Chattogram Maa-O-Shishu Hospital Medical College Journal
Volume 19, Issue 1, January 2020

Original Article

Cytomorphometric Analysis of Exfoliated Buccal Mucosal Cells in Diabetic Patients

Mahmuda Jahan[1*]
Zillur Rahman[2]
Nasrin Sultana[3]
Mahmuda Begum[4]
M Shahab Uddin Ahamed[2]
Syed Ahmed[1]

[1]Department of Pathology
Southern Medical College
Chattagram, Bangladesh.

[2]Department of Pathology
Chattogram Medical College
Chattagram, Bangladesh.

[3]Department of Pathology
Chattagram International Medical College
Chattogram, Bangladesh.

[4]Department of Pathology
Cumilla Medical College
Cumilla, Bangladesh.

*Correspondence to:
Dr. Mahmuda Jahan
Assistant Professor
Department of Pathology
Southern Medical College
Chattagram, Bangladesh.
Mobile : +88 01819 34 56 23
Email: drmahmudajahan@gmail.com

Date of Submission : 09.10.2019
Date of Acceptance : 06.12.2019

www.banglajol.info/index.php/CMOSHMCJ

Abstract

Background: *Diabetes mellitus is a common metabolic disease that causes chronic hyperglycemia and disturbance in carbohydrate, lipid and protein metabolism. It causes various changes in the cells of the oral mucosa, which can be determined by exfoliative cytology. The aim of this study was to study the Cytomorphometric alterations of exfoliated buccal mucosal cells in diabetic patients and to establish an additional diagnostic tool in diabetic patients in Chattogram area.*

Materials and methods: *It was a cross-sectional comparative study carried out in the Department of Pathology, Chattogram Medical College, Chattogram. Oral smears were obtained from 88 diabetic patients and 88 healthy individuals. The smears were stained with Papanicolaou solution. The cell diameter and nuclear diameter of 50 clearly defined cells were measured by multi-head microscope (OLYMPUS B51X). The cytoplasmic area and nucleus-cytoplasmic ratio were then calculated. SPSS software (version 17) was used for statistical analysis of the study.*

Results: *In the result, mean Cell Diameter (CD), Nuclear Diameter (ND), cytoplasmic diameter (CyD) and N/C ratio of diabetic patients and healthy were found to be 250.29 ± 29.02 µm, 54.48 ± 4.03 µm, 195.81 ± 27.97 µm, 0.283 ± 0.04 and 287.15 ± 25.23 µm, 47.41 ± 2.52 µm, 239.74 ± 24.45 µm, 0.199 ± 0.02. A statistically significant increase in ND & N/C ratio and decrease in CD & CyD were observed in diabetic patients compared to control. In addition, it was found that age, sex, blood sugar level, duration and treatment history of diabetes do not affect the morphometric changes significantly.*

Conclusions: *Result of this study suggests that diabetes produces definite morphometric changes in the exfoliated buccal mucosal cells. Exfoliative cytology can be useful as an additional tool to aid in diagnosis of diabetes mellitus.*

Key words: Diabetes Mellitus; Exfoliative Cytology; Cytomorphometry; Mean Cell Diameter (CD); Nuclear Diameter (ND); Cytoplasmic Diameter (CyD); N/C ratio.

INTRODUCTION

Diabetes Mellitus (DM) is a growing and massive silent epidemic that has the potential to cripple health services in all parts of the world[1]. It is basically characterized by chronic hyperglycemia, associated with disturbances in the metabolism of carbohydrates, proteins and lipids, as a result of absolute or relative insulin deficiency[2]. Chronic hyperglycemia causes damage to the eyes, kidneys, nerves, heart and blood vessels. The etiology and pathophysiology leading to the hyperglycemia, however, are markedly different among patients with diabetes mellitus, dictating different prevention strategies, diagnostic screening methods and

treatments[3]. Worldwide, millions of people are affected with diabetes and the number is climbing steeply. A study of International Diabetes Federation (IDF) states that the prevalence of diabetes in Bangladesh in 2010 is 6.1%[4].

