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Detection and Comparision of Cytopathological Changes in Smokers and Non-Smokers: A Comparative Study

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Abstract

Background: Oral cancer is one of the sixth most common cancers in the world. Oral exfoliative cytology is one of the popular screening tool for oral cancer. Use of tobacco in any form are documented as the most common cause as initiators for dysplastic changes in oral mucosa. The purpose of the study was to detect the cytological changes in buccal mucosa, tongue and palate among non-smokers & smokers. **Material & Methods:** Smears sample were collected according to site (buccal mucosa, tongue & palate) from 100 subjects among smokers & non-smokers. Smears were then stained using Papanicolaou staining technique. **Results:** Among the smokers and non-smokers the results were statistically significant. **Conclusion:** Recent advances in the clinical visualization and detection of the oral mucosa have made the viability of cytological procedures more specific and sensitive. Contact endoscopy and use of autofluorescence devices are the forerunners in this group. The fluorescence characteristics of tissues depend upon their biochemical composition and histomorphological architecture, both of which undergo a change during malignant transformation. These changes are detectable as an alteration in the fluorescence spectral profile of the tissues21. Due to low feasibilities of such devices the benchmark of diagnosis will be microscopic tissue examination. Hence cytological smears will always be highly specific, sensitive, easy to use and reproducible procedures in routine screening of population for potentially and malignant conditions of the oral cavity.

Keywords:- Oral cancer, Exfoliative cytology, PAP Satin.

INTRODUCTION

In many developing countries oral cancer is a major health problem.[1] Oral cancer is one of the sixth most common cancers in the world, and is one of tenth major causes of death across the globe.[2] India contributing to almost one-third of the total cases and second country having the highest number of oral cancer cases. The increasing cases of oral cancer are the most

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important concern for public health as it is one of the common type of cancers in India. In India, around 77,000 new cases and 52,000 deaths are reported annually.[3] Tobacco smoking attributed to as a major risk factor for carcinogenesis.[1] 30% - 80% of malignancies of the oral cavity arise from premalignant lesions, such as leukoplakia, erythroplakia and oral submucous fibrosis.[4] Despite improvements in surgical and radiation therapy and combined radiochemo-therapy, it has been reported that the 5-year overall survival rate is reduced by 50%.[5] Lack of early detection and treatment has led to poor survival rate among oral cancer patients. Hence, it is necessary to detect potentially malignant lesions at their incipient stage. Early diagnosis and initiation of appropriate treatment of early malignant lesions offer the best hope of improving the prognosis.[1] Rationale of exfoliative cytology lies in the epithelial physiology. Continuous exfoliation of epithelial cells is a part of physiological turnover.[6] oral exfoliative cytology is a simple, noninvasive and painless method that involves microscopic analysis of cells collected from the surface of oral mucosa.[7] The aim of the study is to detect the cytological changes in the buccal mucosa, tongue and palate among smokers and non-smokers.

MATERIAL AND METHODS

This study was conducted in the department of Oral & maxillofacial pathology & Oral microbiology, Indira Gandhi Govt. Dental College, Jammu. Ethical clearance was obtained from the ethical committee of this institution. Informed consent was obtained from all the patients before the cytological smear.

The study included 100 subjects, age 18-70 years and divided into four groups:

Group 1 - Control group comprising 30 non-smokers healthy volunteers with clinically normal mucosa

Group 2 – Study group comprising 30 smokers with duration of smoking less than 10 years without any lesion

Group 3 –Study group comprising 30 smokers with duration of smoking more than 10 years without any lesion

Group 4 – Study group comprising 10 smokers with lesions (irrespective of site)

Inclusion Criteria

1. All the patient with habit of smoking for more than 2 years were included in study.
2. Only male patients, age 18-70 years were included in study.

Exclusion Criteria

1. Patients with habits of alcohol and gutka chewing.
2. Patients with any systemic illness.
3. Female participants are excluded from study.
4. Patients who have exposed to ionizing radiation within past 6 months.

Thorough examination of oral cavity was done. Patient history was taken to record the subject data and habits. In presence of habit, all the details about habits(smoking) including type, frequency and duration was recorded. Before obtaining smears, patients were asked to rinse their oral cavity with tap water. Wooden spatula was use to collect the smears from buccal mucosa, tongue and palate. Excess saliva and surface debris which was scraped firmly across the site under investigation were wiped off from the area to be smeared. Then, the smears obtained using wooden spatula, transferred to dry glass slide and spread, fixed in 95% alcohol. Slides were then stained with Papanicolaou stain.

