



Oral Finding and Cytomorphometric Analysis of Oral Mucosal Cells in Type 2 Diabetic Patients

Rouaa S. Farhan^{1*} and Layla Sabri Yas²

¹Master Student, Department of Oral Diagnosis, College of Dentistry, University of Baghdad, Baghdad, Iraq

²Assistant Professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad, Baghdad, Iraq

*Corresponding e-mail: dr.rouaa_khy@yahoo.com

ABSTRACT

Background/purpose: Patients with diabetes mellitus have been associated with gingivitis, periodontitis, salivary dysfunction, altered taste, and candidiasis. Diabetes can cause considerable cellular changes. The aims of this study were to assess the oral manifestations in type 2 diabetic patients and to detect cytomorphometric measurements of oral mucosal cells in type 2 diabetic patients and healthy control subjects by using exfoliated cytology smears.

Methods: Samples were collected from 50 adults, aged 30-60 years (25 patients with type 2 diabetes mellitus and 25 non-diabetic healthy persons as the control group). Smears were obtained from two sites, normal buccal mucosa and lateral border of the tongue from each subject. The freshly obtained specimens were assessed for cytomorphometric analysis. An eyepiece micrometer was used to take mean values of ND, CyD, and N: C ratio. Comparison of nuclear diameter (ND), cytoplasmic diameter (CyD) and the ratio of two diameters (N: C) among groups was performed by using ANOVA. **Results:** The results showed that statistically significant increase in ND ($p=0.01$ for buccal mucosa and 0.007 for tongue) and N:C ratio ($p=0.01$ for both tongue and buccal mucosa), with a statistically significant decrease in CyD, was found in diabetic patients compared to controls ($p=0.001$ for both tongue and buccal mucosa).

Conclusion: Diabetes produces definite cytomorphometric changes in the oral mucosal cells of patients. The results suggested that nuclear diameter increased while cytoplasmic diameter was decrease in type 2 diabetic patients. The most predominant oral manifestations found in type 2 diabetic patients were periodontal disease and oral dryness.

Keywords: Oral exfoliative cytology, Oral mucosa, Morphology

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by inappropriate blood hyperglycemia, resulting from the failure of the pancreatic beta cells to produce insulin and/or inability of the body to employ the insulin produced because of insulin deficiency in the body cells. In the body, insulin is the only hormone that reduces blood glucose levels while other hormones such as thyroid hormone, glucagon, growth hormone, catecholamine (epinephrine and norepinephrine) and glucocorticoids all elevate the blood glucose levels [1]. The broad categories of DM are designated as type 1 known as insulin dependent diabetes mellitus (IDDM) and type 2 known as non-insulin dependent diabetes mellitus (NIDDM). Type 2 DM is a heterogeneous group of the disorder usually characterized by insulin resistance, impaired insulin secretion and increase glucose production. Distinct genetic and metabolic defect in insulin action and secretion give rise to the common phenotype of hyperglycemia in type 2 DM [2,3]. The main complications associated with DM are retinopathy, nephropathy, and micro/macroangiopathy. It damages tissue repair processes and causes stomatologic problems of dental interest. Several studies suggest a higher prevalence and severity of oral pathologies like gingivitis, periodontitis, candidiasis, and other manifestations such as alteration of salivary flow and burning sensation [4]. Several studies have examined the deleterious effects of diabetes on oral mucosa. It was reported that diabetes adversely affects the morphology of cheek mucosa, which may compromise

tissue function to favor the occurrence of oral infections and neoplasia [5,6]. Oral exfoliative cytology may be more appropriate in a condition like DM where the invasive techniques lose viability. The morphologic and functional changes in oral mucosa can be studied at the cellular level by using exfoliative cytology which can help in diagnosis with better patient acceptability. Exfoliative cytology is the study of superficial cells which have been exfoliated from mucous membrane or which have been scraped or pulled from the surface. These exfoliated cells are stained with various stains for example Papanicolaou (PAP) according to need. It is the painless, non-invasive, and less time-consuming procedure. With the advancement in the field of quantitative exfoliative cytology, there has been a re-emergence of oral exfoliative cytology as a powerful diagnostic tool. By using cytomorphometric analysis various parameters such as cytoplasm diameter, nuclear diameter, and nuclear to cytoplasmic ratio can be evaluated. CD, ND, and N:C ratio has shown to be significant in the diagnosis of oral and systemic diseases [7].

