai¹, K. Sriram¹

Correspondence: Dr. Aesha Imran
Email: draesha24@gmail.com

¹Department of Oral and Maxillofacial Pathology,
Chettinad Dental College and Research Institute,
Chennai, Tamil Nadu, India

ABSTRACT

Objectives: The objectives of the study are: (i) To determine if periodic acid–Schiff (PAS)-Diastase is an effective tool to diagnose DM noninvasively, (ii) to use three different types of staining procedures to identify the staining pattern over the exfoliated cells of normal and diabetic patients, (iii) to study the periodontal health status of DM and normal subjects. **Materials and Methods:** Basic questions regarding the demographic data were asked, following which community periodontal index (CPI) was recorded. Oral smears were collected from clinically normal buccal mucosa of 150 patients using wooden tongue spatulas. The subjects were asked to gargle their mouth with water and the wooden spatula was scraped at the site from buccal mucosa. The smears were spread evenly on a dry clean glass slide and fixed immediately with absolute ethyl alcohol. Three slides were prepared for each of the patients. PAS, PAS with diastase digestion, and Papanicolaou staining were performed and examined under a microscope. **Results:** Average CPI for study subjects was 3.2 and control subjects was 2.1. The average loss of attachment was 1.1 in the study group and 0.2 in the control group ($P = 0.00$) and the result are statistically highly significant. All the 150 cases (100%) were positive for PAS staining, while PAS-diastase (PAS-D) staining showed positivity only for normal subjects and was negative (100%) in the diabetic group ($P = 0.00$) and the result are statistically highly significant. **Conclusions:** Results of our study showed that exfoliative cytology of the oral cavity, when stained with PAS and PAS-D, can be used as an effective screening and diagnostic tool for DM patients.

Key words: Diabetes mellitus, exfoliative cytology, periodic acid–Schiff-diastase, periodontitis

INTRODUCTION

India has currently become the diabetes capital of the entire world.^[1] Asian phenotype is more susceptible to diabetes mellitus (DM).^[2] DM classically presents with an increased glucose level in the blood. Glycogen is the principal storage form of glucose in our body. Glycogen does not stain with

conventional hematoxylin and stains weakly with eosin.^[3] Glycogen, a polysaccharide of glucose, which serves as an energy reserve in cells, is regulated by two hormones – epinephrine and glucagon. In diabetes, these hormones fail to break down glycogen, resulting

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in its erratic accumulation.^[4] Various cytochemical studies have been done to evaluate the expression of glycogen in normal embryonic tissue, wound healing, keratinization, and inflammation, with varying results, but hardly are there studies that aim to determine the presence of glycogen in oral epithelium.^[5]

DM is widely existing endocrine disorder, which is known to alter the delicate balance in tissues, causing an exaggerated tissue damage whenever challenged with a disease like periodontitis.^[6] Periodontitis is one of the most widely noted oral manifestation among DM patients. In fact, several researchers have advanced the notion that there is a bidirectional relationship between periodontitis and DM.^[7] The mechanisms linking these two conditions are not understood completely. It is hypothesized to involve aspects of immune function, neutrophil activity, and cytokine biology.^[8] Currently, evidence supporting the existence of a two-way relationship between DM and periodontitis is emerging. DM increases the risk for periodontitis and periodontal inflammation, thereby negatively affecting glycemic control.^[9] Community periodontal index (CPI) is most commonly used to assess the periodontal status of individuals which includes CPI score and loss of attachment.

The race for the next generation of noninvasive, precise, and painless techniques in evaluating glycemic status has begun. Exfoliative cytology, a simple, quick, and inexpensive procedure, which is used to study exfoliated desquamated cells, has been proved to be an excellent diagnostic adjunct in various oral lesions.^[9] Exfoliative cytology is based on epithelial physiology. A normal epithelium is exposed to regular exfoliation, namely, the loss of cell surface and the thickness of the epithelium are constant.^[10] The current study was performed to determine the periodontal health status of DM patients, to demonstrate glycogen within the oral exfoliated cells and to evaluate associated cellular changes in buccal smears of Type 2 diabetics and correlate the findings with their serum glucose levels.

