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COMPARATIVE CYTOMORPHOMETRIC ANALYSIS OF ORAL MUCOSA IN PATIENTS WITH DIABETES, PATIENTS WITH ASSOCIATED ORAL HABITS BUT WITH APPARENTLY NORMAL MUCOSA AND CONTROL GROUP

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Abstract:

Aim: The aim of this study was to evaluate the dysplastic changes that occur in the cell, nuclear morphology and diameter in Buccal smears of patients in the study groups.

Background: Oral exfoliative cytology is a simple, non-invasive, and painless method that is well accepted by patients and involves microscopic analysis of cells collected from the surface of oral mucosa. Many factors affect the cytomorphology of the cells collected from oral mucosa. Some of these factors may be systemic disease; like anaemia and diabetes mellitus; radiotherapy; alcohol consumption and smoking. Cigarette and tobacco generally contain many carcinogenic substance mostly DNA-toxic carcinogens, however alcohol itself is not carcinogenic, but when combined with cigarettes or tobacco they have a worse impact on health. In exfoliative cytology various parameters such as nuclear size, cell and nuclear pleomorphism, nuclear membrane discontinuity, degenerative changes of nucleus and nuclear cytoplasmic ratio can be analysed.

Materials and Method: Smears were taken from the buccal mucosa of 15 patients with Diabetes, 15 patients with adverse oral habits but with apparently normal mucosa and 15 patients with normal mucosa. All the smears were stained using H & E stain and Papanicoloau stain and evaluated using research microscope and image analysis software.

Reason: To explore the potential of Exfoliative cytology as a diagnostic adjunct in patients with Diabetes and in patients with Associated habits but apparently normal mucosa.

Introduction:

Oral exfoliative cytology is a simple, non-invasive, and painless method that is well accepted by patients and involves microscopic analysis of cells collected from the surface of oral mucosa.(1) Many factors affect the cytomorphology of the cells collected from oral mucosa. Some of these factors may be systemic disease; like anaemia and diabetes mellitus; radiotherapy; alcohol consumption and smoking. Cigarette and tobacco generally contain many carcinogenic substance mostly DNA-toxic carcinogens (2), however alcohol itself is not carcinogenic, but when combined with cigarettes or tobacco they have a worse impact on health. It has been reported that substances present in cigarette smoke alter the charge and other properties of oral epithelial surfaces, allowing the growth of certain pathogenic bacteria like some species of Neisseria and certain gram-positive bacteria like Staphylococcus aureus and Streptococcus pneumonia.(3) Diabetes mellitus is a complex metabolic disease which is often followed by disorders in the metabolism of carbohydrate, lipid, protein.(4) Oral problems of people suffering from diabetes include xerostomia; salivary gland dysfunction; increased susceptibility to bacterial, viral and fungal infec-tions; increase in teeth decay; inflammation of gingiva; periodontitis; periodical abscess; loss of teeth; lichen plants and burning mouth syndrome.(5) There are several methods to evaluate the oral mucosa of patients with diabetes but the best method with low cost and less aggressive characteristics and also lack of damage to oral tissue of the patient is using exfoliative cytology or brush cytology.(6) The Papanicoloau stain is regarded as the universal stain for cytological preparations since it imparts a different color to the cytoplasm of epithelial cells based on their degree of cellular differentiation.(7) The goal of the present study was to examine the cytomorphometric and morphometric analysis of nucleus of patients with diabetes and patients with associated habits but apparently normal mucosa and compare them to each other and to the healthy patients.

Materials and Method:

The study group consisted of 45 patients; 15 patients with diabetes, 15 patients with associated ha-bits but with apparently normal mucosa and 15 controls. Subjects of both the study and the control groups were informed of the procedure and a written consent was obtained.

Patients with Diabetes:

Inclusion Criteria: Patients aged 45 years and above.

Medical history of diabetes for a minimum period of 5 years prior to commencement of the present study. Diagnostic criteria- Random serum glucose concentration > 200mg/dl, or Fasting serum glucose level > 126mg/dl.

Exclusion Criteria:

- ✓ Patients suffering from other systemic diseases.
- ✓ Known cases of anemia and malignancy.
- ✓ Patients who have undergone radiation therapy and chemotherapy.
- ✓ Patients with smoking or pan chewing habits and alcohol dependency.
- ✓ Patients with poor oral hygiene.
- ✓ Denture wearers.

