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Review Article

Micronucleus scoring: An available approach in the evaluation of genomic damage in exfoliative cervicovaginal cells

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Abstract

Micronucleus is small chromatin extranuclear bodies when chromosomes or chromosomal fragments are not included in the nucleus during cell division. Micronucleus formation usually serves as a sensible indicator of genotoxic damage and also a morphological marker of chromosomal instability. Genomic damage is crucial for the development of degenerative diseases, including cancer. MN assay is a reliable and applicable method on many cell types such as erythrocytes, leucocytes and epithelial cells and it represents an important tool for evaluating DNA damage and defects in mitosis. The purpose of this article is to provide an overview of the current status of the micronuclei scoring in exfoliated epithelial cells and to highlight the importance of this procedure.

Abbreviations

DAPI: 4',6-Diamidino-2-Phenylindole, Dihydrochloride; LBC: Liquid Based Cytology; MN: Micronucleus; PAP: Papanicolaou test

Review of the literature

Micronucleus (the plural is micronuclei) was first identified by Howell and Jolly in red cell precursors and was also referred as Howell–Jolly bodies [1]. At that time, these bodies were described as nuclear remnants (clusters of DNA) in the circulating erythrocytes. After a while, MN was described in lymphocytes, exfoliated buccal and cervicovaginal epithelial cells [1-3]. Extranuclear cytoplasmic bodies of the damaged chromosome fragments and/or whole chromosomes form MN. It is not incorporated into the nucleus after cell division [2]. MN is also a sensible indicator of the genetic damage and linked to various chromosome aberrations such as defective mitotic figures, chromosome fragmentations, mitotic cell death and catastrophe, giant nuclear, and especially genome chaos [4-7]. Genomic damage may occur due to medical factors such as radiation and chemicals and also due to the deficiency of some micronutrients. Lifestyle factors such as alcohol, smoking, stress and genetic factors are also important for the development of genomic damage [6-13]. MN assay can be applied to exfoliative epithelial cells to screen population groups at risk for cancer [14-16]. It can also be used as a biomarker to examine the effects of infection agents [17-19].

History of micronuclei

Micronucleus test was suggested for the first time by Boller, Schmidt and Heddle in the early 1970s. A few years later Countryman and Heddle could be used for the micronucleus approach in the peripheral blood lymphocytes [3]. In 1982, MN technique was first described by Stich and colleaques in exfoliative cells of the buccal mucosa for human biomonitoring studies [11]. Exfoliated cells can be used in several types of cell obtained from buccal and cervical mucosa, bronchi, urinary bladder etc. MN assay seems to represent a useful 'internal dosimeter' for estimating exposure to genotoxic agents [8,14,20].

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Micronuclei formation

The basal layer of squamous epithelium contains the basal cells which are cuboidal-shaped stem cells. During nuclear division, genetic damage may express as MN in these cells [2,3]. According to the knowledge accumulated in literature, some genetic and epigenetic mechanisms effects the formation of MN [2]. There are concisely three mechanisms that may contribute to this process. These are chromosomal breakage, dysfunction of the mitotic apparatus and broken anaphase bridges [16,19]. Agents that stimulate aneuploidy cause centromere division errors and mitotic spindle failure. The classtogens also contribute to MN formation lead to chromosome breaks [2].

Criteria for the evaluation of micronuclei in exfoliated cells

One of the most important criteria for the evaluation of MN in exfoliated epithelial cells is the counting of nuclei and cells with intact boundaries [18,19]. Heddle (1973) initially described the well-established criteria for identifying MN but he did not provide the cell inclusion criteria. Stich and Rosin (1983) established the criteria for inclusion of cells for MN frequency, and later Tolbert, et al. developed the criteria [12,15]. Tolbert, et al. suggested that the criteria for identifying micronucleus should be as follows:

- The diameter of the MN should be less than one-third of the main nucleus, but large enough to discern shape and color
- MN should have similar texture and staining as the main nucleus
- MN should have the same focal plane as nucleus
- MN should have rounded smooth perimeter suggestive of a membrane
- MN should be separated from or marginally overlap with main nucleus as long as there is a clear identification of the nuclear boundary.

