International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Evaluation of Buccal Mucosal Cell in Type 2 Diabetic Patients: (Cytomorphometrical Study)

Rouaa Shakir Farhan[1], Layla Sabri Yas[2]

[1]Master Student, Oral Medicine, Department of Oral Diagnosis, College of Dentistry/ University of Baghdad, Iraq

[2]B.D.S., MSc Assistant Professor, Department of Oral Pathology, College of Dentistry/ University of Baghdad

Abstract: ***Background: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, associated with disturbances in the metabolism of carbohydrates, lipids, and protein.Diabetes can cause considerable cellular changes. Objectives: The Objective of this study was to detect the cytomorphometric measurements (the cytoplasmic diameter, nuclear diameter, and nucleus: cytoplasm ratio) forexfoliated buccal mucosal cells of type 2 diabetic patients and healthy control subjects by use the cytological smears, and to compare thesemeasurements in different study groups. Methods: The total sample composed of 75 adult, aged 30-60 years.The study sample was divided intothree groups. Group 1 was Control healthy, Group 2 was controlled diabetics with HbA1c ≤ 7.0% and Group 3 was uncontrolled diabetics with HbA1C ≥ 7.0% .Smears were obtained from normal buccal mucosafrom each subject. Thefreshy obtained specimens stained with Papanicolaou technique for cytomorphometric analysis. An eyepiece micrometer was used to take mean values of ND, CyD, and N: C ratio. Twenty (20) clearly defined cells were measured in each case in a step wise manner, to evade quantifying cells once more. Comparison of Nuclear Diameter (ND), Cytoplasmic Diameter (CyD) and ratio of two Diameters (N: C) among three groups was performed by using ANOVA. Results: The results showed that statistically significant increase in ND (P=0.001) with statistically significant decrease in CyD(P=0.001)was found in diabetic patients compared to controls. Degree of glycemic control significantly affected ND (P=0.007) and N: C ratio (P=0.001). In general, as the severity of diabetesincreases, ND and N: C ratio rise gradually. Conclusion: Diabetes produces definite cytomorphometric changes in the buccal mucosaof patients. The results suggested that nuclear diameter of buccal mucosal cells increased while cytoplasmic diameter was decrease in type 2 diabetic patients.***

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized byinappropriate blood hyperglycemia, resulting from the failure of the pancreaticbeta cells to produce insulin and/or inability of the body to employ the insulinproduced 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 Type1 known asInsulin Dependent diabetes mellitus (IDDM) and Type 2 known as Non-Insulin Dependent diabetes mellitus (NIDDM). Type 2 DM is a heterogeneous group of 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 common phenotype of hyperglycemia in type 2 DM [(2, 3)].The main complications associated with DM are retinopathy, nephropathy andmicro/macro angiopathy. It damages tissue repair processes and cause 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)].A significant indicator of common condition and possible diabetes complications is metabolic control, which is monitored through the blood glucose level (BGL) and glycosylated hemoglobin level (HbA1c). In 2009, an international committee for the diagnosis of diabetes recommended use of glycated haemoglobin (HbA1c), an index of average plasma glucose over several weeks, as a marker for the disease [(5)].This recommendation was also made by theAmerican Diabetes Association (ADA) in 2010, which suggested that HbA1c > 6.5% (48 mmol/mol) be considered diagnostic of diabetes [(6)].HbA1c is considered equal to fasting plasma glucose (FPG) as a predictor of diabetes [(7)].Several studies have examined the deleterious effects of diabetes on oralmucosa. It was reported that diabetes adversely affects the morphology of cheek mucosa, which may compromise tissue function to favour the occurrence of oral infections and neoplasia [(8,9)].Oral exfoliative cytology may be more appropriate in condition like DM wherethe invasive techniques lose viability. The morphologic and functional changes in oral mucosa can be studied at 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 exfoliatedfrom mucous membrane or which have been scraped or pulled from surface. The rationale of exfoliative cytology lies in epithelial physiology. Normal epithelium undergoes continuous exfoliation or shedding of superficial cells, and it is replenished by a new crop of cells from the basal layer. These exfoliated cells are stained by various stains for example Papanicolaou (PAP) according to need. With the advancement in the field of quantitative exfoliative cytology there has been a reemergence 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 NCD ratio have shown to be significant in diagnosis of oral and systemic diseases [(10).]

