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Original Article

A Comparative Study Of Oral Epithelium In Tobacco And Alcohol Consumers In Central Rajasthan Population

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ABSTRACT

ABSTRACT: Aim: The study was conducted to observe the impact of tobacco and alcohol on the nuclear changes in oral epithelial cells in 800 subjects ranging from 20 to 70 years in age. Methods: The nuclear aberrations such as multinucleation, binucleation, condensed chromatin, pyknosis, karyorrhexis and karyolysis in Papanicolaou stained buccal smears of the patients attending OPD at ENT, Gastroenterology, TB & Chest Disease, Medicine, Surgery and Radiotherapy departments, JLN Medical College and attached hospitals, Ajmer, were evaluated. Results: The consumption of the tobacco and alcohol was observed to be relatively high among poor income group and subjects educated up to 8th grade. A highly significant ($p<0.001$) increased frequency of nuclear changes was observed in relation to the all habit groups. The most common nuclear pathology seen was karyolysis in all groups and the least common observed was the condensed chromatin. Conclusion: Microscopically examined nuclear changes are a useful tool in early diagnosis of the oral carcinoma.

1. Introduction

Oral cancer is one of the ten most common cancers as stated by WHO and each year 5, 75,000 new cases and 3, 20,000 deaths occur worldwide. In India oral cancer is a major health problem, which accounts for 50-70% of all carcinomas diagnosed [1].

The buccal cell nuclear changes were first proposed in 1983 [2] and it continues to gain popularity as a biomarker of genetic damage in numerous applications. The assay provides information on the cytogenetic damage in the tissues, that are targets of human carcinogens and from which carcinomas can develop [3].

Oral cytopathology is a simple technique that is non-aggressive, relatively painless, and readily accepted by the patient. It is used to obtain cells from the oral epithelium [4].

Microscopic observation of nuclear aberrations is performed in cytopathologic samples. It is believed that the number of nuclear changes is related to increase the effects of carcinogens. It is important to emphasize that this event has already occurred before the clinical symptoms of cancer appear [5].

A lot of research work relating to the examination of the oral epithelial cells has been done in last decade [6]. This growing interest may be explained by several factors, including its relative technical simplicity and the variety of complementary toxicological end points evaluated. These include nuclear anomalies such as nuclear buds (indicative of gene 3 amplification), binucleation (caused by cytokinesis-failure or arrest), and various forms of cell death measured as condensed chromatin, karyorrhexis, karyolysis, pyknosis as well as the frequency of basal and fully differentiated cells [7].

The regular use of tobacco and alcohol cause similar effects on oral mucosa like loss of cell cohesion, hyperkeratosis, and increased incidence of nuclear anomalies.

The primary aim of the study was to investigate the impact of tobacco and alcohol on chromosomal damage in oral mucosa cells of alcoholics, smokers and tobacco chewers and monitor the effect of type of addiction on induction of nuclear anomalies including multinucleation, binucleation, condensed chromatin, pyknosis, karyorrhexis and karyolysis in exfoliated buccal cells.

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2. MATERIALS AND METHODS:

The present study was carried out on individuals selected from the patients attending the ENT, Gastroenterology, TB & Chest, Medicine, Surgery and Radiotherapy outdoors. The subjects were classified in 8 habit groups viz, alcoholics (A), smokers(S), tobacco chewers(TC), alcoholics and smokers(A+S), alcoholics and chewers(A+C), smokers and chewers(S+C), alcoholics and smokers and chewers(A+S+C) and control (C, persons were not habituated to any form of tobacco consumption or alcohol intake).

After seeking consent and recording the history, the patients were then subjected to sample collection. The subject was asked to rinse the mouth with drinking water. Taking all aseptic precautions, a clean wooden spatula was used to scrape the sample area (inner side of the cheek) three to four times with firm pressure. The slides were coded before scraping the mucosa to avoid confusion and the sample was then spread on the slide.

These slides were stained according to Papanicolaou staining technique [8]. The smears were observed under 40X and 100X magnifications. From each subject, a minimum of 1000 cells were screened for calculating frequency of nuclear anomalies which includes multinucleation, binucleation, condensed chromatin, pyknosis, karyorrhexis and karyolysis.