MATERIALS AND METHODS

Patients attending at the Diabetic Clinic in AL-Mawani General Hospital (Diabetic-Endocrinology Center) in Basra city during the period from (February 2017 to June 2017). Informed consent was obtained from each individual and a data sheet was completed, detailing the name, age, sex, relevant medical history, dental history, etc. Only patients with a known history of diabetes were included in the study group. Patients were included irrespective of whether they were under any medications for diabetes or not. Control group included normal healthy adult individuals with no history of diabetes or any other illnesses. All participants in this study were examined to see if there are any oral manifestations. Patients with habits like tobacco or alcohol intake; those with anemia or any other systemic illnesses or those who were under any medications other than for diabetes were excluded because previous studies have shown that cell and nuclear sizes are influenced by these factors [8]. A total of 25 diabetic patients and 25 control subjects were included in the study.

Smear Collection

The smear was taken from two sites buccal mucosa and lateral border of the tongue. Before sample was taken the patients were asked to wash their mouth with tap water to remove any debris, then cells from buccal and tongue mucosa were collected by using a cytobrush of Pap smear (disposable kit of pap smear). Uncontaminated, new, dry glass slides were used to fix the smears. Scrapings were placed in the middle of a glass slide and spread over a large area to avoid clumping of cells. The smears were fixed in 96% ethanol and stained by the Papanicolaou method for cytomorphometric analysis. Lastly, smears were dried in 95% absolute ethanol, cleared in xylene, and formerly mounted in the DPX (Di-N-butyl phthalate in xylene).

Cytomorphometric Assessment

Twenty clearly defined cells were measured in each case. An eyepiece micrometer was used to obtain the nuclear diameter (ND), Cytoplasmic diameter (CyD) and nucleus to cytoplasm ratio (N:C).

Statistical Analysis

Continuous variables were presented as the mean \pm standard deviation (SD) whereas categorical variables were expressed as percentages. Data were analyzed using SPSS version 22 for Windows and p-values less than 0.05 were considered statistically significant.

RESULTS

Out of the total 50 participants who were included in the study (25 patients were with type 2 diabetes and 25 were healthy control patients). All participants in this study were examined to see if there are any oral manifestations. Table 1 showed different oral findings in patients with type 2 DM. Oral manifestation in DM group showed highest frequency was for periodontal disease (76%) followed by dry mouth (52%) while the lowest frequency was for altered taste, oral candidiasis, and periodontal abscess (8%, 8%, 4%) respectively.

Table 1 Prevalence of oral manifestation in type 2 diabetes patients

Groups	DM	
	No.	%
O.M		
Periodontal diseases	19	76
Periodontal abscess	1	4
Dry mouth	13	52
Altered taste	2	8
Oral candidiasis	2	8

O.M: oral manifestation

Cytomorphometry showed that nuclear diameter (ND) was significantly larger in patients with diabetes (for both the tongue (p=0.007) and buccal smear (p=0.01) (Table 2 and Figure1).

Table 2 Cytomorphometric analysis results of ND of oral exfoliative cytology smear in both site for control patients and DM2 (Mean ± SD)

Variables	Nuclear Diameter	
	Buccal mucosa	Tongue lateral border
Group		
Control healthy	7.720 ± 0.559	8.410 ± 0.405
Diabetic	9.390 ± 0.368	9.719 ± 0.226
p-value	0.01	0.007

