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A CLINICAL, MICROSCOPIC AND CYTOMORPHOMETRIC ANALYSIS OF ORAL MUCOSA IN TYPE II DIABETES MELLITUS PATIENTS

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ABSTRACT

Background- Diabetes mellitus (DM) is associated with many oral manifestations along with changes in the oral mucosa. The aim of the present study was to correlate the clinical signs of diabetes mellitus with microscopic and cytomorphometric analysis of smears obtained from buccal mucosa, dorsum of tongue and floor of mouth. Materials and methods- Total 100 individuals were included in the study of both sexes after measuring their HbA1c and recording their oral symptoms.50 were normal individuals, 50 were suffering from diabetes mellitus, out of these 20 were having well controlled diabetes, 20 poorly controlled and 10 with uncontrolled diabetes. The smears were stained and analysed by image analysis software. Nuclear area, cytoplasmic area and cytoplasm: Nuclear areas were calculated. The observations were recorded and one way ANOVA was performed. Result- It showed xerostomia, burning mouth and ulcerations were more in diabetes patients. Also nuclear area and cytoplasm: nuclear area showed statistically significant changes from normal to uncontrolled diabetes whereas the cytoplasmic area also showed increase but was not statistically significant. Other findings like candidiasis and inflammation were also found to be more in diabetes patients. Conclusion- The present study shows the importance of exfoliative cytology in diagnosis of diabetes mellitus along with routine diagnostic tests.

KEYWORDS: xerostomia; diabetes mellitus; exfoliative cytology; cytomorphometry.

INTRODUCTION

Diabetes mellitus (DM) is the most common metabolic disorder that produces multiple systemic complications with multiple aetiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism [1] .Oral pathologies like infection, gingivitis, periodontal disease, increment of severe candidiasis incidence, apical root abscess of the tooth, burning mouth syndrome, impairment of taste, proliferation of pathogenic microorganisms, incidence of coated tongue, halitosis, and many other lesions caused by hyperglycaemia were reported in patients with diabetes. Sores of oral squamous epithelium and gingivitis are the most common symptoms in patients with diabetes [2]. So as a dentist it is relatively easy to get to diabetes suspicion through a good anamnesis and a simple clinical examination of the patient.

Diagnosis of diabetes is made according to the American Diabetes Association values settled. Among them are the classic symptoms of diabetes, blood glucose levels taken at any time of day, greater than or equal to 200 mg/dl, unrelated to the time since the last meal [3]. Another criteria, also linked to blood sugar, but breakfast abstaining (with no caloric intake for at least 8 hours) is associated with a value which should be greater than or equal to 126 mg/dl, status that should be confirmed with a second blood glucose on another day [4]. Measurement of glycosylated haemoglobin (HbA1c) is another test that is done for people with diabetes. It is based on measuring the percentage of glucose bounded to haemoglobin in a specific manner, whereby high levels of glucose in blood contribute to an increased binding and thus, higher levels of HbA1c [5]. Every 3 months this test is done and main goal of diabetes care is to maintain the HbA1c levels less than or equal to 7%, [6].

There are several methods to evaluate the oral mucosa of persons with diabetes which one of the most prevalent of these methods is the application of biopsy as incisional or excisional but using of biopsy is usually not applied due to being aggressive and creating psychological problems for the patient [7]. It seems that the best method with low cost and less aggressive characteristic and lack of damage to oral tissues of the patient, is using of cytology exfoliative or brush cytology which was initially applied for rapid diagnosis of precancerous lesions but today, it is used for diagnosis and evaluation

of the quantitative and qualitative changes in epithelial cells of the oral mucosa is suspected.

On literature review we found very few studies which have correlated clinical presentations of oral mucosa in diabetes mellitus and quantitative as well as qualitative changes in oral mucosa by exfoliative cytology. Any observed changes in oral cytomorophology of well controlled DM may possibly attribute to therapy effect.

