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A COMPARATIVE STUDY OF ORAL EPITHELIUM IN TOBACCO AND ALCOHOL CONSUMERS BASED ON HABIT INDEX

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ABSTRACT: BACKGROUND AND OBJECTIVE: 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 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, 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: Mean Habit Index of alcohol and tobacco users observed was 235485.82±450581.39. A statistical significant result was found between frequency of nuclear changes and various habit indices viz: multinucleation with chewing index, alcoholic + smoking index, smoking+ tobacco chewing index; binucleation with alcoholic +tobacco chewing index; karyorrhexis with alcoholic +tobacco chewing index, smoking+ tobacco chewing index; karyolysis with chewing index, smoking+ tobacco chewing index; pyknosis with smoking index, alcoholic + smoking index, smoking+ tobacco chewing index, alcoholic + smoking + tobacco chewing index. Conclusion: Microscopically examined nuclear changes are a useful tool in early diagnosis of the oral carcinoma.
KEY WORDS: Buccal smear; Habit index; Micronuclei; Oral epithelium; Oral cytology; Papanicolaou.

INTRODUCTION: Alcohol and Tobacco are the most common drugs of abuse, and consumed as one of the chief sources of variety of pleasure of life. Since there is a close association between presence of chromosomal aberrations and carcinogenic exposure on the buccal mucosa, examination of nuclear changes has been used since 1937 as an indicator of genotoxic exposures (1).

After the evolution of pap smear and pap stains, a number of studies have been carried out which evaluate papanicolaou stain for studying nuclear changes in buccal cells under field conditions and concluded that pap stain are the preferred method in field studies for scoring and detecting the nuclear anomalies in cells of buccal mucosa (2,3).

Oral cytology is becoming increasingly important in the early diagnosis of oral cancers, as a procedure for obtaining cell samples that can then be analysed by sophisticated diagnostic techniques (4).

A lot of research work has been carried out to observe the nuclear changes associated with the use of tobacco and alcohol (5-10).

In order to evaluate cellular changes induced by tobacco and alcohol consumption, exfoliative cytology is used for the analysis of mucosal smears obtained from the buccal mucosa. The purpose of this study involves examination of epithelial smears to determine the prevalence of cells containing nuclear anomalies.

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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 during the period from February 2008 to February 2011. The sample size was calculated using decision analyst software at 95% confidence level alpha error 5%. 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 ethical acceptance from the review board committee of the institution, a proper consent and the history of the patient were recorded. The patients were then subjected to sample collection. The subject was asked to rinse the mouth with drinking water. Taking all the aseptic precautions, a wooden spatula was then 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 so as to avoid confusion and the sample was spread on the slide.

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

Analysis-
Calculation of various habit indices:
Use during the previous year or last year of usage was applied to estimate lifetime exposure, which was computed with the following formula (11).
Exposure to chewing tobacco was calculated as:
(Chewing Index)
Total Chaws in grams/Day X 365 days/Year X Years =Total Chaws of chewing tobacco use.
[A chaw was defined as the typical portion size of chewing tobacco (Quantity of chewing tobacco used in grams per day) used by the subject.]
Cigarette consumption was determined by the following:
(Smoking Index)
Total Cigarettes/day X 365 days/Year X Years = Total Cigarettes in lifetime
Alcohol concentration was computed as follows:
(Alcohol Index)
Total Episode/Day X 365 Days/year X Years = Total Episode Life exposure
The data thus generated were analyzed using chi square test, the significance level was considered at p< 0.05.
Study design: cross sectional study.

RESULTS: In the present study most of the subjects were in the age group of 40-49 years (26.22%), 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 the total number of males was 761 (95.12%), and females were 39 (4.88%) (Table1).

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Mean Habit Index of alcohol and tobacco consumption observed was 235485.82±450581.39. It ranged from 501397.20± 592069.90 (Alcoholic group) to 13742.54±18610.38 (Tobacco Chewer group). (Table 2)

Table 3 depicts the distribution of subjects according to various habit indices and frequency of nuclear changes and shows the results as follows –

➢ The presence of multinucleation (Plate 3) was significant (p<0.05), when it was compared with the Chewing, Alcohol+ Smoking and Smoking+ Chewing habit indices. In these habit indices, frequency of multinucleation ranged from:

    (1) 38.37%-71.43% (Chewing Index);
    (2) 15.79%-37.50% (Alcohol+ Smoking index);
    (3) 47.44%-72.73% (Smoking+ Chewing Index);

➢ The occurrence of binucleation(Plate 2) among different ranges of Alcoholic +Tobacco Chewing Index, varied in between 13.04% - 29.03%, and this finding was on significant level.(p<0.05).

