https://doi.org/10.33805/2689-6737.109
Volume 1 Issue 1 | PDF 109 | Pages 6

EDELWEISS PUBLICATIONS
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Edelweiss: Cancer Open Access

Review Article 2689-6737

Micronuclei (MN), an Important Cancer Biomarker

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Citation: Adhikari A. Micronuclei (MN), an important cancer biomarker (2019) Edelweiss Cancer OA 1: 37-42.

Received: Nov 01, 2019
Accepted: Nov 18, 2019
Published: Nov 25, 2019

Abstract

Micronuclei (MN) formation has been observed (cancer and pre-cancerous lesions) of the oral cavity among betel quid chewers. Micronuclei act as a cancer biomarker which is related with smokeless tobacco associated genetic mutations. Micronuclei are a sensitive indicator of genetic damage. These are small, extra nuclear bodies that are formed during mitosis from lagging chromosomes. The test is used as a tool for genotoxicity and easily detectable without affecting the cancer patients. The present review focuses on the various types of cancer of the human body with micronuclei study.

Keywords: Micronuclei, Oral Cancer, Cancer Biomarker.

Abbreviations: MN-Micronuclei, CN-Cirrhotic Nodules, LRN-Large Regenerative Nodules, LG-DN-Low-Grade Dysplastic Nodules, HG-DN-High-Grade Dysplastic Nodules, HCC-Hepatocellular Carcinoma, CRC-Colorectal Cancer, PBL-Peripheral Blood Lymphocytes, CKD-Chronic Kidney Disease, NBs-Nuclear Buds, BN-Binucleated Cells, CC-Condensed Chromatin, KR-Karyorrhectic, PK-Pyknotic, BEC-Buccal Epithelial Cells, RB-Retinoblastoma, pRb-Retinoblastoma Protein, RT-Radiotherapy, BC-Breast Cancer, BE-Broken Eggs, CC-Condensed Chromatin, KL-Karyolysis, CP-Cyto Pathological, DBD-Dielectric Barrier Discharge.

Introduction

A biological marker is most useful for identification of various hazards and risk assessment. Many biological markers have been developed to estimate exposure and to assess the risk of adverse effects. In general, micronucleus analysis is utilized in both genotoxicity testing with its exposure which effects in human population. These MN assay allows the detection of both aneugenic agents (numerical chromosome alterations) and clastogenic agents (chromosome breakage). Micronuclei can be measured by the feulgen reaction, giemsa or fluorescence dyes.

History of Micronuclei

In the early 1970s, micronucleus was first suggested by Boller and Schmidt. Heddle showed that it is a easy method to detect genotoxic potential of mutagens using bone marrow erythrocytes of animals [1,2]. Stich and co-workers developed a protocol for micronucleus assay with exfoliated human epithelial cells about 25 years ago [3]. A few years later, Countryman & Heddle showed that peripheral blood lymphocytes could be used for micronucleus approach and they both recommended using micronuclei as a biomarker in testing purpose.

T. Boveri observed abnormal nuclear morphologies which is mainly found in cancer. Micronuclei are also referred to Howell-Jolly bodies; discovered by hematologists William Henry Howell and Justin Marie Jolly in erythrocytes. Micronucleus induction by a chemical (which is mainly treated with colchicine) was first reported in Ehrlich on ascites tumor cells. This results in parts of the chromatids or chromosomes being broken off and developed as an extra nucleus in one of the daughter cells. This is the process by which micronuclei are formed.

Relationship with Micronuclei with Different Types of Cancer

Liver Cancer with MN: Liver cancer is one of the important cancers of human body. The final event in a cascade of genetic changes having their phenotypic counterpart in a spectrum of morphological alterations such as Cirrhotic Nodules (CN); Large Regenerative Nodules (LRN); Low-Grade Dysplastic Nodules (LG-DN); High-Grade Dysplastic Nodules (HG-DN) and full-blown Hepatocellular Carcinoma (HCC) [4-7]. Chromosome instability, including structural chromosome anomalies and allele loss or gain, has been demonstrated in HCC and found to be closely associated with hepatitis B virus [8-11]. Micronuclei are chromosomal fragments left out of the daughter nuclei during nuclear division [12,13].

