Research Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2016;5:1:50-56.

Comparative Evaluation of Micronuclei in Oral Lesions: An in Vivo Cytological Study

Avanindra Kumar[1*] Mukesh Kumar[2] Swati Priya[3] Vaibhav Kamal[4] Soumen Mandal[5] Vijay Prakash[6]

[1]Reader, Department of Oral and Maxillofacial Pathology, Dr. B R Ambedkar Institute of Dental Sciences and Hospital, Patna, India.
[2]Reader, Department of Conservative & Endodontics, Dr. B R Ambedkar Institute of Dental Sciences and Hospital, Patna, India.
[3]Lecturer, Department of Pedodontics, Dr. B R Ambedkar Institute of Dental Sciences and Hospital, Patna, India.
[4]Senior Lecturer, Department of Pedodontics, Dr. B R Ambedkar Institute of Dental Sciences and Hospital, Patna, India.
[5]Private Consultant, Department of Oral and Maxillofacial Surgery, Kolkata, West Bengal, India.
[6]Senior Lecturer, Department of Periodontology, Dr. B R Ambedkar Institute of Dental Sciences and Hospital, Patna, India.

ABSTRACT

Aim: Micronuclei in exfoliated buccal cells are a useful and minimally invasive method for monitoring genetic damage in humans. To evaluate study of micronuclei in exfoliative cytology of premalignant & malignant lesions affecting the oral mucosa.

Materials and method: Oral exfoliative cytological smears were taken from control and study groups. Group-I included 15 cases of Oral Submucous Fibrosis, Group- II included 15 cases of Oral Squamous Cell Carcinoma and 15 cases of age, sex and site matched healthy subjects having no habits of consumption of tobacco, other tobacco related substances or alcohol were considered as control group. Smears were stained by using the Papanicolaou (PAP) staining procedure. The smears were placed on Labomed ATC 2000 microscope and 1000 cells with intact nuclei and cell boundaries were counted. The zigzag method was followed for screening of slides.

Results: The result showed that the mean no. of micronuclei was statistically significantly increased (p = 0.000) from control group to Oral Submucous Fibrosis to Oral Squamous Cell Carcinoma.

Conclusion: Micronuclei are the results of aberrant mitosis and consist of acentric chromosomes, chromatid fragments, or aberrant chromosomes caused by genotoxic damage. These increased no of micronuclei suggest that micronuclei are a product of early events in human carcinogenic process, especially in oral regions. Assessment of the number of MN can be used as a strategy to identify the genotoxic damage in epithelial cells which are exposed to carcinogens or mutagens.

Keywords: Micronuclei, Exfoliative Cytology, Oral Squamous Cell Carcinoma, Oral Submucous Fibrosis.

INTRODUCTION

Modernization of human beings in this fast growing world are also responsible for stress and tension, which make them getting involve into so called “relieving” habits like smoking, alcoholism, tobacco/ betel nut chewing, drug addiction etc. These habits produce untoward effects on human body apart from being addictive. Tobacco has been proved carcinogenic. Chromosomal rearrangements play a central role in human neoplasia. Changes in nuclear DNA content occur quite early in the evolution of cancer and may develop cell lines with abnormal amounts of DNA at an early stage in carcinogenesis.[1]

Received: Apr. 19, 2015: **Accepted**: Aug. 12, 2015
*Correspondence Dr. Avanindra Kumar.
Department of Oral and Maxillofacial Pathology, Dr. B R Ambedkar Institute of Dental Sciences and Hospital, Patna.
Email: dentistavanindra@gmail.com

pISSN 2278-0076
eISSN 2321-9270

Oral cancer is one of the 10 most common cancers in the world. The primary sites of occurrence include buccal mucosa (36%), tongue (20%), alveolus (20%), gingiva ((9%), palate (5%), lip (4%), floor of the mouth (4%) and the maxillary sinus (2%). Oral cancer is a major health problem in terms of patient's morbidity and mortality. The five years survival rate is approximately 50-55%.[2]

