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Study the cytologic changes In Oral Mucosa of Normal and Diabetic patient using H&E, PAP, PAS and PAS-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 elderly. An estimated 100 million people are affected by diabetes mellitus world-wide. 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 (PAP) stain, periodic acid Schiff (PAS) stain and PAS-Diastase (PAS-D) stain.

Results: Glycogen is stained magenta with PAS and was absent on PAS(D) stained slide because of glycogen digestion by 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.

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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 oral mucosa occurs due to alteration 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 (OLP), recurrent aphthous stomatitis, increased tendency to infections, and defective wound healing. The intensity of diabetic complications is usually proportional to the degree and duration of hyperglycemia [3]. Therefore, early diagnosis of the 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 s[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 Glucose-oxidase method. Both fasting and postprandial blood sugar levels 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 like anemia and presence of oral sepsis.

Inclusion Criteria

- Fasting blood sugar level above 120mg\dl.
- Postprandial blood sugar level above 200mg\dl.

Smears were taken from 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 gentle scraping motion cell were scraped from buccal mucosa using 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 the groups.

Results

Glycogen is stained magenta with PAS (Figure 1) and was absent on PAS(D) (Figure 2) stained slide because of glycogen digestion by diastase enzyme.

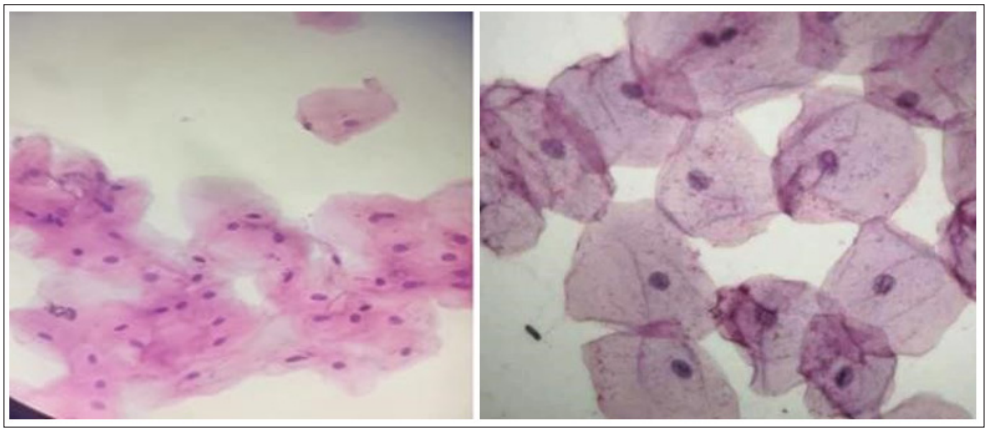

Figure 1: 40X PAS-Stain

Figure 2: 40X PAS-Diastase Stain

All data was collected, analyzed and then tabulated accordingly. Student's t test was applied to find significant values and p-value of ≤0.05 was considered to be statistically significant.

Mean and standard deviation values of Blood sugar level (BSL) in control and study group comprising of fasting and Postprandial BSL were compared and was found that high BSL were seen in study group as compared to control group.

Mean and Standard deviation of Fasting and PP Blood sugar level in control group (n-10) was 79.9±4.80 and 96.3±8.85 and Blood sugar level in fasting and PP in study group (n-10) was 220.8±48.84 and 333±65.24. (Table 1)

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

BSLmg\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

Mean values and Standard deviation of Fasting and PP Blood sugar levels in Control group when compared to that of Study group was found to be highly significant (P≤0.5) 't' value of Fasting in control group and study group was 9.07 and 't' value of PP in control group and study group was 11.36. (Table 2)

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

BSL	Mean+SD (n=10)		't' value	P value	Significance
	Control	Study	Study		
Fasting	79.9±4.80	220.8±48.84	9.07	0.0001	Extremely significant
Postprandial	96.3±8.85	333±65.24	11.36	0.0001	Extremely significant

SD: Standard deviation, BSL: Blood sugar level

Mean and S.D of Fasting when compared with Mean and S.D of PP in Control group was found to be highly significant with 't' value of 5.15.

Mean and S.D of Fasting when compared with Mean and S.D of PP in Study group was also found to be highly significant with 't' value of 4.35. (Table 3)

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

	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

Cellular changes that were observed in the study group were mainly binucleation decreased cytoplasmic/nuclear ratio, nuclear enlargement and enucleation in PAP-stained slides as compared to that of control group. Inflammatory cells were seen in both study and control group However no significant differences were found in H and E-stained slides.

