Evaluation of the Histological Changes as Effect of Type 2 Diabetic Mellitus on the Oral Epithelium (Buccal Mucosa and Gingiva)

Mahmood N. Mustafa [1]
Shaymaa A. Mahdi [2]
Ban I. Seddeeq [3]
Mohammed F. Jwammer [4]
Shujaa N. Ahmed [5]

[1,2,3] Tikrit University, College of Dentistry, Tikrit, Iraq. [4] Specialized Dental Center in Diyala, Iraq. [5] Specialized Dental Center in Tikrit, Iraq.

Abstract
Background: Exfoliative cytology considered as one of the best technique to obtain the epithelial cells lining buccal mucosa in oral cavity. Diabetes mellitus type 2 is one of the most distributed disorder wild world which cause deferent histological and cytological defects in all body organs.
Aim: To realize the histological changes in oral epithelium (of buccal and gingival mucosa) in patients suffering from type 2 diabetic mellitus. Material and methods: Sample composed of two groups, the study group represented by ten male diabetic patients, their age between (50-65) years attending the teaching hospital of college of Dentistry/ Tikrit university, and the control group (10 male 50-65 years old), the smear taken from oral epithelia from buccal mucosa, gingiva and saliva using cotton swab. The samples were carried on glass slides and stained by using Giemsa dye.
Results: The microscopical examination revealed a number of histological changes, as presence of binucleated cells, budding in nuclei and decay (karyolysis) of other nuclei with the observation of fragmentation (karyorrhexis) of others, besides, some of vasicular degeneration seen with the cytoplasm of number of cells and hypertrophy in others.
Conclusion: Diabetes mellitus type 2 cause many histological changes in oral epithelia of buccal mucosa and gingiva which detected by exfoliative cytology.

Introduction:

Diabetes Mellitus (DM) is a number of metabolic disturbances characterized by chronic hyperglycemia due to disturbances in the fundamental production of insulin leading to abnormal metabolism of fat, sugar & protein and it is known to affect

oral disease progression [1,2]. Diabetics patients suffer from Macrovascular changes, that develop over 10 - 15 years & are first diagnosed as changes in each of kidney, retina & nervous system. Also changes on the level of small vessels can be seen in the oral tissues [1].

The technique which named exfoliative cytology can be considered as a diagnostic technique based on a microscopical estimstion of epithelial cells after a process of their fixation and staining [2]. Exfoliative cytology is a noninvasive technique and the smears gained can be analyzed quantitatively and qualitatively. Quantitative cytomorphometric assessments of exfoliated oral mucosal cells have shown clear changes in the cells gained from malignant and pre-malignant lesions [3]. The uses of oral exfoliative cytology were limited because of their nature of its explanations and the altitude of false-negative results. These limitations were overcome via the introduction of quantitative methods such as image analysis systems, particularly in the evaluation of cyto-morphometric changes [4]. Studies on oral mucosal cells of type 2 diabetic patients have shown qualitative and quantitative ulterations upon cellular levels compared to healthy normal individuals (Rats). little research was done in this field. Therefore, in this study decided to estimate the quantitative and qualitative histological changes in oral epithelial of type 2 DM patients, using the exfoliative cytology [3]. The disease process of diabetes results in many problems of dental interest. Many studies pointed out a higher spread and severity of some diseases in the oral tissues of diabetic patients like, candidiasis, gingivitis, periodontitis and some opportunistic infections [3]. It has been proven that diabetes may also be the cause of various ulterations in the cells of the oral mucosal tissues, that can be discovered by using of exfoliative cytology technique [5].

Materials and Methods
The study was conducted on patients attending the teaching hospital of college of dentistry / Tikrit University, the rang of patients age were (50-65) years, samples were taken from 10 patients with type 2 diabetes mellitus, for the control group, samples were taken from other 10 healthy individuals and the smokers and alcoholic drinkers were excluded. obtaining a pre-approval from them to participate in this study. Two samples were taken from each participate, the first sample by using the cotton swab passing on buccal mucosa and the second was saliva sample by collected from the patient. Both samples were placed on glass slides immediately and directly stained with Giemsa dye [6] remain for 3-5 minutes and then washed with distilled water gently and examined under the light microscope.