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The smears were then observed under 10x & 40x magnification. For each slide, 100 epithelial cells with strong staining were selected randomly, each slide observed under 40x from different field. The cytological features in the smears studied were cellular and nuclear pleomorphism, binucleation, micronuclei, clumping of squamous cells and normal epithelial cells.

Data were analysed using student "t" test and "p" value of <0.05 were considered to be statistically significant.

RESULTS

In this study, exfoliated cells from buccal mucosa, tongue and palate of smokers & non-smokers were observed. Results were statistically analysed after data calculation. Cytological parameters i.e., Pleomorphism, Binucleation, Micronuclei, Clumping and presence of normal cells were calculated and tabulated from tongue, palate and buccal mucosa in non-smokers and in smokers according to duration of smoking i.e., less than 10 years & more than 10 years without lesion. Cytological changes were than compared in non-smokers according to site [Table 1] and in smokers less than 10 years [Table 2] and more than 10 years [Table 3]. All together changes were than compared between smokers and non-smokers and results of which were statistically significant [Table 4].

Table 1: Comparison of cytological parameters in non-Smokers according to site (buccal mucosa, tongue and palate)

Cytological Changes	Site	Mean	Standard Deviation	"t"	"p"
Pleomorphism	Buccal mucosa	1.25	0.70	2.73	0.01**
	Tongue	0.4	0,69		
	Buccal mucosa	1.25	0.70		
	Palate	0.5	0.52	2.71	0.01**
	Tongue	0.4	0.69	0.36	0.71
	Palate	0.5	0.52		
Binucleation	Buccal mucosa	0.3	0.48	1.97	
	Tongue	0	0		0.06
	Buccal mucosa	0.3	0.48	1.97	
	Palate	0	0		0.06
	Tongue	0	0	0	0
	Palate	0	0		
Micronuclei	Buccal mucosa	0.6	0.96	0.28	
	Tongue	0.5	0.52		0.77
	Buccal mucosa	0.6	0.96	0.88	
	Palate	0.3	0.48		0.38
	Tongue	0.5	0.52	0.89	0.38
	Palate	0.3	0.48		
	Buccal mucosa	1.3	1.25	1.55	
	Tongue	0.6	0.69		0.13

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Clumping	Buccal mucosa	1.3	1.25	1.26	
	Palate	0.7	0.82		0.22
	Tongue	0.6	0.69	0.29	0.77
	Palate	0.7	0.82		
Normal	Buccal mucosa	41.9	9.64	1.85	
	Tongue	35.5	5.12		0.08
	Buccal mucosa	41.9	9.64	0.87	
	Palate	38.8	5.76		0.39
	Tongue	35.5	5.12	1.28	0.21
	Palate	38.8	5.76		

Table 2: Comparison of cytological parameters in Smokers (<10 years) according to site (buccal mucosa, tongue and palate)

Cytological changes	**Site**	**Mean**	**Standard deviation**	**"t"**	**"p"**
Pleomorphism	Buccal mucosa	31.6	6.70		
	Tongue	35.3	23.34	1.86	0.07
	Buccal mucosa	31.6	6.70		
	Palate	32.1	4.70	2.42	0.02
	Tongue	35.3	23.34	1.16	0.25
	Palate	32.1	4.70		
Binucleation	Buccal mucosa	3.2	1.75	1.34	
	Tongue	3	1.63		0.19
	Buccal mucosa	3.2	1.75		
	Palate	42	1.03	1.10	0.91
	Tongue	3	1.63	0.10	0.91
	Palate	42	1.03		
Micronuclei	Buccal mucosa	9.7	3.33		
	Tongue	11.6	1.71	6.37	0.10
	Buccal mucosa	9.7	3.33		
	Palate	11.5	2.06	1.72	0.10
	Tongue	11.6	1.71	2.67	0.01
	Palate	11.5	2.06		
Clumping	Buccal mucosa	13.6	4.92		
	Tongue	14.6	4.40	1.80	0.08
	Buccal mucosa	13.6	4.92	8.68	0.08
	Palate	13.4	2.87		
	Tongue	14.6	4.40	2.18	0.04
	Palate	13.4	2.87		
	Buccal mucosa	41.9	9.64		
	Tongue	35.5	5.12	1.85	0.08

