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Original Article

To Study the Cytologic Changes in Oral Mucosa of Normal and Diabetic Patients using H&E, Papanicolaou, Periodic Acid Schiff, and Periodic Acid Schiff-Diastase Stain

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Abstract

Introduction: Diabetes mellitus is the fifth most common chronic condition and the sixth most frequent cause of death among the elderly. An estimated 100 million people are affected by diabetes mellitus worldwide. It represents an extreme disturbance in glucose metabolism with severe hyperglycemia and insulin deficiency.

Objective: The objective of this research was to study the effect of diastase enzyme on the glycogen content of the epithelial cells of normal and diabetic patients in oral exfoliative cytology.

Materials and Methods: Ten control subjects and ten diabetic patients (study group) were taken, four oral smears for both control and study group from the buccal mucosa were taken, and stained with hematoxylin and eosin stain, Papanicolaou stain, periodic acid Schiff (PAS) stain, and PAS-diastase (PAS-D) stain.

Results: Glycogen is stained magenta with PAS and was absent on the PAS(D) stained slide because of glycogen digestion by the diastase enzyme. Such detection of glucose can help in the diagnosis of diabetes.

Conclusion: 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 non-invasive and relatively cheap, and hence can be recommended as a routine test for DM patients.

Key words: Diabetes mellitus, Exfoliative cytology, Periodic acid Schiff, Periodic acid Schiff-diastase

INTRODUCTION

Diabetes mellitus is the fifth most common chronic condition and the sixth most frequent cause of death among the elderly. An estimated 100 million people are affected by diabetes mellitus worldwide. It represents an extreme disturbance in glucose metabolism with severe hyperglycemia and insulin deficiency.[1] Hyperglycemia can be associated with severe oral complications. Tissue repair is damaged, and dysfunction of the oral mucosa occurs due to alterations in salivary flow and constituents, changes in nutrition, and reduced immune defenses, leading to changes in microbial oral flora and a greater tendency to infections.[2] The oral manifestations and complications related to DM include dry mouth (xerostomia), tooth decay (including root caries), periapical lesions, gingivitis, periodontal disease, oral candidiasis, burning mouth (especially glossodynia), altered taste, geographic tongue, coated and fissured tongue, oral lichen planus, recurrent aphthous stomatitis, increased tendency to infections, and defective wound healing. The intensity of diabetic complications is usually proportional to the degree and

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duration of hyperglycemia.[3] Therefore, early diagnosis of diabetes mellitus is an important aspect of health care. The aim of our study was to study the effects of diastase enzyme on the glycogen content of the epithelial cells of mucosa of normal and diabetic patients in exfoliative cytology and to compare the staining quality in mucosa of normal and diabetic patients using hematoxylin and eosin (H and E) stain; Papanicolaou (PAP) stain, periodic acid Schiff (PAS), and PAS-Diastase (PAS-D) stains, respectively.[1]

MATERIALS AND METHODS

The present study involved a total of 20 subjects (10 study and 10 controls). Diagnosed cases of diabetes were selected using the Glucose-oxidase method. Both fasting and postprandial blood sugar levels (BSL) were measured after taking informed consent from the patient. Approval from the institutional ethical committee was also taken.

Exclusion Criteria

- Patients with habits of smoking, chewing, and alcohol intake.
- Patient taking medications for malignancies or any systemic diseases other than diabetic medications.
- Nutritional deficiencies, such as anemia and the presence of oral sepsis.

Inclusion Criteria

- Fasting BSL above 120 mg/dL.
- Post-prandial BSL above 200 mg/dL.

Smears were taken from the mucosa of normal (control group) and diabetic patient (study group) after instructing the subjects to rinse their mouth with water to remove any debris. With a gentle scraping motion, cells were scraped from the buccal mucosa using a wooden spatula and transferred onto clean glass slides. The glass slides were then immediately fixed in 95% ethyl alcohol and stained using H and E (10 slides), PAP (10 slides), PAS (10 slides), and PAS-D (10 slides) stains for both groups.

RESULTS

Glycogen is stained magenta with PAS [Figure 1] and was absent on the PAS(D) [Figure 2] stained slide because of glycogen digestion by diastase enzyme. Such detection of glucose can help in the diagnosis of diabetes.

All data were collected, analyzed, and then tabulated accordingly. Student's *t*-test was applied to find significant values, and $P \leq 0.05$ was considered to be statistically significant.