*A significant difference if p-value less than 0.05

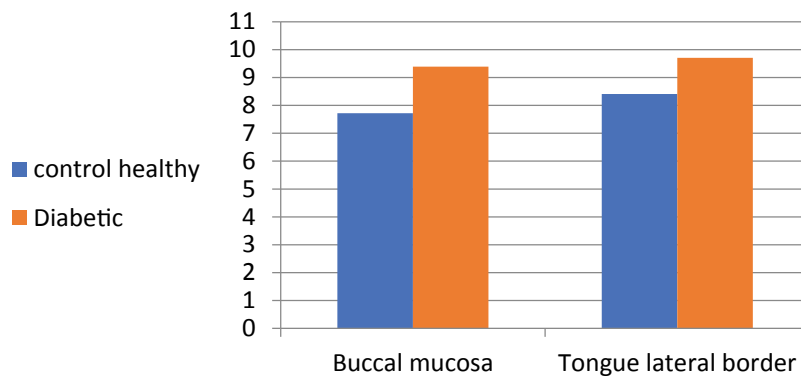


Figure 1 Mean nuclear diameter (ND) value in control and diabetic group

Cytoplasmic diameter (CyD) was also significantly smaller in patients with diabetes (p=0.001) for both the tongue and buccal smear (Table 3 and Figure 2).

Table 3 Cytomorphometric analysis results of CyD of oral exfoliative cytology smear in both site for control patients and DM2 (Mean ± SD)

Variables	Cytoplasmic Diameter	
	Buccal mucosa	Tongue lateral border
Group		
Control healthy	50.931 ± 0.456	51.488 ± 0.583
Diabetic	47.269 ± 0.507	48.319 ± 0.397
p-value	0.001	0.001

*A significant difference if p-value less than 0.05

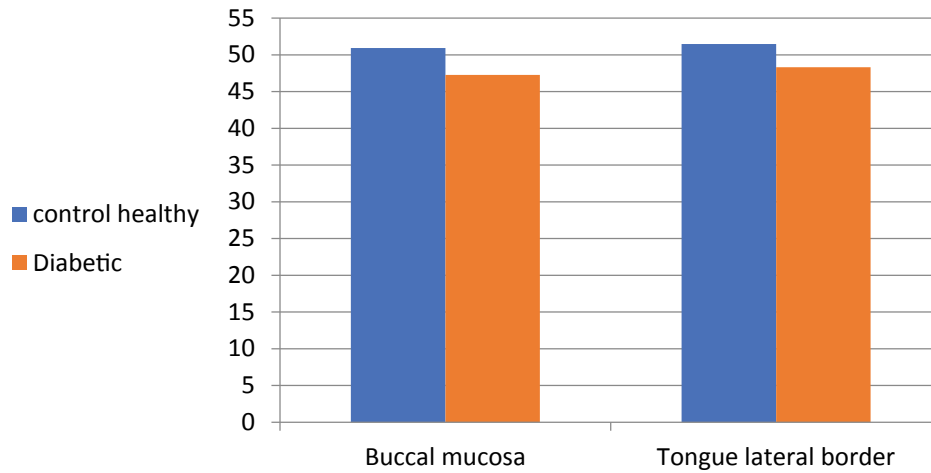


Figure 2 Mean cytoplasmic diameter (CyD) value in control and diabetic group

Nuclear-cytoplasmic (N:C) ratio was significantly larger in diabetes (p=0.01 for both smear sites) (Table 4 and Figure 3). Figures 4 and 5 indicate the nuclear and cytoplasmic diameters in normal healthy and diabetic groups.

Table 4 Cytomorphometric analysis results of N:C ratio of oral exfoliative cytology smear in both site for control patients and DM2 (Mean ± SD)

Variables	Nuclear/Cytoplasmic ratio	
	Buccal mucosa	Tongue lateral border
Control healthy	0.151 ± 0.013	0.163 ± 0.004
Diabetic	0.199 ± 0.032	0.202 ± 0.006
p-value	0.01	0.01

