Global Journal of Medical Research: J
Dentistry & Otolaryngology
Volume 16 Issue 2 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Assessment of Chromosomal Damage and Apoptosis in Exfoliated Buccal Cells of Potentially Malignant Disorders and Oral Cancer

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Materials and methods: Our study included 90 subjects which were divided into three groups of 30 each, Group A-potentially malignant disorders, Group B-Oral cancer and Group C-control.

Results: A significant increase in the frequency of micronucleated cell from lesions than in cells from normal areas. We also observed a gradual decrease in apoptosis from normal mucosa to precancerous lesions to carcinoma.

Conclusion: Thus oral cancer is associated with a very high frequency of chromosomal damage and impaired apoptosis in the exfoliated buccal cells. Perhaps, beside the micronucleus assay, the inclusion of degenerative nuclear alteration indicative of apoptosis can be a useful tool for biomonitoring oral cancer patients.

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GJMR-J Classification: *NLMC Code: WU 158*

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I. Introduction

Oral Cancer is one of the malignant neoplasia of highest incidence worldwide and is particularly common in developing countries.[1] Other potentially malignant lesions or conditions include erythroplakia, lichen planus, submucous fibrosis, and chronic immunosuppression.[2] Cytogenetic biomarkers are the most frequently used end points in human population studies. One of the cytogenetic biomarkers for predicting cancer risk in humans is the micronucleus (MN) test. The MN test in exfoliated buccal cells is an attractive candidate for the genotoxic biomonitoring of human populations and individuals, especially because of its non-invasive application nature. It is considered to be a useful biomarker of genetic damage caused by lifestyle habits, exposure to environmental pollutants, medical procedures and also inherited genetic defects in DNA repair [3-8]. Oral cancer results from alterations that includes point mutations and chromosomal abnormalities in genes that control the cell cycle or in genes that are involved in DNA repair. With the evidence of metastasis, cancer is also characterized by its loss of ability of the cells to evolve to death when genetic damage occurs (apoptosis)[9]. However, oral exfoliative cytology is a minimally invasive test for sampling tissues and does not cause undue stress to study subjects[10,11]. Thus, micronuclei (MNi) are suitable internal dosimeters for revealing tissue specific genotoxic damage in individuals exposed to carcinogens. Thus, this could be used as a biomarker for the detection of early oral mucosal malignant transformations[12].

II. Materials and Methods

The present study consisted a total of 90 subjects, with an age ranging from 20 to 60 years inclusive of both the genders. Relevant case history was recorded including their oral habits, frequency and duration. Detailed clinical examination was carried out. Subjects with oral lesions suspected to be Potentially Malignant Disorders and Oral cancer were included. Selected cases were confirmed with histopathological diagnosis. The study was approved by the Ethical Review Board of V S Dental College and Hospital, Bengaluru. Written informed consent from the selected patients were taken for the procedures to be carried out on them subsequently. The study samples were divided into three groups: Group A–30 cases of Potentially Malignant Disorders (PMD's) (Leukoplakia, Lichen Planus and Oral Submucous Fibrosis). Group B–30 cases of Oral Cancer (Oral Squamous Cell Carcinoma). Group C–30 cases of normal healthy subjects as Controls.

Sample collection and preparation: The sample for analysis was taken from the buccal mucosa without lesions in case and control groups; and from areas with lesion by gentle scraping of the epithelium using a cytobrush. From the collected sample smears were prepared on the clean slides onto which two drops of saline solution was placed priorly. The smears were fixed in a methanol/ acetic acid solution (3:1) and after 24hrs it was stained using the Schiff reagent and counterstained with 1% fast green.

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Cytological analysis: These slides were analysed and a minimum of 1000 cells presenting intact cytoplasm were counted. In which:

a. The number of pyknotic, condensed chromatin, karyorrhectic cells indicating apoptosis were counted.
b. The number of micronucleated cells indicating chromosomal damage were counted.

Criteria for inclusion of cell in the total cell count was based on Tolbert et al[49] and protocol by Thomas et al was followed for identification of micronucleated cell, condensed chromatin, pyknotic and karryohectic cells.

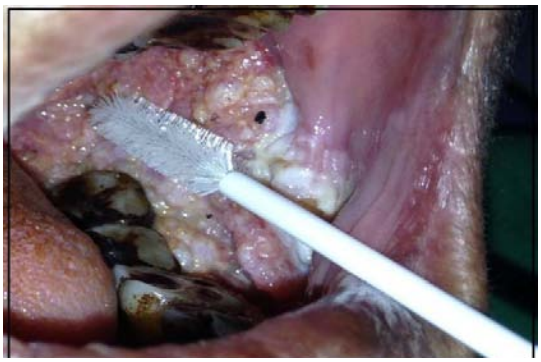

Fig. 1 : Smear from carcinoma of buccal mucosa

III. Results

Table 1 : Micronucleus analysis in group A

[LA] lesion area, [NA] normal area, [a] significant, [b] nonsignificant, N=sampe size