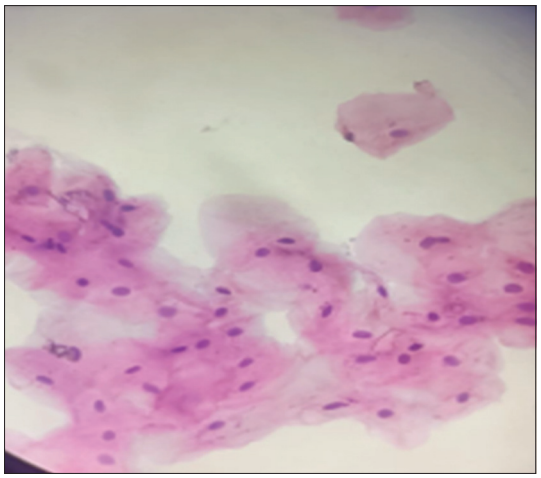

Figure 1: ×40 periodic acid Schiff-stain

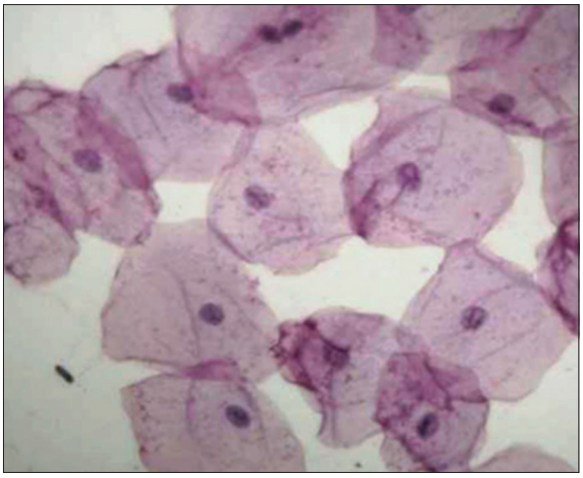

Figure 2: ×40 periodic acid Schiff-diastase stain

Mean and standard deviation values of BSL in the control and study group, comprising fasting and post-prandial BSL, were compared, and it was found that high BSL was seen in the study group as compared to the control group.

Mean and standard deviation of fasting and PP BSL in the control group ($n = 10$) are 79.9 ± 4.80 and 96.3 ± 8.85, and BSL in fasting and PP in the study group ($n = 10$) is 220.8 ± 48.84 and 333 ± 65.24 [Table 1].

Mean values and standard deviation of fasting and PP BSLs in the control group, when compared to those of the study group, were found to be highly significant ($P \leq 0.5$) "*t*" value of fasting in the control group and study group was 9.07 and "*t*" value of PP in control group and study group was 11.36 [Table 2].

Mean and S.D of Fasting when compared with mean and S.D of PP in the control group was found to be highly significant with a "*t*" value of 5.15.

Mean and S.D of fasting when compared with mean and S.D of PP in the study group were also found to be highly significant with a "*t*" value of 4.35 [Table 3].

Cellular changes observed in the study group included: Binucleation, as shown in PAS stain [Figure 3] and PAP stain [Figure 5]; Normal and decreased cytoplasmic-to-nuclear ratio in both normal and diabetic patients, observed using PAP stain [Figure 7]; Nuclear enlargement in PAS diastase stain [Figure 4]; Enucleation in H and E-stained slides [Figure 6]. These changes were observed in comparison to the control group. Inflammatory cells were seen in both the study and control groups; however, no significant differences were noted in the H and E-stained slides.

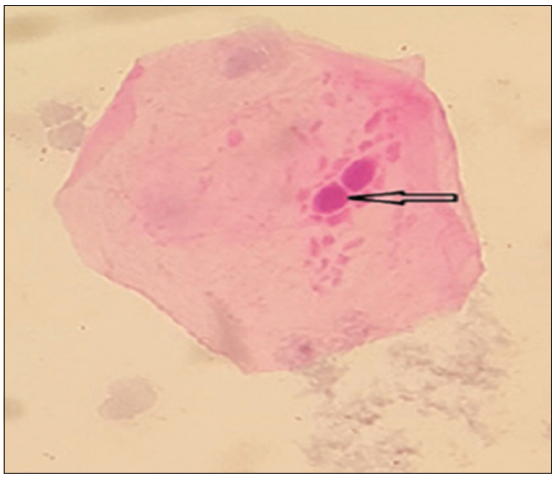

Figure 3: Binucleation shown in periodic acid Schiff stain (×40)

Table 1: Mean and standard deviation values of blood sugar level in the control and study group

BSL mg\dL	Mean±SD	
	Control	Study
Fasting	79.9±4.80	220.8±48.84
Post-prandial	96.3±8.85	333±65.24