*A significant difference if p-value less than 0.05

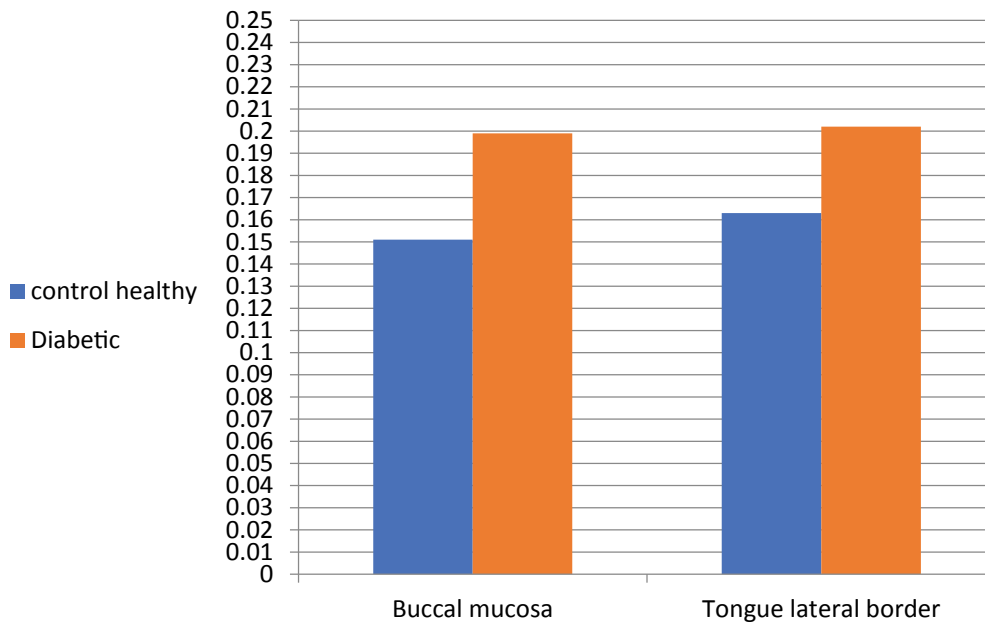


Figure 3 Mean nuclear-cytoplasmic (N:C) ratio value in control and diabetic group

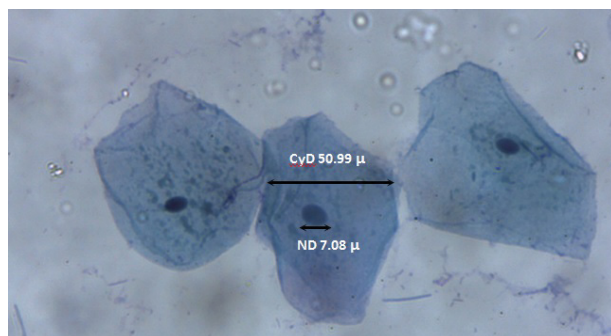


Figure 4 PAP stained cytological smear of normal healthy group ($\times 40$) showing nuclear and cytoplasmic diameters

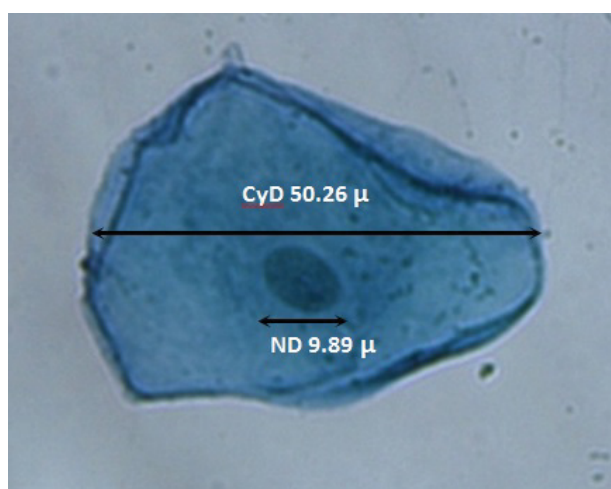


Figure 5 PAP stained cytological smear of diabetic group ($\times 40$) showing nuclear and cytoplasmic diameters

DISCUSSION

Diabetic patients suffer many oral manifestations among which gingival and periodontal diseases are main. Diabetes mellitus is usually associated with various oral changes. Some authors believe that it might be due to altered immunological response in diabetes such as lower chemotaxis and phagocytosis, and due to the involvement of microcirculation because of reduction in blood supply. This makes diabetic patients more prone to oral infections and alterations [9].