Results
Through microscopial examination of the smears obtained from buccal mucosa of control group, the cells appear in its normal size without any defects in the cytoplasm or nucleus. While that obtained from the diabetic patients (in both buccal mucoa and gingiva) noticed many morphological changes. These changes were in the form of binucleation, karyolysis, karyorrhexis, micronuclei, perinuclear halos, vesicular degeneration and cellular swelling (hypertrophy) (Fig. 1-7).

Discussion
Many research have studied the effects of DM type II on oral mucosal tissues, and they reporting its pernicious effects on the morphology of these tissues, and in turn may affect on their function to favor the occurrence of either oral infections or even oral neoplasm. In diabetes, there are losses of oxidation equilibriums, which will lead to a weakening of the activity of antioxidant enzymes and their scavenging agents due to the high concentration of glucose, thus, this is what causes an excessive increase in free radicals, and protein glycation. These harmful processes can cause severe injuries to biological structures at the molecular level which can be matured by oral exfoliative cytology technique [7].

The primary defect to change any cell starts at the molecular level which leads to chain reactions and thus affect the entire cell systems, and thus its shape. The general biological activities of the cells are better reflected in the nuclei and functional activities are reflected in the cytoplasms [8]. Delay in the keratinization is attributed to glycation changes. The continuous hyperglycemia leads to larger accumulation of advanced glycation end products via an abnormal degredation of lipids, proteins, and nucleic acids in the walls of large blood vessels as well as in the basal lamina of smaller vasculature [9]. The gradual narrowing of the lumen of the blood vessel leads to a decrease in the perfusion of the injured tissues and thus a decrease in the rate of cell turnover, which explains the delay in the keratinization pathway of the epithelial cells. This delay in the epithelial differentiation process increases the number of mature cells showing a large nucleus as an initial characteristic. [10].

Changes in oral mucosa have been also associated with nutritional deficiencies, it is observed in patients with type 2 diabetes, where an insufficincy of folic acid and vitamin B12 delays the synthesis of DNA, which is the basic material of cell nucleus, and thus may alter the size of both cytoplasm and nucleus [11].

Conclusion

Diabetes mellitus type 2 is a real threat on oral tissues in general, and in particular cause many histological changes in oral epithelia especially of buccal mucosa and gingiva which detected by exfoliative cytology that was a beneficial technique to determine these chamges.
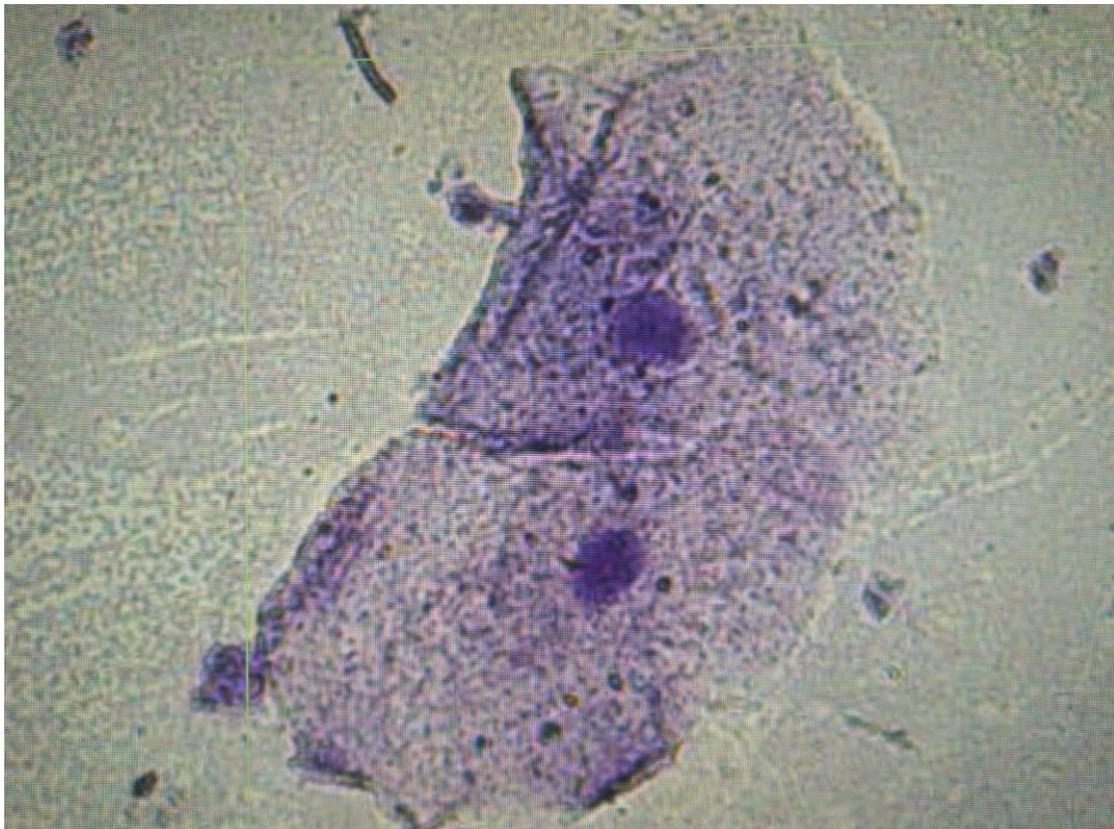