SD: Standard deviation, BSL: Blood sugar level

DISCUSSION

Diabetes mellitus is one of the most common chronic disorders characterized by hyperglycemia. This disease can have many complications in various regions of the body, including the oral cavity. The important oral manifestations and complications related to diabetes include xerostomia, dental caries, gingivitis, periodontal disease, increased tendency to oral infections, burning mouth, taste disturbance, and poor wound healing. Oral complications in diabetic patients are considered major complications and can affect patients' quality of life. There is evidence that chronic oral complications in these patients have negative effects on blood glucose control, so prevention and management of the oral complications are important.[3]

The most accepted clinical technique for the diagnosis of lesions of the oral mucosa is an incisional or excisional biopsy. In specific clinical conditions, such as diabetes, a great many invasive techniques lose viability as a result of variations in blood glucose and the disease itself. In these cases, oral exfoliative cytology may be more appropriate.[1]

A cytologic smear is an advantageous diagnostic procedure because it is non-invasive, relatively painless, inexpensive, and requires a minimum of technical skill. It is useful when a patient refuses to have a biopsy performed or when medically compromised patients would be exposed to unnecessary surgical risks, and anxious patients can be reassured quickly about the nature of oral mucosal changes, especially when a fear of cancer or a family history of

Table 2: Comparison of mean values of blood sugar level in the control and study group

BSL	Mean±SD (*n*=10)		"*t*" value	*P*-value	Significance
	Control	Study			
Fasting	79.9±4.80	220.8±48.84	9.07	0.0001	Extremely significant
Post-prandial	96.3±8.85	333±65.24	11.36	0.0001	Extremely significant

SD: Standard deviation, BSL: Blood sugar level

Table 3: Comparison of mean values and Standard deviation of both fasting and post-prandial blood sugar levels in the control and study group

BSL	Mean±SD		"*t*" value	*P*-value	Significance
	Fasting	PP			
Control group (*n*=10)	79.9±4.80	96.3±8.85	5.15	0.0001	Extremely significant
Study group (*n*=10)	220.8±48.84	333±65.24	4.35	0.0004	Extremely significant

SD: Standard deviation, BSL: Blood sugar level, PP: Post-prandial

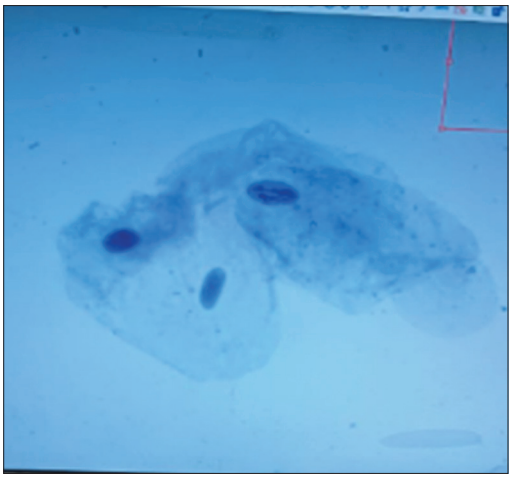

Figure 4: Nuclear enlargement shown in periodic acid Schiff diastase stain (×40)

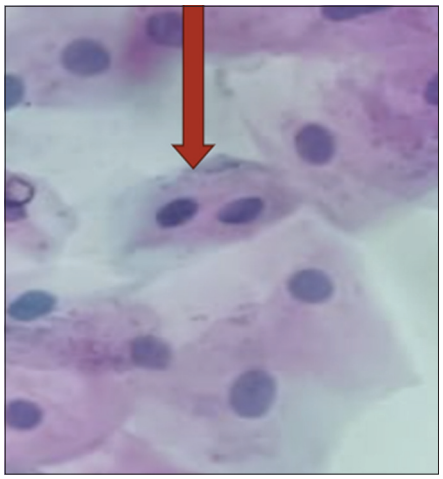

Figure 5: Binucleation shown in PAP-stain (×40)

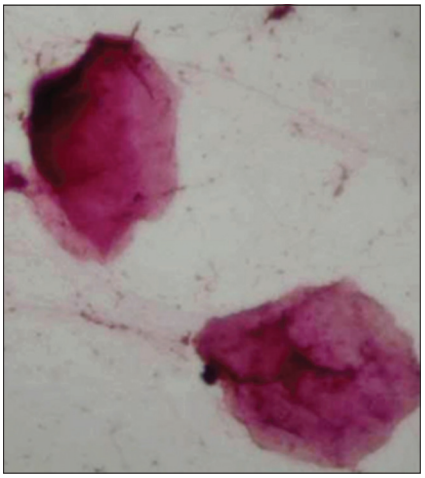

Figure 6: Enucleation shown in H&E stain (×40)

cancer accounts for their apprehension.[1]