Group	N	MN	MN(%)Mean ± SE	Total cells	Comparison	X^2 (DF=1)
Case [LA]	30	112	2.07 ± 0.81	30,107	Case [LA] vs control	74.449(<0.001) [a]
Case [NA]	30	43	1.63 ± 0.31	36,420	Case [LA] vs case [NA]	61.362(<0.003)[a]
Control	30	28	0.36 ± 0.03	33,530	Case [NA] vs control	0.671(0.217) [b]

Table 2 : Micronucleus analysis in group B

[LA] lesion area, [NA] normal area, [a] significant, [b] nonsignificant, N=sampe size

Group	N	MN	MN(%)Mean± SE	Total cells	Comparison	X^2 (DF=1)
Case [LA]	30	277	8.16 ± 2.01	32,436	Case [LA] vs control	77.582(<0.0001) [a]
Case [NA]	30	107	3.11 ± 0.69	35,480	Case [LA] vs case [NA]	11.917(<0.009) [a]
Control	30	28	0.36± 0.03	33,530	Case [NA] vs control	1.67(0.321) [b]

Micronucleus Analysis: Micronucleus occurrence was significantly higher in smears obtained fromlesions in group A than that obtained from without lesins in group A and C(P <0.001). No significant difference was observed in cells obtained from the group C and from normal areas in group A (P = 0.217) as presented in Table 1. Micronuclei were significantly high in cells obtained from areas with lesions in the group B than in cells obtained from areas without lesions in both the group B and C(P <0.0001). A significant difference was noted in comparing cells from group C and from normal areas in the group B (P = 0.009) as presented in Table 2.

Apoptosis analysis: The occurrence of the cells representing apoptosis were significantly less in lesion areas than that obtained from group C (P < 0.0001). It was also less frequent in cells from normal areas in the group A than in normal areas in group C (P < 0.0001). There was no difference in apoptosis occurrence between the lesion areas and normal areas in group A(P = 0.957). Apoptosis occurred significantly less frequently in cells obtained from lesion areas than from group C (P < 0.0001). There was a significant difference in apoptosis occurrence between the lesion areas and the normal areas in the group B(P = 0.0001). And there was also a significant difference from normal areas in the group B than in normal areas in the control group C (P < 0.0001).

IV. Discussion

Genomic damage is one of the important cause of developmental and degenerative diseases. The genomic damage may be produced by certain genotoxins, various medical procedures that includes radiation & chemicals, micronutrient deficiency, lifestyle factors and genetic factors such as inherited defects in DNA metabolism or repair. To evaluate the genotoxic risks, DNA damage can be assessed by cytogenetic markers like chromosomal aberrations, sister chromatid exchanges and micronuclei. Epidemiological studies reveal a positive correlation between micronutrient deficiencies and development of cancer. Thus the measurement of frequency of micronuclei becomes a valuable tool to study the link between nutrition and DNA damage. This in turn will assist in stepping up implementation of public health strategies to reduce diseases of ageing and cancer.[13]

The presence of Micronucleated cell (MNC) in exfoliated buccal cells reflects the carcinogenic exposure on the target tissue from which carcinoma arises .This increase in frequency may indicate that the individuals are at high risk of progressing to malignancy. Our results are similar to those conducted by Delfino V et al14, Kamboj M et al[15], Giovanini AF et al[16], Mahimkar MB et al[17], Grover et al[18]. They concluded that there is highly significant increase in the mean micronuclated cells in PMD as compared to their control group. High frequency of mean MNCs was found in OSCC patients. This reflects the there is genomic instability associated with malignant lesion. It could be considered as to continuous use of the habits with increased frequency and duration. It is apparent that buccal cells of OSCC patients possess higher degree of genetic damage manifested in the form of micronucleated cells. The multiple micronucleation in the target tissue indicates extensive genetic damage resulting in chromosomal instability which is a hallmark of human tumors. It seems likely that the genomic damage is directly proportional to its exposure to carcinogens. Thus the overall values of the mean MNCs obtained from the study groups reveal that there was an increase in MNCs from normal mucosa to PMDs and then to carcinoma suggesting a link of this biomarker with malignant neoplastic progression.

We also observed a gradual decrease in apoptotic cells from normal mucosa to PMDs and then to carcinoma. These results are in accordance with Jain et al[19], Macluskey *et al*[20] and Bentz *et al*[21]. Thus apoptosis may play a vital role in preventing the genetic abnormalities associated with cells progressing to neoplasia[22]. Tumor growth is a summation of mitosis or the cell production and cell loss or death.

V. Conclusion

The present study observed a stepwise increase in the frequency of MNCs from normal buccal mucosa to PMD and then to carcinoma and also a gradual decrease in apoptosis from normal to PMDs and then to carcinoma. Therefore, micronuclei assay holds a promising specific biomarker for exposure to various carcinogens, and can also be used as screening test in oral health centers. It is therefore a simple, reliable, technically easy with minimal expenditure test that aids in serving as a excellent tool for educating people regarding the ill effects of the habits and its consequences.