➢ The range of occurrence of pyknosis (Plate 4) in the following habit indices was on significant level (p<0.05):

    (1) 15.19% -47.62% (Smoking Index);
    (2) 17.1% - 45.83% (Alcohol+ Smoking Index);
    (3) 48.72% - 86.36% (Smoking+ Chewing Index);
    (4) 37.7% - 64.41% (Alcohol+ Smoking+ Chewing Index);

The relation between presence of pyknosis and Alcohol+ Chewing Index was found to be on highly significant level (p<0.001). The frequency varied from 36.23% to 77.4%.

➢ On categorization of subjects according to their degree of use i.e. habit index and frequency of karyorrhexis (Plate 1), significant results were obtained with respect to Alcohol+ Chewing Index (p<0.01) and Smoking+ Chewing Index (p<0.05)

The range of frequency of Karyorrhexis in the above mentioned habit indices varied from:

    (1) 27.54% - 51.61% (Alcohol+ Chewing Index);
    (2) 33.33% - 59.09% (Smoking+ Chewing Index);

➢ The relation between occurrence of Karyolysis (Plate 3) with respect to Alcohol Index and Alcohol+ Smoking+ Chewing Index was found to be statistically highly significant (p<0.001). In these habit indices the variation of frequency was as follows:

    (1) 36.90% - 87.50% (Alcohol Index);
    (2) 52.46% - 89.74% (Alcohol+ Smoking+ Chewing Index);

This study showed significant result when we compared occurrence of karyolysis with   Smoking Index, Alcohol+ Smoking Index, Alcohol+ Chewing Index (p<0.01) and the variation in the frequency of karyolysis was observed as follows:

    (1) 43.04% - 76.09% (Smoking Index);
    (2) 52.63% - 79.17% (Alcohol+ Smoking Index);
    (3) 52.17% - 80.64% (Alcohol+ Chewing Index);

DISCUSSION: Nuclear aberrations are either acentric chromosome fragments or whole chromosome lagging in the normal karyokinesis (12, 13). These laggards or fragments of chromosomes are excluded from the post mitotic daughter cells and form nuclei.

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The induction of nuclear anomalies in oral mucosal cells seems to be due to the presence of genotoxic agents in the tobacco and alcohol. According to Livingston et al (14), saliva soluble compounds present in tobacco could diffuse into the basal cell layer and disturb the reproductive mechanism of the underlying proliferating cell population, thereby bringing about genotoxicity and formation of nuclear aberrations. Nagler and Dayan (15) have also reported that the interactions between redox active metals in saliva and the low reactive free radicles during chewing and smoking of tobacco enhance the potency of genotoxicity.

Alcohol, acting both independently as well as synergistically with smoking has been implicated in oral carcinogenesis (16). More importantly, alcohol may act as a solvent and enhance the penetration of carcinogens into target tissues. Alcohol is metabolized by an enzyme alcohol dehydrogenase and to some extent, by cytochrome p ^{450} to acetaldehyde which is found to be carcinogenic (17, 18). The growing evidences associate increased alcohol consumption with risk of developing oral carcinoma (19, 20).

Earlier reports suggest that the microscopic examination of buccal smears provides evidence of exposure to carcinogens and measure of degree to carcinogen in the tissue from which cancer develops. Increased frequency of nuclear aberrations in buccal mucosal cells of tobacco and alcohol users indicates a high risk group of oral cancer (21-24).

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. The study indicates that amount of use of tobacco and alcohol, calculated as habit index has a significant effect on the oral mucosa. The limitation of this study was the sample size which could have been larger. This technique is primitive and further research by using relationship between years of use of tobacco and alcohol and frequency of nuclear aberrations is recommended.

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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)

TABLE 1: DISTRIBUTION OF SUBJECTS ACCORDING TO AGE & SEX

*figure in paranthesis showing percentage.

Group	Number of subjects	Mean $\pm$ SD
Alcoholic	100	501397.20 $\pm$ 592069.90
Smoker	100	66882.60 $\pm$ 104215.40
Tobacco chewer	100	13742.54 $\pm$ 18610.38
Alcoholic + Smoker	100	333796.50 $\pm$ 414673.90
Alcoholic + Tobacco chewer	100	264371.40 $\pm$ 350213.20
Smoker + Tobacco chewer	100	137263.20 $\pm$ 527677.80
Alcoholic + Smoker + Tobacco chewer	100	330947.30 $\pm$ 553865.90