In our study, we compared the slides of H and E, PAP, PAS, and PAS-D in both the control and study groups and found

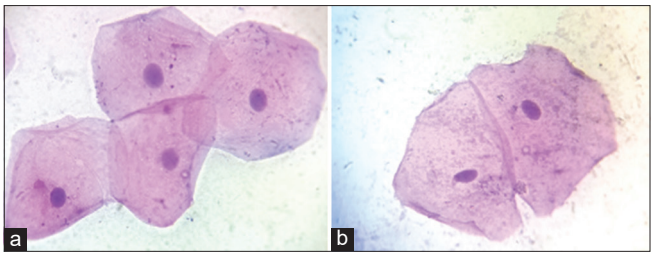

Figure 7: (a) PAP-stained cytological smear representative of diabetic group buccal cells (×100), (b) PAP-stained cytological smear representative of normal healthy control group buccal cells (×100)

that the cells in the study group exhibited binucleation, decreased cytoplasmic/nuclear ratio, nuclear enlargement, enucleation, and inflammation as compared to that of control group, which was similar to the findings of Jajaram *et al*. Shareef *et al*. and Alberti *et al*., who also found that diabetes mellitus can produce alterations in oral epithelial cells, detectable by microscopy and cytomorphometry, which can be used in the evaluation of this disease. We compared the mean values of BSL in the control and study group and found that the mean values of BSL fasting and PP are higher in the study group as compared to the control group, which is highly significant with a $P < 0.01$. Because of the increased glucose in diabetics, their epithelial cells showed less staining when stained with PAS-D, since glycogen is digested by the diastase enzyme treatment before PAS staining.[4]

We found that nuclear changes were significantly higher in the diabetic group than in the control group, which was similar to the findings of Jajaram *et al*.[2] This could be related to increased cellular age in patients with diabetes. Decreased cellular turnover might be a secondary reaction to ischemia caused by atherosclerosis in diabetic patients.[4]

We found a significant increase in inflammation in the diabetic group in comparison with the control group. This might result from decreased salivary flow in diabetes, due to hypofunction of the salivary glands secondary to adverse hormonal, microvascular, and neuronal changes.[5]

Our study showed a significant increase in the number of PAS-positive exfoliative cytology smears prepared from the study group as compared to the smears prepared from healthy controls, with a statistically significant difference. The glycogen content was also greater in the diabetic group. These results are consistent with previous studies of Hallikerimath S, Sapra G, Kale A, and Khandelwal RL.[6]

Our study showed that PAS staining was positive in all cases. This result was similar to the results of Latti *et al*.[1]

In our study, oral epithelium in Type 2 diabetic patients on PAP smears was found that there is an increase in the

nuclear area and binucleation among the diabetic group, which could be probably because of a deficiency of Vitamin B12 or folic acid. These results were consistent with the previous studies of Alberti *et al*., Jajaram *et al*., Shareef *et al*., and Nandita *et al*.[7]

The present study showed an increase in nuclear area, but the cytoplasmic area did not present a statistically significant difference, whereas the cytoplasmic nuclear ratio was significantly diminished in diabetics. These results were inconsistent with the studies done by Alberti *et al*.,[8] Shareef *et al*.,[9] Prasad *et al*.,[10] Sonawane *et al*.,[11] and Suvarna *et al*.[12] An increase in the nuclear area in the buccal mucosa of a Type 2 diabetic patient could be due to a delay in the keratinization process caused by decreased cellular turnover. In diabetics, the glycation of proteins, lipids, and nucleic acids increases with sustained hyperglycemia, causing much greater accumulation of advanced glycation end products in the walls of large vessels as well as the basement membrane of microvasculature. The effect of this is a progressive narrowing of the vessel lumen, decreased perfusion of affected tissues, and decreased turnover, which may cause a delay in the keratinization process of the epithelium. This delay in the differentiation process of epithelium leads to an increase in the cells that present a large nucleus as a primary characteristic.[13]

Another possible hypothesis for explaining the increase in mean NA is as follows: An increased glucose level directly favors cell growth because of its pivotal role in metabolic processes. An actively growing cell is characterized by a prominent and large nucleus.[12]

Ogden *et al*. revealed cytomorphometric changes in the buccal mucosal cells of cigarette smokers similar to those noted in diabetics.[14] Their findings were supported by Ramaesh *et al*.[15]

CONCLUSION

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. However, larger sample size studies should be performed to come to a definite conclusion. This technique, moreover, has the advantage of being non-invasive and relatively cheap, and hence can be recommended as a routine test for DM patients.

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