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Micronuclei in Exfoliated Cells: A biomarker of Genotoxicity in high risk cancer groups

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Abstract
Background: Micronuclei are chromatin particles derived from acentric chromosomal fragment, which are not incorporated into the daughter nucleus after mitosis. Exfoliated cells from the oral cavity of carcinogen-exposed individuals have demonstrated increased frequencies of micronuclei.
Aim: The aim of this study was to assess the frequency of micronuclei in exfoliated cells from oral precancerous and cancerous lesions and to evaluate the micronucleus test as a screening procedure for precancerous and cancerous lesions.
Methods: 120 participants consisting of 40 cases each of controls, oral leukoplakia and oral squamous cell carcinoma comprised the study. All the 120 subjects were subjected to cytosmear for evaluation of frequency of micronucleated cells.
Results: Highly significant differences in the MNC frequency were observed between controls, oral leukoplakia and oral squamous cell carcinoma ($p < 0.001$).
Conclusion: Micronuclei assay of exfoliated oral cells could be used in assessing the genomic or cellular damage in individuals who are at high risk for cancer. However, due to overlapping values of the percentage of micronucleated cells (MNC %) in the control and study group, it may be difficult to assess the prognosis of a lesion based on this MNC% alone. Hence MN should be evaluated in combination with other biomarkers for the assessment of increased cancer risk.

Keywords: micronuclei, genotoxicity, leukoplakia, cancer

1. Introduction

Head and neck cancer remain a significant public health problem throughout the world. These account for about 40% malignancies in eastern countries including India. The high mortality rate of this disease might be reduced by early detection of the disease and treatment of the condition at an early stage [1].

Oral squamous cell carcinoma is believed to progress through sequential stages of pre malignancies to invasive cancer [2]. Even though only a small proportion of potentially malignant disorders actually progress to oral cancer, this development forms a source of about 70% of oral cancers in India [3]. The overall incidence of progression of leukoplakia to invasive squamous cell carcinoma ranges from 2.7 to 17.5% [4].

Unfortunately it is not possible to determine with surety from clinical examination alone, which particular lesion will show evidence of dysplasia. Although exfoliative cytology has been valuable in early detection of cervical cancer, its value in detection of oral cancers or potentially malignant disorders have been limited owing to high false negative results [5].

There have been numerous attempts to improve the evaluation of exfoliative cytology by additional techniques like identification of tumor markers, cytomorphometry etc. One such technique is the micronucleus test which determines the chromosomal damage and thus the mutagenic influences on the mucosa [5, 6].

Micronuclei are chromatin particles derived from acentric chromosomal fragment, which are not incorporated into the daughter nucleus after mitosis. It can be visualized by chromatin stains. Exfoliated cells from the oral cavity of carcinogen exposed individuals have demonstrated increased frequencies of micronuclei [5].

The present study was carried out to study the role of the micronucleus test applied to exfoliated cells, as a screening method in assessing the genomic or the cellular damage in individuals who are at high risk for cancer and use it as a predictor of progression of precancerous lesions to malignancy.

2. Materials and Method

2.1 Source of Data

The study population was drawn from patients attending Outpatient Department of Oral Medicine and Radiology. Informed consent was obtained from all the participants of the study. The experimental group comprised of 40 cases of leukoplakia and 40 cases of squamous cell carcinoma. In each case smears were obtained from the lesion in cases of leukoplakia and from the periphery of the lesion in cases of squamous cell carcinoma. This was followed by incisional/excisional biopsy of the lesion. Only those cases with adequate smears and which were confirmed histopathologically were included in the study.

The control group comprised of apparently healthy subjects age and sex matched with the experimental group, who had never consumed tobacco, alcohol in any form or had no history of viral disease or any medication during the preceding six months. They were subjected to clinical examination and cytosmear.

