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Cytomorphometric Analysis of Exfoliative Cytology in Buccal Mucosa of Smokers and Non Smokers in Diabetic Patients in Salem Population – A Comparative Study

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Abstract

Introduction: Altered cellular microenvironment of oral cavity is a not an uncommon finding in diabetes mellitus patients. Environmental factors also play a pivotal role in cellular changes where tobacco smoking is one of the major risk factor. **Aim & Objective:** The aim of the study is to find the cytological changes in oral mucosal cells of diabetic people with and without smoking by using cytomorphometric analysis. **Materials and Methods:** Oral smears were taken from 50 diabetic patients where 25 with smoking habit and 25 without smoking habit from the oral pathology department of Vinayaka mission's Sankarachariyar dental college and hospital. The smears were fixed with 95%ethyl alcohol and stained with hematoxylin and eosin stain. The stained section observed under image analyzer for cytomorphometric analysis. **Results:** Cellular and nuclear alterations was significantly increased in diabetic patients with smoking habit when compared to diabetic patients without smoking, with a significant P value of 0.001. **Conclusion:** Diabetes mellitus influences a definite changes in cellular microenvironment in oral mucosa of smokers. However, further research is required to analyze the significance of these findings as a tool for identifying precancerous condition of oral tissues at an early stage.

Keywords: Buccal Mucosa, Cytomorphometric Analysis, Diabetic Patients, H&E Staining, Non-smokers, Oral Exfoliative Cytology, Smokers

1. Introduction

Oral cavity is an index to general health status of an individual.[1] In India oral cancer represents more than 50% of all malignancies, which is associated with various tobacco habits and environmental factors.[2] Smoking tobacco can cause DNA changes and increases the potential for premalignant and malignant changes.[3] In tobacco smoking >60 known carcinogens has been isolated when compared to smokeless tobacco, among them tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons and aromatic amines have potent carcinogenic effect.[4] Apart from cancer initiation smoking tobacco alters the

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cellular microenvironment where it develops reduced efficacy and stimulates clearance of anti-cancer drugs, and increases the potential of second primary tumor development.[5]

Diabetes mellitus is one of the common endocrine disorder, characterized by hyperglycemia and associated with various metabolic irregularities of proteins, carbohydrates and lipids.[6] Frequent alteration observed in oral cavity of diabetic patients include dental caries, increased prevalence of dental caries, impaired healing of oral wounds, burning mouth syndrome and various opportunistic infections.[7] Recently researchers have highlighted the cellular and nuclear alteration in oral mucosa of diabetic patients and the possible association with premalignant and malignant conditions.[8] Oral exfoliative cytology is easy, quickly and painless approach in patients with systemic ailments where biopsy is contraindicated. The entire procedure is based on epithelial physiology. In normal circumstances the epithelium thickness is maintained by periodic exfoliation of dead cells. In case of systemic and environmental changes cells lose their cohesiveness and forced to exfoliate. This collection of exfoliated cells can be examined microscopically to observe the early changes taking place in the cellular microenvironment.[9] These changes can be accurately evaluated by cytomorphometric analysis which is a non-invasive approach for screening and tracking of the condition in diabetic patients.[10]

In the present study the oral epithelium of diabetic patients with and without smoking habit was evaluated and analyzed by cytomorphometric analysis, to determine the changes caused by diabetes and tobacco in oral cavity.

2. Materials and Methods

This study was done among the patients who have visited, Department of oral pathology and oral microbiology, for routine blood investigations. Among them, the individuals were divided into two groups. In group A the individuals with known diabetes with smoking habit were included and in group B individuals with known diabetes without tobacco smoking habit were involved in this study. Diabetic patients for minimum one year without smoking habit were taken as controls. Diabetic patients for minimum one year with smoking habits for two years and above were taken as study group. Both males and females were included. Patients who were non-diabetic and with any other systemic diseases were excluded. Oral smears were taken from 50 patients (25 with tobacco and 25 without tobacco) (Figure 1). The smears were transferred to 95% ethyl alcohol and fixed for 1 hour (Figure 2). The smeared slides were stained with H&E stain and studied under image analyzer software SCOPE IMAGE 9.0. Hundred cells were randomly observed for normal and abnormal cells under microscope; hence 50 x 100 = 5000 cells were observed in both smoking and non-smoking group. The results were analyzed statistically with SPSS version 16.