WHO distinguishes between oral pre cancerous lesions and oral pre cancerous conditions.[3] A pre cancerous lesion consists of morphological altered tissue that is more likely to be transformed into cancer than its normal counterpart such as leukoplakia, erythroplakia, and changes associated with reverse smoking of cigarettes.[3] A pre cancerous condition is a state associated with a significantly increased risk for cancer, such as syphilis, oral sub mucous fibrosis, sideropenic dysphagia.[3] Diagnosis of oral pre cancer and cancer lesions can be established with the aid of invasive procedures like biopsy. Cytogenic changes have been widely used as biomarkers of exposure to mutagens and carcinogens. Buccal epithelial cells provide a source of tissue for human monitoring to occupational and environmental toxic exposure. Since the buccal mucosa is in close contact with bioactive components of betel quid and is the site affected by oral cancers, chromosomal alterations in this particular cell type may serve as an early biomarker of cancer predisposition and provide insights into oral carcinogenesis. [2, 5, 6] Bigger micronuclei result from exclusion of whole chromosome following damage to the spindle apparatus of the cell whereas smaller MN results from structural aberrations causing chromosomal fragments.[7]

With this view in mind, the present study was carried out to assess the MN frequencies in normal, premalignant and malignant oral epithelial cells.

AIM AND OBJECTIVES

AIM: A quantitative study of micronuclei in exfoliative cytology of premalignant & malignant lesions affecting the oral mucosa.

OBJECTIVES:

- To find out the frequency of micronuclei in oral exfoliated cells of healthy control subjects and patients with premalignant & malignant lesions by Papanicolaou (PAP) staining method.
- To compare the micronucleus frequencies between the control group and the patients with premalignant & malignant lesions.
- To compare the micronucleus frequencies between OSMF and OSCC patients.
- To compare the micronucleus frequencies between the control group and the patients with different clinical stages of OSMF.
- To compare the micronucleus frequencies between the control group and the patients with different histopathological grading of OSMF & OSCC.

MATERIALS AND METHOD

The present study was conducted in the department of Oral Maxillofacial Pathology, Dr. B. R. Ambedkar Institute of Dental Sciences and Hospital, Bihar.

Study Group:

Each group consists of 45 subjects. Control group consists of age, sex and site matched healthy subjects having no habits of consumption of tobacco, other tobacco related substances or alcohol. These cases were selected from the patients visiting the Outpatient Department of Oral Medicine & Radiology at Dr. B. R. Ambedkar Institute of Dental Sciences and Hospital.

Armamentarium for clinical examination, cytology and biopsy procedures:

1. Conventional dental chair with illumination facility
2. A pair of disposable gloves
3. Disposable mouth mask
4. Stainless steel kidney trays
5. Two Plain mouth mirrors
6. Straight probe
7. Tweezers
8. Sterile gauze piece and cotton
9. Disposable glass with water
10. Cytosmear kit
11. Microscopic slides
12. Coupling jars

13. 2 ml disposable syringe with 21 gauge needle
14. Tissue holding forceps
15. Small pointed surgical scissor
16. 5cc sterile screw capped glass bottles with 10% formalin.

Method of collection of data:

After obtaining a written informed consent, examination of cases was recorded for each individual in study proforma. Patients were asked to rinse the oral cavity with normal saline. Oral exfoliative cytologic smears were taken from both control & lesional group patients after the oral mucosa was dried with gauze swab to remove surface debris and excess saliva. For Control group the exfoliative cytology was taken from buccal mucosa & lesional group, the exfoliative cytology was taken from lesional area. Smears were obtained using a gentle scraping motion exerting little pressure with a wooden spatula moistened with normal saline.

Preparation of smears:

For the preparations of smears, clean, fresh and dry glass slides were used the scraps were smeared onto the center of glass slide and spread over a large area preventing clumping of cells. Then immediately fixed with 95% ethyl alcohol. The slides were stained by using the Papanicolaou staining procedure.

Biopsy procedure:

Incision biopsies were taken from the representative sites taking all aseptic precautions. The tissue specimen were labeled and immediately fixed in 10% formalin for 24 hours. The specimens were processed as per the procedure laid down by Bancroft and Stevens. Two tissue sections of 5µm thickness were cut out of each paraffin block. The sections were stained by Haematoxylin and Eosin (H& E) stain as per the procedure laid down by Bancroft and Stevens.