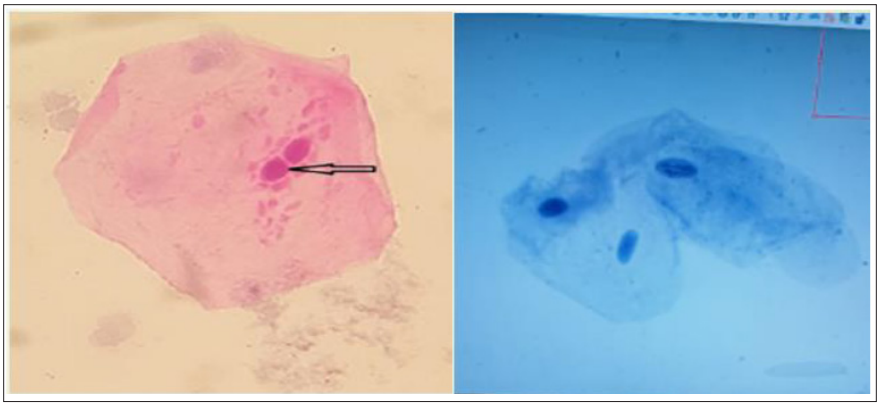

Figure 3: Binucleation shown in PAS-Stain (40X)

Figure 4: Nuclear enlargement shown in PAS Diastase stain (40X)

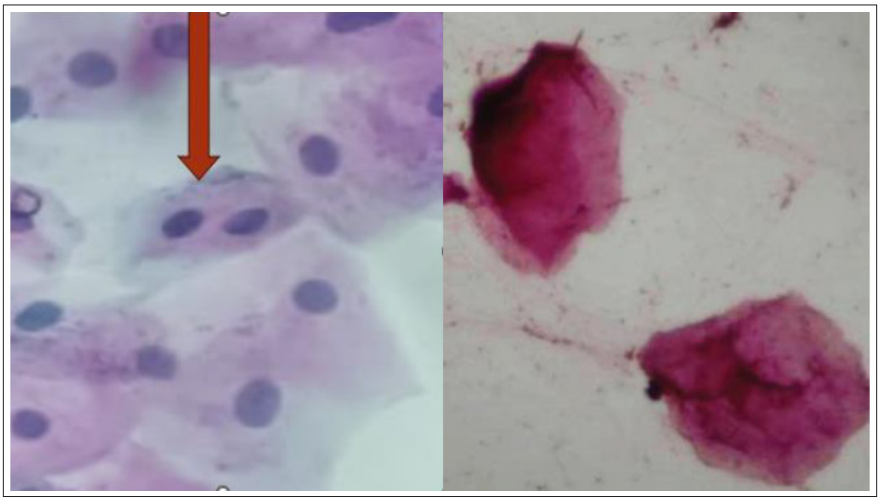

Figure 5: Binucleation shown in PAP Stain (40X)

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

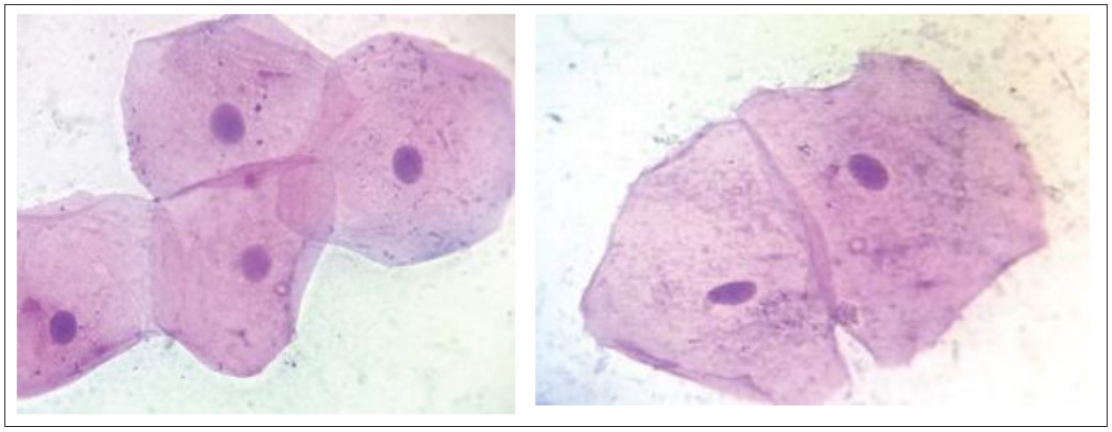

Figure 7: (a) Pap-stained cytological smear representing diabetic group buccal cells (×100), (b) Pap-stained cytological smear representing normal healthy control group buccal cells (×100)

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 cancer accounts for their apprehension [1].