2.2 Method of collection of smear

Subjects were instructed to rinse their mouth with water. A disposable pre moistened wooden spatula was used to obtain cell samples from the mucosa. The cells were smeared over a precleaned coded microscopic slide, air dried and fixed in

95% ethanol. They were refrigerated at 4 degree centigrade. All observations were completed within one week of sample collection.

2.3 Staining procedure and MN Scoring

The fluorescent genochrome acridine orange was used. All staining procedures were carried out in subdued light. Fixed slides were stained for 3 minutes in freshly prepared staining solution. Slides were rinsed thrice in buffer, air dried and mounted in the same buffer. Observations were carried out using LEICA DMRB microscope equipped with a band pass filter of 450-490 nm excitation range blue- (515-565 nm).

Micronuclei were scored only in intact epithelial cells. The criteria for identification and scoring of micronuclei were as established by Tolbert P.E *et al.* [7] Code slides were read at 40X magnification. In each slide 500 intact epithelial cells were scored for the presence of micronuclei, since 2 slides per region was scored, a total of 1000 cells were scored per subject.

2.4 Statistical Analysis

Percentage of MNCs was presented as mean, standard deviation and minimum and maximum values (range).95% confidence limits were calculated for each group. Multiple groups were analysed by one factor ANOVA and pair wise comparisons by Mann Whitney and student t-test. P- Value of less than 0.5 was considered for statistical significance.

3. Results

The age range, mean age, gender, mean MNC% in each group and comparison of MNC percentage between controls, leukoplakia and squamous cell carcinoma is presented in table 1.

The mean value of MNC percentage in controls, cases of leukoplakia and SQCC was found to be 0.42 ± 0.33, 2.38 ± 1.49 and 4.18 ± 1.58 respectively. The difference between the groups were statistically significant (<0.001).

Comparison between clinical and histologic types of leukoplakia is presented in table 2 and 3.Comparison between MNCs in various grades of squamous cell carcinoma are presented in table 4.

Comparison of MNC percentage with respect to tobacco use is presented in table 5. The distribution of MNC percentages in control, leukoplakia and squamous cell carcinoma is depicted in the chart -1.

Table 1: Demographic characteristics and comparison of MNC% in control and study groups

Groups	n	Age range in years	Mean age In years	males	females	MNC (%) Mean	MNC (%) SD	Difference Between Groups * Groups Compared	Mean Difference	t	p
I. Control	40	30-70	50.1±12.5	24	16	0.42	0.33	I – II	1.96	8.12	<.001
II. Leukoplakia	40	32-77	51.4±11.2	34	6	2.38	1.49	I – III	3.78	14.91	<.001
III. Squamous Cell Carcinoma	40	35-40	54±12	23	17	4.18	1.58	II – III	1.80	5.30	<.001

Table 2: Clinical types of Leukoplakia

Clinical Type	No. of Cases	MNC (%) Range	Mean	SD
Homogenous Leukoplakia	7	0.6 – 5.0	2.30	1.58
Non-Homogenous Leukoplakia	33	0 – 5.5	2.39	1.49

Table 3: Histopathologic Diagnosis

Histopathologic Diagnosis	No. of Cases	Range	Mean	SD
No Dysplasia	1	0.8	0.80	-
Mild dysplasia	27	0.3 – 5.1	2.13	1.41
Moderate dysplasia	11	0 – 5.5	3.07	1.57
Severe dysplasia	1	3.10	3.10	-

ANOVA, F = 1.58, p = 0.21, Not significant
SD = Standard Deviation

Table 4: Squamous Cell Carcinoma

Histopathologic Diagnosis	No. of Cases	Percentage of Micronucleated Cell Range	Mean	SD	Groups Compared	Mean Difference	p-Value*
WD	18	1.2 – 6.3	3.41	1.54	WD – MD	1.08	0.04, S
MD	17	2.0 – 6.7	4.49	1.20	WD – PD	2.51	0.01, S
PD	5	4.5 – 7.3	5.92	1.00	MD – PD	1.43	0.02, S