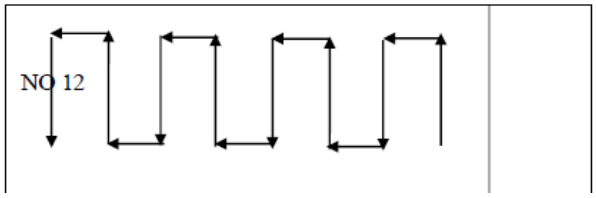
Clinical staging:

Khanna J.N. and Andrade N.N (1995)[7] has classified clinical findings of OSMF into 4 groups.

Histopathological grading:

Histopathological grading of Oral submucous fibrosis done according to Khanna JN and Andrade NN (1995)[7] and the carcinoma sections were graded according to the Pindborg et al (WHO 1997)[8]

Scoring method:



The most commonly used method i.e. the zigzag method was followed for screening of slides. The screening of slides was started from the lower right corner going across the shorter dimension of the slides. The field of vision was moved slightly laterally and screening was done vertically downwards along the slide. In this way screening was done along vertical columns till 1000 intact epithelial cells were counted.

Scoring Criteria:

1000 cells with intact nuclei and cell boundaries were counted. The criteria for designating an extra nuclear body as 'micronucleus' were as follows:

1) Diameter less than one third of the main nucleus.
2) Staining intensity similar to, or slightly weaker than, that of the nucleus.
3) Round to oval shape.
4) Texture same as that of the main nucleus.
5) Close proximity but no actual contact with the nucleus.
6) Plane of focus same as that of the main nucleus.

Only those structures fulfilling the above mentioned criteria were regarded as micronuclei. Micronuclei cells were counted out 0f 1000 intact epithelial cells (Figure 1).

Statistical analysis:

Statistical analyses were carried out by applying non parametric test (Wilcoxon test) within group and Mann-Whitney Test between groups.

Fig 1: Photograph showing exfoliated cells with single micronucleus (PAP Stain, X400)

RESULTS

Out of 15 patients of control group, no patient was in the age range of 0-9 years & 10-19 years, 4 (26.7%) were in the age range of 20-29 years , 3 (20%) were in the age range of 30-39 years , 3 (20%) were in the age range of 40-49 years , 3 (16.7%) were in the age range of 50-59 years and 2 (16.7%) were in the age range of 60 above. The mean age of patients was 42.53 years.

Table 1: Age wise distribution of the Control and Study Groups.

Group	Total No. of Patients	Age Range (in years)							Mean Age (in years)
		0-9	10-19	20-29	30-39	40-49	50-59	60 above	
Control Group	15 (100%)	0 (0%)	0 (0%)	4 (26.7%)	3 (20%)	3 (20%)	3 (16.7%)	2 (16.7%)	42.53
Group I (OSMF)	15 (100%)	0 (0%)	0 (0%)	5 (33.3%)	5 (36.7%)	1 (6.7%)	1 (3.3%)	3 (20%)	38.97
Group II (OSCC)	15 (100%)	0 (0%)	0 (0%)	1 (6.7%)	3 (23.3%)	6 (43.3%)	2 (10%)	3 (16.7%)	45.47

Out of 15 patients of oral submucous fibrosis (OSMF), no patient was in the age range of 0-9 years & 10-19 years, 5 (33.3%) were in the age range of 20-29 Years , 5 (36.7%) were in the age range of 30-39 years , 1 (6.7%) were in the age range of 40-49 years , 1 (3.3%) was in the age range of 50-59 years and 3 (20%) were in the age range of 60 above. The mean age of patients was 38.97 years. Out of 15 patients of oral squamous cell carcinoma (OSCC), no patient was in the age range of 0-9 years & 10-19 years, 1 (6.7%) were in the age range of 20-29 years , 3 (23.3%) were in the age range of 30-39 years , 6 (43.3%) were in the age range of 40-49 years , 2 (10%) were in the age range of 50-59 years and 3 (16.7%) were in the age range of 60 above. The mean age of patients was 45.47 years.

Table 2: Site wise distribution of the Control and Study Groups.