Chromosome breakage and mitotic apparatus dysfunctions are involved in the morphogenesis of MN, closely associated with chromosome instability [14]. MN has been demonstrated in every stages of liver carcinogenesis in an experimental model [15-17]. MN may activate the p53-mediated cell cycle checkpoint [18]. Micro nucleated hepatocytes were consistently found in significantly lower numbers in normal liver than in any of the types of non-neoplastic and neoplastic liver nodules; a frequency of ≤ 4.50, 0.76 MN/1,000 hepatocytes unequivocally identified normal liver tissue. MN (markers of chromosome instability) progressively increases over the course of human liver carcinogenesis.

Colorectal Cancer with MN: Genetic instability with chromosomal instability, microsatellite instability, and abnormal DNA methylation as the mayor path described for Colorectal Cancer (CRC) [19].

The diagnosis of CRC at early stages is one of the proven strategies resulting in a higher cure rate [20]. Micronucleus frequency in (PBL) Peripheral Blood Lymphocytes has emerged as one of the most reliable biomarkers for cancer risk assessment, including CRC [21]. MN frequency has the potential to be a sensitive and non-invasive biomarker for CRC risk assessment which is highly effective for research purpose.

Kidney Cancer with MN: Chronic Kidney Disease (CKD) is a major public health problem in recent years [22]. Significantly increases in MN frequencies and basal DNA damage (determined by comet assay) in peripheral blood lymphocytes of children with CKD. Frequencies of MN and other nuclear anomalies, such as Nuclear Buds (NBs), Binucleated Cells (BN), Condensed Chromatin (CC), Karyorrhectic (KR) and Pyknotic (PK) cells in Buccal Epithelial Cells (BEC) in children with CKD.

In each CKD subgroup, either the MN frequency or the micronucleated cell frequency in BEC was significantly (5 to 7 fold) elevated than control. Scoring MN and other nuclear anomalies such as nuclear buds (for DNA damage), Binucleated Cells (for cytokinetic defects), condensed chromatin, and karyorrhectic and pyknotic (for cell death) cells in BEC, known as the cytome approach, has also been found to be associated with down syndrome and Alzheimer's disease [23]. Limited number of studies on nuclear anomalies other than the MN frequency, it has been suggested that the predictive value of the MN assay in BEC is most important [24].

Retinoblastoma (RB) with MN: Formation of micronuclei caused by aneugenic events leading to chromosome loss, likely originating from erroneous kinetochore attachment, and not spindle assembly checkpoint dysfunctions are the trigger for aneuploidy in (pRb) Retinoblastoma Protein depleted primary human fibroblasts. Micronuclei are considered a marker of chromosome damage (chromosome loss or chromosome breakage events) formed during mitosis and identified in interphase as small bodies of extra chromatin in the cytoplasm of mammalian cells.

MN might originate by the presence of lagging chromosomes observed during anaphase in human fibroblasts after RB acute loss. MN, which remains separate from the nucleus after nuclear division, might be the most likely way by which pRb-depleted cells become hypo diploid. MN is considered a possible cause of chromosome loss during mitosis resulting in the generation of hypodiploid cells that lack few chromosomes. Indeed immunofluorescence microscopy performed in pRb-depleted cells and in RB/AURORA-A and RB/PLK1 knockdown cells showed the presence of micronuclei containing whole chromosomes.

An alternative hypothesis might be that micronuclei originate in RB silenced cells by defects in kinetochore assembling that generates dysfunctional centromere unable to contact correctly mitotic microtubules. Altered expression and dosage of some centromere components by promoting defects in kinetochore assembling could trigger micronuclei generation resulting in unfaithful chromosome segregation during mitosis. pRb plays a critical role in regulating chromosome stability in human primary fibroblasts and its deficiency triggers defects in chromosome segregation via micronuclei formation. Thus, micronuclei could represent a mechanism underlying chromosome instability following RB acute loss [25].

Blood Cancer with MN: Micronucleus is formed from acentric chromosome fragments or whole chromosomes during metaphase or anaphase phase of cell division [26]. They reflect chromosome damage and may thus provide a marker of early-stage carcinogenesis [27-29]. A number of studies have been designed to evaluate the potential influence of factors such as gender, age, smoking habit, alcohol, genotoxic agents, chemical substances and radiation had a remarkable effect on the frequency of MN [28,30]. MN test can be performed for different cell types such as lymphocytes, fibroblast and epithelial cells to check any abnormalities [31]. Consequently, MN formation is a reliable biomarker of exposure to radiation [32-34]. In our study, the MN frequency was also compared with WBC count. The MN frequency showed an increase during RT. MN level was showed greater frequency in the elderly control group (non-smokers) with a mean age of 52.6 ± 2.9 years than the young controls with a mean age of 23.5 ± 1.3 years (non-smokers). MN formation was not observed in the young controls, but a low frequency of MN (0.05%) was determined in the elderly controls.