In our study, we compared the slides of H and E, PAP, PAS, PAS-D in both control and study group and found 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 Jajarm 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 evaluation of this disease. We compared the mean values of BSL in control and study group and found that mean values of BSL fasting and PP is more in study group as compared 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 prior to PAS staining [4].

We found that nuclear changes were significantly higher in the diabetic group than in the control group that was similar to the findings of Jajarm 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 numbers of PAS-positive exfoliative cytology smears prepared from the study group as compared to the smears prepared form healthy controls with statistically significant difference. The glycogen content was also greater in diabetic group. These results are consistent with previous studies of Hallikerimath S et al [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 found that there is an increase in the nuclear area and binucleation among the diabetic group which could be probably because of deficiency of Vitamin B12 or folic acid. These results were consistent with the previous studies of Alberti et al, Jajarm et al, Shareef et al and Nandita et al [7].

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

increase in the cells which present a large nucleus as a primary characteristic [13].

Other 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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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Latti BR, Birajdar SB, Latti RG (2015) Periodic Acid Schiff-Diastase as a key in Exfoliative cytology in diabetics: A pilot study. J Oral Maxillofac Pathol 19: 188-891.
2. Jajaram HH, Mohatsham NRingian A (2008) Evaluation of oral mucosa epithelium in type-2 diabetic patients by an exfoliative cytology method: Journal of oral science 50: 335-340.
3. Rohani B (2019) Oral manifestations in patients with diabetes mellitus. World J Diabetes 10: 485-489.
4. Morris HF, Ochi S, Winkler S (2000) Implant survival in patients with type 2 diabetes: Placement to 36 months. Ann Periodontol 5: 157 165.
5. Conner S, Iranpour B, Mills J (1970) Alteration in parotid salivary flow in diabetes mellitus. Oral Surg Oral Med Oral Pathol 30: 55 59.
6. Salih MM, El-Esawy BH, Abd El hafez A, Abd El-Hafez A (2018) Cytomorphologic patterns of Pap and PAS-stained oral exfoliative cytology smears in adult Saudi diabetic patients as compared to healthy controls. Diagnostic Cytopathology 00: 1-6.
7. Imran A, Parakh MK, Kumar SM, Nachiammai N, Sriram K (2016) Periodontal health status and implication of periodic acid–Schiff diastase - a key in exfoliative cytology among a diabetics' mellitus patients: A case–control study. Eur J Dent 10: 475-479.
8. Alberti S, Spadella CT, Francischone TR, Assis GF, Cestari TM, et al. (2003) Exfoliative cytology of the oral mucosa in type II diabetic patients: Morphology and cytomorphometry. J Oral Pathol Med 32: 538-543.
9. Shareef BT, Ang KT, Naik VR (2008) Qualitative and quantitative exfoliative cytology of normal oral mucosa in type 2 diabetic patients. Med Oral Patol Oral Cir Bucal 13: E693 E696.
10. Prasad H, Ramesh V, Balamurali P (2010) Morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients. J Cytol 27: 113-117.
11. Sonawane K, Jain S, Gupta I, Karthik BV, Singaraju S, et al. (2011) Cytomorphometric analysis of oral mucosa in diabetic patients in Bhopal region an in-situ study. Int J Clin Dent Sci 2: 12 15.
12. Suvarna M, Anuradha C, Kumar KK, Shekhar PC, Chandra KL Reddy (2012) Cytomorphometric analysis of exfoliative buccal cells in type II diabetic patients. J NTR Univ Health Sci 1: 33-37.
13. Sahu M, Suryawanshi H, Nayak S, Kumar P (2017) Cytomorphometric analysis of gingival epithelium and buccal mucosa cells in type 2 diabetes mellitus patients. J Oral Maxillofac Pathol 21: 224 228.
14. Ogden GR, Cowpe JG, Green MW (1990) Quantitative exfoliative cytology of normal buccal mucosa: effect of smoking. J Oral Pathol Med 19: 53-55.
15. Ramaesh T, Mendis BR, Ratnatunga N, Thattil RO (1999) The effect of tobacco smoking and of betel chewing with tobacco on the buccal mucosa: a cytomorphometric analysis. J Oral Pathol Med 28: 385-388.