Patients with Associated Habits but With Apparently Normal Mucosa:

Inclusion Criteria:

- ✓ Patients aged 45 years and above.
- ✓ Patients who smoked at least 10 cigarettes a day for the last 10 years, with apparently normal mucosa.
- ✓ Patients with daily drinking or regular drinking for at least 10 years, with apparently normal mucosa.

Exclusion Criteria:

- ✓ Patients who suffered from systemic diseases like anaemia or diabetes.
- ✓ Patients who had undergone radiation therapy or chemotherapy.
- ✓ Patients with malignant or potentially pre malignant oral lesions, such as leukoplakia or erythroplakia.
- ✓ Patients diagnosed with cancer.

Control Group:

- ✓ Patients aged 45 years and above.
- ✓ Patients with clinically healthy mucosa.
- ✓ Patients who were non-smokers and non-alcoholics.
- ✓ Patients who did not suffer from any systemic diseases.
- ✓ Patients who did not have any oral lesions.
- ✓ Patients with poor oral hygiene were excluded.

Method for Collection of Data:

Smear Collection: Smears were collected from clinically normal buccal mucosa of the patients using a wooden spatula moistened in distilled water. Three smears from each site were obtained. The scraping were thinly and uniformly transferred to a clean glass slide. The smears were then immediately fixed with 95% 2-propanol. Two smears were stained with H&E stains and one smear was stained with Papanicolaou stain to visualize under the microscope for cytomorphometric analysis of the cells i.e., nuclear area, cellular area, cytoplasmic area and the nucleus: cytoplasm ratio. The unfolded cells in a field were taken as representative of the cytological picture and evaluated. The entire slide was screened in a reister pattern for the existence of micronuclei. The collected data was then compared, where the control group was compared with the diabetic group and the associated habits group, and the diabetic and associated habit groups were also compared.

Result:

Table 1: Nuclear area of patients with diabetes, adverse oral habits and normal mucosa

Descriptives

NUCLEAR AREA (µm2)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NORMAL	15	126.00	15.409	3.979	117.47	134.53	104	146
ADVERSE ORAL HABITS	15	140.07	6.756	1.744	136.33	143.81	132	153
DIABETES	15	123.87	12.999	3.356	116.67	131.07	102	145
Total	45	129.98	14.024	2.091	125.76	134.19	102	153

Table 2: Comparison of the nuclear areas of patients with diabetes, adverse oral habits and normal mucosa

ANOVA

NUCLEAR AREA (µm2)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2324.311	2	1162.156	7.713	.001
Within Groups	6328.667	42	150.683		
Total	8652.978	44			

Table 3: Cytoplasmic area of patients with diabetes, adverse oral habits and normal mucosa

Descriptives

CELL AREA (µm2)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NORMAL	15	3567.40	482.556	124.596	3300.17	3834.63	2977	4237
ADVERSE ORAL HABITS	15	3694.93	522.789	134.984	3405.42	3984.44	3018	4626
DIABETES	15	3275.13	691.134	178.450	2892.40	3657.87	2583	4819
Total	45	3512.49	587.044	87.511	3336.12	3688.86	2583	4819

Table 4: Comparison of the cytoplasmic area of patients with diabetes, adverse oral habits and nor-mal mucosa

ANOVA

CELL AREA (µm2)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1389583	2	694791.489	2.119	.133
Within Groups	13773704	42	327945.340		
Total	15163287	44			

Table 5: Comparison of micronuclei in patients with diabetes, associated oral habits and normal mucosa

Multiple Comparisons

MICRONUCLEI Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diabetics	Adverse Oral Habits	11.400	4.800	.062	-.50	23.30
	Normal	15.900*	4.800	.007	4.00	27.80
Adverse Oral Habits	Diabetics	-11.400	4.800	.062	-23.30	.50
	Normal	4.500	4.800	.622	-7.40	16.40
Normal	Diabetics	-15.900*	4.800	.007	-27.80	-4.00
	Adverse Oral Habits	-4.500	4.800	.622	-16.40	7.40

*. The mean difference is significant at the 0.05 level.

Table 6: Comparison of other nuclear changes in patients with diabetes, associated oral habits and normal mucosa.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Karyolysis	Equal variances assumed	2.885	.115	.950	12	.361	1.571	1.654	-2.032	5.175
	Equal variances not assumed			.950	8.886	.367	1.571	1.654	-2.177	5.320
Karyorhexis	Equal variances assumed	.158	.705	1.567	6	.168	.750	.479	-.421	1.921
	Equal variances not assumed			1.567	4.973	.178	.750	.479	-.483	1.983
Nuclear Bud	Equal variances assumed	.	.	-2.000	2	.184	-2.000	1.000	-6.303	2.303

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Karyolysis	Equal variances assumed	2.885	.115	.950	12	.361	1.571	1.654	-2.032	5.175
	Equal variances not assumed			-2.00	1.000	.295	-2.000	1.000	-14.706	10.706
Condensed Chromatin	Equal variances assumed	.	.	.	0	.	2.000	.	.	.
	Equal variances not assumed			.	.	.	2.000	.	.	.
Pyknosis	Equal variances assumed	.	.	.115	2	.919	.333	2.906	-12.170	12.837
	Equal variances not assumed			.	.	.	.333	.	.	.