2. Materials and Methods

Patients attending at the Diabetic Clinic in AL- Mawani General Hospital (Diabetic –Endocrinology Center) in Basra city during the period from (Febrewary-2017 to June

2017) for checking the glucose level using HbA1c which was done in the laboratory. Informed consent was obtained from each individual and a data sheet was completed, detailing the name, age, sex, relevant medical 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. Determine the HbA1c concentration were done in all the subjects included in the study. 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 [(11)]. A total of 50 diabetic patients and 25 control subjects were included in the study. Diabetic patients were also grouped into two categories for further analysis based on their HbA1c levels, which indicates the degree of glycemic control achieved:

- controlled diabetics – HbA1c ≤ 7.0%
- Uncontrolled diabetics – HbA1C ≥ 7.0%

The smear was taken from buccal mucosa. Before sample was taken the patients was asked to wash their mouth with tap water to remove any debris, then cells from right and left buccal 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 on the middle of glass slide and spread over a large area to avoid clumping of cells. The smears were fixed in 95% ethanol and stained by the Papanicolaou method forcytomorphometric analysis. Lastly, smears were dried in 95% absolute ethanol, cleared in Xylene and formerly mounted in the DPX (Di-N-Butyle Phthalate in Xylene).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). Continuous variables were presented as Mean ± standard deviation (SD) .Analysis of variance was applied to associate means of quantitative data in 3 groups. Data were analyzed using SPSS version 22 for Windows and p values less than 0.05 were considered statistically significant.

3. Results

Out of the total 75 participants who were included in the study (25 patients werewith uncontrolled diabetes, 25 were controlled diabetes patients which came to the diabetic clinic randomly and 25 werehealthy control patients).

Table 1 showed Cytomorphometric Comparison of ND between control and two groups of diabetes (controlled and uncontrolled) of buccal smear. It was noted that the mean nuclear diameter ND rises steadily from the control group(7.72±0.58) to the uncontrolled diabetic group(9.89±0.52). ND in the UCD group is raised significantly when it is compared with the other groups.

Table 2 showed Cytomorphometric Comparison of CyD between control and two groups of diabetes (controlled and uncontrolled) of buccal smear .The results of CyD showed significant decrease in buccal mucosa for both uncontrolled and controlled diabetes groups (45.33±0.026,49.20±0.027) respectively,whencompared with control healthy group(50.93±0.060). In addition; there was significant decrease in uncontrolled DM when compare with controlled DM.

Table 3 showed Cytomorphometric Comparison of N:C ratio between control and two groups of diabetes (controlled and uncontrolled) of buccal smear .The present study showed statistical significant increase in N: C ratio in buccal mucosa for uncontrolled DM(0.210±0.026) and controlled DM groups(0.180±0.014) when compared with control healthy group(0.151±0.013). Also there's significant increase for uncontrolled DM when compare with controlled DM.The N: C ratio again showed similar trends as the ND. It is raised significantly in the UCD group when it is compared with the other groups (P value=0.001)

Table 1: Cytomorphometric Comparison of ND between control and two groups of diabetes of buccal smear (Mean± SD)

Cell parameter	Nuclear Diameter (ND)		
Groups	Buccal mucosa	multiple comparison	p-value
Control healthy	7.72 ±0.58	Control healthy vs Controlled D.M.	0.001
Controlled DM	8.88 ±0.36	Control healthy vs Uncontrolled D.M	0.003
Uncontrolled DM	9.89 ±0.52	Controlled D.M. vs Uncontrolled D.M	0.007

*** A significant if p-value less than 0.05 (p<0.05)**

Table 2: Cytomorphometric Comparison of CyD between control and two groups of diabetes of buccal smear (Mean± SD)

Cell parameter	Cytoplasm Diameter (CyD)		
groups	Buccal mucosa	multiple comparison	p-value
Control healthy	50.93±0.060	Control healthy vs Controlled D.M.	0.001
Controlled DM	49.20±0.027	Control healthy vs Uncontrolled D.M	0.001
Uncontrolled DM	45.33±0.026	Control D.M. vs Uncontrolled D.M	0.004

*** A significant if p-value less than 0.05 (p<0.05)**

Table 3: Cytomorphometric Comparison of Nuclear/Cytoplasmic ratio between control and two groups of diabetes of buccal smear (Mean± SD)

Cell parameter	Nuclear/Cytoplasmic ratio (N:CD)		
Groups	Buccal mucosa	multiple comparison	P-value
Control healthy	0.151±0.013	Control healthy vs Controlled D.M.	0.005
Controlled DM	0.180±0.014	Control healthy vs Uncontrolled D.M	0.001
Uncontrolled DM	0.210±0.026	Controlled D.M. vs Uncontrolled D.M	0.001