The observation of MN in these healthy persons without Radiotherapy (RT) and chemotherapy clearly indicates that MN formation is related to donors' age. These findings suggest that age can influence the formation of MN in WBC. The effect of aging on spontaneous MN frequency has been reported by various authors [30,31,33,35-37]. Higher frequencies of MN have previously been reported in females than in males [38]. Anna, et al. investigated the level of cytogenetic damage in peripheral blood lymphocytes of patients undergoing chemotherapy [39]. As a result, it has been showed that highest level of cytogenetic damage was observed at the end of therapy. They determined the frequencies of increased MN during the first half of therapy and declined thereafter. Moreover, they observed the leukocyte count strongly decreased at the beginning of therapy with an upward trend at the end. Boreham, et al. reported the relationship between radiation dose and radiation-induced the apoptosis and MN formation [40].

They found that apoptosis and the MN frequency decreased in low dose rate of radiation, but apoptosis and the MN frequency in binuclear cells increased with increasing of applied radiation dose. Hubert, et al. used MN test to determine the effects of radiation on 99 workers studied in Belgium Doel Nuclear Center [41]. They reported an increase in the frequency of MN with increase in the annual exposure to radiation. In another study, MN formation and cell proliferation in human lymphocytes exposed to 50 Hz magnetic fields for 72 h was investigated. As a result, 50 Hz magnetic fields have no effect on MN formation, and a significant increase in cell proliferation was not observed [42]. Widel, et al. investigated the frequency of MN in peripheral blood samples were taken before and after RT in patients with cervical cancer [43].

As a result, they reported a significant increase in the MN frequency compared with the controls. Maes, et al. reported effect of 2450 MHz microwave on the MN frequency in human blood lymphocytes in vitro where they stated an increase in the MN frequency with increasing of exposure duration of microwave [44].

Breast Cancer (BC) with MN: Breast Cancer is the most frequently diagnosed form of cancer and the leading cause of cancer-related deaths among females in the world. It is leading cause of cancer-related deaths among females in the world, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400 subjects) of the total cancer deaths in 2008 [45]. In Mexico, the incidence and mortality of BC have risen in the last years.

Results of several studies showed that the frequencies of MN in the lymphocytes of BC patients are significantly increased compared with healthy women [46-49]. A minimally invasive and potentially useful method for monitoring genetic damage in humans is the MN assay in exfoliated buccal mucosa cells [50,51]. MN and Broken Eggs (BE) phenomenon are considered as genotoxic events, BN as a spindle disturbance (aneugenic effects) and Condensed Chromatin (CC), KR, Karyolysis (KL) and pyknosis as acute cytotoxic effects [50]. Bonassi, et al. analyzed all the data concerning MN assay in

buccal cells of cancer patients and postulated that a diagnosis of cancer significantly increased MN and other endpoints frequencies [52].

Especially high correlation was found for oro-pharyngeal cancers, respiratory system cancers, and for all the other cancers pooled together. Two Indian research groups also reported about increased number of MNi in oral mucosa cells of BC patients compared with healthy controls and patients with benign breast lesions. Hence, several groups of investigators reported increased frequencies of MNi in buccal mucosa cells of BC patients. We also investigated possible differences of nuclear anomalies between BC patients with stage I and II and healthy females. It can be noted that all parameters (reflecting both genotoxicity and cytotoxicity) in cells of stage I BC patients were higher compared to controls.

It is also noteworthy that 72% of fragile site are coincidental with oncogene loci on human chromosomes. Highly significant differences were found between BC patients with stages I, II and III and the control subjects in the numbers of lymphocytes with MN. Defective DNA repair leads to genetic instability which appears in the elevation of MN in somatic (epithelial) cells Increase in MN radiation induced in small groups of healthy women carrying a BRCA 1/2 mutation compared with matched control groups suggested a close relationship between the presence of these mutations and the MN induction by ionizing radiations [53-55].

It has showed an increased MN frequency associated to in vitro radiation exposure in sporadic BC patients [56]. MN frequency was higher in cases than in controls. Overall, in the BRCA-negative group, there was a difference of 52.8 MN/1000. The MNT has been evaluating the chromosomal instability in selected groups of patients affected by cancer or degenerative diseases, compared with control [57-59].

