

Evaluation of Oral Mucosal Cells in Patients with type 2 Diabetes by Exfoliative Cytology Method

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Abstract

Background: Exfoliative cytology is a simple, non-invasive clinical technique which can be used to determine the morphology and cytomorphometric changes in exfoliated cells. Studies on oral mucosal cells of type II diabetes have shown qualitative and quantitative cellular changes compared to that of normal healthy individuals.

Aims: To evaluate alterations in the morphology and cytomorphometry of oral cells in type 2 diabetics and healthy individuals using exfoliative cytology technique.

Materials and Methods: Smears from scrapings of clinically healthy oral mucosa (buccal mucosa and tongue) of 25 type 2 diabetics and 25 healthy controls were stained by Papanicolaou staining method and were observed under microscope for cytomorphological assessments. In each slide, the cytoplasmic area (CA), nuclear area (NA) and cytoplasmic area to nuclear area ratio (CA:NA) of 50 cells were measured. The data was statistically analysed. The p value was calculated using student 't' test.

Results: Out of 25 diabetics patients 9 (36%) were males and 16% were females and were found to be in the age range of 30-80yrs. The cytoplasmic area of the cells from diabetic patients was reduced in comparison to the normal patients and was statistically significant with a p value of less than 0.001. Exfoliated cells from diabetic patients showed an increase in the nuclear area in comparison to the normal with a p value of 0.001 which is statistically significant. The nuclear cytoplasmic ratio of cells of diabetic patients was increased in comparison to the normal cells and was found to be statistically significant with p value of 0.001. In 25 cases of diabetic smears obtained 14 cases were from buccal mucosa and 11 cases from tongue. However, we found no statistical significant difference in the cytoplasmic area, nuclear area and nuclear cytoplasmic ratio between the two sites.

Conclusion: Exfoliated cells reveal definitive morphological changes which can be assessed morphometrically. Exfoliative cytology can be a reliable tool to aid in the diagnosis of diabetes mellitus.

Keywords: Cytomorphometry, Diabetes Mellitus, Oral Mucosa, Exfoliative Cytology.

Introduction

The unprecedented economic development and rapid urbanization in Asian countries, particularly in India has led to a shift in health problems from communicable to non-communicable diseases such as Diabetes. Diabetes mellitus is a common metabolic disease that causes chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism as a result of absolute or relative insulin deficiency. Studies have shown that the prevalence of diabetes in urban Indian adults is about 12.1%, the onset of which is about a decade earlier than their western counterparts and the prevalence of Type 2 diabetes is 4-6 times higher in urban than in rural areas.⁽¹⁾ Higher prevalence of Diabetes Mellitus is seen in men than that in women.⁽²⁾ Diagnosis of diabetes is made according to the classic symptoms of diabetes and blood glucose levels. Glycosylated haemoglobin (HbA1c) is another laboratory test that is used to diagnose and assess control in persons with diabetes. HbA1c levels less than or equal to 7%, is considered good control and is one of the main goals in diabetes care. A number of oral lesions associated with DM have been reported in literature.⁽³⁾ Among the most common oral abnormalities are gingivitis, periodontitis,

abscesses, candidiasis and other opportunistic infections.⁽⁴⁾

Interest in the field of oral exfoliative cytology has re-emerged following advancements in the field of quantitative oral exfoliative cytology as a powerful non-invasive diagnostic tool. Exfoliative cytology is a simple, non-invasive clinical technique which can be used to determine the morphology and cytomorphometric changes in exfoliated cells. Studies on oral mucosal cells of type II diabetes have shown qualitative and quantitative cellular changes compared to that of normal healthy individuals. Although diabetes can cause considerable cellular changes, this field has attracted little research. We therefore decided to evaluate the quantitative and qualitative changes in oral epithelial cells using an exfoliative cytology method in type 2 diabetic patients.

Materials and Methods

The study was carried out in the department of Oral Medicine and Radiology, Vydehi Institute of Dental Sciences, Bangalore. In this case-control study, 25 subjects in the age group of 30-70 years with type II diabetes mellitus for a minimum period of 1 year were selected. Patients were included irrespective of type of

medication used for glycemic control. The age, gender, disease duration of diabetes, their medical history was recorded. Random blood sugar level, hemoglobin percentage, Glycosylated hemoglobin (HbA1c) levels were measured. The control group consisted of 25 healthy subjects with clinically normal mucosa without any history of diabetes mellitus (assessed by random blood sugar).

Persons who were smokers, alcoholic persons, persons with anaemia, exposure to radiation, pregnancy, malignancy and any kind of systemic problem and other medications that affect the assay in each group were excluded.

