Cytomorphometric Analysis of Oral Mucosa in Diabetic Patients in Bhopal Region an In-Situ Study.

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Abstract

Background: Diabetes is a common endocrine metabolic disorder and prevalence is increasing worldwide. In condition like diabetes, premalignant lesions and iron deficiency anemia; oral exfoliative cytology may be more appropriate as the invasive techniques lose viability.
Aim: The study was conducted to analyze the cytomorphometric changes in exfoliated cells of oral mucosa as an adjunct to diagnosis of diabetes.
Method: Samples were collected from buccal mucosa and divided into 2 groups; 100 diabetic patients (study) and 100 healthy individuals (control). The smears were stained with rapid Papanicolaou stain (PAP). Nuclear area (NA), cytoplasmic area (CA) and cytoplasmic to nuclear ratio (CNR) were evaluated in 50 cells in each smear using Image analysis software (Magnus pro™) and research microscope (Lawrance & Mayo™).
Results: Mean NA was significantly higher ($p < 0.001$) in study group whereas mean CA didn't exhibit any statistically significant difference ($p > 0.001$). The mean CNR was significantly lower in study group ($p < 0.001$).
Conclusion: Clinical observations and results suggested morphologic and functional alterations in oral epithelial cells in diabetic patients; detectable by microscopic and cytomorphometric analysis using exfoliative cytology and can be used in diagnosis.

KEYWORDS: Cytomorphometry; Diabetes mellitus; Exfoliative cytology; Papanicolaou stain.

Introduction

Diabetes mellitus is a common endocrine metabolic disorders and its prevalence is increasing all over the world.[1] In 1997, an estimated 124 million people worldwide were suffering from diabetes. By 2010 around 221 million people will be suffering from diabetes worldwide, and in certain regions (like - Asia, Africa) diabetes rates are expected to rise twofold or threefold. In general diabetes has shorter life span and quality of life is not good as compared to a healthy population.[2] There is no definitive and complete cure for diabetes mellitus.[3] The major classical findings of diabetes are polyphagia, polydypsia, polyuria, fatigue and continuous weight loss.[4] The common oral complications are xerostomia, increased incidence of dental caries, gingivitis, periodontitis, periapical abcess, and parotid enlargement, candidiasis, lichen planus, lichenoid reactions, burning mouth syndrome, traumatic ulcers, glossodynia, neurosensory dysesthesias, irritational fibromas, and taste dysfunctions etc.[1,3,5]
Early diagnosis of the diabetes mellitus could be an important aspect for health care.[7] Exfoliative cytology is a moderate, straight forward and non-invasive technique compared to conventional examination.[4] Cytomorphometric analysis of exfoliated cells can be established as a non-invasive diagnostic marker for Diabetes Mellitus. Thus, the purpose of this study was to demonstrate cytologic changes using morphometric analysis of the exfoliated oral mucosal cells of diabetic patients in Bhopal region, to establish its role as diagnostic criteria.

Materials and Methods-

The study was conducted on 100 diabetic patients (study group) and 100 control group who visited the clinical laboratory of the Department of Oral Pathology, Rishiraj Dental College and Research Center, Gandhinagar, Bhopal. The patient's detailed case history was recorded and written informed consent was taken. All subjects were examined clinically, biochemically and hematologically to exclude the possibility of any other oral or systemic disease. The subjects with habits like tobacco smoking / chewing, betel and quid chewing, alcoholism and other oral and systemic diseases or under medications other than the diabetic medications were excluded from this study.

Preparation of smears-

Clean, fresh, dry glass slides were used to prepare the smears. The scraps were smeared onto the centre of glass slide and spread over a large area so that there will be no clumping of cells. The slides were immediately sprayed with Biofix™ spray fixative to ensure proper fixation. The smears were stained using Papanicolaou (PAP) staining technique.