Group	Total No. of Patients	Site of Lesion	
		Buccal Mucosa	Other Sites
Control	15 (100%)	8 (50%)	7 (50%)
Group I (OSMF)	15 (100%)	15 (100%)	0 (0.0%)
Group II (OSCC)	15 (100%)	8 (50%)	7 (50%)

Out of 15 patients of control group, 8 (52.33%) were on buccal mucosa and 7 (46.66%) were on other sites. Out of 15 patients of oral submucous fibrosis (OSMF), all 15 (100%) lesions were on buccal mucosa. Out of 15 patients of oral squamous cell carcinoma (OSCC), 8 (52.33%) lesions were on buccal mucosa and 7 (46.66%) lesions were on other sites.

Table 3 : Distribution of cases according to Clinical Stages of Oral Submucous Fibrosis & its correlation

Group	Total No. of Patients	Clinical stages	No. of Patients	Mean No. of Micronuclei ± SD
Group I (OSMF)	15 (100%)	Stage-I	2 (13.33%)	5.25±1.71
		Stage-II	4 (26.67%)	5.13±1.89
		Stagee-III	8 (53.33%)	5.25±1.84
		Stage-IV	1 (6.67%)	6.5±2.12

Out of 15 patients of oral submucous fibrosis (OSMF), 2 (13.33%) were in clinical stage I, 4 (26.67%) were in clinical stage II, 8 (53.33%) were in clinical stage III and 1 (6.67%) were in clinical stage IV. Out of 30 patients of oral submucous fibrosis, the mean number of MN was 5.3 of which, 5.25 in stage I, 5.13 in stage II, 5.25 in stage III & 6.5 in stage IV.

DISCUSSION

The present study was undertaken to identify a feasible and economical method which could be used as a screening test in high risk population for identifying the effects of genomic instabilities and to introduce timely interventional strategy in order to treat and control the epidemics. So, the present study was aimed at finding out the frequencies of micronuclei in exfoliated oral cells from controls, oral sub mucous fibrosis and in oral squamous cell carcinoma.

A total of 45 cases were included in the present study. Group-I consist of 15 cases of oral sub mucous fibrosis, Group- II consist of 15 cases of oral squamous cell carcinoma and 15 cases were control group having no habits of consumption of tobacco, other tobacco related substances or alcohol.

In the present study, the oral submucous fibrosis (group-I) approximately half of the patient were in the age group of 20 – 40 years, which coincides with the findings of Pindborg JJ and Sirsat SM (1966) and Dr. Rajenderan (2003) who also found most of the cases in the age range of 20 – 40 years.[4] Youngest patient in our study is 20 years old.

In the present study, the oral squamous cell carcinoma (group II) more than half (53.33%) of the patients were in the age range of 40-60 years, which is similar to the findings of Kazi R.A(2003) who found most frequent age group in his study, being 40-50 years.[9] Yazdi I. & Khalili M (1996) found 52.8% of patients being in the age range of 18 to 75 years.[10]

The Present study showed that the patients of oral sub mucous fibrosis (group I) were younger (mean age 38.97) as compared to OSCC (group II) patients (mean age 45.47). In a study Trivedi & Adhvryu (2000) also observed that the oral cancer patients were older (mean age 44.1 years) than Oral sub mucous fibrosis patients (mean age 30.3 years).[11].

In the present study, buccal mucosa was the major site involved as compared to rest of the sites. Buccal mucosa was involved in all the cases of OSMF. In 50% cases of OSCC showed involvement of buccal mucosa & remaining cases showed involvement of other sites. This characteristic site distribution of the lesions in both the study groups directly reflects the regular placement and maximum contact of the carcinogenic agent to the involved site. In a review by Palve H.D et al. (2008) who told maximum no. of cases were observed to involve the buccal vestibule[7], which was actually the site of placement of betel quid in most of the patients & according to Napier S et al.

(2008) the potentially malignant lesions may be located in any part of the oral mucosa but buccal mucosa is most common site for the development of potentially malignant lesions. National cancer registry program, India (2002) also found the buccal mucosa is most common site for OSCC in India.