In general, the most commonly used method is the zigzag method for screening of the slides. Tolbert et al. also recommended the scoring of at least 1000 cells. If less than 5 micronucleated cells were found after counting 1000 cells, he suggested to count 2000-3000 cells [6,21].

Exfoliative cytology and Papanicolaou test (Pap test)

The cervicovaginal epithelium is composed of several distinct layers or strata. This rapid turnover of epithelial tissue brings the cells to the surface, where they exfoliate [3,22]. Exfoliation of cells is one of the main mechanisms of cell loss participating in the homeostatic control of cell population size. Exfoliated epithelial cells have some advantages such as a good definition of nuclear detail, cytoplasmic transparency [23]. General appearances of cervicovaginal samples and epithelial cells with different infection agent were seen in Figure 1.

In Figure 2, micronucleated exfoliated epithelial cells with different infection agents and cervical intraepithelial neoplasia were shown.

Pap test is an easily performed, quick, noninvasive, inexpensive and safe methods to screen for preinvasive, invasive cervical cancer and the effects of gynecological infection agents [24]. Cervical cancer is the fourth most common cancer in female. Pap test can help prevent cervical cancer [25-27].

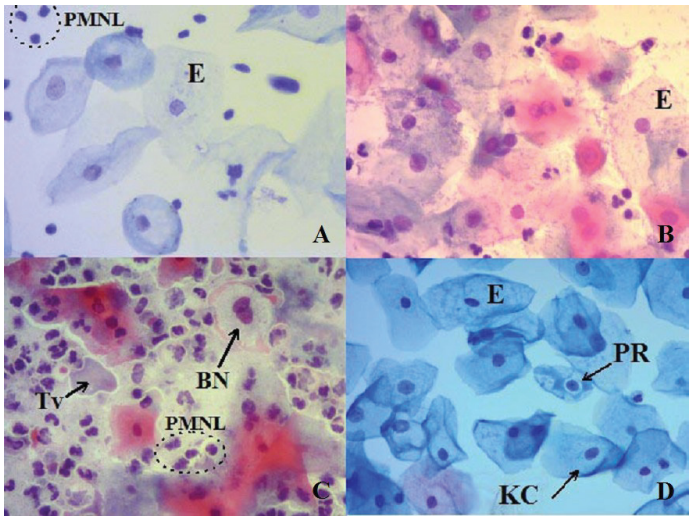

Figure 1: General appearance of cervicovaginal samples and epithelial cells with different infection agent (Pap X 40).
A. Exfoliated cervicovaginal epithelial cells with clear nuclear detail and cytoplasmic transparency (E) and Polymorphonuclear leucocytes (PMNL) in HPV 16 infected liquid based sample
B. Epithelial cell (E) in Candida spp. infected conventional sample.
C. Trichomonas vaginalis (Tv), Binucleated epithelial cell (arrow, BN) and Polymorphonuclear leucocytes (PMNL) in conventional sample.
D. Exfoliated epithelial cells (E), koilocyte (KC) and perinuclear halo (PR) in HPV 18 infected liquid based sample.

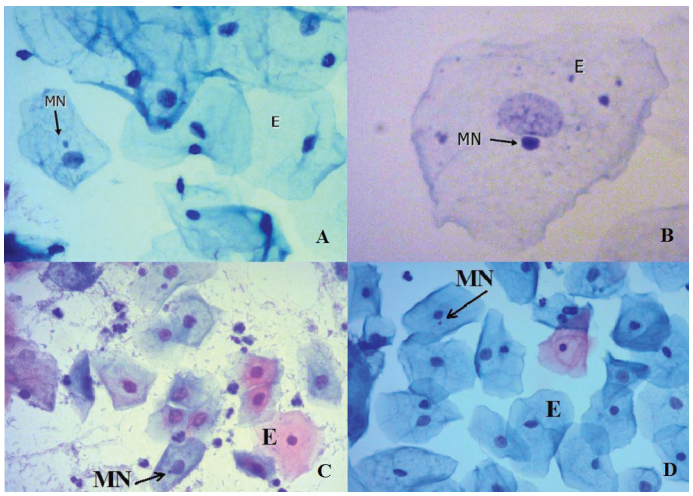

Figure 2: Micronucleated exfoliated cervicovaginal epithelial cells with different infection agents and cervical intraepithelial neoplasia (Pap X 40)
A. Micronucleus (arrow, MN) and epithelial cells (E) in cervical intraepithelial neoplasia
B. Cervicovaginal epithelial cell (E) and micronucleus (arrow, MN) in cervical intraepithelial neoplasia (Pap X 100).
C. Micronucleus (arrow, MN) and exfoliated epithelial cells (E)) in Candida spp. infected conventional cervicovaginal sample.
Micronucleus (arrow, MN) and exfoliated epithelial cells (E) in HPV 18 infected liquid based cervical sample.

