ECRONICON OPEN ACCESS

EC DENTAL SCIENCE
Review Article

Role of Micronucleus as a Potential Biomarker in Oral Carcinogenesis

Snehi Kaisar[1]* and Ujwala Kale[2]

[1]*Dental Consultant, Department of Oral Medicine, Garud Cancer Hospital and Research Centre, Ahmednagar, India*

[2]*Senior Lecturer, Department of Oral Medicine and Radiology, Rural Dental College, Loni, Ahmednagar, India*

***Corresponding Author:** Snehi Kaisar, Dental Consultant, Department of Oral Medicine, Garud Cancer Hospital and Research Centre, Ahmednagar, India.

Received: March 01, 2018; **Published:** March 27, 2019

Abstract

Genotoxic effects of chemical carcinogens can cause Cancer, which is a complex disease with altered expression, abnormal growth and disruption of normal function of cells. Oral exfoliative cytology can help detect high-risk patients' extent by detection and quantification of certain 'biomarkers' in non-invasive and painless procedures that can also help improve patient compliance. Micronucleus (MN) is a small additional nucleus and is readily identified by light microscopy. Biologically, micronuclei are the chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division. MN scoring can be used in various clinical setting such as to supervise biomonitoring of diseases, genotoxicity, screening of pre-neoplastic lesions and identification of high risk patients as MN occurs due to genetic damage of the cell and the MN scoring is the indicator of the genetic damage. In this brief review, the morphology, etiology and applications of MN have been discussed.

Keywords: *Cancer; Gentoxicity; Biomarkers; Micronucleus*

Introduction

At least 90% of all oral malignancies comprises of Oral Squamous cell carcinoma [1]. It is estimated that more than one million new cases are being detected annually in the Indian subcontinent. 92 - 95% of all oral malignancies are oral squamous cell carcinomas (OSCC) [2]. It is a complex disease with altered expression, abnormal growth pattern and disruption of normal function of cells that occurs due to genotoxic effects of chemical carcinogens [3]. The main reason for the poor prognosis of OSCC is that oral cancers are diagnosed in advanced stages and hence treated late. The survival rate can be improved with early detection of a premalignant or cancerous oral lesion. Gold standard in diagnosing OSCC is histopathological examination but biopsy being an invasive technique, it has limitations [4].

Micronuclei (MN) are small chromatin bodies in the cytoplasm [5] formed by condensation of acrocentric chromosomal fragments or by whole chromosomes, lagging behind the cell division and are sensitive indicators of genetic damage [6]. The prevalence of MN in epithelial cells has been considered a potential tissue-specific indicator of cancer risk [7].

Oral carcinogenesis is a highly complex multifocal process that takes place when squamous epithelium is affected by varying genetic alterations [9]. It is a multi-step process of accumulated genetic damage thereby causing cell dysregulation with disruption in cell signalling, DNA-repair and cell cycle which are fundamental to homeostasis [4].

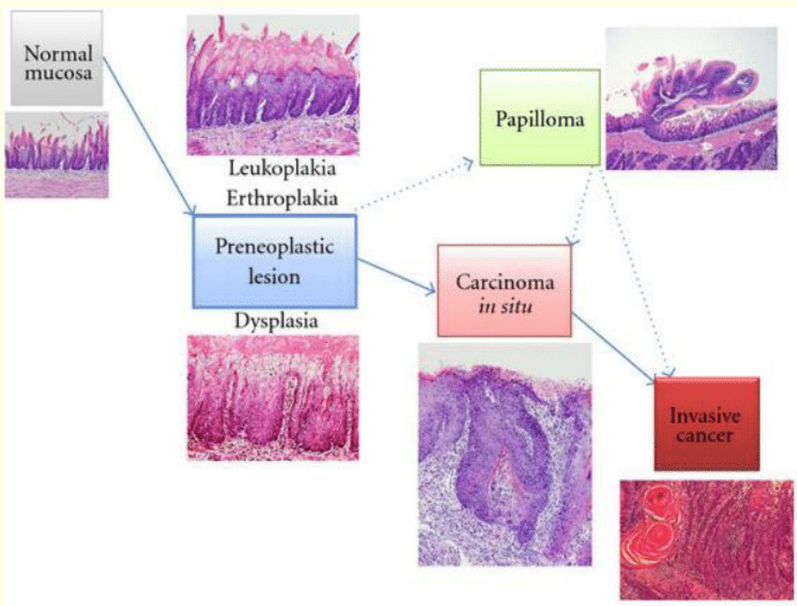

Figure 1: *The natural history of oral carcinogenesis.*

Biomarkers (Molecular Marker/ Signature Molecule): The United Nations' World Health Organization defines a biomarker as any substance, structure or process that can be measured in the body or its products and influences or predicts the incidence of outcome or disease [10].