The data thus generated was analyzed using chi square test, the significance level was considered at $p < 0.05$.

3. RESULTS AND DISCUSSION:

In the present study most of the subjects were in the age group of 40-49 years (26.62%), followed by 50-59 years (20.50%), 30-39 years (19.75%), 60-70 years (19.12%), and 14% within the age group of 20-29 years. Out of 800 subjects, 761 (95.12%) were males, and 39 (4.88%) females (Table1).

Mean age in different habit groups varied from 44.13±13.49 years for group A to 50.10±13.49 years for group A+S+C (Table 2).

In the present study variations in different habits were observed according to the educational status, family structure and occupation. The level of consumption of alcohol and tobacco was in inverse proportion to their educational level [9]. 70.88% of subjects belonged to joint family and 29.12% from nuclear family structure. The consumption of tobacco and alcohol, singly or in combination was observed as 0.63% in house wives, 7.37% in unemployed category, 13% in farmers, 15.37% in laborers, 22.88% in business class, and 39.25% in salaried class people (Table 3).

The subjects under study were divided into 5 income groups based on per capita family income. Most of the cases (59.25%) were in poor income group, followed by 21% in lower middle class, 9.5% in upper middle class, 5.5% in below poverty line group and 4.75% in higher income group (Table 4). This is in accordance with the previous study done by Jha et al in 1999 [10].

Table 1. Distribution of subjects according to age and sex

Age group (in years)	Sex		Total
	Male	Female	
20-29	104 (13.00)	8 ((1.00)	112 (14.00)
30-39	149 (18.62)	9 (1.12)	158 (19.75)
40-49	203 (25.37)	10(1.25)	213 (26.62)
50-59	158 (19.75)	6(0.75)	164 (20.50)
60-70	147 (18.38)	6(0.75)	153 (19.12)
Total	761 (95.12)	39(4.88)	800 (100.00)

***Figures in parenthesis showing percentages**

Table 2. Mean ± SD of age of Various habit group subjects

Group	Number of Subjects	Mean ± SD
A	100	44.13 ±13.49
S	100	45.75±16.80
TC	100	47.75±15.56
A+S	100	45.86±13.16
A+C	100	45.34±13.84
S+C	100	48.85±16.17
A+S+C	100	50.10±13.49
C	100	42.55±15.44

A= alcoholics, S= smokers, TC= tobacco chewers, A+S= alcoholics and smokers, A+C= alcoholics and chewers, S+C= smokers and chewers, A+S+C= alcoholics and smokers and chewers, C= control

The frequency of nuclear changes in different habit groups (table 5) shows that incidence of multinucleation ranged from 20% (group A) to 53% (group S+C). The incidence of binucleation varied from 6% (group A+S) to 26% (group A+C). The presence of karyorrhexis was highest in group A+S+C and group S+C i.e. 39% and lowest in group A and group S i.e. only 11%. The occurrence of karyolysis varied from 45% (group A) to 67% (group A+S+C). The frequency of pyknosis was observed to be lowest (22%) in group S and highest (61%) in TC group. Thus p value clearly depicted that nuclear changes in the different habit groups were highly significant. As seen in the study, the incidence of condensed chromatin varied from 6% (group S+C and group A+S+C) to 1% (group A, group TC, and group A+S). It was present only in the 2.5% of subjects; hence this observation could not be put in statistical analysis.

Table 3-Distribution of subjects according to habit groups and educational status, type of family, occupation

	ES			TOF			Occupation				
Groups	M	9^{th}-12^{th}	PSE	N	J	Sl	Bs	Lb	Fm	Ue	Hw
A	51%	26%	23%	24%	76%	45%	16%	23%	8%	8%	0%
S	57%	22%	21%	35%	65%	26%	19%	20%	20%	13%	1%
TC	46%	32%	22%	38%	62%	37%	27%	16%	6%5	5%	1%
A+S	35%	44%	21%	24%	76%	38%	31%	13%	13%	5%	0%
A+C	49%	37%	14%	26%	74%	48%	25%	13%	13%	1%	0%
S+C	53%	27%	20%	30%	70%	32%	25%	14%	19%	9%	0%
A+S+C	46%	18%	36%	10%	90%	2%	20%	9%	15%	4%	0%
C	41%	36%	23%	46%	54%	36%	20%	15%	10%	14%	3%
Total	47.25%	30.25%	22.50%	29.12%	70.88%	39.25%	22.88%	15.37%	13.00%	7.37%	0.63%

