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Original Research Paper

Dental Science

MORPHOLOGICAL ANALYSIS OF EXFOLIATED BUCCAL AND TONGUE DORSUM CELLS IN TYPE II DIABETIC PATIENTS

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ABSTRACT

Diabetes mellitus type 1 that results from immunologically mediated damage to the β-cells in the pancreas .Diabetes mellitus is characterized by recurrent or persistent hyperglycemia. Hyperglycemia can be associated with salivary gland dysfunction and alterations in the oral epithelial cells

Aim: To study the morphologic changes of exfoliated epithelial cells from dorsal surface of tongue and buccal mucosa in Type II Diabetics.

Methodology: We performed light microscopic analysis of the buccal and tongue dorsum smears in thirty type II diabetic patients and thirty healthy individuals. The oral smears were stained using Papanicolaou method for cytological examination. Morphological changes in the stained smears were identified by careful examination of the images captured under 40x magnification.

Results: Diabetic patients showed an increased number of binucleated cells ($p<0.01$),intracytoplasmic inclusions($p<0.05$),perinuclear halo($p<0.01$).An increase in number of inflammatory cells ($p<0.01$) were also found in Type 2 Diabetic patients from dorsum of tongue.

Conclusion: The light microscopic study indicates that type II diabetes can produce morphological changes in the oral mucosa that are noticeable with exfoliative cytology.

KEYWORDS : Diabetes Mellitus, Morphologic, Exfoliated Cells

Introduction

Diabetes mellitus is a common endocrine-metabolic disorder. It causes hyperglycemia, associated with disturbances in the metabolism of carbohydrate, fat, and proteins, as a result of absolute or relative insulin deficiency[1].Type II Diabetes mellitus (DM) accounts for approximately 95% of diabetic cases and occurs usually in patients over 40 years of age[2]. It has emerged as a major health care problem worldwide. According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken3.

Hyperglycemia can be associated with salivary gland dysfunction and alterations in the oral epithelial cells[4,5,6]. The biopsies are available for evaluation of oral mucosal changes[1,7].However, in diabetes, many invasive techniques lose viability as a result of variations in blood glucose and delayed healing processes[4,6]. In these cases, exfoliative cytology may be more appropriate. Previous studies have examined morphological changes suffered by oral epithelial cells in diabetics, results of which are higher and significant when compared to healthy patients. These changes are represented by binucleation, karyorrhexis, karyolysis, intracytoplasmic inclusions, perinuclear halo etc which may assist in the diagnosis of Diabetes. Till date only very few studies have used exfoliative cytology to evaluate morphological changes in the oral mucosa in Diabetes. Hence the present study is focused to assess morphological changes of the exfoliated oral mucosal cells in Type II diabetic patients.

AIMS

1. To study the morphologic changes of exfoliated epithelial cells from dorsal surface of tongue and buccal mucosa in Type II Diabetics.
1. To compare and correlate the cellular changes in Type II Diabetes with control group.

OBJECTIVES

1. To identify morphologic changes of oral epithelial cells by exfoliative cytology using papinicolaou staining of patients with Type II Diabetes and compare it with the control group.

MATERIALS AND METHODS

The study was an observational type of comparative study done in the Department of Oral Pathology & Microbiology Govt. Dental College, Kozhikode, Kerala in collaboration with Department of Internal medicine, Govt. medical college, Kozhikode. Instituitional Ethical committee clearance was obtained prior to the study. The duration of the study was 1 year. Subjects were randomly selected from the patients attending Diabetic clinic, Department of Internal medicine, Govt. medical college, Kozhikode. Study subjects comprised 30 patients with age ranging from 25-60 years inclusive of both the genders with a known history of Type 2 DM for the past 1 year prior to the commencement of study, irrespective of the mode of medical therapy for glycemic control.

- **Diagnostic criteria for Type 2 DM are as follows**

a. Fasting plasma glucose ≥126mg/dl
b. 2-hour plasma glucose ≥200 mg/dl.
c. HbA1C ≥6.5%

Exclusion criteria of Cases

1. Habits of smoking or betel nut chewing.
2. Systemic diseases other than DM
3. Diagnosed cases of anemia and malignancy
4. Patients who have undergone radiation therapy and chemotherapy.
5. Denture wearers.
6. Clinical evidence of pre malignancy.
7. Alcohol dependency.
8. Pregnancy and lactation.

Control group comprised 30 healthy individuals without any history of DM and other systemic diseases selected from staffs and students of Govt. Dental College, Kozhikode.

Exclusion criteria of controls

b. Negative for clinical signs of systemic diseases
c. Negative for presence of Diabetes and anemia

Written informed consent was obtained from the patients participated in the study .The selected subjects were instructed to

gargle the mouth with water. The oral mucosa was dried with gauze swab to remove surface debris and excess saliva. smears were taken by using a sterile metal spatula with a gentle scrapping motion, exerting little pressure from clinically normal buccal mucosa either left or right and dorsum of tongue. The scrapings were evenly smeared on to the centre of the glass slides. The slides were immediately immersed in Ether alcohol to ensure proper fixation for half an hour. Smears were stained by conventional Papinicolaou technique. Morphological analysis was performed by capturing images using MICAPS High definition camera attached to Lx 400 Labomed Trinocular Research Microscope. Morphological changes in the stained smears were identified by careful examination of the images captured under 40x magnification. Morphological changes noted in the diabetic patients were binucleation, karyorrhexis, perinuclear halo and Intracytoplasmic inclusions. Inflammatory cells were frequently found in association with the exfoliated cells from dorsum of tongue in diabetic patients.