References Références Referencias

1. Dorea L T M, Meirles J R C, Lessa J P R, Oliveira M C, Pereira C A B, Campos A P et al. Chromosomal Damage and Apoptosis in Exfoliated Buccal Cells from Individuals with Oral Cancer. Int J Dent 2012; 1-6.
2. Halder A, Chakraborty T, Mandal K, Gure PK, Das S, Raychowdhury R, *et al*. Comparative Study of Exfoliated Oral Mucosal Cell Micronuclei Frequency in Normal, Precancerous and Malignant Epithelium. Int J Hum Genet 2004. p. 257-60.
3. Martins, R. A., Gomes, G. A., Aguiar, O., Jr and Ribeiro, D. A. (2009) Biomonitoring of oral epithelial cells in petrol station attendants: comparison between buccal mucosa and lateral border of the tongue. Environ. Int., 35, 1062–1065.
4. Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., Knasmueller, S. and Fenech, M. (2008) The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. Mutat. Res., 659, 93–108.
5. Nersesyan, A. K. and Adamyan, R. T. (2004) Micronuclei level in exfoliated buccal mucosa cells of patients with benign and malignant tumors of female reproductive organs and breast. Tsitol. Genet., 38, 72–75.
6. Celik, A., Cavasx, T. and Ergene-Go¨zu¨ kara, S. (2003) Cytogenetic biomonitoring in petrol station attendants: micronucleus test in exfoliated buccal cells. Mutagenesis, 18, 417 421.
7. Burgaz, S., Erdem, O., Cakmak, G., Erdem, N., Karakaya, A. And Karakaya, A. E. (2002) Cytogenetic analysis of buccal cells from shoeworkers and pathology and anatomy laboratory workers exposed to nhexane, toluene, methyl ethyl ketone and formaldehyde. Biomarkers, 7, 151–161 Erratum in: Biomarkers, (2006) 11(4): 383.
8. Burgaz, S., Karahalil, B., Bayrak, P., Tasxkin, L., Yavuzaslan, F., Bo¨kesoy, I., Anzion, R. B., Bos, R. P. and Platin, N. (1999) Urinary cyclophosphamide excretion and micronuclei frequencies in peripheral lymphocytes and in exfoliated buccal epithelial cells of nurses handling antineoplastics. Mutat. Res., 439, 97–104.
9. D. Hanahan and R. A. Weinberg, "The hallmarks of cancer,"*Cell*, vol. 100, no. 1, pp. 57–70, 2000.
10. Jois H S, Kale A D, Kumar K.P M. Micronucleus as Potential Biomarker of Oral Carcinogenesis. IJDA (2010); 2(2): 197-202.
11. Holland N, Bolognesi C,Volders M K , Bonassi S, Zeiger E, Knasmueller S et al. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. Mutat Res 659 (2008) 93–108.
12. Samanta S, Dey P. Micronucleus and Its Applications. Diag Cytopathol (2010); 40(1):84-90.
13. Lal A, Ames BN. Association of chromosome damage detected as micronuclei with haemato-logical diseases and Micronutrient Status. Mutagenesis 2011; 26(1): 57-62.

14. Delfino V, Casartelli G, Garzoglio B, Scala M, Mereu P, Bonatti S et al.Micronuclei and p53 accumulation in preneoplastic and malignant lesions in the head and neck. Mutagenesis (2002); 17(1): 73-77.
15. Kamboj M, Mahajan S. Micronucleus—an upcoming marker of genotoxic damage. Clin Oral Invest (2007) 11: 121–126.
16. Giovanini AF, Vieira DC, Franco LB, Zielak JC, Pizzatto E, Gonzaga CC. Analysis of micronuclei in exfoliation cytology of oral leukoplakia. Perspect. Oral Sci. (2009 ; 1(1): 19-23.
17. Mahimkar MB, Samant TA, Kannan S, Patil T. Influence of genetic polymorphisms on frequency of micronucleated buccal epithelial cells in leukoplakia patients. Oral Oncol 46 (2010) 761–766.
18. Grover et al ; Evaluation of diagnostic reliability of micronuclei in potentially malignant disorders of oral cavity;; Journal of Health and Research /Vol 1/Issue 1/Jan-Apr 2014.
19. Anshu Jain, Veena Maheshwari, Kiran Alam, Ghazala Mehdi, S. C. Sharma., Apoptosis in premalignant and malignant squamous cell lesions of the oral cavity: A light microscopic study; Indian journal of pathology and microbiology, april –june 2009 52(2).
20. Macluskey M, Chandrachud LM, Pazouki S, Green M, Chisholm DM, Ogden GR, *et al*. Apoptosis, proliferation and angiogenesis in oral tissues, possible relevance to tumor progression. J Pathol 2000; 199: 368-75.
21. Bentz BG, Chandra R, Haines GK, Robinson AM, Shah P, Radosevich JA. Nitric oxide and apoptosis during human head and neck squamous cell carcinoma development. Am J Otolaryngol 2002; 23: 4-11.
22. Langlois NE, Eremin O, Heys SD. Apoptosis and prognosis in cancer: Rationale and relevance. J R Coll Surg Edinb 2000; 45: 211-9.