Cytomorphometric analysis

The cytomorphometric analysis of PAP stained smears was done using (Magnuspro3.0) image analysis software with Research microscope (Lawrence & Mayo). The images were captured by CCD (Closed circuit device) camera which was attached to the research microscope. The final images had a magnification of 400X on monitor. In a stepwise manner fifty clearly defined cells (with good staining) were selected by systematic sampling moving the microscope stage from left to right, and then down and across in order to avoid repetition of cells. Digital cursor was used to obtain the nuclear area (NA) and cytoplasmic area (CA) by drawing around the nuclear and cell boundaries. The cytoplasmic ratio & nuclear ratio (CNR) were calculated using the software.(Fig.1 A,B)

Results

Table-1 shows the cytomorphometric analysis of oral smears of control and diabetic groups.

The data were compared between both the groups using the student's t-test. The mean values of NA, CA and CNR between both the groups were compared using Students "t" test. The period of disease was longer than 5 year in 85% of the diabetic patients, and they were on medication for the same. The level of glycosylated haemoglobin for the control group was 91.65 ± 7.91 mg/dl, while the level for the diabetic group was 158.10 ± 17.62 mg/dl. The mean NA in control group was 66.543 µm2, and in diabetic group was 86.182 µm2. In study group Mean NA showed statistically significant increase as compared to control group ($p<0.001$). The mean CA in diabetic group was 2613.736 µm2 and in control group was 2734.712 µm2. Mean CA did not show any significant difference in between study group and control group ($p > 0.001$). In study group Mean CNR showed statistically significant decrease as compared to control group ($p<0.001$).

Discussion-

Diabetes mellitus is a complex metabolic disorder. Hyperglycemia is the hallmark for diabetes mellitus. Diabetes mellitus is a syndrome characterized by abnormalities in metabolism of carbohydrate, lipid, and protein, which results either from a profound or an absolute deficiency of insulin (IDDM) or from target tissue resistance to its cellular metabolic effect (NIDDM).[7] Many researcher have examined the effects of Diabetes mellitus on oral mucosa. Adversely affects of Diabetes mellitus was reported on morphology of oral mucosa, which may compromise tissue functions to favor the occurrence of oral infections & neoplasia.[8] Exfoliative cytology is a simple, painless, non-invasive, low cost and rapid diagnostic tool.[9] With the advancement in the field of quantitative exfoliative cytology, oral exfoliative cytology has come out as a powerful diagnostic tool. It is a nonaggressive technique and easily accepted by the patients. Therefore it can be a very good option for the early diagnosis of epithelial atypia, oral cancer and squamous cell carcinoma.[10] After recent advancements; planimeters are replaced by semiautomatic image analysis techniques, which gives faster, more accurate and more reproducible results. Modern image analysis software can encompass morphometry, densitometry, neural network and expert system with better accuracy and in a very less time period.[11]

In current study we made an attempt to study morphometric and cytologic changes in the exfoliated cells of buccal mucosa of normal controls and diabetic patients. We selected 100 known diabetic patients as study group, and 100 healthy individuals as control group (which were matched according to age and sex). In current study we considered American Diabetic association 2000 criteria for diagnosis of Diabetes mellitus. Patients were considered as diabetic when the fasting blood sugar (FBS) level was greater than or equal to 126 mg/dl. The patients whose FBS levels were less than 110mg/dl; considered normal. Patients with FBS level greater than 110 mg/ dl but less than 126 mg / dl were not included in the study and considered as impaired fasting glucose. In this present study on NA of the exfoliated cells from the buccal mucosa of control group ranged from 65.432 µm2 to 69.912 µm2 (mean=66.543µm2), and in study group it ranged from 85.782 µm2 to 89.123 µm2 with a (mean=89.123µm2).

On statistical analysis significant difference was found in mean values between the study and control groups ($P < 0.001$). The NA values were in close relation with the study done by Alberti S et al. (diabetic group was 86.5 ± 12.30 µm2 and that of control was 61.3 ± 16.60 µm2). But Hassan H J Noorshin M. (2008) reported that mean NA for diabetic group was 60.48± 0.62 µm2 and for control was 40.90±0.68 (significantly higher in diabetic group) and Ban T. Shareef, Kok T.A. reported that the median NA for diabetic group was 170.18 and for control was 142.83. This difference could be due to delay in

Table-1 Cytomorphometric analysis of oral smears of control and diabetic groups.