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This test can be performed via the conventional method and liquid based cytology. In the conventional method, exfoliated epithelial cells were layered on a glass slide and immediately fixed with appropriate fixative. In LBC, cells are stored in a vial containing a special liquid. LBC had better performance than conventional Pap test [26,28]. Light microscopic analysis of MN in cervical smears increases the sensitivity and specificity of cytology in the evaluation of cellular pictures due to genomic instability [19].

Micronucleus scoring and the interaction between MN and gynecological infections

MN scoring can be performed immediately by using microscopy and it can be screened in various diseases, infection agents, cervical intraepithelial lesions and carcinoma [17-19,29]. MN scoring on the epithelial cells of cervix can be used as supplemental in cervical cancer screening. MN requency appears to increase in carcinogen-exposed tissues long before any clinical symptoms are evident [27].

MN assay is a simple, reliable, convenient, cheap and applicable method that can be performed on many cell types and is a noninvasive technique for the evaluation of genetic damage. A variety of different stains can be used for the analysis of micronuclei. Among the most DNA-specific stains, Feulgen is the one which is being most widely used. Acridine orange, propidium iodide 4,6-diamidino-2-phenylindole (DAPI) are also being used for the same purpose. Nonspecific stains (May-Grunwald's Giemsa, Giemsa, and less frequently Orcein) were used in MN scoring about 30% of the studies on epithelial cells [30]. Palaskar and Jindal have shown that Pap is a better stain for counting MN due to the fact that MN was easily seen in the clear cytoplasm when compared to other stains like Giemsa stain [31]. Ayyad, et al. indicated that Pap test was the preferred method for detecting MN in the oral epithelial cells after comparing it with May-Grünwald-Giemsa stain [32].

Many studies were published related to the MN assay in exfoliated cervicovaginal epithelial cells with preneoplastic lesions and cancer. The large majority of these studies demonstrated a significant increase in MN frequency in these patients [16,33]. Safi Oz, et al. demonstrated that the frequencies of micronucleated cells were significantly higher in genital Candidiasis and Trichomoniasis [18,19]. Cortés Gutiérrez, et al. demonstrated that MN frequencies were higher in HPV infected Mexican females [17]. Rosin and Anwar stated that MN frequencies were higher in the S. haematobium infected group [34]. In some studies, the limitations of the micronuclei scoring has been reported [3]. A few studies reported at possibility of false-positive results as the bacteria or bacterial colonies, nuclear debris, small stain deposits and keratohyalin granules may resemble to MN [3,29,30]. Safi, et al. indicated that bacteria and bacterial colonies can be differentiated from MN by their characteristic shape, color, staining intensity and smaller size, which is also apparent in the background. Also stain deposits are polymorphic granules in the smear, generally over the cells. Pap staining ensures that the nucleus and cytoplasm of the epithelial cells are clearly stained, ensuring visible and identifiable MN [30]. MN monitoring could be incorporated into routine screening procedures as an additional criterion for the early detection of cytogenetic damage as used in evaluation of cancer and infectious agents [35].

Conclusion

This review article summarizes the literature about the current status of the micronuclei scoring and the importance of this procedure in exfoliated cervicovaginal epithelial cells in human beings as an approach for genomic damage. DNA damage can produce a wide variety of effects on human health. Exfoliated epithelial cells have been used in cytology to detect chromosome fractures, morphology disorder, premalignant and malignant changes and infection agents and also for evaluating MN. Because MN assay is a fast, safe and a reliable technique to assess genomic instability and also does not require too much expensive equipment, this test could be incorporated into routine Pap test an additional criterion for the early detection of genomic instability. Consequently, the combination of MN assay and Pap test will increase the sensitivity and specificity of the Pap test by providing information about genomic instability as well as evaluation of cytological parameters.

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