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Normal cells	Buccal mucosa	41.9	9.64		
	Palate	38.8	5.76	0.87	0.39
	Tongue	35.5	5.12	1.28	0.21
	Palate	38.8	5.76		

Table 3: Comparison of cytological parameters in smokers (>10 years) according to site (buccal mucosa, tongue and palate)

Cytological changes	**Site**	**Mean**	**Standard deviation**	**"t"**	**"p"**
Pleomorphism	Buccal mucosa	34.8	5.49	1.78	0.09
	Tongue	38.5	3.59		
	Buccal mucosa	34.8	5.49	0.77	0.44
	Palate	36.5	4.24		
	Tongue	38.5	3.59	1.13	0.26
	Palate	36.5	4.24		
Binucleation	Buccal mucosa	5	1.41	0.05	
	Tongue	5.1	1.59		0.95
	Buccal mucosa	5	1.41	0.86	
	Palate	5.5	1.17		0.39
	Tongue	5.1	1.59	0.22	0.82
	Palate	5.5	1.17		
Micronuclei	Buccal mucosa	10.7	3.74	1.49	
	Tongue	13	3.09		0.15
	Buccal mucosa	10.7	3.74	0.89	
	Palate	11.9	2.02		0.38
	Tongue	13	3.09	0.94	0.35
	Palate	11.9	2.02		
Clumping	Buccal mucosa	14.1	3.51	0.07	
	Tongue	14	2.26		0.94
	Buccal mucosa	14.1	3.51	0.83	0.41
	Palate	12.6	4.50		
	Tongue	14	2.26	0.87	0.39
	Palate	12.6	4.50		
Normal cells	Buccal mucosa	35.4	3.59	3.19	
	Tongue	29.4	4.74		0.005**
	Buccal mucosa	35.4	3.59	0.69	
	Palate	33.5	7.80		0.49
	Tongue	29.4	4.74	1.42	0.17
	Palate	33.5	7.80		

Table 4: Comparison of cytological parameters in smokers and non-smokers

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Cytological Changes	"t"	"p"
Pleomorphism	21.91	<0.00001**
Binucleation	6.73	0.00026**
Micronuclei	16.31	<0.00001**
Clumping	29.50	<0.00001**
Normal Cells	24.10	<0.00001**

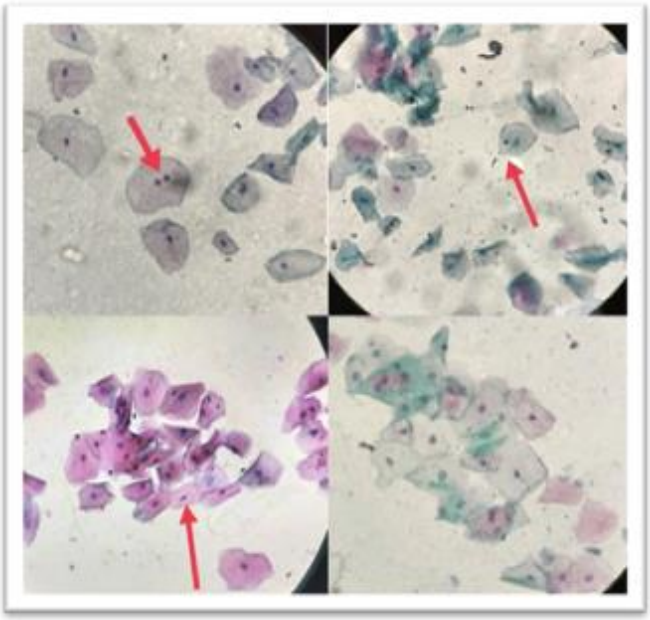

Figure 1: A) Binucleation, B) Micronuclei, C) Pleomorphism, D) Clumping.