The results of present study exhibited a higher percentage of periodontitis in DM group (76%). There were some studies that agree with this current finding reporting that severity of gingival bleeding and periodontal disease was more in individuals with poorly controlled type 2 diabetes, but contradict with the study of Nichols, et al., that suggest glycemic control is not correlated with periodontal status in diabetic patients [10-12]. Chandna, et al., also showed periodontitis to be a recognized complication of diabetes and it was more common in individuals with elevated glucose levels [13]. Rees, et al., suggested that the incidence and severity of periodontitis were influenced by the presence or absence of DM, as well as the severity of hyperglycemia [14]. A number of surveys have suggested that numerous contributing factors are responsible for increased susceptibility of diabetes mellitus to periodontal diseases with increase in the risk threefold because of compromised polymorph nuclear leukocyte function resulting from impaired neutrophil adherence, chemotaxis, and phagocytosis prevent destruction of bacteria in the periodontal pocket and markedly enhance periodontal destruction, angiopathy, altered microbial flora, abnormal collagen metabolism, alterations in salivary flow and composition [15]. Abnormalities of collagen metabolism, impaired proliferation of osteoblasts and weakened mechanical properties of newly formed bone have been documented in hyperglycemic patients [16-18].

Also, the result of the present study showed that xerostomia was more frequent in diabetics group (52%). Various studies have reported that xerostomia is one of prominent manifestation in diabetic patients. In this study, the data

revealed that xerostomia is a common oral manifestation among diabetes. This result is in agreement with other studies [19-21].

This very common symptom of the disease and has been linked with dysfunction of the parenchyma of the major salivary glands and with polyuria. The substitution of the functioning tissue by adipose tissue has been suggested to quantitatively and qualitatively modify saliva production, facilitating hyposalivation and burning mouth symptoms.

In addition, the result of the current study showed other oral findings were less frequently such as altered taste, oral candidiasis, and periodontal abscess. Taste dysfunction has been reported in a study to occur more frequently in patients with poorly controlled diabetes compared to healthy controls [16]. Candidiasis may result from xerostomia, hyperglycemia or deficient leukocyte function and is more prevalent in diabetics with poor blood glucose control [22], for periodontal abscess it has been statistically proven that diabetes is one of the predisposing factors for the development of periodontal disease and more prevalent in the uncontrolled and controlled diabetics than in the non-diabetic [23].

The present study performed cytomorphometric analysis on both sites smears obtained from patients with diabetes and compared the slides with those from healthy individuals. The first parameter assessed was a nuclear diameter, which was increased in diabetics in the exfoliated cells of normal buccal and tongue mucosa in type 2 diabetic patients. This finding is consistent with several studies where nuclear change was significantly higher in the diabetic group [4,24-28]. The reason for ND increase among the study group might be related to sustained hyperglycemia which could be explained by the delay in keratinization of oral epithelium, effects of aging, dehydration/atrophy, and inflammatory process. Delay in the keratinization is attributed to glycation changes. Sustained hyperglycemia causes a greater accumulation of advanced glycation end products by abnormal glycation of proteins, lipids, and nucleic acids in the walls of large blood vessels as well as in the basement membrane of the microvasculature. The progressive narrowing of the vessel lumen leads to decreased perfusion of the affected tissue and consequently decreases cell turnover, thereby explaining the delay in the keratinization process of the epithelium. This delay in the process of epithelial differentiation leads to an increase in the number of mature cells, which present a large nucleus as a primary characteristic [29,30].