Diabetes diagnostic confirmation by blood test is the most accepted and validated. But this creates a stressful situation for the patient and requires more time and resources. So, it would be useful to find a new tool that could replace, complement or compare the results giving greater patient comfort and tranquillity. Studies have been carried out to find an additional tool in this regard for years.

It has been observed that diabetes causes various changes in the cells of the oral mucosa, which can be determined by exfoliative cytology[5]. Application of quantitative techniques has largely improved the potential accuracy of exfoliative cytology[6]. Therefore, a study of cytomorphometry in oral exfoliative cytology can enable, its use as additional diagnostic tool of simplified nature, for diabetes mellitus.

Exfoliative cytology is a relatively easy, straight-forward, simple and non-invasive clinical technique[7]. It has the potential to be developed as a more practical technique for routine investigation to evaluate the oral mucosa in diabetes. At this context, this study has been carried out to evaluate the method of cytomorphometric alterations in oral epithelial cells in diagnosis of diabetic patients in Diabetic General Hospital and Chattogram Medical College Hospital situated in Chattogram, Bangladesh.

MATERIALS AND METHODS

It was a cross-sectional comparative study which was carried out in Diabetic General Hospital and Department of Pathology in Chattogram Medical College, Chattogram, Bangladesh. Study period was one year starting from January 2012 to December 2012.The optimum sample size for the thesis was kept in two (02) groups namely Diabetic Group and Control Group. Both the groups had 88 (Eighty Eight) samples. As a result, the study was conducted on 176 samples taken by non-probability convenience sampling.

Inclusion Criteria

Followings were the inclusion criteria of study population of the thesis study

i) Patients with Diabetes for at least past 06 (six) months and giving consent
ii) Diabetic Patients irrespective of medication for diabetes or not
iii) Control group included normal healthy adult with no history of diabetes/other illness.

Exclusion Criteria

Followings were the exclusion criteria of study population for both (Diabetic/ Control) Groups

i) Study population under the age of 18 (Eighteen) years
ii) Study population with habits of betel nuts & leaf / tobacco / alcohol
iii) Study population with anaemia / any other systemic illness / buccal mucosal problems
iv) Diabetes patients under any medication other than for diabetes.

Subjects of both groups had clinically healthy oral mucosa. The age range of study subjects were 21-70 years. A total of 450 (Four hundred and fifty) persons were primarily selected for the study considering the exclusion and inclusion criteria. A detailed history was taken along with meticulous physical examination. A data sheet was completed, detailing the name, age, sex, relevant medical history, etc. Fasting blood sugar (FBS) value and blood sugar value 2 hours after breakfast were collected from their medical records.

Before collecting smear, each person was asked to rinse the mouth with drinking water. A wooden spatula (moistened in distilled water) was then used to scrape the sample area (inner side of the cheek) three to four times with firm pressure. The scrapings were then transferred to clean glass slides previously marked with the patient's reference number and spread thinly and uniformly with a circular motion over the middle third of the slide (Figure-1).

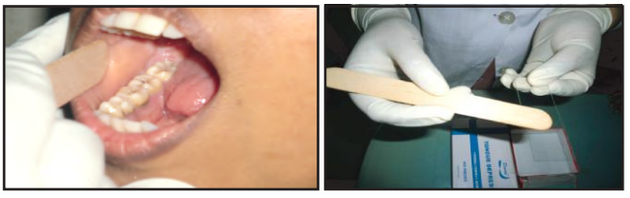

Figure 1: Collection of Buccal Mucosa and Slide Preparation

The smears were then immediately fixed in 95% ethanol and stained by the Papanicolaou method. Fifty clearly defined cells were measured in each case imaging from slides with 100X magnification.The Cell Diameter (CD) and Nuclear Diameter (ND) of 50 cells were measured (In micrometer, µm) from the images.