Therefore, a study of cytomorphometry in oral exfoliative cytology was taken up to assess the usefulness of this procedure in the diagnosis and follow-up of diabetes patients. The goal of our study was to identify the quantitative nuclear and cytoplasmic changes of buccal mucosa, tongue and floor of mouth in type II DM at different hyperglycemic status (uncontrolled, well controlled and poorly controlled) and assess their relation with xerostomia, burning mouth sensation and oral ulceration.

MATERIALS AND METHODS

In the present study, a total of 50 type II DM patients were selected. According to their HbA1c levels (which indicates the degree of glycemic control achieved), they were subdivided into 3 groups; uncontrolled (HbA1c >12%), well-controlled (HbA1c =8%) and poorly controlled (HbA1c >10% and =12). The control group included 50 non-diabetic healthy volunteers with no risk factor for diabetes and their HbA1c< 6.5%. All participants' age ranged between 40-50 years. The exclusion criteria were: 1) smokers (18-19) or alcoholic patients, 2) systemic diseases or other medications that affect the assay , and 3)ladies who were pregnant or during menstrual period or taking contraceptives.

The study was approved by the local ethical committee and all patients signed a written consent form. Patient's name, age, sex, medical history, presence of burning mouth sensation (oral mucosal pain related to DM and not to other medical or dental cause) and xerostomia (subjective feeling of oral dryness) as described by the patients were recorded. Oral ulceration (presence of mucosal discontinuity) was assessed clinically by specialist using mouth mirror and under good light vision).

Methods of collection of data

Smear procedure

After detailed clinical examination, the subjects were requested to rinse the mouth with normal saline. Smears were obtained from the buccal mucosa (BM), the dorsum of tongue and floor of mouth of each subject using a wooden spatula moistened in distilled water. Two smears from each site were obtained. The smears were transferred onto grease-free glass slides and fixed with 95% ethyl alcohol.

Two smears each from all the three sites were stained with Papanicolou stain to visualize under compound light microscope for cytomorphometric analysis of cells (for nuclear area (NA), cytoplasmic area (CA), and cytoplasm : nucleus (N/C) ratio.

Cytomorphometric assessment

To evaluate cytomorphometry, the computer connected to camera and Photoshop software and the analysis system type Motic Image plus 2 (micro- optic industrial group co LTD1) was used. Imaging from slides was performed with 40-fold magnification by light microscope. In each slide, an average of 50 cells with strong staining and the determined cellular limits were selected. If placing cells on top of one another was observed and their membranes were not obvious, they were excluded the study. In order to prevent the errors of measurement and enumeration of cellular samples again, the movement of microscope is performed from left to right, then to up and down. After that, the determination of the average sizes of the nucleus and cytoplasm of each cell (in µm2) and the ratio of nuclear to cytoplasm size were performed and the results were reported in Mean ± SD.

Qualitative evaluation of cytomorphometry

In each slide, cytology by a number of 50 cells in 5 microscopic fields with the magnification of 40- fold were examined in terms of the characteristics of the nucleus (the presence of bi-lobed or multi-lobed nucleus, karyorrhexis, vacuolization of cytoplasm, the presence of inflammation in persons with diabetes types II and the healthy persons. but these were not subjected to any statistical analysis.

Statistical analysis

SPSS was used for statistical analysis. The significance of the results obtained from the control and study groups were statistically analyzed by one way ANOVA test. The p-value < 0.05 was considered to be statistically significant.

RESULTS

The number of subjects in each group according to their HbA1c were distributed in relation to sex and oral symptoms are illustrated in Table-1.

The well controlled group included 20 individuals, out of which 50% were suffering from xerostomia, 20% from burning and 30% with oral ulcerations. The poorly controlled group included 20 individuals, out of which 80% were suffering from xerostomia, 30 % from burning and 25% with oral ulceration. The uncontrolled group included 10 individuals, out of which 100% were suffering from xerostomia,80 % from burning and 20% with oral ulceration.

Concerning cytomorphometric measurements, cytoplasmic area (Table 2) reduced from well controlled to uncontrolled, also there was difference seen in the sites which showed more cytoplasmic area in the cells obtained from buccal mucosa, followed by floor of

mouth, which was further followed by tongue being the least. Although the results were statistically insignificant.