One Way ANOVA (F = 7.59, p<.01, Significant)
* Mann-Whitney Test
WD: Well differentiated
MD: Moderately differentiated
PD: Poorly differentiated
S: Significant

Table 5: Habits and MNC%

	No. of Cases	Range	Mean	SD	Groups Compared	p-Value
I. No habits	6	2.04 – 4.5	3.35	1.01	I-II	0.73, NS
II. Only Chewing	36	0.8 – 7.3	3.32	1.67	I – II	0.09, NSI – IV
III.Only tobacco smoking	23	00 – 5.3	2.21	1.53	II – III	0.02, S
IV.Chewing + Smoking	15	0.8 – 6.7	4.80	1.49	II – IV	<.01, S
					III – IV	<.001, HS

One Way ANOVA (F = 8.32, p<.001, HS)

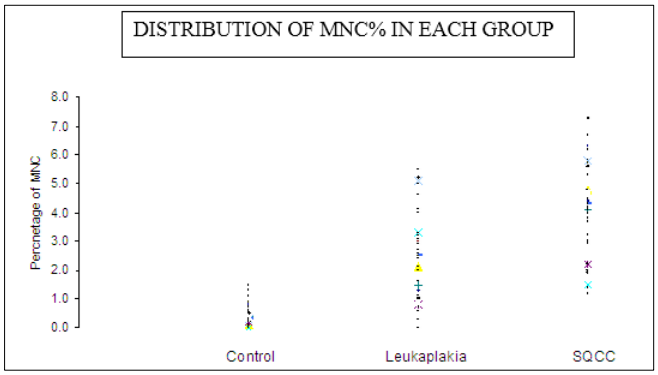

Fig 1: Distribution of MNC%

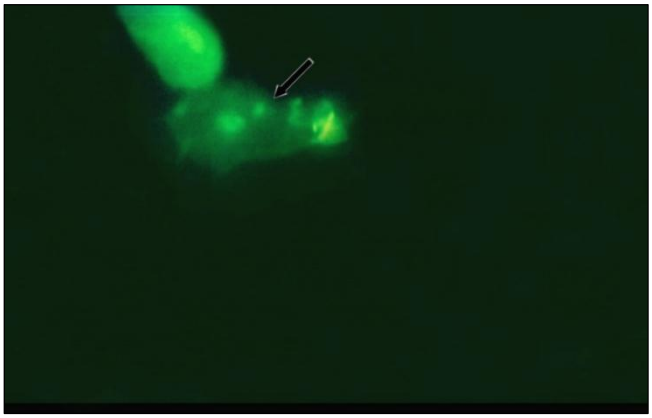

Fig 2: Photomicrograph showing micronuclei (Magnification 100X)

4. Discussion

Oral squamous cell carcinoma is a multistage process from normal to dysplastic lesions and ultimately to squamous cell carcinoma.[8] Development of oral cancer proceeds through discrete molecular genetic changes that are acquired from the loss of genomic integrity after continued exposure to environmental risk factors [9].

Elevated frequencies of MNC reveal the genotoxic action of carcinogens and may indicate an elevated probability for the formation of particular chromosome changes, which in turn, via the effect of such alterations on oncogene expression, could be associated with neoplastic transformation [10]. MN formation is relevant in cancer risk only if the DNA breakage that it reflects, or some related process is commonly involved in atleast one of the rate determining "stages" of carcinogenesis. This is suggested by the striking parallels between the causes of oral cancer: both conditions are affected strongly and synergistically by tobacco and alcohol use, x-radiation and by betelnut use [11].

Burkhardt A. (1985) [5] suggested that the MN test applied to exfoliated cells could provide valuable information and improve the evaluation of premalignant lesions and carcinomas by exfoliative cytology.

However very few studies have compared MNC counts in premalignancies and malignancies, to assess the value of MN as a predictor of progression of precancerous lesions (leukoplakia) to malignancy.

