



MICRONUCLEI AND ITS SIGNIFICANCE SUBJECT: ORAL PATHOLOGY/MOLECULAR BIOLOGY

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ABSTRACT

Micronucleus is conveniently the name given to the small nucleus that forms whenever a chromosome or a fragment of it is not incorporated into one of the daughter nuclei during cell division. It is known to be a sign of chromosomal instability and genotoxic events that are predominantly seen in cancerous cells, and eventually indicate genomic damage to result in degenerative diseases, or diseases of developmental aetiology. The term micronucleus may also be alluded to as the Paramecium, a ciliate protozoan. In earlier studies it stated that the micronucleus induction by a chemical was apparently, first reported in Ehrlich ascites tumour cells that got treated with colchicine. Also, the apparent relationship between formation of micronuclei and exposure to environmental factors was first recognised in root tip cells that got exposed to ionising radiation. It's formation has been suggested to be observed in cells undergoing nuclear division, that basically results from acentric chromosome fragments that are not included in the daughter nuclei. Several micronucleus tests or assays are performed in which the genotoxicity of chemical compounds are tested. These tests provide important information about a chemical's ability to interfere with chromosome structure and function. The purpose of this review article is to learn about Micronuclei and its fundamental role in causing oral cancer. The origin, formation and identification, the significance and the different micronucleus assays performed have been described briefly.

KEYWORDS: *Micronuclei, erythrocytes, lymphocytes, chromosome aberrations, micronucleus assay.*



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Received on : 26.10.2016

Revised and Accepted on : 30.01.2017

DOI: <http://dx.doi.org/10.22376/ijpbs.2017.8.1.b717-720>

INTRODUCTION

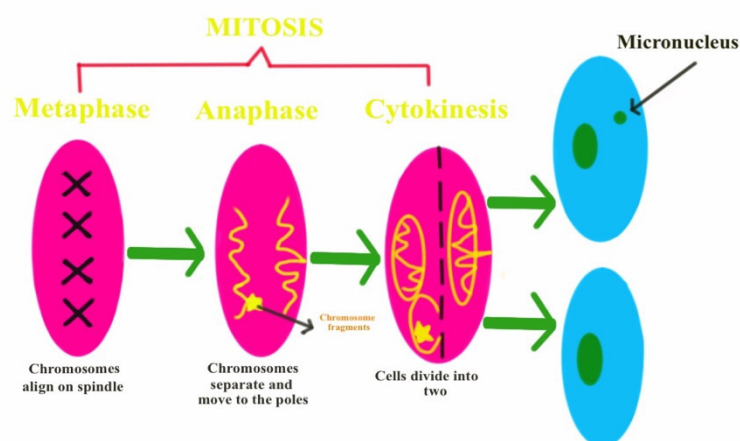
Micronuclei also known as Howell-Jolly bodies is found to be associated with deficiencies in vitamin B12 and folate and has been described in erythrocytes by William Howell and Justin Jolly. They are induced by genotoxic stress such as clastogen and aneugen. Micronuclei induction by clastogen involves induction of either chromosome fragments or a chromatin bridge between chromosomes while aneugen induces whole chromosomes that were not bound to mitotic spindle at anaphase probably by disrupting the spindle checkpoint. Micronuclei is one of the four cytogenetic endpoints the remaining three being structural chromosome aberrations, aneuploidy, and sister chromatid exchanges. These are most frequently used in hazard

identification assays as the first step in the risk assessment process.¹

ORIGIN

At present it is well established that micronuclei mainly originate from acentric chromosome fragments, acentric chromatid fragments or whole chromosome that doesn't get included in the daughter nuclei during mitosis. Malsegregation of whole chromosomes at anaphase may also lead to micronuclei formation as a result of defects in kinetochore proteins, dysfunctional spindle and defective anaphase checkpoint gene.² The diagrammatic representation of micronucleus formation is given in figure 1.

FIGURE 1
MICRONUCLEUS FORMATION



DISRUPTION OF MICRONUCLEI

The nuclear envelope recruited from missegregated chromosomes during mitotic exit, form micronuclei that have reduced functioning compared to primary nuclei. Here micronuclei undergoes an irreversible loss of compartmentalisation due to nuclear envelope collapse. This disruption of micronuclei induced by defects in nuclear lamina assembly is associated with chromatin compaction and invasion of ER tubules into the chromatin. Disrupted micronuclei can however, be a useful objective biomarker for genomic instability in solid tumours.³

IDENTIFICATION

The criteria for identifying micronuclei are

- Diameter less than one-third the main nucleus.
- Be on same plane of focus.
- Have same colour, texture and refraction as main nucleus.
- Have smooth, oval or round shape.
- Be clearly separated from main nucleus.⁴

MICRONUCLEUS TESTS

The micronucleus assay or micronucleus test provides a simple and straight-forward measure of aneugenic and

clastogenic agents that result in chromosomal damage. Some of the micronucleus tests and analysis options include