Biomarkers for Oral Cancer- Applications [9]

1. Biomarkers helps in evaluating the preventive or therapeutic measures and the detection of the earliest stages of oral mucosal malignant transformation.
2. Reveal the genetic and molecular changes related to early, intermediate, and late end-points in the process of oral carcinogenesis.
3. Refine the ability to increase the prognosis, diagnosis, and treatment of oral carcinomas - Monitor progression/ recurrence, treatment compliance.
4. Can be used in early stages of cancer drug development.
5. Can be used to determine the efficacy and safety of chemo-preventive agents.

What is micronucleus?

A micronucleus is a small additional nucleus easily identified by light microscopy because it is morphologically similar to but smaller than the main nucleus. It is usually located around the main nucleus, within inner half of the cytoplasm except signet ring cells or mucin-filled cells where it can be seen at the periphery of the cell [12]. Chromatin texture and staining intensity resemble to the main nucleus [13]. The cells can't be called bi-nucleated cells as the diameter of micronucleus is less than 1/3rd of that of the main nucleus [14]. It is also different from a broken-egg cell because it does not have any connection with the main nucleus [15].

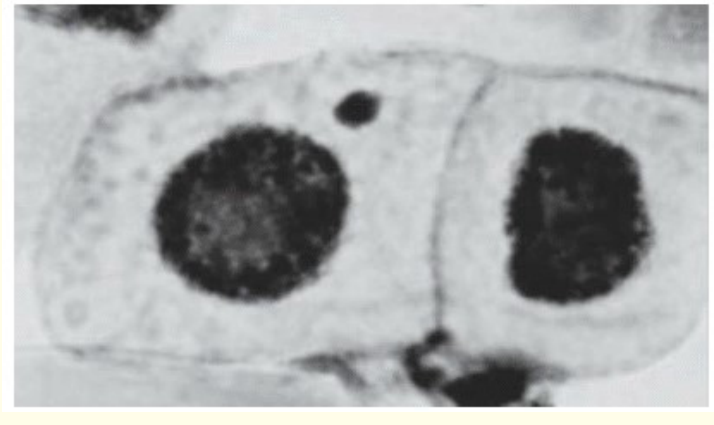

Figure 2: *Micronucleus.*

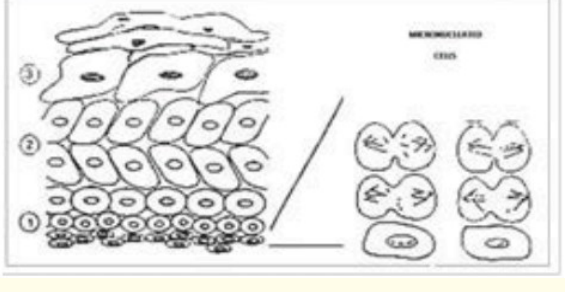

Figure 3: *Proposed theory for formation of MN.*

Biologically, micronuclei results from failure of the mitotic apparatus and is detected in the cytoplasm of the interphase cell as a small additional nucleus or an acentric chromosome (fragment) because of chromosome breakage [16,17].

Theories on origin of MN

Micronuclei are one of such biomarkers that are cytoplasmic chromatin masses with the appearance of small nuclei arising from lagging chromosomes at anaphase or from acentric chromosome fragments.10, formed by chromosomal damage in the basal cells of the epithelium. MN is one of the cell's manifestations of DNA damage occurring from chromosomal breakage or loss. Asymmetrical structural aberration of a chromosome or chromosome breakage because of unrepaired or mis-repaired DNA lesions or chromosome mal-segregation that occurs due to mitotic malfunction can cause MN formation. Acentric fragments are usually seen after irradiation of cells, whereas entire chromosomes are more frequent and frequently occurring MN or after induction by spindle (apparatus) poisons without any clastogenic treatment. The two basic mechanisms that are responsible for the formation of MN are chromosome breakage and disturbance of the chromosome segregation machinery. So, MN expression requires a mitotic or meiotic division of the cell even if the cell has already undergone genetic damage. This could be expected in rapidly proliferating cells like mucosal epithelial cells [19]. Otherwise any stable cell should be stimulated mitotically. This is the basis for *in-vitro* MN assay with the cytokinesis-blocking methodology.