The mean value of MNC percentage in the 40 control groups was 0.42 with a standard deviation of 0.33. This value is consistent with the mean value of 0.47% reported by Stich H.F. *et al.* (1982) [12] in non-chewers tobacco, in Bihar. Another study by Stich H.F. *et al.* (1992) [13] conducted in Orissa also gives a comparable value of 0.4%. This value is also consistent with the mean value of 0.45 ± 0.05 as reported by Bagwe A.N. and Bhisey R.A. (1993) [14].

However these values are higher than those reported by Nair V. *et al.* (1991) [15] which was 0.26% and Tolbert P.E. *et al.* (1992) [16] which was 0.16%.

These variations could be attributed to inter individual differences in scoring, inclusion criteria and differences in staining procedures, the so called 'controls' may not actually provide smany different genotoxic agents. Depending on their exposures to background levels of carcinogens and mutagens, the frequencies of MNCs will already be elevated in these individuals. Exposure to excessive solar radiation, chemicals, curried food, viral infections are known to cause an increase in MNC percentage. [10, 17, 18].

In the present study, it was not possible to include the above mentioned confounding factors in the criteria for selection of the control group.

Of the 44 cases of leukoplakia, 2 were excluded due to inadequate smear and further 2 cases were excluded as they showed features of lichenoid reaction and tobacco pouch keratosis.

38 of 40 cases of leukoplakia (95%) occurred on buccal mucosa and retrocommissural area. The influence of tobacco on the development of oral leukoplakia varies by anatomic site [19].

The mean value of MNC percentage was 2.38 with a standard deviation of 1.49. This value is lower than that reported by Stich H.F *et al.* (1988) in leukoplakic lesions of fishermen from Kerala. They found MNC% in 3 groups of patients with leukoplakia before chemopreventive intervention to be 3.69 ± 1.22, 4.09 ± 1.10 and 4.01 ± 1.05 respectively [20]. This difference could be attributed to the variability in staining procedure, inclusion criteria for MN, habits of subjects, environmental factors, diet etc.

Smears were obtained from 52 cases which were clinically diagnosed as malignancies. 1 case was diagnosed as angiosarcoma histopathologically, hence it was excluded. 11 cases were excluded due to insufficient number of epithelial cells and abundance of necrotic cells and blood cells. Hence only 40 out of 52 (78.8%) smears were adequate. Even in these cases, the site to be smeared had to be cleaned of debris and slough thoroughly prior to sampling and multiple samples had to be obtained around the lesion.

Dave B.J. *et al.* (1992) [21] also mention about the difficulty in obtaining scorable smears in patients with oral cancer. Rosin (1992) [22] suggested that studies to be done with the MN assay should involve repeated sampling of each examined individual.

The mean MNC% for oral squamous cell carcinoma was 4.18 ± 1.56. Grade I had a mean of 3.41 ± 1.54, Grade II 4.49 ± 1.20 and Grade III 5.92 ± 1.00. These values are comparable with the findings of Kumar V. *et al.* (2000) [23] who in their study detected mean of 2.93%, 4.61% and 5.36% in Grade I, II and III respectively.

Tobacco contains potent carcinogens, including nitrosamines (nicotine), polycrylic aromatic hydrocarbons, nitrosodiethanolamine, nitrosopropane and polonium [8].

S Stich H.F. *et al.* (1982), [12] Nair U. *et al.* (1991), [15] Stich H.F. *et al.* (1992) [13] Bagwe A.N. and Bhisey R.A. (1993)[14] have all found an increased MNC frequency in tobacco users in various forms. Hence in the present study, MNC% was compared in 80 patients of leukoplakia and SQCC with respect to their habit of tobacco use.

The mean value of MNC% was significantly higher in the

group who chewed tobacco as well as smoked. It is possible that the combined effect of direct contact of the oral tissue with tobacco, mechanical irritation due to tobacco chewing and the smoke, which generates heat and contains potent genotoxins could have resulted in this effect. Tobacco smoking and chewing act synergistically in oral carcinogenesis and that persons with mixed habits form a substantially high-risk population [24].