*** A significant if p-value less than 0.05 (p<0.05)**

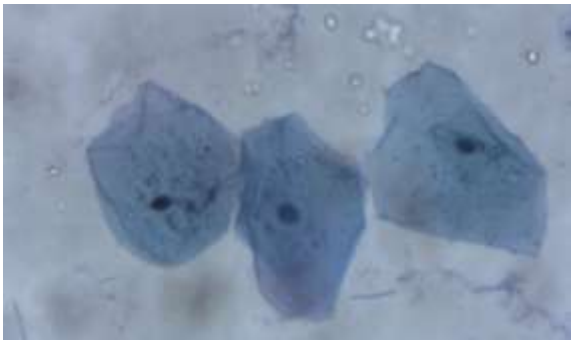

Figure 1: *Pap stained cytological smear of normal healthy group (×40).*

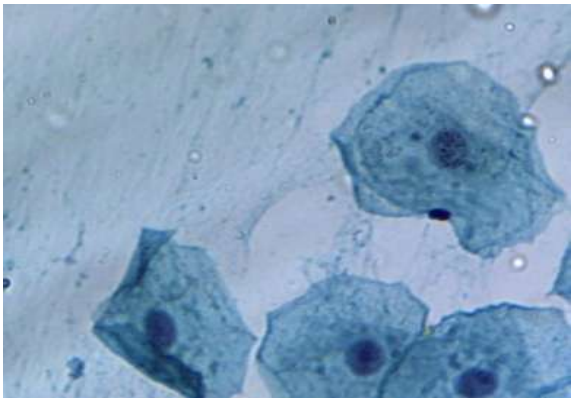

Figure 2: *Pap stained cytological smear of diabetic group (×40)*

4. Discussion

The present study was conducted to assess cytomorphometric changes in the mucosal cells which were taken from buccal site in type 2 diabetic patients. The analysis tool used for these exfoliative cells was the exfoliative cytology followed by cytomorphometric analysis of these cells, which is already accepted as a very simple, low cost, rapid and painless tool for the diagnosis of oral pathologies [(14)].In this study measured the ND, CD, CyD and N:C ratio of exfoliated buccal mucosa cells using a micrometer in the diabetic patients (*n*=50) and control subjects (*n*=25).The diabetic patients were grouped under grades of glycemic control, based on their HbA1c levels. Analysis of the three study parameters between these groups showed that glycemic control definitely influenced ND, CyD and N:C ratio, each parameter was individually compared across control group and various grades of diabetes.

The main parameters for the cytomorphometric analysis are the diameter of nucleus and cytoplasm.

The first parameter assessed was nuclear diameter, which was increased in diabetics.The NDshowed a consistent and uniform increase in diameter from control to uncontrolled diabetic group.This increase in ND is highly significant in uncontrolled diabetic patients when compared to controlled DM2 patients and to control healthy group. This finding concurs with the study by *Prasad et al.* where he found that diabetes severity (or in other words, the amount of glycemic control), measured with Hb1Ac, definitely influenced ND [(12)].Also agreewith other study by *Sadia et al.* that gave the close results when compared to this study for the variable of ND and found the nuclear diameter showed gradual increase in size from control to uncontrolled diabetic group [(13)].This finding are also in consistent with several studies [(4,14-18)].Where found the nuclear change was significantly higher in diabetic group.

The reason for ND increase among the study group might related to sustained hyperglycemia which could be explained by delay in keratinization of oral epithelium, effects of ageing, dehydration/atrophy, and inflammatory process. Delay in the keratinization is attributed to glycation changes. Sustained hyperglycemia causes 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 [(19,20).]

Another parameter assessed was Cytoplasm diameter, the results of the present study showed the difference in mean of CyD between study groups was statistically significant decrease in buccal mucosa cells from patients with uncontrolled and controlled diabetes when compared to control patients.This is similar to the findings of Prasad *et al*. noted a clear and definite decrease in cytoplasmic diameter in uncontrolled diabetes [(12)].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 [(16)].But contradictory to the findings of Jajarm*et al*. who reported a significant increase in cytoplasmic area the in diabetic patients [(15)].

In the present study, a significant increase was found in the mean values of N:C ratio of the exfoliated cells from the buccal smear with uncontrolled and controlled diabetes when compared to control patients. A gradual increase in N:C ratio is noticed as progress from control healthy group to uncontrolled diabetics which is highly significant.The NCR values are coincident with the study realized by *Prasad et al.*[(12)].This finding inconsistent with the findings by *Jajarm et al.* according to him the mean N:CR was significantly lower in diabetic group as compared to controls [(15)].