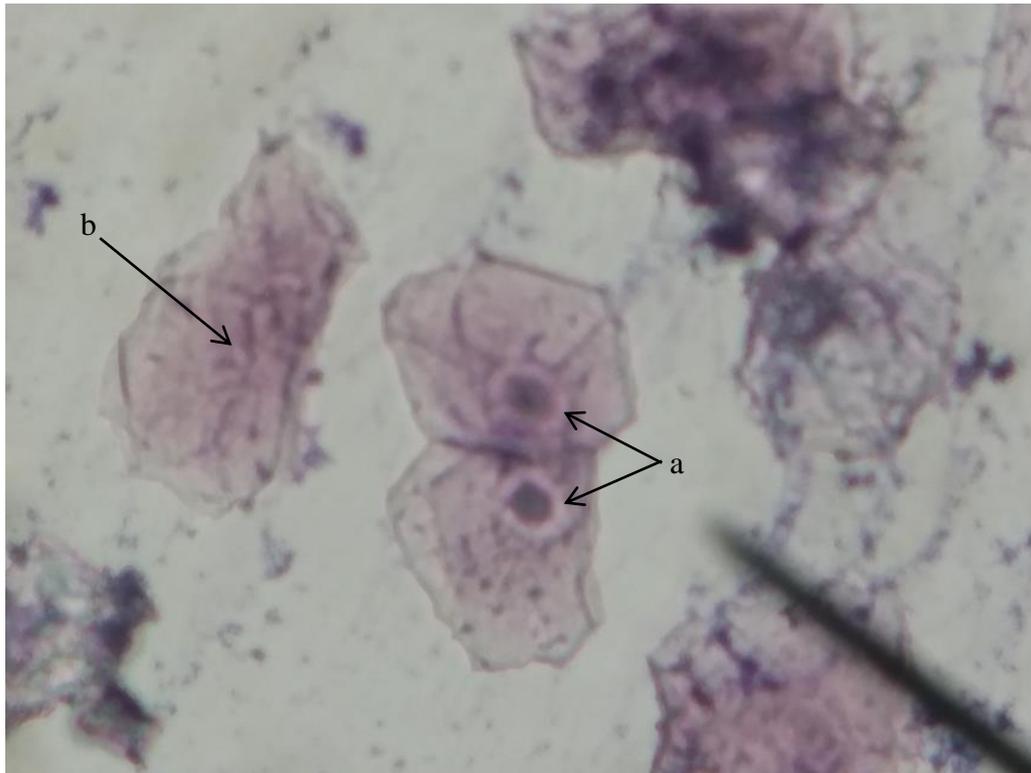Fig 1: Normal epithelial cell from buccal mucosa of control group . Giemsa stain (400x).