Morphology of MN [19]

Location	Intracytoplasmic; near the main nucleus; generally within inner half of cytoplasm. However, in glandular epithelial cells or mucin-filled cells like signet-ring cells, it can be seen in the periphery of the cytoplasm. If the main nucleus is oval or spindly, Micronuclei is generally close to one end of it. MN is always separate from the main nucleus; however, can overlap it.
Size	It is $1/16^{th}$ to $1/3^{rd}$ the diameter of the main nucleus.
Staining	Generally, with same intensity or of more intensity in relation to main nucleus. Occasionally paler.
Texture	Similar to the main nucleus. May be more clumped. Perimeter is smooth suggestive of a membrane. Non-refractile.
Shape	Mostly oval or round; may be pyramidal, hemispheric, elliptical, cylindrical or very rarely irregular.
Number	Generally, occur singly in a cell. Very occasionally double, especially in HSIL and IC cases. Triple or more not seen or rare.
Others	Plane of focus coincides or nearly same as the main nucleus.
Cells of Occurrence	Seen in the benign-appearing cells as well as frankly malignant or dysplastic cell.
Mimickers	Stain deposits, bacteria, nuclear dusts, clumped cytoplasmic fragments, partial karyorrhexis or necrotic nucleus, carried over nuclear fragments from other cells.

Causes of MN [19]: MN can occur spontaneously which provides an index of accumulated genetic damage occurring during the lifespan of the cells under evaluation [18]. This is called the baseline MN frequency for the particular individual.

Category	Examples	Possible explanations
Spontaneous	Seen in all otherwise normal persons	Exposure to environmental pollutants, radiation, bio-hazard materials, drugs, other poisonous chemicals, food/drink habits, free radical injury, etc [20].
Chronic Inflammation	Tobacco, Para-amino hippuric acid, colorants, toxic gases like Ethylene oxide Formaldehyde etc. Crohn's disease, Oral Lichen Planus	Chronic free radical injuries of DNA, Metabolic dysfunction due to aneugens and clastogens, Mitotic spindle dysfunction due to toxic substances [14].
Genotoxicity	Lead paints, solvents, benzene Ethylene oxide, Paraamino hippuric acid, Formaldehyde, ethanol, Antineoplastic drugs, Pesticide mixtures Arsenic	Direct DNA damage, cell cycle inhibition, mitotic spindle toxins, aneuploidy, DNA synthesis and cell cycle interference [21,22].
Chemotherapy	Cancer patients	
Radiation injuries	Increased MN frequency after radiotherapy Radiation-induces cancers	Direct DNA damage/breakage, accumulated in consecutive populations [23].
Neoplastic conditions	Cervical cancer Oral carcinomas Breast carcinoma Urothelial cancer Colon cancer Pleural Malignant Mesothelioma	Primary or secondary DNA damage, Aneuploidy, mitotic dysfunction genetic instability [25-27].
Preneoplastic conditions	Oral submucosal fibrosis Oral leukoplakia Oral lichen planus First female relatives of breast cancer patients Precancerous oral lesions Cervical intra-epithelial	Chromosomal aberrations, chromosome loss/breakage, mitotic apparatus dysfunctions, aneuploidy, genetic instability DNA damage – congenital or acquired [24,25].
Genetic diseases	Down syndrome Xeroderma pigmentosa Reproductive failure	Various chromosomal aberrations, DNA damage/breakage, genetic instability [28].
Infective	Genital Chlamydiasis Schistosoma haematobium infection Human papilloma virus Infection	Mechanisms exactly not known; possibly interfere with cellular division and produce free radicals etc. leading to DNA damage. HPV is a DNA virus and interferes with genes involved in cell cycle Metabolic stress caused by tumor growth, clastogenic products released from tumor cells and the presence of HPV [29,30].
Metabolic	Megaloblastic anemia, Vitamin deficiencies, Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, beta-carotene and high intake of pantothenic acid, biotin and riboflavin	Interference with DNA synthesis; damage to DNA possibly secondary to other associated causes like reactive oxygen species and activated human neutrophils; increased genome instability [31,32].
Appliances used in orthodontic and pedodontic patients	Orthodontic appliances Metal crowns	Do not expose healthy patients to an increased risk of genotoxic damage in oral mucosa cells [33]. Odontological exposure to metal crowns results in genotoxic damage at the cellular level of the oral mucosa and an increase in the urinary excretion of Ni within 45 days of exposure [34].