Subjects of both the study and control groups were informed of the procedure and written consent was obtained. After detailed clinical examination, the subjects were instructed to rinse the mouth with water. The oral mucosa was dried with gauze to remove surface debris and excess saliva. Smears were obtained from clinically healthy oral mucosa (buccal mucosa and tongue) of diabetic patients and non diabetic healthy controls using a wooden spatula moistened in distilled water. The scrapings obtained were transferred on to a clean, dry glass slides and a smear was obtained and were fixed using with 95% ethyl alcohol spray fixative. The slides were processed for cytomorphological assessments using the Papanicolaou staining procedure.

Cytomorphometric Analysis: The Papanicolaou stained slides were observed under research microscope (Olympus 20i) with 40x magnification. Individual cells with clear cellular and nuclear outlines without overlapping and clumping were selected. 50 such selected cells in each slide were counted from left upper corner to right and then down in order to avoid repeated assessing of the same cells. The images were captured and cellular area (CA), nuclear area (NA) and measured using Magnus Pro 4.2 image analyzing software which was calibrated to micrometer (μm). The cellular and nuclear areas were measured using the freehand option in the software the values of which would be displayed

immediately. The measured values were automatically transferred on to an excel sheet and used to in turn calculate the nuclear cytoplasmic ratio. The excel sheet were then submitted for statistical analysis. The p value was calculated using student 't' test.

Results

The current study comprised of 25 normal controls and 25 diabetics patients. Out of 25 diabetics patients 9 (36%) were males and 16% were females and were found to be in the age range of 30-80yrs (Table 1). Exfoliated oral mucosal cells were subjected to cytomorphometric analysis to measure their cytoplasmic area, nuclear area and nuclear cytoplasmic ratio. The values obtained were analyzed with unpaired student 't' test using SPSS package. The cytoplasmic area of the cells from diabetic patients was reduced in comparison to the normal patients and was statistically significant with a p value of less than 0.001. Exfoliated cells from diabetic patients showed an increase in the nuclear area in comparison to the normal with a p value of 0.001 which is statistically significant. The nuclear cytoplasmic ratio of cells of diabetic patients was increased in comparison to the normal cells and was found to be statistically significant with p value of 0.001. Cytomorphometric results of oral smears in types 2 diabetes and control groups is shown in Table 2. In 25 cases of diabetic smears obtained 14 cases were from buccal mucosa and 11 cases from tongue. However, we found no statistical significant difference in the cytoplasmic area, nuclear area and nuclear cytoplasmic ratio between the two sites.

Table 1: Number of subjects according to study groups and gender

Study Groups	Male (%)	Female (%)	Total
Control	12 (48%)	13(52%)	25
Types 2 Diabetes	9(36%)	16 (64%)	25
Total	21	29	50

Table 2: Cytomorphometric results of oral smears in types 2 diabetes and control groups

Variable	Control group (n = 25)			Diabetic group(n = 25)		
	Mean	Standard deviation	Standard error	Mean	Standard deviation	Standard error
Nuclear area (NA)	70.5830	13.6412	4.3137	163.8960	28.6097	9.0472
Cytoplasmic area (CA)	333.0300	73.0595	23.1034	760.0960	153.1348	48.4255
Cytoplasmic/ Nuclear ratio (C/N)	0.22100	0.07244	0.02291	0.22600	0.07405	0.02342

Discussion

Diabetes mellitus is the most common and important pathologies affecting worldwide estimated to roughly around 100 million population. In India, diabetes is fast growing disease with more than 62 million people currently diagnosed and it is predicted to increase to 79 million by 2030.⁽⁵⁾ It is noted that there is a continuous significant increase in its incidence. It is a chronic disease made up of two types namely Type 1: Insulin dependent diabetic mellitus [IDDM] and Type 2: Non- Insulin dependent diabetic mellitus [NIDDM]. Type 2 is more common than in type 1 in population over 40 yrs, associated with a strong familial history and obesity.⁽⁶⁾

It is a metabolic disorder with hyperglycemia and characterized by polyuria, polyphagia and polydipsia. It affects and damages multisystem ranging from heart, kidney, liver, eye, brain etc. patients with diabetes usually have complications like diabetic neuropathy, diabetic nephropathy, diabetic angiopathy and diabetic ketoacidosis. It is associated with a high risk of mortality and morbidity and usually related to cerebrovascular diseases, chronic renal failure, uncontrolled infections, septicemia, coma etc.

The most common oral finding seen in diabetic patient is gingivitis, periodontitis, abscesses, candidiasis and other opportunistic infections.⁽⁷⁾ There is rapid cellular aging in diabetics which may be a reason for their short span of life.