However the non-chewers showed a slightly higher value as compared to 'only chewers' and 'only smokers'. The reason for this is not clear. The leukoplakia and SQCC groups have been combined to form the 4 groups with various habits.

Majority of 'only smokers' belonged to the 'leukoplakia' group and most of the 'only chewers' belonged to the 'squamous cell carcinoma' group. These lesions already represent sites of chronic exposures to genotoxic agents. In case of non- users of tobacco genotoxic agents other than tobacco could have played a role. This group does not represent normal individuals with normal mucosa. This could partly explain the unexpected results.

Besides, the inconsistency in revealing the history of adverse habits and the smaller sample size in the subgroup I should also be considered.

Duration and frequency of habits was not taken into consideration due to unreliability of data. A considerable amount of deviation might exist between the given estimates and the actual duration and frequency of habits. Stich H.F. *et al.* (1982) [12] and Nair *et al.* (1991) [15] failed to find any influence of duration of habits and MN frequency in exfoliated cells.

The mean values of MNC% for the 33 cases of non-homogenous type (2.39) were higher than the 7 cases of homogenous type (2.30). However no statistical significant difference was observed between non-homogenous and homogenous type of leukoplakia.

Inclusion of larger samples in each group could give a clearer picture of MN frequency in each group. Again although the mean value of MNC% increased with the degree of dysplasia, no significant difference was observed between the groups.

This could be attributed to the subjective nature of diagnosing the degree of dysplasia (Fischer D.J. *et al.* 2004) [25], less number of cases in each group and site wise variations.

The present study showed significant increase ($P<0.01$) in the percentage of MN in Grade III (mean = 5.92%) as compared to Grade II and Grade I SQCC (4.49% and 3.41% respectively). This finding is consistent with that of Kumar V. *et al.* (2000) [23]. who in their study detected mean values of 2.93%, 4.61% and 5.36% in Grade I, II and III respectively.

The mean values of MNC% for the control group, leukoplakia and squamous cell carcinoma groups were 0.42, 2.38 and 4.18 respectively and a highly significant difference ($P<0.001$) existed between the groups. The MNC% of SQCC group was almost ten times that of the control group.

Stich H.F. *et al.* (1984) [26] mentioned that MN frequency can be increased tenfold by carcinogenic stimuli.

Ramirez A. *et al.* (2002) [27] reported a seven-fold increase in MN frequency in the region of squamous cell carcinomas as compared to controls with normal mucosa. A Chinese study by Sun Z. *et al.* (2000) [28] reported a higher frequency of MNC of exfoliated oral cells in leukoplakia compared to controls.

Castartelli G. *et al.* (2000) [29] also observed a similar increase of MNC frequencies in exfoliated cells in precancerous lesions as compared to normal mucosa and further increase in carcinomas. The relation between production of MN cells and production of oral leukoplakia or carcinomas remains unresolved. Perhaps the formation of specific abnormal chromosome complements and the appearance of specific chromosome translocation in human cancer cells may require not only years of random chromatid breakages and reshuffling of chromatid segments, but also some more specific insult. Since, however chromosome breakages may be associated with chromatid translocations, which may in turn lead to the transposition and activation of oncogenes, there could well be a direct relation between the causes of MN, which are a byproduct of these breakages and some causes of one or more of the cellular "stages" that will eventually culminate in full neoplastic transformation [26].

Considerable overlapping of values have occurred between the three groups. As emphasized by Stich H.F. and Rosin M.P. (1984) [10] increase in frequencies of MNC does not necessarily indicate the formation of preneoplastic lesions or carcinomas. Elevated frequencies of MNCs reveal the genotoxic action of carcinogens, and may indicate an elevated probability for the formation of particular chromosome changes, which, in turn, via the effect of such alterations on oncogene expression, could be associated with neoplastic transformation [10].