Another parameter assessed was cytoplasm diameter. The results of the present study showed the difference in mean of CyD between study groups was a statistically significant decrease in tongue and buccal mucosa cells from patients with type 2 diabetes when compared to control patients. This is similar to the findings of Hallikerimath, et al. [27]. The results also agree with Shareef, et al., in a similar study found a statistically significant decrease in CyA, which could be due to the cell shrinkage caused by dehydration [26]. But contradictory to the findings of Jajarm, et al., who reported a significant increase in the cytoplasmic area the in diabetic patients [25].

In the present study, a significant increase was found in the mean values of N:C ratio of the exfoliated cells from both smears sites with diabetes when compared to control patients. The NCR values are coincident with the study realized by Hallikerimath, et al. [27]. This finding inconsistent with the findings by Jajarm, et al., according to him the mean N:C ratio was significantly lower in diabetic group as compared to the controls [25].

CONCLUSION

There is an increase in ND in patients with type 2 diabetes compared to normal individuals. In contrast to the increase in ND, there is a decrease in CyD in patients with diabetes. The N:C ratio increases in diabetics. From the present study, we conclude that type 2 diabetes produces morphometric changes in the exfoliated oral mucosal cells.

DECLARATIONS

Conflict of Interest

The authors declare no potential conflict of interest.

REFERENCES

- [1] Wild, Sarah, et al. "Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030." *Diabetes Care*, Vol. 27, No. 5, 2004, pp. 1047-53.
- [2] Braunwald, Eugene, et al. *Harrison's Principles of Internal Medicine: Textbook, Self-Assessment and Board Review*. 15th ed., McGraw-Hill, 2001.

- [3] Gardner, David G., Dolores M. Shoback, editors. *Greenspan's Basic and Clinical Endocrinology*. 9th ed., McGraw-Hill Medical, 2011.
- [4] Alberti, Sandra, et al. "Exfoliative cytology of the oral mucosa in type II diabetic patients: morphology and cytomorphometry." *Journal of Oral Pathology & Medicine*, Vol. 32, No. 9, 2003, pp. 538-43.
- [5] Caldeira, Eduardo José, et al. "Morphometric analysis and ultrastructure of the epithelium of the oral mucosa in diabetic autoimmune NOD mice." *Brazilian Journal of Morphological Sciences*, Vol. 21, No. 4, 2004, pp. 197-205.
- [6] Auluck, Ajit. "Diabetes mellitus: an emerging risk factor for oral cancer?" *Journal Canadian Dental Association*, Vol. 73, No. 6, 2007, pp. 501-503.
- [7] Nayar, Amith K., and B. Sivapatha Sundharam. "Cytomorphometric analysis of exfoliated normal buccal mucosa cells." *Indian Journal of Dental Research: official publication of Indian Society for Dental Research*, Vol. 14, No. 2, 2003, pp. 87-93.
- [8] Ogden, Graham Richard, Andrew John Wight, and Jonathan George Cowpe. "Quantitative oral exfoliative cytology. Effect of alcohol on normal buccal mucosa." *Analytical and Quantitative Cytology and Histology*, Vol. 21, No. 2, 1999, pp. 126-30.
- [9] Beikler, Thomas, and Thomas F. Flemmig. "Implants in the medically compromised patient." *Critical Reviews in Oral Biology & Medicine*, Vol. 14, No. 4, 2003, pp. 305-16.
- [10] Javed, Fawad, et al. "Comparison of periodontal and socioeconomic status between subjects with type 2 diabetes mellitus and non-diabetic controls." *Journal of Periodontology*, Vol. 78, No. 11, 2007, pp. 2112-19.
- [11] Bridges, Raymond B., et al. "Periodontal status of diabetic and non-diabetic men: effects of smoking, glycemic control, and socioeconomic factors." *Journal of Periodontology*, Vol. 67, Vol. 11, 1996, pp. 1185-92.
- [12] Nichols, Chris, Larry L. Laster, and Levente Z. Bodak-Gyovai. "Diabetes mellitus and periodontal disease." *Journal of Periodontology*, Vol. 49, No. 2, 1978, pp. 85-88.
- [13] Chandna, S., et al. "Diabetes mellitus-a risk factor for periodontal disease." *Internet Journal of Family Practice*, Vol. 9, No. 1, 2010, pp. 181-84.
- [14] Rees, Terry D. "Periodontal management of the patient with diabetes mellitus." *Periodontology*, Vol. 23, No. 1, 2000, pp. 63-72.
- [15] Mealey, Brian L., and Thomas W. Oates. "Diabetes Mellitus and Periodontal Diseases." *Journal of Periodontology*, Vol. 77, No. 8, 2006, pp. 1289-1303.
- [16] Lalla, Rajesh V., and Joseph A. D'ambrosio. "Dental management considerations for the patient with diabetes mellitus." *The Journal of the American Dental Association*, Vol. 132, No. 10, 2001, pp. 1425-32.
- [17] Ship, Jonathan A. "Diabetes and oral health: an overview." *The Journal of the American Dental Association*, Vol. 134, 2003, pp. 4S-10S.
- [18] Azodo, C. C. "Current trends in the management of diabetes mellitus: the dentist's perspective." *Benin Journal of Postgraduate Medicine*, Vol. 11, No. 1, 2009.
- [19] Malicka, Barbara, Urszula Kaczmarek, and Katarzyna Skośkiewicz-Malinowska. "Prevalence of xerostomia and the salivary flow rate in diabetic patients." *Advances in Clinical and Experimental Medicine*, Vol. 23, No. 2, 2014, pp. 225-33.
- [20] Quirino, M. R., Esther G. Birman, and Claudete R. Paula. "Oral manifestations of diabetes mellitus in controlled and uncontrolled patients." *Brazilian Dental Journal*, Vol. 6, No. 2, 1995, pp. 131-36.
- [21] Chávez, Elisa M., et al. "A longitudinal analysis of salivary flow in control subjects and older adults with type 2 diabetes." *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics*, Vol. 91, No. 2, 2001, pp. 166-73.
- [22] Guggenheimer, James, et al. "Insulin-dependent diabetes mellitus and oral soft tissue pathologies. II. Prevalence and characteristics of Candida and candidal lesions." *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics*, Vol. 89, No. 5, 2000, pp. 570-76.