3. Results

The present study was carried out in 50 diabetic patients out of which 25 diabetic patients were smokers and 25 diabetic patients were nonsmokers. And the results showed that in smokers the cell size and nuclear size were randomly increased in diabetic smoker patients. In non-smokers the cell size and nuclear size were not that increased in diabetic non-smokers.

The comparison of nuclear size in diabetic smokers and nonsmokers statistically showed a p value of 0.001* using Mann-Whitney U test. The mean value of nuclear size in smoker and diabetic was 28.09 and standard deviation of nuclear size in smoker and diabetic was 4.28. The mean value of nuclear size in non-smoker and diabetic was 15.90 and standard deviation of nuclear size in non-smoker and diabetic was 0.41.

The comparison of cell size statistically in diabetic smokers and non-smokers showed a p value of 0.001* using t test. The mean value of cell size in smoker and diabetic was 181.44 and standard deviation of cell size in



Figure 1. Armamentarium.

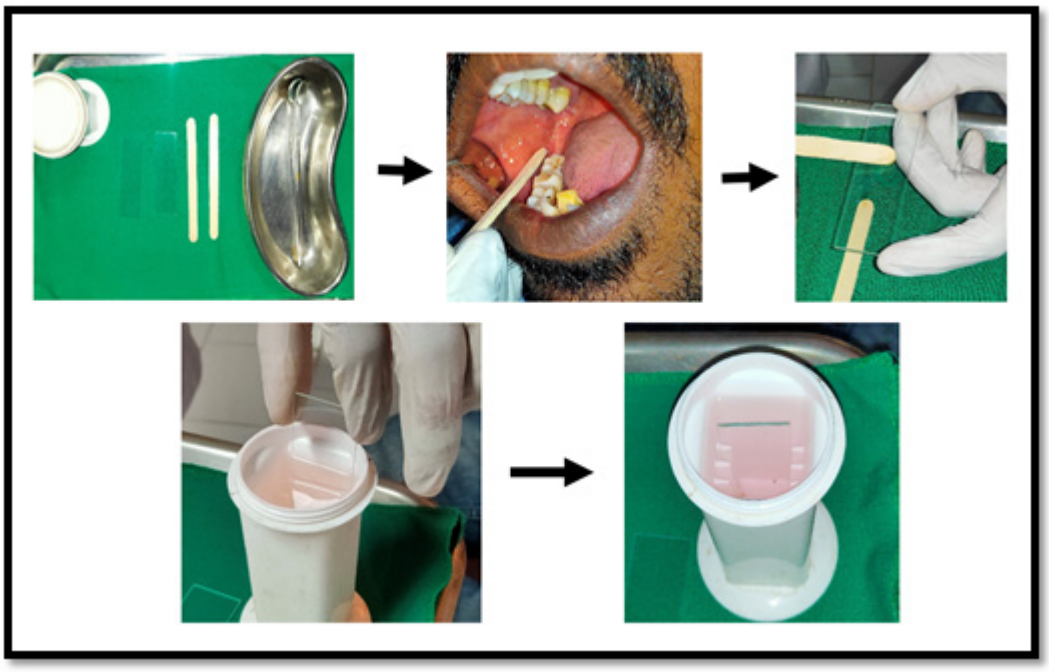
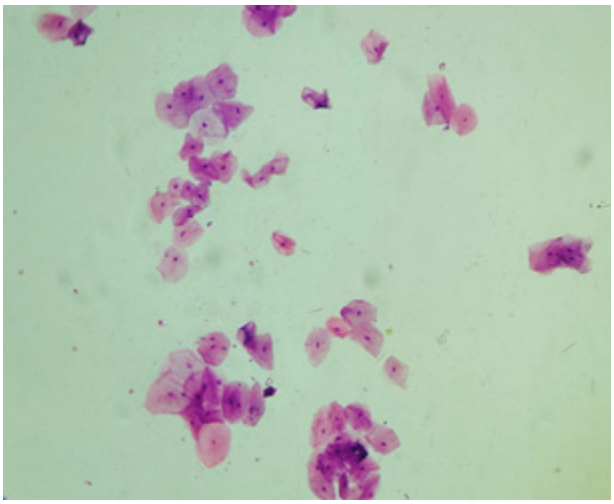


Figure 2. Armamentarium (Procedure).

smoker and diabetic was 12.19. The mean value of cell size in non-smoker and diabetic was 150.65 and standard deviation of cell size in non-smoker and diabetic was 5.05.