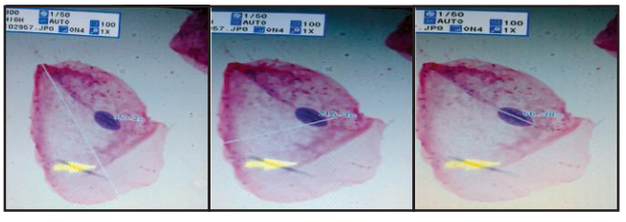

Figure 2: Cell Measurement- CD Maximum, CD Minimum and ND

The values of Cytoplasmic Diameter (CyD) and Nucleus / Cytoplasm ratio (N/C) were then derived using simple mathematical calculations. The data were finally analysed by computer using SPSS software (version 17). In relevant cases, ANOVA test and t-test were carried out. The level of significance was set at $p<0.05$.

RESULTS

The age range of DM group was 21 to 70 years with a mean age of 45.34 ± 10.58 years. On the other hand, the age range of control group was 21 to 52 years with a mean age of 38.15 ± 8.05 years. In DM group, there were 46 male and 42 female patients whereas in control group, there were 57 male and 31 female. The average FBS value of the diabetic patients and the

control group was 8.192 mmol/l and 4.03 mmol/l respectively. The duration of DM in diabetic group was 0.5 years to 22 years. Cytomorphometry showed that the cell diameter and cytoplasmic diameter in DM group were lower than Control group. The difference was found to be statistically significant ($p<0.05$). On the other hand, the nuclear diameter and N/C ratio in DM group were found higher than Control group and the difference was statistically significant ($p<0.05$) (Table-1).

Table 1: Cytomorphometrical analysis of exfoliative cytology between DM and control group

Parameter	Group	Mean ± SD	t value	p value
Cell Diameter, CD (µm)	DM	250.29 ± 29.02	-8.993	<0.001
	CONTROL	287.15 ± 25.23		
Nuclear Diameter, ND (µm)	DM	54.48 ± 4.02	13.957	<0.001
	CONTROL	47.41 ± 2.52		
Cytoplasmic Diameter, CyD (µm)	DM	195.81 ± 27.97	-11.092	<0.001
	CONTROL	239.74 ± 24.45		
N/C Ratio	DM	0.283 ± 0.04	16.647	<0.001
	CONTROL	0.199 ± 0.02		