- ✓ In vitro micronucleus tests.
- ✓ In vivo micronucleus tests.
- ✓ Micronucleus evaluation by flow cytometry.
- ✓ Micronucleus evaluation by microscopy.⁵

Uses

The purpose of micronucleus assay is to detect those agents which modify chromosome structure and segregation in such a way that leads to induction of micronuclei in interphase cells.⁶ As micronuclei arise from lagging chromosomes, there is the potential to detect aneuploidy-inducing agents that are difficult to study in conventional chromosomal aberration tests. eg OECD Test Guideline 473. Manual scoring of micronucleus assays is time consuming and subject to operator variance, bias and error while automated analysis of micronucleus assays is significantly faster and enables consistent objective scoring.⁷ The other putative advantages of MN assay are greater accuracy, high reliability, reduced invasiveness of cell collection, minimal cost, ability to score a statistical power of thousands of cells in in-vitro chromosomal aberration test and providing facility for storage and slide preparation.⁸

SIGNIFICANCE

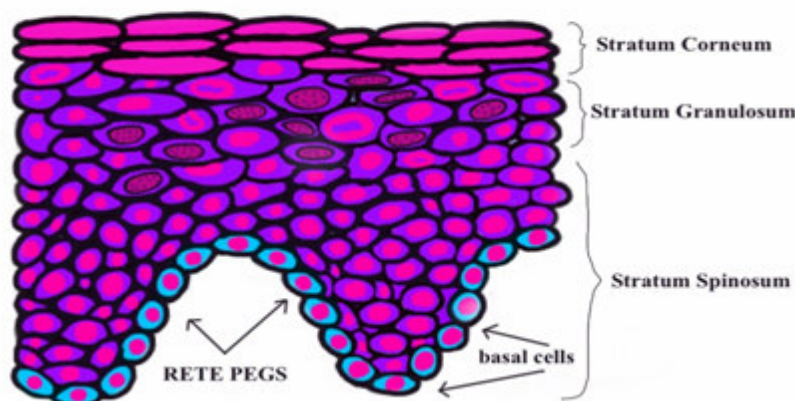
• Micronuclei in Oral cancer

Micronuclei in exfoliated oral epithelial cells can represent a preferred target site for the genotoxic events to occur that are induced by the carcinogenic agents. The severity of this genotoxic damage and the correlation of the frequency of micronuclei are shown in various studies. The measurement of severity of genotoxic damage is done in terms of grading of the lesions. This especially holds true for oral squamous cell carcinoma (OSCC). The significant increase in the frequency of micronuclei compared to normal counterparts, indicated that strong cytogenetic damage secondary to genotoxic and carcinogenic agents are released by tobacco and areca products. After obtaining these results it was shown that micronuclei in oral exfoliated cells can be a candidate for grading of OSCC.⁹

• Micronuclei in oral submucous fibrosis

Various studies have revealed that the carcinogenic nature of gutkha, which is a cheap and convenient betel quid substitute, leads to oral submucous fibrosis and also increases the chances of oral cancer. Areca nut is one of the main component of gutkha that is responsible for oral submucous fibrosis (OSMF) and gutkha is known to be a cheap source of betel quid substitute. OSMF develops within a very short span of time and becomes incurable ultimately leading to oral cancer. Areca nut is known to cause increased chances of formation of precancerous lesion and OSMF. An elevated micronucleated cell frequency is found in the buccal mucosal epithelium of areca nut chewers. The micronucleus assay in buccal cells can be used to detect cancerous or precancerous lesions and also to monitor the effects of a number of chemopreventive agents. In the present study the effect of gutkha was also studied on the micronucleus frequency in buccal epithelial cells.¹⁰ The diagrammatic representation of the cross section of normal buccal mucosa is given in figure 2.

FIGURE 2
Cross section of normal Buccal mucosa
ORAL CAVITY



• Micronuclei in smokers

It was seen among cigarette smokers that the buccal cell micronuclei and the degenerative nuclear alterations were more frequent than non-smokers. It is learnt from recent studies that cytogenetic damages showed significant and positive correlation between intensity of smoking and the number of nuclear alterations. The years of smoking had a significant influence on the number of nuclear alterations. The years of smoking had a significant influence on the number of nuclear alterations in micronuclei and nuclear buds in buccal cells. Cigarette smoking significantly increases the frequencies of micronuclei and nuclear buds, pyknotic, karyolytic, and karyorhectic cells.¹¹

DISCUSSION

The micronuclei assay in exfoliated buccal cells is an innovative genotoxicity technique, which holds promise for the study of epithelial carcinogens. The micronucleus

test has been receiving increasing attention as a rapid, simple and sensitive short term assay for studying the effects of environmental genotoxicants. Therefore micronucleus assay in exfoliated cells has proved to be a site-specific biomarker of exposure to genetic toxins and also for cancer it is used as a screening prognostic tool in community centre of oral cancer and precancer.¹²

CONCLUSION

The significance of micronuclei in oral cancer and some precancerous lesions are clearly understood, along with the uses of different micronucleus tests performed.

CONFLICT OF INTEREST

No Conflict of interest to declare.

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