Data obtained were entered in the Microsoft Excel sheet and statistical analysis was performed by using SPSS version 18.0.

Results

Pearson chisquare test was done to compare the morphological alterations in type 2 diabetic patients with the controls. It was found that diabetic patients showed an increased number of binucleated cells ($p<0.01$),intracytoplasmic inclusions($p<0.05$),perinuclear halo($p<0.01$).An increase in number of inflammatory cells ($p<0.01$) were also found in Type 2 Diabetic patients from dorsum of tongue.(Table2)

Table 1: Percentage distribution of Morphologic changes in Exfoliated cells of Type 2 Diabetic patients and controls (N=30)

	Binucleation	Karyorhexis	Intracytoplasmic inclusions	Perinuclearhalo	Polymorphonuclear leucocytes
Cases	43.3%	36.7%	66.7%	36.7%	60.0%
Buccal mucosa					
Tongue	36.7%	20.0%	43.3%	50.0%	80%
Controls	0%	16.7%	23.3%	0%	0.2%
Buccal mucosa					
Tongue	0%	0%	20.0%	0%	0.4%

Table 2: Pearson chi square test

Morphological Features	Value	df	P value	
			BM	TG
Binucleation	16.596	1	0.000	0.000
karyorrhexis	3.068	1	0.080	0.010
Intracytoplasmic inclusions	11.380	1	0.001	0.052
Perinuclear halo	13.469	1	0.000	0.000
Polymorphonuclear leucocytes	25.714	1	0.000	0.000

MORPHOLOGICAL ALTERATIONS SEEN IN ORAL EPITHELIAL CELLS OF TYPE II DIABETIC PATIENTS

Figure 1: PERI NUCLEAR HALO

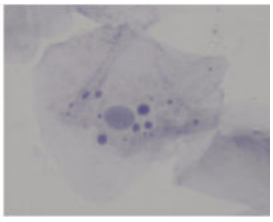

Figure 2:INTRACYTOPLASMIC INCLUSIONS

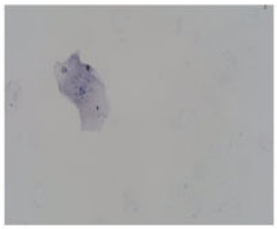

Figure 3: KARYORRHEXIS

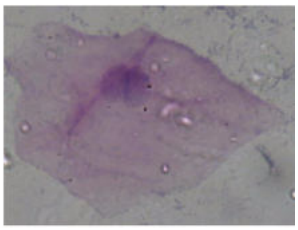

Figure 4: BINUCLEATION

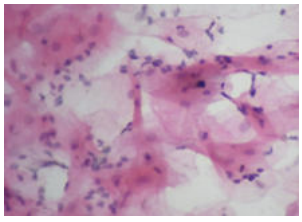

Figure 5: INFLAMMATORY CELLS

Discussion

Morphological alterations were studied in buccal and tongue dorsum smears obtained from type II diabetic patients which included Binucleation, Karyorhexis, intracytoplasmic inclusions and perinuclear halo. Inflammatory cells were frequently found in smears from dorsum of tongue in Type II diabetic patient which is in agreement with study by Oz etal[8]. In the present study binucleation was significantly increased in buccal smears.

The cellular changes which are observed in our study involved superficial and intermediate squamous cells of oral squamous epithelium. The genesis of cellular changes is somewhat puzzling[9]. Karyorrhectic cells appear to have no nucleus. It is possible that they represent a very late stage in the cell death process.[9]

Karyopyknotic cells may be undergoing a form of cell death although this has not been convincingly investigated[10]. It is possible that the disruption of cell skeletal filaments also induces binucleation, and perinuclear halo which is a clear area around the nucleus formation[11]. Type 1 diabetes leads to cytoskeleton changes. Insulin has been suggested to play a key regulatory role in the functional organization of actin microfilaments[11]. The actin network is an essential mediator in the action of insulin. The microtubules are also targets of insulin. The microtubules are seen in greatest density around the nucleus[11]. Thus, many cellular effects of insulin may depend on the functional integrity and organization. A chronic insulin deficiency could lead to impairment in the organization of the cytoskeleton[11]. Microtubule cytoskeleton may function in the positioning and initiation of the cleavage furrow, and the actin filament cytoskeleton may play key roles in the initiation and ingression of the furrow[11]. It is possible that a chronic insulin deficiency could lead to disruption of the microtubules, and this might result from perinuclear halo formation[11].We are of the opinion that similar mechanism may be responsible for cytological alterations in Type II diabetes.

A bilobed nucleus was suggested of ageing cells[12]. On the other hand, minor nuclear abnormalities such as slight-to-moderate nuclear enlargement, slight irregularities of the nuclear contour, and increase in granularity of the chromatin indicate reactive or regenerative state[12].

In the present study, inflammatory cells showed a statistically significant increase in tongue dorsum smears. In diabetic patients, it is a well-established fact that there is a neutrophil chemotactic defect[2]. In an attempt to overcome this deficiency, a positive feedback mechanism acts, resulting in an increase in the inflammatory component[2]. Increased inflammation may also be secondary to adverse hormonal, micro vascular, and neuronal changes[2].

Although the qualitative and quantitative changes found in the oral smears of type II diabetic patients are features that point to

malignancy, it can be differentiated from the latter by the diminished C/N ratio and uniformity in the nuclear configuration2.

conclusion

From the present study, we conclude that type II diabetes can produce morphological alterations in oral epithelial cells that are detectable by microscopy with exfoliative cytology method. Furthermore studies with a greater sample size is required to compare the described cellular changes to similar cellular changes caused by other diseases.

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