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MICRONUCLEI THE REVIEW

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ABSTRACT
Genomic damage is considered is as prime cause for various malignancies. Such genetic damage may give rise to small fragment of nucleus. Micronuclei are biomarker of genotoxic events and chromosomal instability. The aim of this article is to provide an overview of the historical research, current status of the micronuclei, along with procedure of collection, staining, and scoring of oral exfoliated mucosal cells in micronucleus assay.

KEYWORDS: biomarker, degenerative diseases, genetic damage, malignancy, micronuclei.

INTRODUCTION

Genomic damage is considered is as prime cause for development of various malignancies. Such genetic damage is produced by toxic agents, radiation & chemicals, micronutrient deficiency, lifestyle factor and genetic factors such as inherited defects in DNA metabolism or repair.[1]

To evaluate the genotoxic risks, DNA damage can be assessed by chromosomal aberrations, sister chromatid exchanges and micronuclei. Out of all these, micronucleus test is preferable for screening as it is a non-invasive and cost effective procedure. Literature have shown that Micronuclei test is one of the best indicator which shows mitotic interference, chromosomal mutations and breakages.[2]

Micronuclei are biomarker of genotoxic events and chromosomal instability. Such micronuclei are collectively measured in micronucleus cytome assay. Presence of these nuclear anomalies could increase the risk of developmental and degenerative diseases along with various types of malignancy.[1]

People who were exposed to organic solvents, genotoxic drugs, polycyclic aromatic hydrocarbons, drinking water contaminated with arsenic and paints with lead content shows significantly higher frequencies of MNs in exfoliated mucosal cells.[3] The lifestyle factors like smoking, alcohol consumption and diet, especially vitamin deficiencies are also associated with the genetic damage, in turn with increase frequency of Micronucli.[4]

Epidemiological studies have shown a positive correlation between micronuclei frequency, degenerative disease and development of cancer. This in turn will assist in stepping up implementation of public health strategies to reduce degenerative diseases and cancer.[5]

The aim of this article is to provide an overview of the historical research, current status of the micronuclei, along with procedure of collection, staining, and scoring of oral exfoliated mucosal cells in micronucleus assay.

Historical Background

In the early 1970s, the term micronucleus was first time suggested by Boller and Schmidt.[6] The buccal cell micronucleus assay was proposed in 1983, thereafter it gained popularity as a biomarker of genetic damage in numerous applications.[4]

Other Nuclear Abnormalities and Micronuclei Identification.

The consideration should be given to the various nuclear abnormalities. Such nuclear abnormalities can get confused with Micronuclei. Various nuclear abnormalities are Binucleated cells.

Binucleated cells: Presence of two nuclei within the cell which is indicative of failed cytokinesis. It was found that higher frequency of chromosomal disjunction occurs such binucleated cells than those cells with completed cytokinesis.[7]

Broken eggs or cells with nuclear buds: They contain nuclei with a sharp constriction at one end of the nucleus.

Such nuclear bud and the nucleus are usually appear to be attached to one another. The morphology and staining properties of the nuclear bud are same as that of the nucleus; however its size may range from a quarter or less to that of the main nucleus.[8]

Cells with condensed chromatin: A roughly striated nuclear pattern in which the aggregated chromatin is intensely stained. When chromatin aggregation is extensive the nucleus may appear to be fragmenting.[8]

Pyknotic cells: Cells characterized by a small shrunken nucleus which contains a high density of nuclear material. They may represent as an alternative mechanism of nuclear disintegration that is distinct from the process leading to the condensed chromatin and cell death stages.[7]

Karyorrhectic cells: Cells with nuclear disintegration and the loss of integrity of the nucleus.[7] They have nuclei that are characterized by more extensive nuclear chromatin aggregation relative to condensed chromatin cells.[8]

Karyolytic cells: Cells in which the nucleus is completely depleted of DNA and is apparent as a ghost like image.[8]

In mitotic cells, Micronuclei could arise from the dysfunction of the mitotic apparatus along with the chromosome breakage.[9] It is now well established that micronuclei mainly originate from the acentric chromosome fragment, acentric chromatid fragments or whole chromosome that fail to be included in the daughter nuclei at the completion of telophase.[1]

Also larger size micronuclei results from the damage to the spindle apparatus of the cell resulting in the exclusion of whole chromosome (aneugenic effect) whereas smaller MN results from structural aberrations causing fragments of chromosomes (clastogenic effect).[10] It is evident that multiple molecular mechanisms can lead to the formation of micronuclei and other nuclear abnormalities.[1]

The collected exfoliated cells are smeared immediately on pre-cleaned microscopic slides followed by fixation in 80% methanol or absolute ethanol.[6] There are lot many staining methods out of which DNA specific stains are ideal for staining.

Scoring Criteria

Since the first publications of Stich and Rosin for the basic criteria for identification of MN,[11] a number of studies have been done by different authors to standardize the MN identification criteria. In 1992 Tolbert et al developed the criteria for choosing the cells which consists of the following parameters.[4]

A. Parameters for cell inclusion in the cells to be scored.

i) Intact cytoplasm and relatively flat cell position on the slide.
ii) Little or no overlap with adjacent cells.
iii) Little or no debris.
iv) Nucleus normal and intact, nuclear perimeter smooth and distinct.

B. Parameters for identifying micronucleus.

i) Rounded smooth perimeter suggestive of a membrane.
ii) Less than a third the diameter of associated nucleus, but large enough to discern shape and color.
iii) Staining intensity similar to nucleus.
iv) Texture similar to nucleus.
v) Same focal plane as nucleus.
vi) Absence of overlap with bridge to nucleus.

Tolbert et al, also recommended the scoring of at least 1000 cells, which can be increased to 2000-3000 if less than 5 micronucleated cells are observed after counting 1000 cells.[6]

Association of Micronuclei Assay with Oral Cancer

Since the frequencies of micronuclei are increasingly being used as a biomarker also various factors influence their frequency in health and diseased states. Epidemiological studies reveal a strong association between micronuclei frequency and various factors that include.

1. Environmental and occupational exposures,
2. Radiotherapy,
3. Chemoprevention,
4. Lifestyle and habits,
5. Vitamin supplement trials,
6. Cancer and other diseases.

Oral cancer, one of the 10 most common cancers as stated by World Health Organization, is a complex disease with abnormal growth, altered gene expression and disruption of normal function of cells caused by genotoxic agents. It results in genomic instability at an early stage of cancer, which is often reflected as leukoplakia, erythroplakia, lichen planus and oral submucous fibrosis.[2]

Micronucleus assay is useful for screening the populations under the risk of mutagenic agents that may cause oral neoplasms, and also for the identification of pre-clinical steps of carcinogenic process.[18] Casartelliet al observed that there is a gradual increase in MN counts from normal to precancerous lesions to carcinoma, and suggested a link of this biomarker with neoplastic progression.[3]

CONCLUSION

Micronuclei assay is non-invasive technique that offers a very simple method for obtaining information on status of the epithelial cells, particularly DNA damage, proliferative potential of basal cells and cell death. Although many studies have shown a statistically significant increased micronuclei frequency in the buccal cells of populations with premalignancy and oral cancer.

Micronuclei made it attractive in the past and will ensure a key role in the evaluation of mutagenicity and primary prevention in the future.

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