In this current study we opted for Hb1Ac to measure the blood glucose level as it not affected by factors like diet or medication intake, and it gives an accurate and objective measure of glycemic control over the past 3 months, thus acts as a more reliable parameter.⁽⁸⁾

Most of the oral changes occurring in diabetics are due to xerostomia which causes mucosal atrophy. Hence the oral mucosa becomes dehydrated and more susceptible to trauma and damage.⁽⁹⁾ The changes in the oral mucosa are usually reflected as variations in the cell, nucleus and their ratio.⁽¹⁰⁾ Such changes can best be assessed by biopsying the tissues. But medical condition and post surgical complication may not permit obtaining a biopsy. In such cases the morphological alterations of the cells can be studied using an alternative technique known as exfoliative cytology.

Exfoliative cytology is the branch that deals with study of exfoliated cells under a microscope. It is based on the simple fact of normal shedding or exfoliation of cells in the body including oral cavity. It can also be brushed or scrapped from the body and examined for morphological changes of the cell like size of cell and nucleus, changes in nuclear number, pattern, nuclear cytoplasmic ratio etc. Exfoliative cytology is a simple, non-invasive technique which yields quick results hence is considered an adjunct or alternative to biopsy.

Different stains can be used to stain the cells in the exfoliative cytology like H&E, Pap, toluidine blue, acridine orange, PAS etc. Among these Papanicolaou stain (PAP) is a commonly used cytopathological stain used in most of the oral pathology laboratories. PAP stain was developed by George Nicolas Papanikolaou, the father of cytopathology in 1942. It is a multichromatic stain used specially to demonstrate keratin. The cytoplasm of the superficial cells appear pink, cytoplasm of intermediate cells appears pale greenish-blue, cytoplasm of parabasal cells appear deep greenish-blue, and nuclei appear dark blue. It also gives good differential staining with cytoplasmic transparency and clear nuclear details, hence is the preferred stain in routine cytopathology. For the above

reason we opted to use PAP stain in our present study.⁽¹¹⁾

Variety of lesions and conditions show changes in the cellular and nuclear area, which can be studied by exfoliative cytology. The most common ones are aging, atrophy, systemic diseases, endocrinopathies like diabetes, respiratory illness, smoking nutritional deficiency like vit B 12 and folic acid and also in premalignant and malignant disorders.^(12,13)

Aging pattern in diabetes is directly related to atherosclerosis leading to ischemia which results in altered rate of cell turnover.^(14,15) Accumulation of end products of advanced glycation is involved in pathogenesis of diabetes as well as aging.⁽¹⁶⁾

The changes noted in the cells and nucleus in exfoliative cytology of diabetic is attributed to a) Cellular atrophy: occurs as a compensatory mechanism to loss of its intracellular contents and is seen as decreased cellular area.⁽¹⁷⁾ b) Enlarged nucleus: There is increase in nuclear size seen in diabetics. But contrarily exfoliated cells of atrophic mucosa shows basal and parabasal cells thus presenting with enlarged nuclei. Secondary infections owing to xerostomia may stimulate chronic inflammatory response thereby resulting in increased nuclear size. Xerostomia also causes mucosal atrophy hence results in increased nuclear diameter and increase in N:C.

The findings of our study were similar to the above mentioned features and similar to the previous studies of Prasad H, Ogden et al, Ramesh et al etc. These overall findings observed in our study should be attributed to diabetes alone and not to factors like age, sex, smoking habit and systemic diseases. Although, literature suggests contrasting findings by Jajarm *et al* showed an increase in cytoplasmic area in diabetic patients.⁽¹⁸⁾ Alberti *et al* concluded that cell area did not show any significant difference in diabetic individuals and also showed additional changes in cellular and nuclear morphology like binucleation and karyorrhexis, even we found very few such alterations.⁽¹⁹⁾

Alterations seen in the cellular, nuclear and N:C seen in exfoliated epithelial cells of diabetics may be confusing with that of dysplastic or malignant lesions. Clinical findings such as a normal site of exfoliation and histological finding of nuclear uniformity inspite of an increase in N:C will play a crucial role in arriving at a proper diagnosis.⁽²⁰⁾

It is noted that uncontrolled diabetes is associated with a significant decrease in cellular diameter and may be associated with dehydration secondary to xerostomia. N:C generally increases from controls to poorly controlled diabetics with a steep rise in N:C in uncontrolled diabetics. This reflects the earlier findings of increase in nuclear diameter and decreased in cellular diameter.

Conclusion

Exfoliative cytology is a simple, rapid and non-invasive diagnostic tool to detect changes in cells especially where biopsy is contraindicated. Exfoliated cells reveal definitive morphological changes which can be assessed morphometrically. Hence, exfoliative cytology can be a reliable alternative to tissue biopsy especially in diabetics where biopsy may not always be advisable or contraindicated and is considered to be extremely statistically significant.

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