- [23] Ogunbodede, E.O., et al. "Oral health status in a population of Nigerian diabetics." *Journal of Contemporary Dental Practice*, Vol.6, No. 4, 2005, pp. 75-84.
- [24] Jajarm, Hassan Hosseinpour, Nooshin Mohtasham, and Afsaneh Rangiani. "Evaluation of oral mucosa epithelium in type II diabetic patients by an exfoliative cytology method." *Journal of Oral Science*, Vol. 50, No. 3, 2008, pp. 335-40.
- [25] Shareef, Ban Tawfeek, Kok Teong Ang, and Venkatesh Ramasamy Naik. "Qualitative and quantitative exfoliative cytology of normal oral mucosa in type 2 diabetic patients." *Medicina Oral Patologia Oral y CirugiaBucal*, Vol. 13, No. 11, 2008, pp. E693-E696.
- [26] Prasad, H., V. Ramesh, and P.D. Balamurali. "Morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients." *Journal of Cytology/Indian Academy of Cytologists*, Vol. 27, No. 4, 2010, p. 113.
- [27] Hallikerimath, Seema, et al. "Cytomorphometric analysis and assessment of periodic acid Schiff positivity of exfoliated cells from apparently normal buccal mucosa of type 2 diabetic patients." *Acta Cytologica*, Vol. 55, No. 2, 2011, pp. 197-202.
- [28] Suvarna, M., et al. "Cytomorphometric analysis of exfoliative buccal cells in type II diabetic patients." *Journal of Dr. NTR University of Health Sciences*, Vol. 1, No. 1, 2012, p. 33.
- [29] Greenberg, Mark S. *Burket's Oral Medicine: Diagnosis and Treatment*. 10th ed., Elsevier, 2003.
- [30] Kumar, Vinay, et al. *Robbins Basic Pathology*. 8th ed., Elsevier Health Sciences, 2007.