5. Conclusion

- There is a clear and definite increase in ND as we progress from normal individuals to patients with uncontrolled diabetes.
- In contrast to the increase in ND, there is a decrease in CyD in uncontrolled diabetes.
- The N:C ratio gradually and steadily increases from normal individuals to uncontrolled diabetics.

From the present study, we conclude that type2 diabetes produces definite morphometric changes in the exfoliated buccal mucosal cells.

References

[1] World Health Organization . Global Prevalence of Diabetes: Estimatesfor the Year 2000 and Projections for 2030. Geneva: World HealthOrganization; 2009.
[2] Braunwald E, Fauci A, Kasper D. Harrison's principles of internal Medicine. 16th Edition. Mc Graw Hill Company; 2003.
[3] Shoback, edited by David G. Gardner, Dolores. Greenspan's basic &clinical endocrinology (9th ed.). New York: McGraw-Hill Medical. Pp.Chapter 17. 2011.
[4] Alberti S, Spadella TC, Francbiscbone TRCG, Assis GF, Cestari TM, Taveira LAA. Exfoliative cytology of the oral mucosa in type II Diabetic patients: Morphology and cytomorphometry. J Oral Pathol Med 2003; 32:538-43.
[5] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33Suppl 1:S62–S69.
[6] Selvin E, Steffes MW, Zhu H, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med. 2010;362(9): 800–811.
[7] d'Emden MC, Shaw JE, Colman PG, et al, Australian Diabetes Society; Royal College of Pathologists of Australasia; Australasian Association of Clinical Biochemists. The role of HbA1c in the diagnosis of diabetes mellitus in Australia. Med J Aust. 2012;197(4):220–221.
[8] Eduardo Jose Caldeira, Progresso Jose Garcia, Elaine Minatel1, Jose Angelo Camilli, Valeria Helena Alves Cagnon. 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] AjitAuluck. Diabetes mellitus: an emerging risk factor for oral cancer? JCDA 2007; 73:501-502.
[10] Amit K. Nayar, B Sivapathasundharam. Cytomorphometric analysis of exfoliated normal buccal mucosa cells IJDR 2003; 14 (2):87-93.
[11] Ogden GR, Wight AJ, Cowpe JG. Quantitative oral exfoliative cytology.Effect of alcohol on normal buccal mucosa. Anal Quant CytolHistol1999;21:126-30.
[12] Prasad H, Rames HV, Balamurali P. Morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients. J Cytol2010;27:113-7.
[13] Sadia Sharif, NaureenSarwar, BushraNisar, Muhammad Khalid Masood, Asim Hameed. Detection of Cytomorphological Changes in Buccal Mucosal Cells of Type 2 Diabetic Patients Using Oral Exfoliative Cytology. ANNALS VOL 23, ISSUE 2, APR. – JUN. 2017
[14] Jajarm HH, Mohtasham N, Moshaverinia M, Rangiani A. Evaluation of oral mucosa epithelium in type II Diabetic patients by an exfoliative cytology method. J Oral Sci 2008;50:335-40.
[15] Shareef BT, Ang KT, Naik VR. Qualitative and quantitative exfoliativecytology of normal oral mucosa in type II Diabetic patients. Med Oral Patol Oral Cir Bucal 2008;13:E693-6.
[16] Tozoğlu Ü and Bilge OM. Exfoliative Cytology of Type 1 Diabetic Patients. Eur J Gen Med 2010; 7: 264-268.
[17] Hallikerimath S, Sapra G, Kale A and Malur P. Cytomorphometric analysis and assessment of Periodic Acid Schiff positivity of Exfoliated cells from apparently normal buccal mucosa of type 2 diabetic patients. Actacytologica 2011; 55: 197-202.
[18] Suvarna M, Anuradha C, Kumar K and Sekhar P. Cytomorphometric analysis of exfoliative buccal cells in type II diabetic patients. J Dr NTR Univ Health Sci 2012; 1: 33.
[19] Martin S Greenberg, Michael Glick. Burket's Oral Medicine – Diagnosis & Treatment. 10th Edition. B C Decker Inc: Elsevier India; 2003
[20] Kumar V, Cotran RS, Robbins SL (2003) Robbins basic pathology. 7th ed, Saunders, Philadelphia, 42-1198