MN scoring on smears

MN scoring has been done on (buccal) smears by several investigators in recent few years especially for pre-neoplastic and neoplastic conditions.

Although the spontaneous frequencies of MN are similar in all types of exfoliated cells, these levels can increase significantly at different sites in response to specific exposures. For example, Micronuclei has been especially induced in the buccal cells of betel and tobacco chewers, and in the buccal and nasal mucosal cells of people exposed to formaldehyde. Increased MN frequency in exfoliated buccal mucosal cells have also been seen because of exposure to pesticides or anti-neoplastic drugs, radiotherapy, smoking and arsenic in drinking water.

Oxidative stress, exposure to clastogens or aneugens, genetic defects in cell cycle checkpoint and/or DNA repair genes, as well as deficiencies in nutrients essential as co-factors in DNA metabolism and the chromosome segregation machinery have also been implicated as causes of increased MN frequency. The presence of MN in benign and chronic inflammatory conditions has also been studied. An elevated frequency of micronucleated exfoliated cells (MEC) in atrophic and erosive oral lichen planus (OLP) has been documented. Vitamin B-Complex supplementation has shown significant decrease in MEC frequency in atrophic and erosive OLP.

Role of MN in tumor grading

Role of MN in cancer screening is almost well appreciated because in any malignancy MN score is always very significantly elevated in comparison to benign lesion or people without tumor (normal age-matched control). But the exact role of MN in detecting the pre-neoplastic conditions is probably the most demanding issue in Micronuclei literature. There are several reasons for this:

- First, many conditions like radiation, drugs, pollutants, even normal aging process can cause it, as MN formation is a manifestation of genetic damage or chromosomal breakage. Malignancy is both a cause as well as effect of genetic damage. So increased MN is suggestive but not diagnostic of pre-neoplastic condition and caution should be taken in the form of precise clinical history and examination.
- Second, lack of standardization – there is a term called the base-line Micronuclei frequency. It is a presentation of day to day exposure to radiation, foods and the genetic make-up or ethnicity which again differ around the globe environmental pollutants, infections, nutrition. There must be an upper limit of the base-line MN frequency only beyond which we can label it as increased Micronuclei frequency. This calibration of the upper limit for a given population is one of the toughest issues [9].

Conclusion

For monitoring genetic damage in humans, the micronucleus (MN) assay in exfoliated buccal cells is a helpful and minimally invasive method. Micronuclei frequencies in exfoliated human cells seem to represent a beneficial 'internal dosimeter' for estimating exposure to genotoxic, and by implication, carcinogenic agents in the tissue from which cancers will develop. Future research should consider sources of variability in the assay (e.g. between laboratories and scorers, as well as inter- and intra-individual differences in subjects), and resolve key technical issues, such as the optimal criteria for classification of normal and degenerated cells, method of buccal cell staining and for scoring micronuclei and other abnormalities.

Bibliography

1. Massano J., *et al*. "Oral squamous cell carcinoma: Review of prognostic and predictive factors". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* 102.1 (2006): 67-76.

2. Olshan FA. "Epidemiology, Pathogenesis, and Prevention of Head and Neck Cancer". London, Springer, (2010).

3. Sivasankari NP., *et al*. "Micronucleus index: An early diagnosis in oral carcinoma". *Journal of the Anatomical Society of India* 57.1 (2008): 8-13.

4. Jois H., *et al*. "Micronucleus as Potential Biomarker of Oral Carcinogenesis". *Indian Journal of Dental Advancements* 2.2 (2010): 197-202.

5. Kayal JJ., *et al*. "Incidence of micronuclei in oral mucosa of users of tobacco products singly or in various combinations". *Mutagenesis* 8.1 (1993): 31-33.

6. Murray EB and Edwards JW. "Micronuclei in peripheral lymphocytes and exfoliated urothelial cells of workers exposed to 4,4′-methylenebis-(2-chloroaniline) (MOCA)". *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* 446.2 (1999): 175-180.

7. Bohrer PL., *et al*. "Assessment of micronucleus frequency in normal oral mucosa of patients exposed to carcinogens". *Acta Cytologica* 49.3 (2005): 265-272.

8. Iarmarcovai G., *et al*. "Micronuclei frequency in peripheral blood lymphocytes of cancer patients: A meta-analysis". *Mutation Research* 659.3 (2008): 274-283.

9. Yildirim IH., *et al*. "Micronucleus frequency in peripheral blood lymphocytes and exfoliated buccal cells of untreated cancer patients". *Genetika* 42.5 (2006): 705-710.