Other parameter nuclear area (Table 3) showed increase in area from well controlled to uncontrolled, also there was difference seen in the sites which showed more nuclear area in the cells obtained from buccal mucosa, followed by floor of mouth ,which was further followed by tongue being the least. The results were statistically significant.

The same statistically significant results were obtained when cytoplasm: nuclear area (Table 4) were compared on all the three sites and showed a progressive decrease as the severity of disease increased.

An attempt was made to note the qualitative changes in the exfoliated epithelial cells. It was seen that the inflammatory component was higher in the diabetic group compared to healthy group.

Table 1 Frequency and percentage distributions of healthy subjects and type II DM patients according to their HbA1c in relation to sex and oral symptoms

Group	Hba1c	Total	Male	Female	Xerostomia		Burning		Ulcer	
					No %	Yes %	No %	Yes %	No %	Yes %
Normal	<6.5	50	30	20	0	0	2	4	1	2
Diabetes	Well controlled ≤8	20	9	11	10	50	4	20	6	30
	Poorly controlled >10≤12	20	12	8	16	80	6	30	5	25
	Uncontrolled >12	10	4	6	10	100	8	80	2	20

Table 2: The mean± SD values of cytoplasmic area in control and type II DM patients according to their HbA1c

Group	HbA1c	Cytoplasmic area CA		
		Buccal mucosa	Tongue	Floor of mouth
Normal	<6.5	**3837.5 ± 876.45**	**2984.3±453.4**	**3124.5±435.4**
Diabetes	Well controlled ≤8	**3425.8±675.4**	**2732.7±213.7**	**3045.2±213.2**
	Poorly controlled >10≤12	**3324.3±549.3**	**2676.2±142.8**	**2941.1±132.4**
	Uncontrolled >12	**2743.4±328.3**	**2593.6±132.7**	**2812.6±548.2**
	P VALUE	**>0.05**	**>0.05**	**>0.05**

Table 3: The mean± SD values of nuclear area in control and type II DM patients according to their HbA1c

Group	HbA1c	Nuclear area NA		
		Buccal mucosa	Tongue	Floor of mouth
Normal	<6.5	**62.5±17.60**	**51.9±10.34**	**58.9±15.03**
Diabetes	Well controlled ≤8	**69.7±13.80**	**59.9±12.60**	**67.5±10.20**
	Poorly controlled >10≤12	**75.9±15.90**	**67.8±11.20**	**79.5±12.80**
	Uncontrolled >12	**86.7±12.40**	**75.8±16.20**	**85.5±11.32**
	P VALUE	**<0.05**	**<0.05**	**<0.05**

Table 4: The mean± SD values of cytoplasmic area/ nuclear area in control and type II DM patients according to their HbA1c

Group	HbA1c	Cytoplasmic area/ nuclear area		
		Buccal mucosa	Tongue	Floor of mouth
Normal	<6.5	**66.7±12.98**	**59.7±19.74**	**54.9±23.04**
Diabetes	Well controlled ≤8	**52.7±23.80**	**48.9±73.80**	**47.5±36.42**
	Poorly	**48.7±75.40**	**41.8±75.12**	**39.9±53.90**

	controlled >10≤12			
	Uncontrolled >12	**37.9±72.30**	**36.8±51.90**	**32.5±13.43**
	P VALUE	**<0.05**	**<0.05**	**<0.05**

DISCUSSION

Proper understanding of DM enables early diagnosis that is helpful in control of blood sugar level at an early stage to prevent various complications. Several studies have examined the deleterious effects of DM on oral mucosa with reports stating its adverse effects on the morphology of oral mucosa, which in turn may compromise tissue function to favor the occurrence of oral infections and oral neoplasia.