t-test, p value<0.05

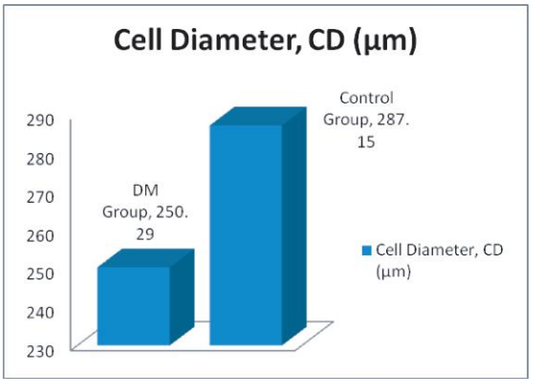

Figure 3 : Comparison of Cell Diameter (CD) between DM and Control Group

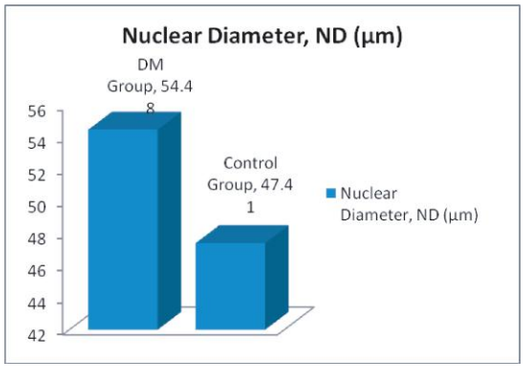

Figure 4 : Comparison of Nuclear Diameter (ND) between DM and Control Group

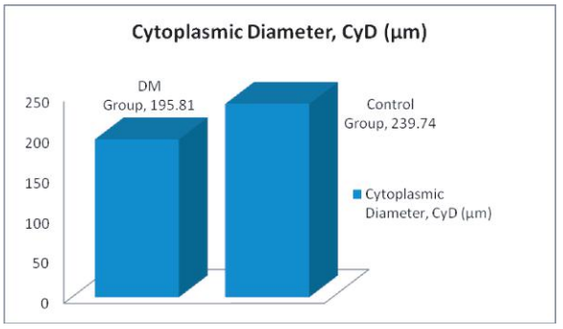

Figure 5 : Comparison of Cytoplasmic Diameter (CyD) between DM and Control Group

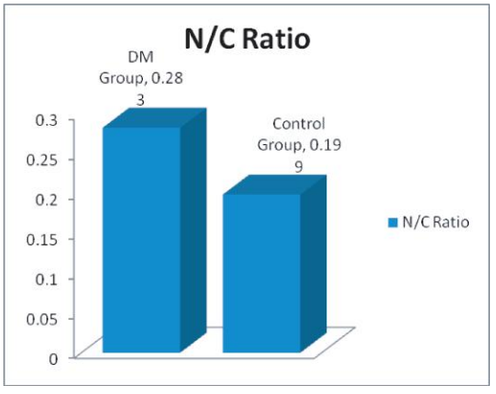

Figure 6 : Comparison of N/C Ratio between DM and Control Group

In addition, it was found that age, sex, blood sugar level, duration and treatment history of diabetes do not affect the morphometric changes significantly.

DISCUSSION

The study was carried out to find the cytomorphometric alterations of exfoliated buccal mucosal cells in diabetic patients. The study group had 88 diabetic patients and 88 healthy persons. The age range of the DM group was 21 to 70 years with a mean age of 45.34 (SD±10.58) years. On the other hand, the age range of control group was 21 to 52 years with a mean age of 38.15 (SD±8.05) years. It was similar to a study conducted by Rivera and Mendoza, where mean age of the DM patients was 45 years[8].

Regarding sex in diabetic group, out of 88 patients there were 46 male and 42 female with male-female ratio 1.09:1. In control group, out of 88 subjects there were 57 male (64.77%) and 31 female (35.23%) with male-female ratio 1.84:1. The study done by Shareef et al., had female predominance where the sample size was 10 (1 male and 9 female)[3].

The FBS range of diabetic group was 4.3-15.8 mmol/l with an averageof 8.19 mmol/l. On the other hand, the FBS range of control group was 3.1-5.8 mmol/l with an average of 4.03 mmol/l. Majority of the diabetic patients 34 were in FBS range 6.1- 8.0mmol/l. It was near to similar to a study conducted by Rivera and Mendoza[8].

In this study, ND in DM group is (54.48 ± 4.02) µm and in Control group is (47.41 ± 2.52) µm. At the same time, the N/C ratio in DM group is (0.283 ± 0.04) and in Control group is (0.199 ± 0.02) which showed significant increase of ND and N/C ratio in DM group compared to Control group (Table-1).

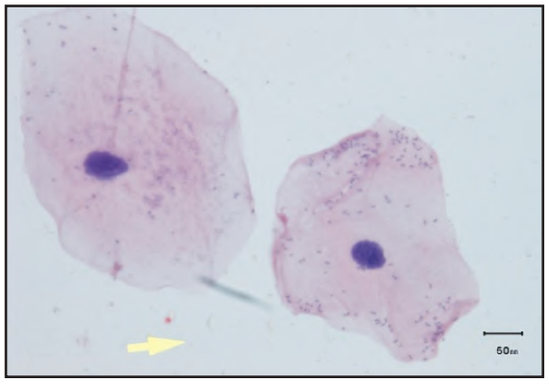

Figure 7 : Oral epithelial cells of the healthy individual showing normal size nuclei