Fig 2: Epithelial cells of buccal mucosa of diabetic patients shows a- perinuclear halos and b-nuclear karyolysis. Giemsa stain (400x).
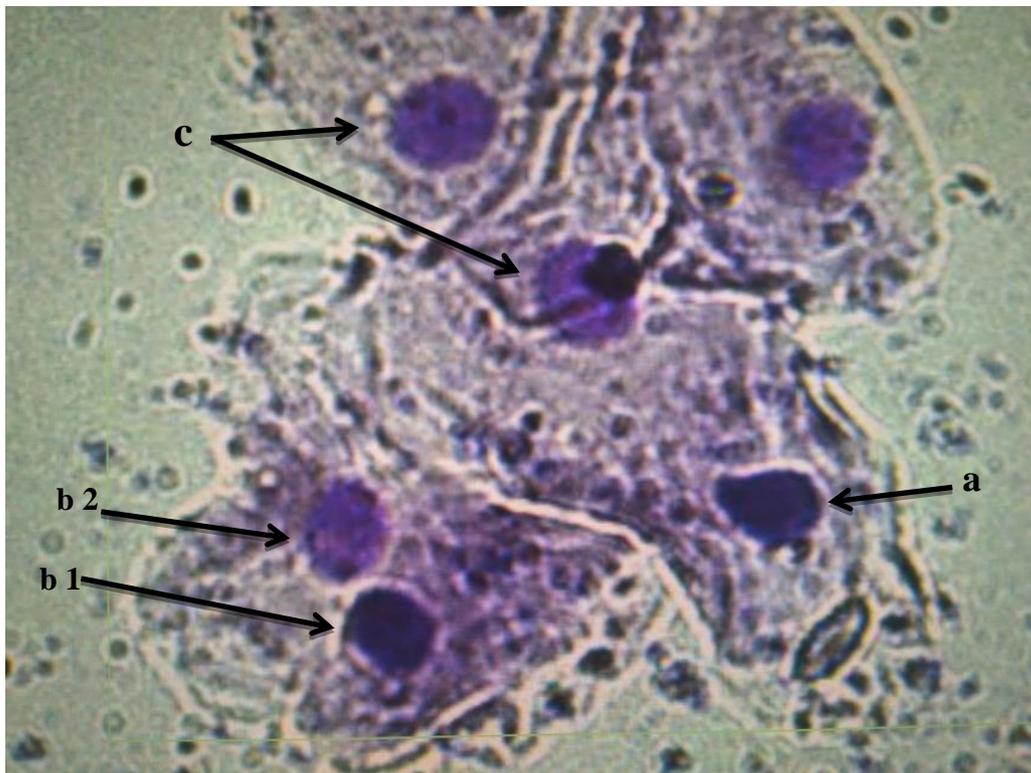

Fig 3: epithelial cells of gingiva in diabetic patient showing a- nuclear budding. b- (b1-nuclear pyknosis and   b2-bi nucleated cell). c- nuclear enlargement (aged cell) . Giemsa stain (400x).
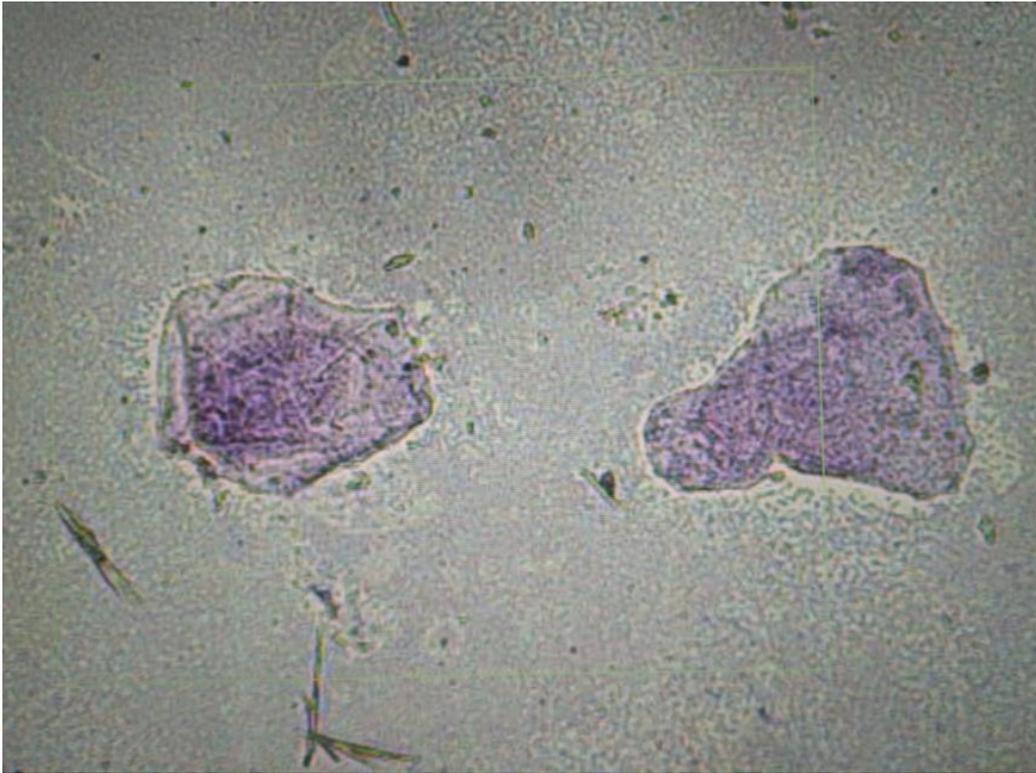
Fig 4: Epithelial cells of buccal mucosa in diabetic patient showing karyolitic nucleus   Giemsa stain (400x).