Table 7: Comparison of the presence of microorganisms in patients with diabetes, associated oral habits and normal mucosa

MICROORGANISMS * GROUP Crosstabulation						
			Group			Total
			Diabetics	Adverse Oral Habits	Normal	
Microorganisms	Present	Count	2	1	0	3
		% within Group	20.0%	10.0%	.0%	10.0%
	Absent	Count	8	9	10	27
		% within Group	80.0%	90.0%	100.0%	90.0%
Total		Count	10	10	10	30
		% within Group	100.0%	100.0%	100.0%	100.0%

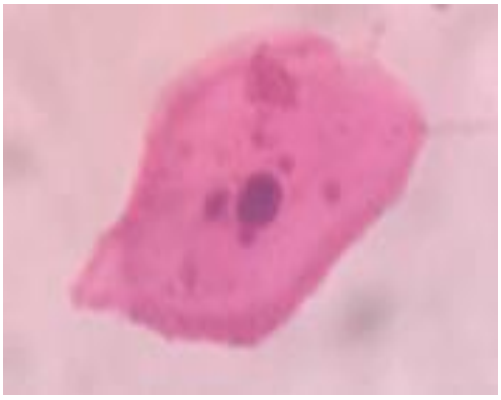

Figure 1: Micronuclei

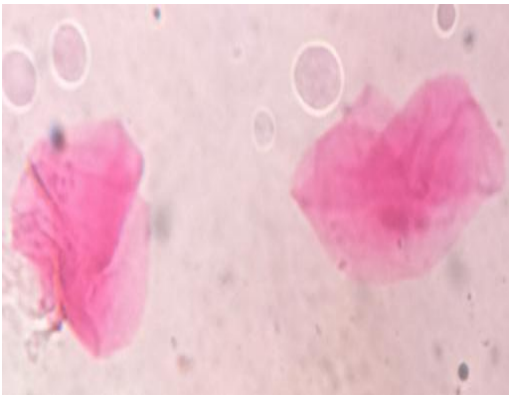

Figure 2: Karyolysis

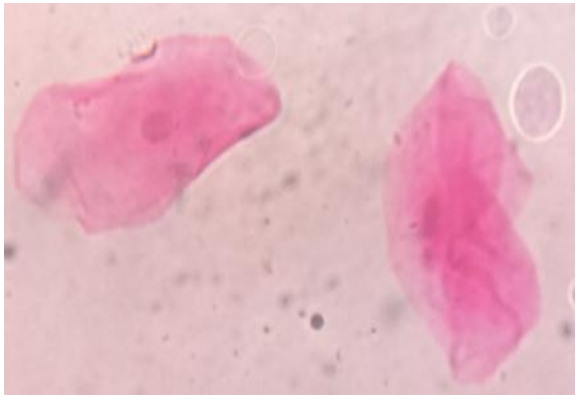

Figure 3: Karyorhexis

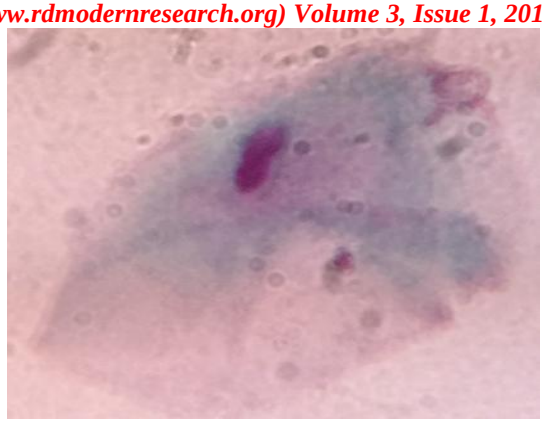

Figure 4: Nuclear bud

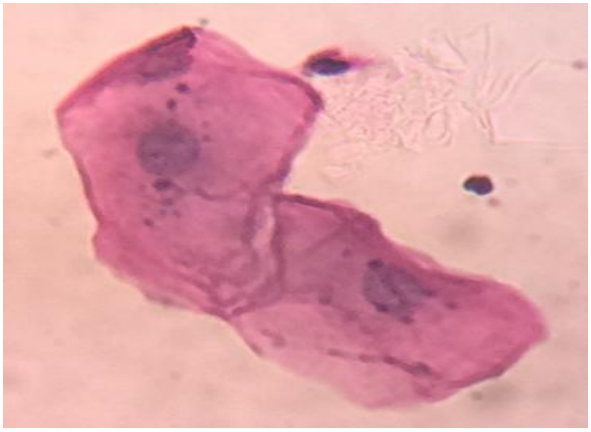

Figure 5: Condensed chromatin

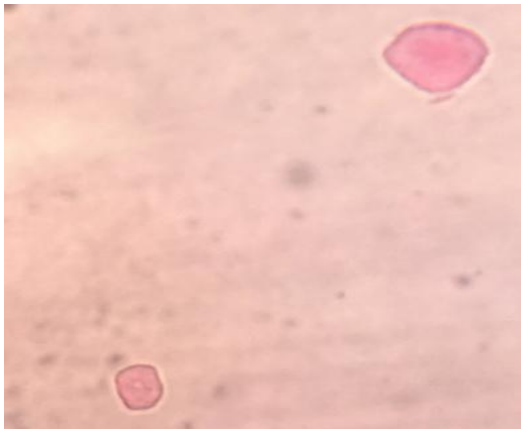

Figure 6: Pyknotic nuclei with a halo

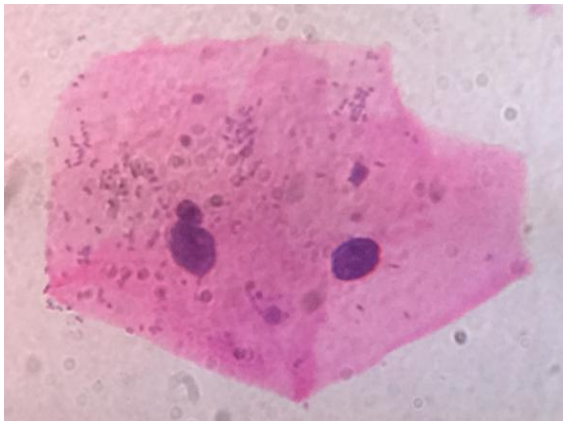

Figure 7: Micronuclei and Microorganisms seen in a patient with diabetes

Discussion:

In our present study, we have measured the ND, CD, and various nuclear changes of exfoliated buccal mucosa cells in the diabetic patients (n=15), associated oral habits patients (15) and control subjects (n=15). The purpose of our study is to determine if any significant differences in these parameters and various nuclear changes are present in diabetic individuals, and if they can be used as a diagnostic criterion. In our study, we found that their was a significant increase in the nuclear dimension in case of both diabetic patients as well as associated oral habits patient. There was an increase in the cytoplasmic diameter seen in patients with associated oral habits. These finding concurs with the study by Alberti et al., [8] and Prasad et al.,[9] who report a significant increase in nuclear area in diabetic patients and Ogden et al.,[10] who also report a