MATERIALS AND METHODS

Ethical clearance

The ethical clearance was obtained from the Institute's Ethical Committee.

Sample selection

Convenience sampling was a method of sample selection used. The patients selected were from outpatients at our institute.

A total of 150 subjects were enrolled in the study after receiving written consent form. The patients were briefed on the study, and a detailed case history and details about the mode of glycemic control were recorded. All the subjects in the study group were diagnosed cases of DM Type 2. The inclusion criterion for the study group was that all the patients should be diagnosed diabetics and on medication. Patients not under medication for diabetes, any frank oral lesions, or other systemic disorders were excluded from the study. Random blood glucose levels were taken, and the subjects were divided into diabetic and control groups.

Periodontal status was assessed for all the subjects using CPI. In CPI, both CPI scores and loss of attachment were recorded. For clinical examination of CPI, a CPI probe with a 0.5 mm ball tip, with a black band between 3.5 and 5.5 mm was used. The teeth examined were 17, 16, 11, 26, 27, 37, 36, 31, 46, and 47. Although ten index teeth were examined, one relating to each sextant was made. When both or one of the designated molar teeth was present, the more severe finding from these tooth surfaces was recorded for the sextant. If no index teeth were present in a sextant qualifying for examination, all the remaining teeth in that sextant were examined. If no teeth were present in the sextant, then it was coded as X.^[11]

Oral exfoliative cytology smears were prepared from clinically normal appearing buccal mucosa of 150 patients (75 diabetic and 75 control patients) using wooden tongue spatulas. The subjects were asked to rinse their mouth with water and the wooden spatula was scraped at the site from buccal mucosa. The smears were spread evenly on a dry clean glass slide and fixed immediately with absolute 95% ethyl alcohol. Three smears were prepared for each of the patients. Periodic acid-Schiff (PAS), PAS with diastase and Papanicolaou (PAP) staining were performed and histologically examined.

Staining procedure

Papanicolaou staining

The prepared smear is immersed in distilled water for 3 min, following which it is immersed in hematoxylin stain for 3 min and washed under running water. The slide is then dehydrated in alcohol for 1 min. This slide is then immersed in equal proportions of orange-G stain and Eosin Azure stain for 3 min, followed by immersing in alcohol for 45 s. The slide is then run under water, dried, and mounted.^[12]

Periodic acid–Schiff staining

The fixed smear is placed into 0.5% periodic acid for 5 min, following this it is rinsed with distilled water. Schiff's reagent is left for 30 min, until deep magenta. The slide is then washed in running tap water for 5 min. Counterstain-hematoxylin is added and left for 3 min. The slide is then washed with tap water, then dehydrated in alcohol, and later mounted.^[13]

Periodic acid–Schiff–diastase staining

The fixed smear is treated with saliva, which is rich in diastase enzyme for 60 min, following which procedure of PAS stain is followed.^[3]

Criteria for evaluation of stain

In each field, about fifty cells were observed. PAP slides showed cells from various layers of the epithelium [Figure 1]. PAS slide which stained positive showed magenta to pink cells with a blue nucleus [Figure 2]. PAS-diastase (PAS-D) slide was stained little lighter shade from magenta to pink cells with a blue nucleus [Figure 3].

Statistical analysis

Data obtained were entered, and statistical analysis was done using social sciences (SPSS) TM software (version 10.05), (SPSS Inc., Chicago, IL, USA). The Student's *t*-test was used to compare the staining between diabetics and nondiabetics. $P < 0.05$ was considered to be statistically significant.

RESULTS

Seventy-five cases of diagnosed DM patients and 75 controls were included in the study. The age of the patients varied from 28 to 60 years (mean \pm standard

deviation 41.28 ± 9.96) with 64 male and 86 female subjects.