Fig 5: Epithelial cells of gingiva of diabetic patiemt showing a- karyorrhexis b- vesicular degeneration Giemsa stain (600x).
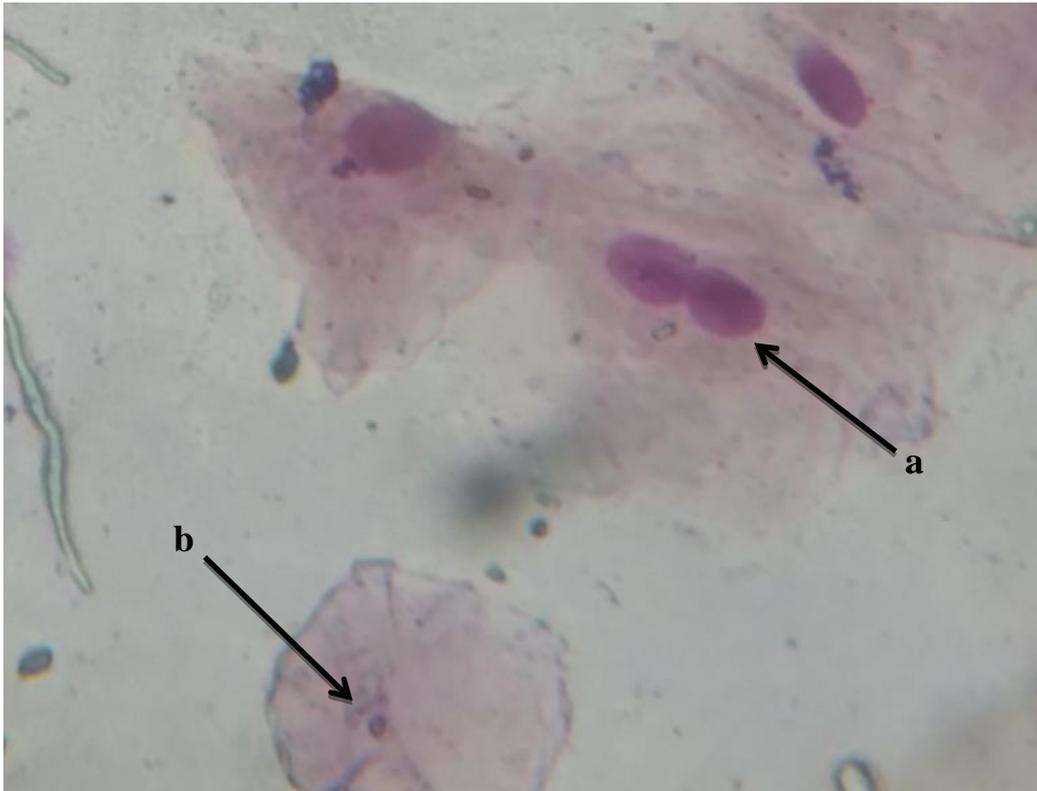
Fig 6: Epithelial cells of buccal mucosa of diabetic patient showing a- binucleation b- micronuclei Giemsa stain (400x).
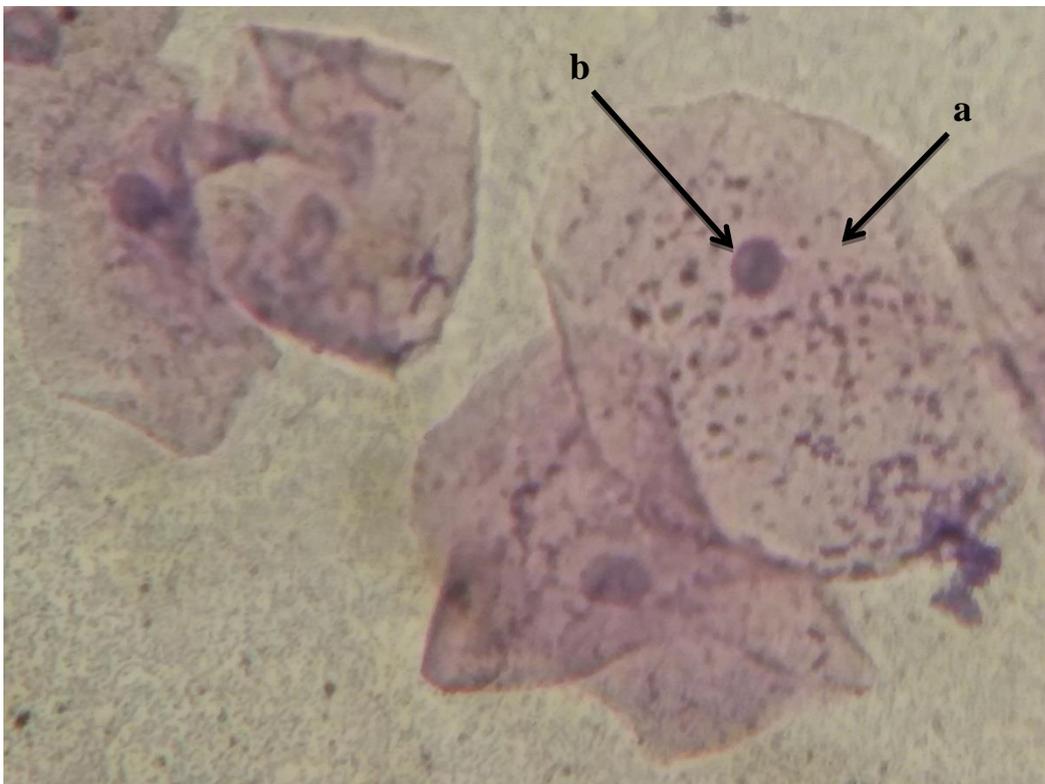