Urinary Cancer with MN: Biomarkers used in health studies of human are generally divided into three classes:

- Biomarkers of exposure
- Biomarkers of effect
- Biomarkers of susceptibility [60,61].

In human bio monitoring the most commonly used materials are blood and urine. Now a day's epithelial cells are becoming more and more popular as they may be obtained from the oral cavity, bladder or nose in a noninvasive way [62,63]. Potential carcinogens enter the body through dermal penetration, ingestion and/or inhalation. Thus, epithelial cells are usually the first and the most significant barrier to absorption of exogenous factors [64]. Micronucleus assay conducted on buccal cells and urothelial cells detected biomarkers of early biological effects. Higher micronuclei frequency was recorded in the group of male smokers from Indian population aged 41 years and above, smoking for more than 20 years [65].

In other studies a statistically higher frequency of micronuclei in urothelial cells was detected in male smokers in comparison to nonsmokers [66,67]. Bonassi, et al. (2011) based on the project HUMNxl results, concluded that the significant increase of micronuclei in buccal cells was associated with heavy smoking exceeding 40 cigarettes per day [68].

Cervical Cancer with MN: Cervical cancer is the third most common cancer among women worldwide and the second cause of cancer mortality in Brazilian female [69,70]. Cervical cancer has some of the best prospects in terms of prevention and cure, when diagnosed early, cervical cancer has up to a 100% chance of cure. About 80% of cervical cancer cases occur in developing countries, where neither population-based screening nor optimal treatment is available to treat this cancer [71].

Complementary methods aimed at increasing the sensitivity of screening for cervical cancer have been described, including high-risk HPV testing and MN identification [72-74]. The presence of MN is a biomarker, used to screen populations at risk of cervical cancer [75].

Evolution from CIN I to CIN III is accompanied by enhanced genetic instability, the presence of MN has been used, in combination with cytological findings, as a biomarker of the risk of cervical cancer [76,77]. MN screening was done in all selected Cyto Pathological (CP) smears by counting 1,000 cervical squamous cells with a light microscope at a magnification of 1000x [78]. The MN frequencies observed here were significantly higher in the groups with cellular changes compared to the control group, in agreement with previous case-control studies [79,80].

Brain Cancer with MN: Induction of micronucleus formation (cytogenetic damage) in brain cancer cells upon exposure of Dielectric Barrier Discharge (DBD) plasma [81]. The effect of DBD plasma on micronucleus formation in brain cancer cells has not been described by scientific community yet. Therefore, major purpose of our present study is to investigate the effect of DBD plasma on the genetic material for different types of cell lines. In the recent years, the in vitro micronucleus assay has become an attractive tool for measuring genotoxicity because of its capacity to detect clastogenic and aneugenic events, simplicity of scoring, accuracy, multipotentiality, and wide applicability [82,83].

Brain tumors represent one of the most treatment refractory forms of cancer. These include different kind of intracranial malignancies, malignant gliomas Grade IV, anaplastic astrocytoma (Grade III), and different types of anaplastic tumors of mixed origins, such as oligoastrocytoma and oligodendroglioma [84]. These are the most common brain tumors in adults and third leading cause of cancer-induced deaths in the age group of 15 to 34 year with 20-25 per cent of pediatric neoplasms [85-87]. Post irradiation presence of LND significantly increased micronuclei formation, particularly in the cells that had been pre-sensitized by 5-Bromo-2-deoxy-Uridine [88]. The efficacy of temozolomide treatment is limited to tumors with relatively lower levels of the enzyme MGMT, and the presence of an intact MMR pathway.

Conclusion

Total 311 subjects screened from different regions of Eastern & North Eastern India and also from RKMSP and ESI Hospital, Kolkata. Out of 311 subjects more than 60% persons chew betel quid. In our study we checked that after black tea supplementation, micronuclei percentages are lower than previous one. It is seen that 5 folds increase MN% than normal, in oral cancer cases who mainly chew betel quid, which is highly statistically significant ($p<0.0001^{*}$).

Acknowledgements

The authors thank the Prof (Dr.) Madhusnata De (PhD. Guide), Secretary of Ramakrishna Mission Seva Pratishthan, for kind permission to use the laboratory for this work and Department of Maxillofacial and ENT Department of RKMSP Hospital and also Dr. P. B. Kar, Dr. A. Das, Dr. Johuri of ESI Hospital, Sealdah, Kolkata.

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