CONCLUSIONS

1. OSMF was more prevalent in males than females (M: F = 6.5: 1) and the mean age was significantly less compared to females.
2. OSCC was more prevalent in males than females (M: F = 4.1: 1) and the mean age was significantly less compared to females.
3. In OSCC, no. of micronuclei increases with age.
4. The mean no. of micronuclei in female was slightly higher than male in control, OSMF and OSCC cases.
5. The mean no. of micronuclei in OSMF was observed to be more in the clinical stage IV (6.5) as compared to stage I (5.25), II (5.13) & III (5.25) respectively.
6. The mean no. of micronuclei in OSMF was observed to be more in the histopathological grade II (5.6) as compared to grade I (5.43), III (4.82) respectively.
7. No significant difference was found in micronuclei frequencies when comparing grade I OSCC to grade II OSCC.(p = 0.85)
8. The mean micronucleus frequency in oral exfoliated cells of healthy control subjects was observed to be 1 ± 1.20.
9. The mean micronucleus frequency in oral exfoliated cells of OSMF (group-I) patients was observed to be 5.3 ± 1.79.
10. The mean micronucleus frequency in oral exfoliated cells of OSCC (group-II) patients was observed to be 10.57 ± 2.10.
11. Statistically significant difference was found when comparing mean micronuclei frequency in exfoliated cells and they showed a stepwise increase of MN frequencies from normal to precancerous to cancer. (p=0.00, 0.00 & 0.00 respectively).

Here by to conclude with the knowledge of the risk status, various interventions can be initiated and the above test can again be performed to assess the cellular improvements & prognosis after the completion of treatment / chemoprevention trials. Further studies upon larger samples are required to assess the exact relationship between these nuclear anomalies and the individual biological behavior of the associated lesions.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Shah JP, Johnson NW, Batsakis JG, editors. Oral cancer. London: Martin Duntiz; 2003.

2. Suhas S, Ganapathy K S, Gayatridevi, Ramesh C. Application of the micronucleus test to exfoliated epithelial cells from the oral cavity of beedi smokers, a high-risk group for oral cancer. Mutation research 2004; 561: 15-21.

3. Rajendran R. Benign and malignant tumors of oral cavity. In: Rajendra R, Shivapathasundaram B editors. Shafer's text book of Oral pathology. 5th Ed. New Delhi (India): Reed Elsevier India Private Limited; 2006. P.143-78.

4. Jens J. Pindborg, Satyavati M. Sirsat. Oral Submucous Fibrosis. Oral Surg Oral Med Oral Pathol 1966; 22(6): 764 – 769.

5. L. Popova, D. Kishkilova, VB Hadjidekova, RP Hristova, P. Atanasova, VV Hadjidekova, D Ziya and VG Hdjidekov Micronucleus test in buccal epithelium cells from patients subjected to panoramic radiography. Dentomaxillofacial Radiology (2007) 36, 168-171.

6. D.S. Rupa and D.A. Eastmond Chromosomal alterations affecting the 1cem-1q12 region in buccal mucosal cells of betel quid chewers detected using multicolor fluorescence in situ hybridization. Carcinogenesis vol. 18 no. 12 pp. 2347-2351, 1997.

7. Palve D H, Tupkari J V: Clinico-pathological correlation of micronuclei in oral squamous cell

carcinoma by exfoliative cytology. Journal of oral and maxillofacial pathology 2008; 12: 2-7.

8. Rnganathan k, Gauri Mishra: An oveerviewww of classification schemes for oral submucous fibrosis. Journal of oral and maxillo facial pathology; Vol.10 iissue 2julyDec 06, 55-58 .Cited by Khanna JN, Andrade NN. Oral submucous fibrosis: a new concept in surgical management – Report of 100 cases. Int J Oral Maxillofac Surg 1995; 24:433- 439.

9. Kazi R A. Carcinoma of tongue, a retrospective study of 110 cases. The internet of otohinolaryngology 2003; 2: 2

10. Yazdi I and Khalili M. Grading of oral cancer, comparison of different systems with respect to lymph node metastasis in tongue squamous cell carcinoma.1996, 43: 523–34.

11. Trivedy CR, Warnakulasuriya KA, Peters TJ et al. Raised tissue copper levels in oral submucous fibrosis. J Oral Pathol Med Jul 2000; 29(6): 241-8.