ES= educational status, TOF= type of family, M= up to grade 8th, PSE= post school education, N= nuclear family, J= joint family, Sl= salaried, Bs= business, Lb= labourer, Fm= farmer, Ue= unemployed, Hw= housewife

Table 4-Distribution of subjects according to habit groups and income groups

	income groups based on per capita family income				
Groups	**BPL (< 500 Rs.)**	**Pr (500-1499 Rs.)**	**LM (1500-2999 Rs.)**	**UM (3000-4999 Rs.)**	**H (5000-9999 Rs.)**
A	7%	54%	23%	11%	3%
S	10%	53%	20%	11%	6%
TC	6%	61%	22%	8%	5%
A+S	1%	65%	24%	8%	2%
A+C	6%	72%	13%	9%	0%
S+C	6%	55%	23%	11%	5%
A+S+C	4%	53%	27%	8%	8%
C	4%	61%	16%	10%	9%
Total	5.50%	59.25%	21%	9.50%	4.75%

BPL = below poverty line, Pr = poor class, LM = lower middle class, UM = upper middle class, H = higher class

Table 5-Distribution of subjects according to habit groups and frequency of nuclear changes

Groups	MN	BN	KR	KL	Pk	CC
A	20%	8%	11%	45%	26%	1%
S	27%	7%	11%	50%	22%	3%
TC	43%	16%	23%	62%	61%	1%
A+S	21%	6%	16%	59%	24%	1%
A+C	26%	26%	35%	61%	49%	2%
S+C	53%	18%	39%	62%	57%	6%
A+S+C	25%	18%	39%	67%	48%	6%
C	10%	9%	4%	13%	12%	0%
Total	28.12%	10.63%	22.25%	52.37%	52.37%	2.50%
$\chi 2$ =	64.345	44.074	78.008	85.306	101.818	–
P value =	<.001	<.001	<.001	<.001	< .001	--

MN= multinucleation, BN= binucleation, KR= karyorrhexis, KL= karyolysis, Pk= pyknosis, CC= condensed chromatin

Stich and Rosin (1983) observed a synergistic effect of alcohol and nicotine, but the two drugs alone did not cause an elevation of nuclear aberration frequencies [2].

Oliveira et al (2012) showed significant alterations in the frequency of binucleation, and karyolysis in chronic smokers, but not in the frequency of karyorrhexis [11].

In a study conducted by Suhas et al (2004) a significant correlation between the habit of smoking and the frequency of the oral mucosal nuclear changes was observed [12].

Research done by Chan Yu Jung (2003) indicates that the frequency of the buccal mucosal nuclear changes had a positive relation with the exposure to consumption of cigarette and alcohol [13].

The study observed by Hashibe (2000) et al [14] reveals a stronger association of karyolysis with chewing habit, lesser association with smoking and least with alcohol drinking.

The most significant changes in buccal cells were associated with the chewing of betel quid with or without tobacco [15, 16].

Sarto et al. (1987) [17] reported an increased frequency of nuclear pathology resulting from chromosome breaks and spindle disturbances in healthy subjects. The result relating to the frequency of nuclear aberrations was approximately twice as high in smokers compared with nonsmokers ($p< 0.01$). No significant statistical correlation was found for any of the variables examined, including alcohol consumption, gender, age, coffee, hot food, spicy food, teeth brushing, oral antiseptics, oral prosthesis, and oral infection.

CONCLUSION:

The increased range of nuclear aberrations and statistical analysis suggest that oral mucosa is susceptible to cancer in those who are consuming alcohol and tobacco in combination as compared to single habit groups. The methodology used in the study is simple, rapid and painless. Such a method is cost effective and can be done in the rural hospitals for the early detection and diagnosis of the oral carcinoma. This will greatly help in early preventive measure.

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