Among 75 study subjects and 75 control subjects, the periodontal status was calculated using CPI. Average CPI for study subjects was 3.2 and control subjects was 2.1. The average loss of attachment was 1.1 in the study group and 0.2 in the control group ($P = 0.00$).

All the 150 cases (100%) were positive for PAS staining, while PAS-D staining showed positivity only for normal subjects and was negative (100%) in the diabetic group ($P < 0.05$). All the study subjects were tested for random blood sugar, and it ranged from 180 to 215 (mean \pm standard deviation 197.20 ± 10.93), while the control group had random blood glucose levels of 80–110 (mean \pm standard deviation 95.88 ± 9.04). This difference in blood sugar levels, when subjected to statistical analyses, was significant ($P = 0.00$).

The study group exhibited various nuclear changes such as binucleation [Figure 4], nuclear enlargement [Figure 5], and enucleation [Figure 6] in PAP-stained smears when compared to that of the control group whose cells appeared normal. Inflammatory cells were noted in both study and control groups. Increased colonies of cocci and bacilli were noted on smears of study group when compared to that of normal subjects.

DISCUSSION

In our study, periodontal status was recorded using CPI, following which oral exfoliative cytology

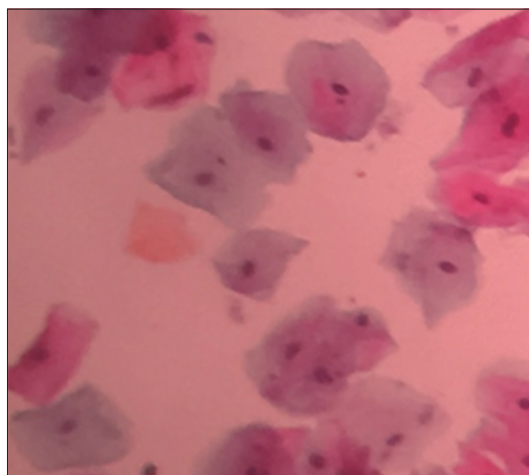


Figure 1: Papanicolaou-stained smear

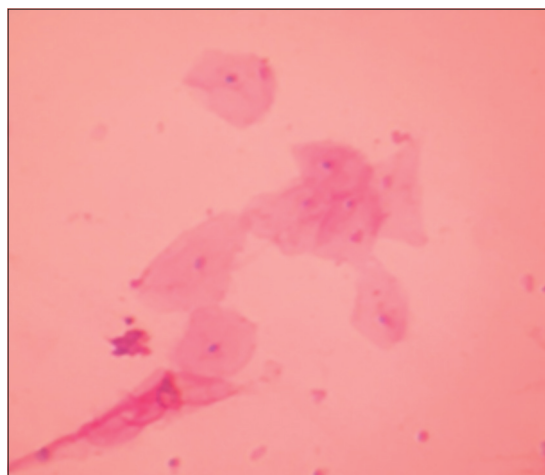


Figure 2: Periodic acid–Schiff-stained smear

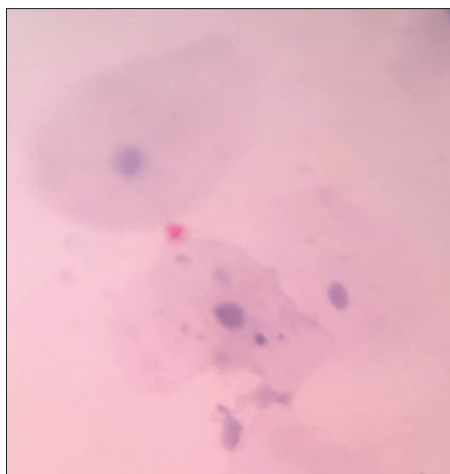


Figure 3: Periodic acid-Schiff-diastase-stained smear



Figure 4: Binuclear cells in study subjects

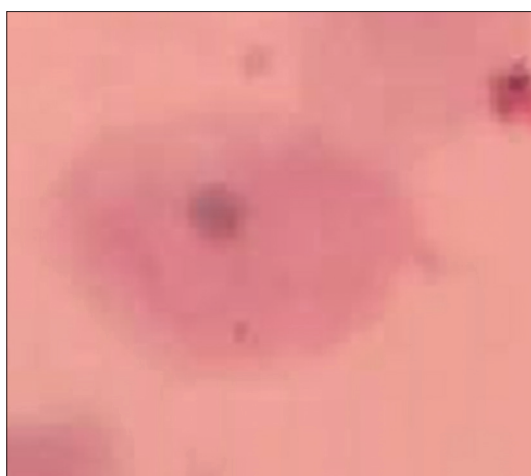


Figure 5: Increase in size of nucleus



Figure 6: Enucleated squamous cells seen in study subjects

smears were made in both DM and control groups. These smears obtained were fixed and subjected to PAP, PAS, PAS-D staining and were examined histopathologically.

Our study showed that diabetes patients had a significant higher CPI score and loss of attachment than control group which was similar to results obtained by Kim *et al.*,^[14] Radhika and Ranganathan,^[15] Popławska-Kita *et al.*,^[16] Das *et al.*,^[17] Prakash *et al.*,^[18] and Gupta *et al.*^[19] in their studies. This higher CPI clearly denotes that they have a higher incidence of periodontitis, probably because there is alteration in chemotaxis of neutrophils and, moreover, diabetics have compromised local immunity which aggravated the periodontal damage. Diabetics have an excessive accumulation of accumulated glycation end-products which impairs the healing process and increases the glucose levels in a gingival crevicular fluid, furthermore aggravating the periodontal status of diabetic patients.