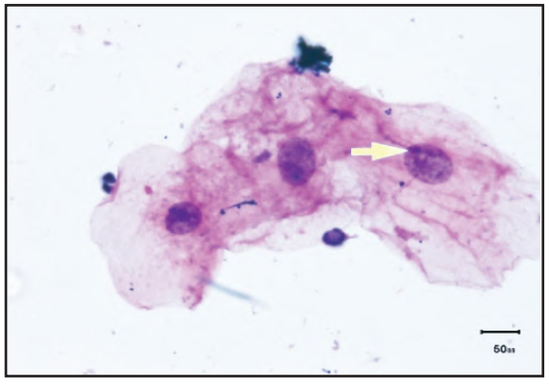

Figure 8 : Oral epithelial cells of diabetic patient showing enlarged nuclei

This result was similar to the studies carried out by Prasad et al, Shareef et al, Albertie et al, Rivera et al, Suvarna et al, Jajarm et al, Sonawane et al, Parmer et al, Seifi et al, Nandita et al and Sankhla et al[1,3,5,8-15].

The study also showed that CD in DM group is (250.29 ± 29.02) µm and in Control group is (287.15 ± 25.23) µm. At the same time, CyD in DM group is (195.81 ± 27.97) µm and in Control group is (239.74 ± 24.45) µm which showed significant decrease of CD and CyD in DM group compared to Control group (Table-1). This result was similar to the studies carried out by Albertie et al., Parmer et al and Seifi et al[5,12,13].

Age, sex, duration of diabetes and history of medication (Oral / injection) for DM patients had no effect the morphometric changes of the cells. Similarly, FBS and blood sugar 2 hours after breakfast in DM group also did not influence the study parameters.This result was similar to the study of Prasad et al[1].

The increased ND in the DM group may be due to delay in keratinization of oral epithelium, effects of ageing, dehydration / atrophy and inflammatory process. 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 which results 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 show a large nucleus as a primary characteristic[14].

Increase in nuclear size might be another indicator of cellular ageing in diabetic patients. Cellular ageing is the result of a progressive decline in the proliferative capacity and lifespan of cells. The effects of continuous exposure to exogenous factors cause the progressive accumulation of cellular and molecular damage[1,8].

Diabetic Patients also suffer from dehydration which may lead to mucosal atrophy. When cytologic samples from atrophic mucosa is made, it is possible that more of basal and parabasal cells may get included, thus leading to an increased ND. From this study, it is found that, in diabetes mellitus, buccal mucosal cell size is decreased and nuclear size is increased. As a result, the cytoplasmic size is decreased and N/C ratio is increased[1,3,12].

In the present study, all possible causes other than diabetes that might increase the value of ND were avoided. For this, subjects with any other systemic disease, clinically apparent oral mucosal lesions and previous benign or malignant lesions were excluded. Both control and diabetic subjects were non-alcoholics and non-smokers. So the changes in this study were obviously due to diabetes.

Results of this study were similar to many studies and deviated from few as well. The differences that were found may be caused due to different sample size, the kind of cytomorphometry measurement software, the microscope magnification applied and strict implication of exclusion criteria.

Result of this study shows that diabetes produces definite morphometric changes in the exfoliated buccal mucosal cells. Exfoliative cytology can be useful as an additional tool in diagnosis of diabetes mellitus. This field has scope for further exploration in our country. Further studies with greater sample size and comparison to other conditions causing similar cytomorphometric changes are needed to determine the predictive value of this method.

CONCLUSIONS

There is increase in nuclear parameters, decrease in cellular parameters and increase in nuclear-cytoplasmratioin smears from diabetics as compared to normal subjects in this study. The cytomorphometric changes in buccal mucosa of diabetic group cannot be attributed to factors such as age, sex, smoking habit, type of treatment and systemic diseases but are due to diabetes mellitus itself. Therefore, detection of these quantitative cellular changes by exfoliative cytology can help in

the diagnosis of diabetes mellitus. Exfoliative cytology is a simple, non-invasive clinical technique which may cause only mild discomfort. It was proven to be well accepted by the volunteers and was easy to use. This study might contribute to the general understanding of the alterations in the cellular pattern of buccal mucosal cells in diabetic patients. Result of this study can be used as an additional tool to aid in the evaluation of oral mucosal alterations in diabetes mellitus.