significant increase in nuclear diameter and cytoplasmic area in patients with associated oral habits. The findings are contrary to the findings found by Ramesh et al., [11] where they found a significant decrease in nuclear and cytoplasmic dimensions. Increase in nuclear size might be an indicator of cellular ageing. Decreased cellular turnover as a result of ischemia following atherosclerosis would result in more number of mature cells with large nuclei in the smear. However, this has to be confirmed by studying the oral epithelial turnover rates. Ageing would also produce various nuclear alterations in cells in the form of pleomorphism, bilobed nuclei,

pyknotic nuclei with halo etc.[11] We were able to see such morphologic variations in cells obtained from both the diabetic patients as well patients with associated oral habits. Diabetic patients also suffer from dehydration and this (when combined with the decreased salivary flow rates) 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. [10,11] Patients who are smokers and have diabetes are more likely to have xerostomia and atrophic oral mucosa due to dehydration caused by the heat and disease process as well as due to reported de-crease in salivary flow rates. This is also associated with superadded infections like candidiasis, which can evoke a chronic inflammatory response in the oral mucosa. We were able to see the presence of microorganisms in a few smears in patients with diabetes.

Conclusion:

In accordance to studies conducted earlier, significant results were obtained in the present study. But the present study is to be continued with a larger sample size in order to obtain clear cut results, thus highlighting the dysplastic changes of the cells as well as the nuclear variations observed in different groups.

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