TABLE 2: MEAN $\pm$ SD OF INDEX OF HABIT OF VARIOUS GROUP SUBJECTS

Habit index		Multinucleation	Binucleation	Pyknosis	Karyorrhexis	Karyolysis
A index	<200000	19.05%	7.14%	23.81%	10.71%	36.90%
	>200000	25.00%	12.50%	37.50%	12.50%	87.50%
	Chi square	0.042	0.049	1.309	0.051	11.932
	P value	> 0.05	> 0.05	> 0.05	> 0.05	< 0.001
S index	<200000	22.78%	6.33%	15.19%	11.39%	43.04%
	>200000	42.86%	9.52%	47.62%	9.52%	43.04%
	Chi square	3.391	0.0008	10.167	0.022	7.293
	P value	> 0.05	> 0.05	< 0.005	> 0.05	< 0.01
C index	<30000	38.37%	13.95%	58.14%	20.93%	60.46%
	>30000	71.43%	28.57%	78.57%	35.71%	71.43%
	Chi square	4.103	0.981	1.341	1.486	0.237
	P value	< .05	> 0.05	> .05	> 0.05	> 0.05
(A+S) index	<500000	15.79%	6.58%	17.10%	9.21%	52.63%
	>500000	37.50%	4.17%	45.83%	37.50%	79.17%
	Chi square	5.182	0.003	8.253	0.022	5.309
	P value	< .05	> .05	< .005	> .05	< .05
(A+C) index	< 300000	27.54%	13.04%	36.23%	27.54%	52.17%
	> 300000	22.58%	29.03%	77.42%	51.61%	80.64%
	Chi square	0.273	3.705	14.520	5.450	7.296
	P value	> .05	<.05	< .001	< .02	< .01
(S+C) index	< 200000	47.44%	14.10%	48.72%	33.33%	57.69%
	> 200000	72.73%	31.82%	86.36%	59.09%	77.27%
	Chi square	4.406	3.649	8.283	4.785	2.792
	P value	<.05	> .05	< .005	< .05	> .05
(A+S+C) index	< 200000	21.31%	9.84%	37.70%	29.51%	52.46%
	> 200000	30.77%	7.69%	64.41%	53.85%	89.74%
	Chi square	1.135	0.00005	5.450	5.923	13.320
	P value	> .05	> .05	< .02	> .05	< .001

**TABLE 3: DISTRIBUTION OF SUBJECTS ACCORDING TO HABIT INDEX
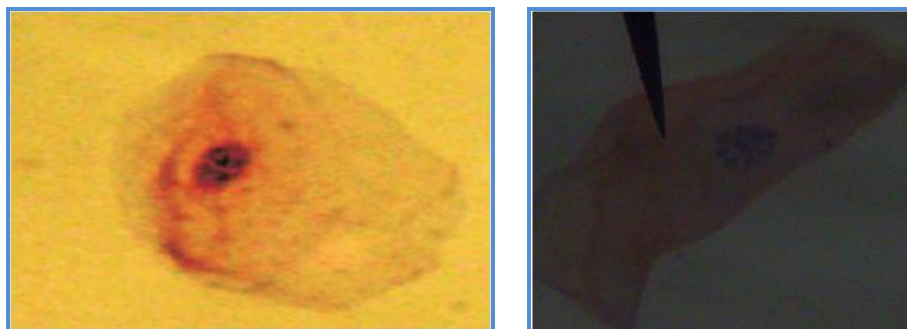AND FREQUENCY OF NUCLEAR CHANGES**

Plate 1. Microphotograph is showing karyorrhexis
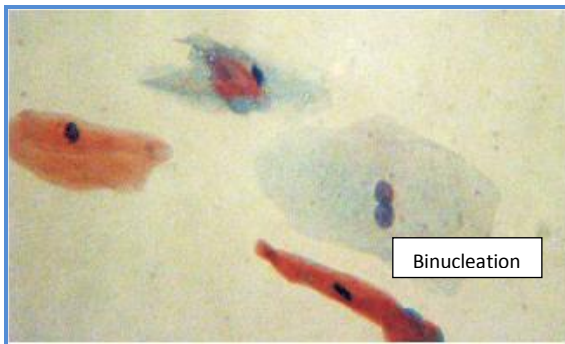
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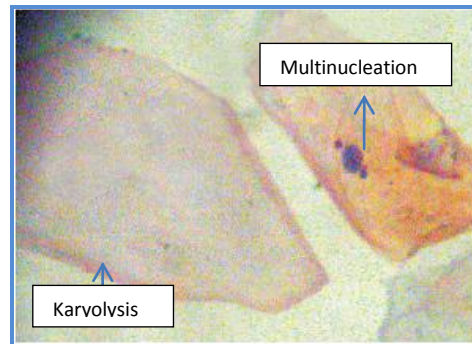Plate 2. Microphotograph is showing binucleation

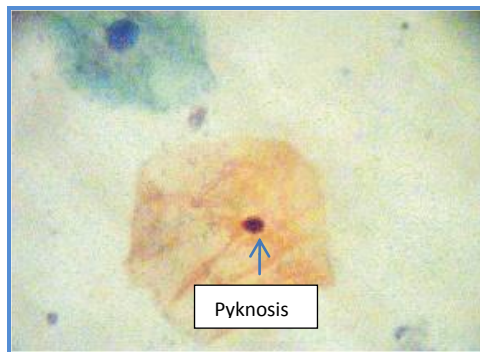Plate 3. Microphotograph is showing karyolysis and multinucleation

Plate 4. Microphotograph is showing pyknosis

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