DISCUSSION

Oral cancer is one of the most devitalizing diseases which afflict humans. The worldwide incidence of cancer is on arise today despite the best efforts of clinicians and researchers.[8] The effect of smoking as a risk factor for oral cancer, depends on the number of cigarettes smoked daily and the duration of smoking. Smoking has led to different changes in the oral mucosa of many individuals. Smoking has been related to many pathologies, which range from harmless and reversible lesions to oral cancer in oral mucous membranes.[9]

Oral exfoliative cytology has been used for the assessment of epithelial atypia and screening for early diagnosis of premalignant and malignant oral mucosa lesions.[10] As per the normal physiology, the oral epithelium renews itself rapidly (probably every 2 weeks). The rationale of oral exfoliative cytology is based on this physiological process, examining cells that are desquamated or abraded from the surface of the oral mucosa.[11] It may be advantageous for the detection and intervention in oral cancer patients at very early stages.[12] The most common cellular morphologic changes are those of pleomorphism, hyperchromatism and altered nuclear cytoplasmic ratio which reflect malignant change.[13]

Twinky Merlin Thomas et al in their cytological comparative study of exfoliated cells in the buccal mucosa of smokers and non-smokers indicated that mild to moderate pleomorphism, clumps of cells, binucleation, micronuclei were seen in the oral cavity of smokers compared to non-smokers.[1] These results were consistent with our study. In a study by Seifi S et al on evaluation of cytological alterations of oral mucosa in smokers and waterpipe users, smoking and waterpipe use were found to be effective in creating some quantitative cytometric alterations in oral mucosa, while smoking shows greater effect than waterpipe use in this regard.[14] In our study only smoking habit of more and less than 10 years of duration

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was selected. Mohammed S. et al, in their study detected cytomorphological changes in oral mucosa among alcoholics and cigarette smokers and proved that alcohol and cigarette smoking are risk factors for oral atypical cellular changes and the degree of change depends on the duration of alcohol consumption and cigarette smoking.[2] Goregen M in their study of cytomorphological analysis of buccal mucosa cells in smokers concluded that dysplastic changes tend to be more in smokers than non-smokers.[15] In our study buccal mucosa, tongue and palate were the selected sites and cytological parameters according to site were not so significant.

Navya BN et al, compared cytogenetic abnormality of exfoliative buccal cells among smokers and non-smokers and concluded that cigarette smoking induces DNA damage and leads to cellular death by increasing the above parameters in buccal mucosa cells and hence these parameters can be considered as indicators in predicting the risk of oral cancer.[8] Hashemipour MA et al, studied exfoliative cytology of oral mucosa among smokers, opium addicts and non-smokers, results of which indicate different rates of epithelial cell keratinization in oral cavity among smokers, opium addicts and non-smokers, thus suggest a possible relationship between the number of cigarettes per day, daily opium consumption and an increase in the rate of cellular proliferation of oral mucosal cells.[16]

R.P. Cancado et al, studied nucleolar organizer region associated proteins in exfoliative cytology of normal buccal mucosa and conclude correlation between the smoking habit and an increased rate of cellular proliferation in the oral mucosal cells.[17] De Castro Sampaio et al, did AgNOR Count in exfoliative cytology of normal buccal mucosa,[18] results of which indicate that AgNOR number in smears of smokers was higher than in smears of non-smokers. S Babuta in their study of cytomorphometrical analysis of exfoliated buccal mucosal cells concluded that increase in NA (nuclear area) and decreased CA (cytoplasmic area) as well as altered N/C ratio (nuclear cytoplasmic ratio) would appear to be due to smoking tobacco.[19] Ahmed HG, Babiker AA. Evaluated assessment of cytological atypia, AgNOR and nuclear area in epithelial cells of normal oral mucosa exposed to toombak and smoking results of which indicate that AgNOR number in smears of smokers was higher than in smears of non-smokers.[20]

CONCLUSIONS

Recent advances in the clinical visualization and detection of the oral mucosa have made the viability of cytological procedures more specific and sensitive. Contact endoscopy and use of autofluorescence devices are the forerunners in this group. The fluorescence characteristics of tissues depend upon their biochemical composition and histomorphological architecture, both of which undergo a change during malignant transformation. These changes are detectable as an alteration in the fluorescence spectral profile of the tissues21. Due to low feasibilities of such devices the benchmark of diagnosis will be microscopic tissue examination. Hence cytological smears will always be highly specific, sensitive, easy to use and reproducible procedures in routine screening of population for potentially and malignant conditions of the oral cavity.

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