10. Tanaka T., *et al*. "Oral Carcinogenesis and Oral Cancer Chemoprevention: A Review". *Pathology Research International* (2011).

11. Mishra A and Verma M. "Cancer biomarkers: are we ready for the prime time?" *Cancers (Basel)* 2.1 (2010): 190-208.

12. Kashyap B and Reddy P. "Micronuclei assay of exfoliated oral buccal cells: Means to assess the nuclear abnormalities in different diseases". *Journal of Cancer Research and Therapeutics* 8.2 (2012): 184-191.

13. Samanta S., *et al*. "The role of micronucleus scoring in fine needle aspirates of ductal carcinoma of the breast". *Cytopathology* 22.2 (2011): 111-114.

14. Holland N., *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". *Mutation Research* 659.1-2 (2008): 93-108.

15. Fenech M., *et al*. "The human micronucleus project-an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans". *Mutation Research* 428.1-2 (1999): 271-283.

16. Fenech M and Crott JW. "Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes-evidence for breakage fusion bridge cycles in the cytokinesis-block micronucleus assay". *Mutation Research* 504.1-2 (2002): 131-136.

17. Hannu N and Ghita CMF. "What do human micronuclei contain?" *Mutagenesis* 18.3 (2003): 221-233.

18. Kirsch-Volders M., *et al*. "Report from the in vitro micronucleus assay working group". *Mutation Research* 540.2 (2003): 153-163.

19. Fenech M. "The Cytokinesis-Block Micronucleus Technique and Its Application to Genotoxicity: Studies in Human Populations". *Environmental Health Perspectives* 101.3 (1993): 101-107.

20. Samanta S and Dey P. "Micronucleus and its applications". *Diagnostic Cytopathology* 40.1 (2012): 84-90.

21. Konopacka M. "Effect of smoking and aging on micronucleus frequencies in human exfoliated buccal cells". *Neoplasma* 50.5 (2003): 380-382.

22. Goud KI., *et al*. "Genotoxicity evaluation of individuals working with photocopying machines". *Mutation Research* 563.2 (2004): 151-158.

23. Lewinska D., *et al*. "Micronucleus frequency in peripheral blood lymphocytes and buccal mucosa cells of copper smelter workers, with special regard to arsenic exposure". *International Archives of Occupational and Environmental Health* 80.5 (2007): 371-380.

24. Cao J., *et al*. "Chromosomal aberrations, DNA strand breaks and gene mutations in nasopharyngeal cancer patients undergoing radiation therapy". *Mutation Research* 504.1-2 (2002): 85-90.

25. Andrew JO., *et al*. "Tetraploidy and chromosomal instability are early events during cervical carcinogenesis". *Carcinogenesis* 27.2 (2006): 337-343.

26. Saran R., *et al*. "Risk assessment of oral cancer in patients with precancerous states of the oral cavity using micronucleus test and challenge assay". *Oral Oncology* 44.4 (2008): 354-360.

27. Delfino V., *et al*. "Micronuclei and p53 accumulation in preneoplastic and malignant lesions in the head and neck". *Mutagenesis* 17.1 (2002): 73-77.

28. Palve DH and Tupkari JV. "Clinico-pathological correlation of micronuclei in oral squamous cell carcinoma by exfoliative cytology". *Journal of Oral and Maxillofacial Pathology* 12.1 (2008): 2-7.

29. Thomas P., *et al*. "The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls". *Mutation Research* 638.1-2 (2008): 37-47.

30. Dimitrijevic A., *et al*. "Micronucleus frequency in women with genital Chlamydia Trachomatis infection before and after therapy". *Mutation Research* 608.1 (2006): 43-48.

31. Rosin MP and Anwar W. "Chromosomal damage in urothelial cells from Egyptians with chronic Schistosoma haematobium infections". *International Journal of Cancer* 50.4 (1992): 539-543.

32. Fenech M., *et al*. "Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, beta-carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability-results from a dietary intake and micronucleus index survey in South Australia". *Carcinogenesis* 26.5 (2005): 991-999.

33. Heravi F., *et al*. "DNA Damage in Oral Mucosa Cells of Patients with Fixed Orthodontic Appliances". *Journal of Dentistry* 10.6 (2013): 494-500.

34. Martinez MJ., *et al*. "Genotoxicity in oral epithelial cells in children caused by nickel in metal crowns". *Genetics and Molecular Research* 12.3 (2013): 3178-3185.

Volume 18 Issue 4 April 2019