Group	Control	Diabetic	P value
Mean nuclear area (µm2)	66.543 ±6.530	86.182±8.736	< 0.001
Mean cytoplasmic area (µm2)	2734.712± 229.376	2613.736±234.325	>0.001
Mean cytoplasmic & nuclear ratio (µm2)	41.096±4.721	30.328±3.241	<0.001

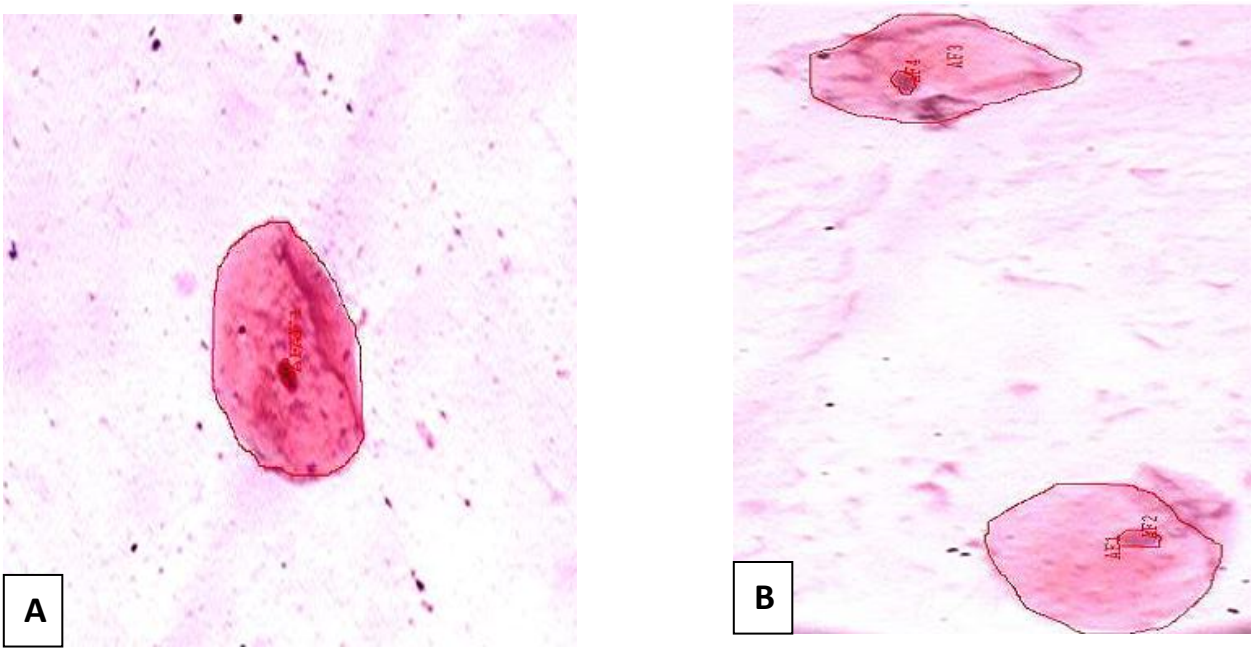

Fig1- Smears showing Oral epithelial cells stained by Papanicolaou (PAP) method:- (A) Nucleus in diabetic group (B) Nucleus in control group. (Nucleus is enlarged in study group then control group)

keratinization process caused by decreased cellular turnover in the buccal mucosa of type 2 diabetic patients. Decreased cellular turnover might be asecondary reaction to ischemia caused by atherosclerosis in diabetic patients. Ischemia can result into progressive narrowing of vessel lumen, decreased perfusion of affected tissues and decrease turnover which may cause delayed keratinizaton of epithelium. Cellular turnover could decrease and young cells will have limited production, so the majority of cells are old or aged.[1] The delay in differentiation process of epithelium will lead to increase in the cells which present a large nucleus as a primary characteristic.[4] In our study, the cytoplasmic area (CA) of the exfoliated cells from the buccal mucosa of the study group ranged from 2510.137 µm2 to 2738.147 µm2 (mean=2613.736 µm2). Whereas in control group the CA ranged from 2678.234 µm2 to 2789.139 µm2 (mean=2734.712 µm2). The difference in mean CA between the two groups was not significant (P > 0.001) on statistical analysis. The CA values are in accordance with that of Alberti S et al. (2003)[4].