Thus, we conducted a study in which clinical , qualitative and quantitative cytomorphometric analysis of oral mucosa in type II Diabetes patients was performed in buccal mucosa, dorsum of tongue and floor of mouth. Our study demonstrated that the cellular alterations are not restricted to the specific site of oral mucosa, as the microscopic and cytomorphometric findings of the diabetic group were found in all the sites studied. The results of present study showed statistically significant increase in nuclear area and the cytoplasmic area did not present statistically significant results.

The diabetic patients were grouped under various grades of glycemic control, based on their HbA1c levels. In order to further clarify the influence of glycemic control on the various study parameters, each parameter was individually compared across control group and various grades of diabetes. The NA showed a consistent and uniform increase in area from control to uncontrolled diabetic group. This increase in NA is significant in poorly controlled and uncontrolled diabetic patients. This finding concurs with the study by Alberti *et al* [7] who report a significant increase in nuclear area in diabetic patients. However, they have not taken the degree of glycemic control into account.

Morphological changes in oral mucosa in diabetes may be related to the metabolic control of the diabetic state and medication beside the previous reported factors that related to the reduction in epithelial nourishment, proliferation and turnover secondarily to micro vascular and metabolic disorders that may be accompanied with reduction in the stimulatory effect of insulin and IGF-I on keratinocyte [8]. Furthermore diabetics are commonly suffering from xerostomia that may alter oral mucosa and predisposing them to microbial colonization with critical reduction in salivary lubricant effect. This leads to atrophic oral mucosa or ulceration [9] that showed cells with large NA which may indicate more basal and parabasal cells. However such finding need to be related to cellular morphological features as Prasad et al [10] suggested that an increase in nuclear size with nuclear pleomorphism, bilobed nuclei, and cytoplasmic vacuolization in DM may related to cellular ageing, which resulted from reduction in cellular turnover and persistence of more number of mature cells. In the present study we also noticed such changes in diabetes individuals.

We did not find any significant difference in the CA between the groups. These results were inconsistent with the studies done by Jajarm HH *et al.*,[11] Alberti S *et al.*[7] and Ban Tawfeek Shareef *et al*·[12] On the contrary, Jajarm HH *et al.*[11] found CA enlargement in the diabetic group and he explained it could be related to the disparity in the number of analyzed cases.

We found a trend for candidiasis and inflammation to be more frequent in the diabetic group than in control group; however statistical analysis was not performed for this criterion. A high percentage of diabetic patients present with xerostomia and the complaint of dry mouth, which was also a finding of present study. This results in a dry, atrophic mucosa with accompanying mucositis as well as opportunistic infection with an increase in polymorphonuclear leucocytes, in response to the microbial colonization. Studies of Chavez EM *et al*., have shown that subjects with poorly controlled Diabetes had significantly lower stimulated parotid salivary flow rates. [13]

Cellular ageing produces various morphologic alterations and genotoxic damages in cells in the form of pleomorphism, bilobed nuclei, micronucleus, nuclear budding, karyorrhexis, which were also observed in the smears from diabetic patients in the present study.

It is extremely beneficial to determine the severity of the DM and the degree of control of glycemia, but the glycated hemoglobin assay is not currently recommended as a screening tool or as an initial test for the diagnosis of diabetes. It is used to monitor glycemic control in patients with previously diagnosed diabetes. Therefore, dentist can use cytology as additional tool in the clinic for screening and referral for diagnosis of previously undiagnosed patients after thorough review of the patient's health history and oral examination, or uncontrolled DM patients and explains the associated oral manifestations to them as well as to seek possible measurements to prevent local complications.

CONCLUSION

In the present study notable changes were seen in cytomorophology of diabetes patients and characterization of these changes make clinicians aware and give them a clearer image of what happens during diabetes. Although these findings cannot be considered predictive or diagnostic of this disease as these are not

unique to diabetes. Other nuclear changes, inflammation and candidiasis can collectively alert clinician for possibility of diabetes. The limitation of this study was that all the cytomorphometric changes were not correlated according to the type of treatment taken by patients.

Further studies with larger sample size and correlating treatment modality with the cytomorphometric changes should be taken up so that patient can receive better therapeutic approach.

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