DISCLOSURE

All the authors declared no competing interest.

REFERENCES

1. Prasad.H, Ramesh, V. andBalamurali, P.D. Morphologic and Cytomorphometric Analysis of Exfoliated Buccal Mucosal Cells in Diabetes Patients; Journal of Cytology. 2010;27(4):113-117.
2. Wild, S., Roglic, G., Green, A., Sicree, R., and Kin, H. Global Prevalence of Diabetes; Diabetes Care. 2004;27(5):1047-1053.
3. Shareef, B T., Ang, K T. and Naik, V R. Qualitative and Quantitative Exfoliative Cytology of Normal Oral Mucosa in Type 2 Diabetic Patients.Med Oral Patol Oral Cir Bucal. 20081;13 (11):693-696.
4. International Diabetes Federation (IDF). Prevalence Estimates of Diabetes Mellitus, by Country; Percentage of adult population with diabetes, ranked highest to lowest. 2010.
5. Alberti, S., Spadella, CT., Francischone, TR., Assis, GF.,Cestari, TM. andTaveira, LA., Exfoliative Cytology of the Oral Mucosa in Type II Diabetic Patients. Morphology and Cytomorphometry. J Oral Pathol Med. 2003;32:538-543.
6. Ogden, G.R., Cowpe, J.G. and Wight, A.J. Oral Exfoliative Cytology: Review of Methods of Assessment. Journal of Oral Pathology Medicine. 1997;26: 201-205.
7. Sugerman, P.B., and Savage, N.W. Exfoliative cytology in clinical oral pathology. Australian Dental Journal. 1996;41:71-74.
8. Rivera, C. and Núñez-de-Mendoza, C. Exfoliative Cytology of Oral Epithelial Cells from Patients with Type 2 Diabetes: Cytomorphometric Analysis. International Journal on Clinical Experimental Medicine. 2013;6(8):667-676.
9. Suvarna, M., Anuradha, C. and Kiran, K. K. Cytomorphometric analysis of exfoliative buccal cell in type II diabetic patients. Journal of Dr NTR University of Health Sciences. 2012;1:33-37.
10. Jajarm, H H., Mohtasham, N. and Rangiani, A. Evaluation of Oral Mucosa Epithelium in Type II Diabetic Patients by an Exfoliative Cytology Method. Journal of Oral Science. 2008;50(3):335-340.
11. Sonawane, K., Jain, S., Gupta, I., Karthik, B.V., Singaraju, S. and Singaraju, M. Cytomorphometric Analysis of Oral Mucosa in Diabetic Patients in Bhopal Region an In-Situ Study. International Journal on Clinical Dental Science. 2011; 2(4):12-15.
12. Parmar, D., Sawke, G K. and Sawke, N. A Cytomorphometric Evaluation of Oral Mucosal Cells in Type II Diabetics; People's Journal of Scientific Research. 2014;7(2):22-27.
13. Seifi, S., Feizi, F., Moazzezi, Z., Mehdizadeh, M., and Zamani, B. Evaluation of Oral Mucosal Epithelium in Diabetic Male Patients by Exfoliative Cytology Method. Journal of Diabetes & Metabolic Disorders. 2014;13(1): 77.
14. Nandita, K.P., Boaz, K., Shrikant, N., Lewis, A.J. and Manaktla, N. Oral epithelium in diabetics: A cytomorphometric correlation. Dent Hypotheses. 2014;5:59–65.
15. Sankhla, B., Sharma, A., Shetty, R S., Bolla, S C., Gantha, N S and Reddy, P, Exfoliative Cytology of Buccal Squames: A Quantitative Cytomorphometric Analysis of Patients With Diabetes. Journal of International Society of Preventive Community Dentistry. 2014;4(3):182–187.