4. Results - Microscopic View

Figure 3 and 4 shows photomicrograph of the exfoliated cells in Diabetic nonsmokers under 10 X and 40 X magnification.



10X

Figure 3. Non-Smokers – Diabetic

Figure 5 and Figure 6 shows photomicrograph of the exfoliated cells in Diabetic smokers under 10 X and 40 X magnification.

4.1 Statistical Analysis

The values were analyzed using by Statistical Package for Social Sciences version - SPSS - 16 software. The MannWhitney U tests and t test was done to evaluate

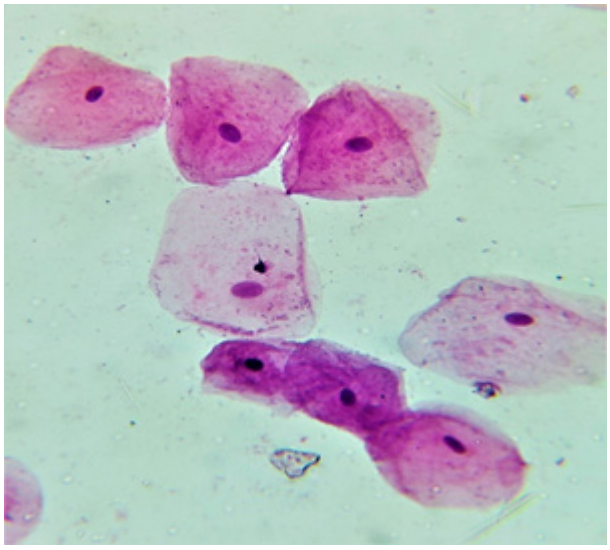
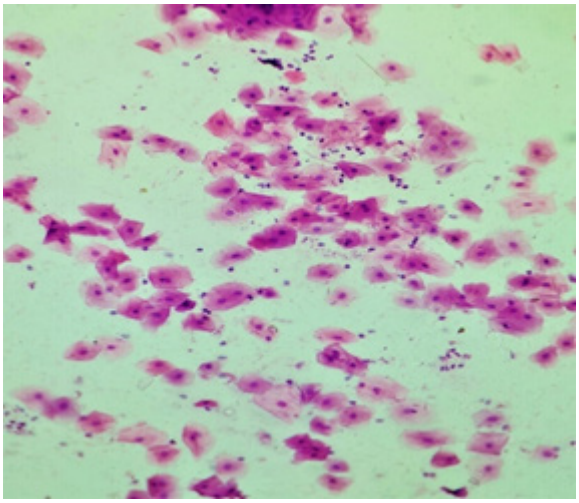


Figure 4. 40 X



10X

Figure 5. Smokers – Diabetic

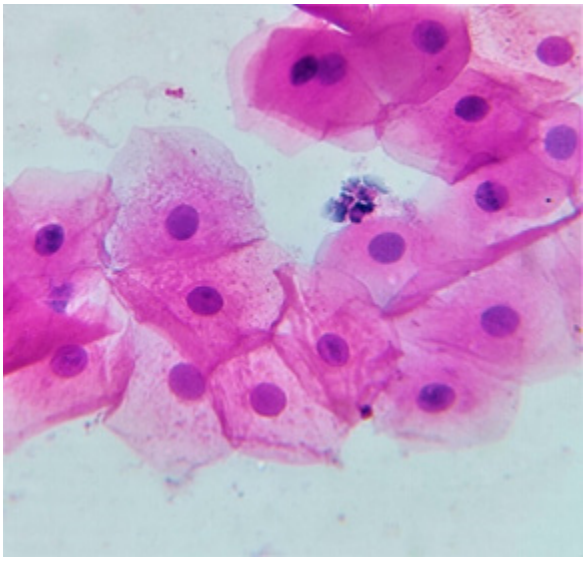


Figure 6. 40 X

the differences in many measures. (NA, CA, N/C ratio) between two groups.