There are two principal methods by which cell control proper proportions and proper quantities of different cellular constituents:[12]

1. The mechanism of genetic regulation and
2. The mechanism of enzyme regulation.

The explanation could be that in a cell the enzymes that are normally inactive; can be activated on need. As mentioned above, there is decreased perfusion of affected tissues and decrease turnover of cells and the cells may remain in a stressful situation. When most of the ATP has been depleted in the cell, a considerable amount of c-AMP is found as a breakdown product of ATP. The c-AMP immediately activates the glycogen splitting enzyme, phosphorylase liberating glucose molecule that are rapidly metabolized and provide energy for replenishment of ATP stores. The c-AMP acts as an enzyme activator for phosporylase and control intracellular ATP concentration, and maintains functions

in stressful condition also. The preservation of CA in diabetes could be a compensatory mechanism for cell functions in stressed conditions like diabetes mellitus. The constant crossfeed between the synthetic systems results in almost equal amount of substance in the cell all the times.[13]

Conclusion-

The present study showed an increase in NA whereas CA did not show any statistically significant difference. The cytoplasmic to nuclear ratio was diminished significantly. The general understanding of the alterations in the cellular pattern of oral mucosal cells in diabetic patients provide health professionals with a non-invasive tool for verification of clinical diabetes. As the association of diabetes mellitus with various oral neoplastic and inflammatory diseases has been reported earlier; the early changes in oral cavity can be ascertained through cytology, more so through cytomorphometry.

References:

1. Jajarm H.H, Mohtasham N, Rangiani A. Evaluation of oral mucosa epithelium in type II diabetic patients by an exfoliative cytology method. J Oral Science 2008; 50(3): 335-340.
2. Shareef B.T., Ang K.T., Naik V.R. Qualitative and quantitative exfoliative cytology of normal oral mucosa in type 2 diabetic patients. Med Oral Pathol Oral Cir Bucal 2008 Nov1; 13(11): E693-6.
3. Ship J.A. Diabetes and oral health: An overview. J Am Dent Assoc. 2003; 134: 4S-10S.
4. Alberti S., Spudella C.T, Francischone T.R.C.G, Assis G.F, Crstari T.M, Taverira L.A.A. Exfoliative cytology in type II diabetes patients- morphology and cytomorphometry. J Oral Pathol Med 2003; 32: 538-543
5. Abate N., Chandalia M. Ethnicity, type 2 diabetes & migrant Asian Indians. Indian J Med Res. 2007; 125: 251-258.
6. Vernillo A.T: Diabetes mellitus: relevance to dental treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 91:263-70.
7. Taylor G.W, Borgnakke W.S. Periodontal disease: associations with diabetes, glycemic control and complications Special Review in Periodontal Medicine. Oral Diseases (2008) 14, 191–203.
8. Caldeira E.J, Garcia P.J, Minatell E, Camilli J.A, Cagnon V.H.A. Morphometric analysis and ultrastructure of the epithelium of the oral mucosa in diabetic autoimmune NOD mice. Braz. J. Morphol. Sci. 2004; 21(4):197-205.
9. Talhari C,Souza J.V.B.D, Parreira J.V Reinel D, Talhari S. Oral exfoliative cytology as a rapid diagnostic tool for paracoccidioidomycosis. Journal Compilation 2007; 51; 177-178.
10. Mehrotra R, Gupta A, Singh M, Ibrahim R. Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions. Molecular Cancer 2006; 5:
11. Ogden G.R, Cowpe J.G, Wight A.J. Oral exfoliative cytology: review of methods of assessment. J Oral Pathol Med 1997, 26:201-5.
12. Guyton A.C, Hall J.E. Textbook of medical physiology. 11th Ed. W.B Saunders; 2006; 36-39.
13. Koss L.G. Diagnostic Cytology. 4th Ed. J.B Lippincott; 1992.