Limitations of the study: Results on counting MN in various publications are difficult to compare because of differences in methods used, especially with regard to microscopic magnification, the number of cells counted and the inclusion criteria for MN. The counting of MNC is a tedious procedure and subject to interobserver variations [16].

Cell turnover varies with different sites in the oral mucosa and its effect on MN frequency is not known. Only 40 cases in each group could be analyzed, which resulted in small sample size when subgroups were examined. Poor cell yield and presence of necrotic matter in smear limits its use in oral carcinomas, which appear clinically as ulcers. The problem of poor cell yield in oral carcinomas may be solved by centrifugation of the smeared material and using cell suspensions for analysis as performed by Ramirez A. *et al.* (2002) [27]. Automation and use of computerized image analysis systems as suggested by Tolbert P.E. *et al.* (1992) [16] could improve intraobserver reproducibility of counting MNC. No single biomarker can predict the risk of malignant transformation [30], Hence MN should be evaluated in combination with other biomarkers for the assessment of increased cancer risk.

5. Conclusion

Micronuclei assay of exfoliated oral cells could be used in assessing the genomic or cellular damage in individuals who are at high risk for cancer.

However due to overlapping values of MNC% in the control and study group, it may be difficult to assess the prognosis of a lesion based on the MNC% alone.

Future studies with larger samples, performed longitudinally could evaluate its role as a predictor of progression of premalignant lesions to cancer.