5. Nucleus Size

When nuclear size between Diabetic Smokers and Diabetic Non Smokers were compare using Mann-Whitney U Test, p value was found to be 0.001 and highly significant (Table 1).

6. Results - Cell Size

When Cell size between Diabetic Smokers and Diabetic Non Smokers were compared using t Test, p value was found to be 0.001 and highly significant.

7. Discussion

Diabetes Mellitus (DM) is a metabolic disease characterized by abnormal carbohydrate, fat, and protein metabolism that results in acute or chronic complications due to absolute or relative lack of insulin. DM is the fifth most common chronic condition and the sixth leading cause of mortality among the elderly worldwide.[11]

Proper understanding of cellular changes in the Oral mucosa of patients with DM enables early diagnosis that is helpful in preventing various complications. Many researchers have tested the deleterious effect of DM on oral mucosa with reviews mentioning its unfavorable outcomes at the cellular morphology of oral mucosa,

Table 1. Mann-Whitney U Test

Nucleus size	N	Min	Maxi	Mean	SD	SE	Mann-Whitney U	P
Smoker and Diabetic	25	21.61	37.49	28.09	4.28	0.855	6.064	0.001**
Non-smoker and Diabetic	25	**15.30**	**17.08**	**15.90**	**0.41**	0.083		

P value < 0.005 highly significant.

Table 2. T Test, P value

Cell size	N	Min	Maxi	Mean	SD	SE	T	P
Smoker and Diabetic	25	163.26	208.20	181.44	12.19	2.437	11.69	0.001**
Non-smoker and Diabetic	25	138.58	158.86	150.65	5.05	1.011		

P value < 0.005 highly significant.

which in turn can initiate incidence of oral infections and oral neoplasia.

Similarly, in the previous year's few researches have also been investigated to know the effects of smoking tobacco on the oral mucosa, using the non - invasive exfoliative cytology technique and it is found that smoking habit, contributes to the damage to the mucosal cells which in turn acts as a risk factor for oral malignancies. This fact mostly depends on the number of cigarettes smoked per day and the duration of exposure to smoking.

Using cytomorphology Ogden *et al.* investigated the effect of smoking on the oral mucosa in individuals over 40 years of age and reported a 5% average increase in the NA values of smokers when compared to non-smokers.[12] Similar findings were also reported by Goregen M *et al.*[13] & Seifi S *et al.*[14]

In the present study, the morphometric and cytological evaluation showed significant increase in the nuclear size and cell size in the exfoliative smear slide of the diabetic patients with habit of smoking when compared to the diabetic patients without smoking habit. These alterations were noted in the buccal mucosa of the subjects which was apparently normal.

The present finding was similar with the studies done by Alberti *et al.* (2003)[15], Jajarm *et al.,* (2008)[16], Shareef *et al.,* (2008)[17], Tozoglu and Bilge (2010)[18], Prasad H *et al.,* (2011)[19] where the mean NA was significantly higher among the diabetic group.

Based on these facts, there is enough evidence that the diabetes along with the habit of smoking may cause significant alterations in the morphology of the oral exfoliated cells, which may further lead to oral premalignant changes.

8. Conclusion

We conclude that the Present exfoliative cytology study clearly states that tobacco smoking is related with abnormal cytological change in cells of buccal mucosa which may progress towards premalignancy and may become malignant. Diabetes on the other hand, may predispose, definite morphologic and cytomorphometric changes in the oral mucosa of patients.[20]

Further studies with large sample size should be carried out to analyze the significance of these findings as a tool for early detection of oral cancer in diabetic patient and its effects on various sites of the oral cavity. This study will help to give awareness to the society about the oral mucosal changes in diabetic persons with and without smoking habits.

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