Fig 7: Epithelial cells of buccal mucosa of diabetic patient showing a- cellular hypertrophy b- micronuclei Giemsa stain (400x).

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care.2004;27:1047–53. [PubMed] [Google Scholar]

2. Rajput DV, Tupkari JV. Early detection of oral cancer: PAP and AgNOR staining in brush biopsies. J Oral Maxillofac Pathol. 2010; 14, 52–58.

3. Maloth1 S, Mukunda A , Padmashree S. Evaluation of Oral Mucosal Cells in Patients with type 2 Diabetes by Exfoliative Cytology Method. International Journal of Oral Health Dentistry; January-March 2017;3(1):34-37

4. Gururaj N. Sivapathasundaram. Cytological findings in iron deficiency anemia. Indian J Dent Res 2004;15:126-8.

5. Ogden GR, Cowpe JG, Wight AJ. Oral exfoliative cytology: review of methods of assessment. J Oral Pathol Med. 1997;26:201–5. [PubMed] [Google Scholar]

6. ADVANCE study. Intensive Blood Glucose Control and Vascular Outcomes in Patients with Type 2 Diabetes - The ADVANCE Collaborative Group 2008,

7. Auluck A. Diabetes mellitus.; An emerging risk factor for oral cancer? J Can Dent Assoc; 2007;73:501-3.

8. Sivapathasundharam B and Kalasagar M. Yet another article on exfoliative cytology. J Oral Maxillofac Pathol.; 2004;8:54–7. [Google Scholar]

9. Nandita K. P., Karen Boaz, Srikant N., Amitha J. Lewis and Nidhi Manaktala. Oral epithelium in diabetics: A cytomorphometric correlation, Dental Hypotheses, 2014;Vol 5 , Issue 2, 59-65.

10. Patel PV and Gujjari SK. Cytomorphometric analysis of the gingival epithelium in type 2 diabetic patients with and without smoking habit. J Cytol.2013;30:109-15.

11. Prasad H, Ramesh V and Balamurali P. Morphologic andcytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients. J Cytol.2010;27:113-7.