Conflict of Interest: None

6. References

1. Hittleman WN. Head and neck field carcinogenesis. (In): Head and neck cancer – Emerging Perspectives. Ensly

J.F., Gutkind J.S., Jacobs J.K., Lippman S.M. (eds.) Elsievier Science (USA). 2003; 16:227-243.
2. Rosin MP, Zhang L, Poh C. Molecular markers of oral premalignant lesion risk (in) Head and Neck cancer – Emerging perspectives. Ensly JF, Gutkind JS, Jacobs JK, Lippman SM (Eds) Elsievier Science (USA). 2003; 17:245-259.
3. Mehta FS, Hamner JE. III: Tobacco related oral mucosal lesions and conditions in India. Basic Dental Research Unit, Bombay, 1993, 28-45.
4. Lonardo F, Saar W. Preneoplastic lesions of the upper aerodigestive tract. (in) Head and Neck cancer – Emerging perspectives. Ensly JF, Gutkind JS, Jacobs JK, Lippman SM (Eds) Elsievier Science (USA). 2003; 4:35-53.
5. Burkhardt A. Advanced methods in the evaluation of premalignant lesions and carcinomas of the oral mucosa. J of Oral Path. 1985; 14:751-778.
6. Ogden GR, Cowpe JG, Wight AJ. Oral exfoliative cytology: Review of methods of assessment. J Oral Pathol Med. 1997; 26:201-205.
7. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutation Research. 1992; 271:69-77.
8. Park NH, Kang MK. Genetic instability and oral cancer. Electronic Journal of Biotechnology. 2000; 3:1.
9. Heddle JA, Whissel D, Body-Cote DJ. Changes in chromosome structure induced by radiations: a test of the two chief hypothesis, 1969.
10. Stich HF, Rosin MP. Micronuclei in exfoliated human cells as a tool for studies in cancer risk and cancer intervention. Cancer Lett. 1984; 22:241-253.
11. Stich HF, Rosin MP. Reduction of Vitamin A and β-carotene administration of proportion of micronucleated buccal mucosal cells in Asian betelnut and tobacco chewers. The Lancet, 1984, 1204-1206.
12. Stich HF, Curtis JR, Parida BP. Application of the micronucleus test to exfoliated cells of high cancer risk groups: Tobacco Chewers. Int J Cancer. 1982; 30:553-559.
13. Stich HF, Parida BP, Brunnmann KD. Localized formation of micronuclei in the oral mucosa and tobacco-specific nitrosamines in the saliva of "reverse" smokers, Khaini-tobacco chewers and gudakhu chewers. Int J Cancer. 1992; 50:172-176.
14. Bagwe AN, Bhisey RA. Occupational exposure to tobacco and resultant genotoxicity in bidi industry workers. Mutation Research. 1993; 299:103-109.
15. Nair U, Obe G, Nair J, Maru GB, Bhide SV, Pieper R, Barisch H. Evaluation of frequency of micronucleated oral mucosal cells as a marker for genotoxic damage in chewers of betel quid with or without tobacco. Mutation Research. 1991; 261:163-168.
16. Tolbert PE, Shy CM. Allen JW. Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutation Research. 1992; 271:69-77.
17. da Cruz AD, McArthur AG, Silva CC, Curado MP, Glickman BM. Human micronucleus counts are correlated with age, smoking and Cesium-137 dose in the Goiania (Brazil) radiological accident. Mutation Research. 1994; 313:57-68.
18. Picker JD, Fox DP. Do curried foods produce micronuclei in buccal epithelial cells? Mutation Research. 1986; 171:185-186.
19. Schepman KP, Bezemer PD, van der Meij EH. Smeele L.e., Van der Waal I.: Tobacco usage in relation to the anatomic site of oral leukoplakia. Oral Diseases. 2001; 7:25-27.
20. Stich HF, Rosin MP, Hornby AP, Mathew B, Sankaranarayanan R, Nair MK. Remission of oral leukoplakias and micronuclei in tobacco/betel quid chewers treated with beta-carotene and with beta-carotene plus vitamin A. Int J Cancer. 1988; 42(2):195-9.
21. Dave BJ, Trivedi AH, Adhvaryu SG. Role of arecanut consumption in the cause of oral cancers – A cytogenetic assessment. Cancer. 1992; 70:1017-1023.
22. Rosin MP. The use of the micronucleus test on exfoliated cells to identify anticlastogenic action in humans : a biological marker for the efficacy of chemopreventive agents. Mutation Research. 1992; 267:265-276.
23. Kumar VS, Rao NN, Nair NS. Micronucleus in oral squamous cell carcinoma. A marker of genotoxic damage. IJDR, 2000; 11:3.
24. Jayant K, Balakrishann V, Sanghvi LD. Quantification of the role of smoking and chewing tobacco in oral and oropharyngeal cancer. Br J Cancer. 1977; 35:232-5.
25. Fischer DJ, Epstein JB, Morton J, Schwartz SM. Interobserver variability in the histopathological diagnosis of oral premalignant and malignant lesions. J Oral Pathol Med. 2004; 33:65-70.
26. Stich HF, Rosin MP. Reduction of Vitamin A and β-carotene administration of proportion of micronucleated buccal mucosal cells in Asian betelnut and tobacco chewers. The Lancet, 1984, 1204-1206.
27. Ramirez A, Saldanha PH. Micronucleus investigations of alcoholic patients with oral carcinomas. Genet Mol Res. 2002; 1(3):246-260.
28. Sun Z, Li N, Zhang Z. The correlation analysis between frequency of MNC of exfoliated oral mucosal cells in different grading of oral leukoplakic lesions. Zhoughua Kou Qiang Yi Xue Za Zhi. 2000; 35(6):439-41 (Article in Chinese).
29. Casartelli G, Bonatti GS, DeFerrari M, Seala M, Mereu P, Margarino G, Abbondandolo A. Micronucleus frequencies in exfoliated buccal cells in normal mucosa, precancerous lesions and squamous cell carcinoma. Anal Quant Cytol Histol. 2000; 22(6):486-92.
30. Delfiino V, Casartelli G, *et al*. Micronuclei and p53 accumulation in preneoplastic and malignant lesions of the head and neck. Mutagenesis. 2002; 17(1):73.