Alberti *et al.*,^[20] Jajarm *et al.*,^[21] Shareef *et al.*,^[22] Nandita *et al.*,^[23] Prasad *et al.*,^[24] and Satpathy *et al.*^[25] in their studies on oral epithelium in Type 2 diabetic patients on PAP smears found that there is an increase in the nuclear area and binucleation among the diabetic group which was similar to results obtained in our study, which is probably because these patients usually have a deficiency of Vitamin B12 or folic acid. We also found that frequency of nuclear changes was higher among the diabetic group than in the control group and these results were similar to the findings of Jajarm *et al.*^[21] and Latti *et al.*^[26]

Satpathy *et al.*^[25] and Kishi *et al.*,^[27] in their study showed increased colonies of cocci and bacilli in PAP-stained smears of the diabetic group, which was similar to results obtained in our study. This increase in bacterial colonies can be probably attributed to decreased salivary flow and reduces overall immunity among diabetes patients.

There was an increase in a number of inflammatory cells on PAP smears seen in the study group when compared to control group, which was similar to results obtained by Satpathy *et al.*,^[25] Latti *et al.*,^[26] and Kishi *et al.*^[27] This is probably because the diabetic population is more prone to mucosal ulceration and has an increased incidence of infections.

Our study showed that PAS staining was positive in all cases. This result was similar to the results of Latti *et al.*^[26] On PAS with diastase digestion, our study showed significantly lighter stain among the study group which was also similar to results obtained by Latti *et al.*^[26] The lighter stains are probably because in DM patients, there is an increase in the level of glycogen in the oral tissues and this glycogen when subjected to diastase enzyme splits into smaller glucose molecules which no longer takes up PAS stain.

CONCLUSIONS

The results of our study showed that exfoliative cytology of the oral cavity, when stained with PAS-D, can be used as an effective screening and diagnostic tool for DM patients. This technique, moreover, has the advantage of being noninvasive and relatively cheap and hence can be recommended as a routine test for DM patients.

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Conflicts of interest

There are no conflicts of interest.

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