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Oral Exfoliative Cytology and Micronuclei

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ABSTRACT

In recent years, there has been a significant resurgence in interest in oral cytology as a diagnostic and prognostic approach for tracking patients with mouth cancer and possibly malignant illnesses. To assess genetic instability, the buccal mucosal micronuclei assay was initially proposed in 1983. Biomarkers exist that indicate the likelihood of an aggressive tumor developing from a potentially malignant illness. According to reports, these genotoxic and carcinogenic substances are strong clastogenic and mutagenic agents, which are believed to be in charge of causing chromatid/chromosomal abnormalities that lead to the formation of micronuclei. According to several research, the steady rise in micronucleus (MN) counts from healthy oral mucosa to possibly cancerous conditions to oral carcinoma indicated a connection between this biomarker and the development of neoplasms.. MN scoring is a biomarker that can be used to detect various preneoplastic disorders far before clinical signs appear. It may be particularly useful in screening high-risk individuals for a particular cancer. As a result, it can serve as a screening, prognostic, and instructional tool in oral cancer community centers.

INTRODUCTION

One of the biggest risks to public health worldwide is cancer. Oral squamous cell carcinoma is the most prevalent of all oral malignancies that either directly develop or are preceded by certain benign lesions or illnesses that are referred to as potentially malignant disorders. Oral cancer is one of the top ten most common types of cancer [1]. Leukoplakia, oral submucous fibrosis, lichen planus, and other potentially malignant conditions might have unpredictable biological activity since some of them have the potential to develop into malignant transformation. According to reports, the probability of malignant transformation ranges from 6.6% to 36.4% [2]. Finding the risk group among them would be practically significant [3]. A great option for a biomarker that can identify chromosomal loss is the micronuclei assay [4].

The most significant underlying cause of degenerative and developmental diseases is most likely genomic damage [5]. Oral carcinogenesis is widely believed to be a multi-step process of cumulative genetic damage that results in cell dysregulation and a breakdown in cell signalling [1]. Although the gold standard for detecting malignancies is the histology of biopsied material, because biopsy is an intrusive procedure, it loses viability due to changes in blood sugars and the disease itself [4]. Biopsies have psychological ramifications for certain

patients and limitations for certain specialists [3,6]. Noninvasive diagnostic testing has recently gained popularity due to the discomfort associated with invasive diagnostic methods [7]. The technique's simplicity and painlessness might encourage more frequent testing [8]. Exfoliative cytology, which can easily be performed chair side during a routine oral examination, would be a rather safe treatment under such circumstances [7]. Oral cytology is making a comeback as a potent screening and diagnostic technique because to the development in quantitative exfoliative cytology [9]. Screening entails determining whether a person without symptoms has a disease [10]. The ability of traditional cytopathology to identify alterations in individual exfoliated cells early has improved [11]. The collection and analysis of cells from the oral mucosa's surface is known as exfoliative cytology [12].

History of Micronuclei

Boller and Schmidt first proposed the term micronucleus (MN) test in the 1970s. Using bone marrow erythrocytes, Heddle demonstrated that this assay offered a straightforward way to identify the genotoxic potential of mutagens following in vivo exposure of animals [4]. In 1976, Countryman and Heddle suggested that the micronucleus technique may be applied to peripheral blood lymphocytes and that micronuclei be employed as a biomarker in testing plans [13]. In 1980, the genotoxic effects of quid and betel nuts were assessed using exfoliated buccal mucosa cells. Additionally, the MN assay in buccal cells was utilized to monitor the effects of several chemopreventive drugs and to investigate malignant and precancerous lesions [6].

Theories of Origin of Micronuclei

There are two predominant mechanisms leading to the MN in a mitotic cell:

- Chromosomal breakage
- Dysfunction of the mitotic apparatus [14].

Chromosome fractures caused by clastogens result in acentric fragments. Micronuclei directly incorporate these chromosomal segments. In the alternative process, aneugenic substances stop the spindle apparatus from forming during mitosis [14]. Interest and engagement in the field of molecular epidemiology are growing [4,15].Genetic and environmental variables affect an individual's susceptibility to cancer, according to molecular biology research. MN, sister chromatid exchanges, and chromosomal abnormalities can all be used to evaluate DNA damage. When compared to other tests, the MN test is the most sensitive of them because it doesn't require time-consuming steps like cell culture or certain DNA stains [16].

A microscopically detectable round to oval cytoplasmic chromatin clump next to the nucleus is called a micronucleus [17]. MN are cytoplasmic entities that are extranuclear. It is located in the inner part of the cytoplasm, surrounding the major nucleus. MN is easily distinguished from a binucleated cell because its diameter is less than one-third that of the main nucleus [14]. Human lymphocytes, exfoliated epithelial cells, and erythrocytes can also undergo micronucleus scoring or test [14]. Micronuclei have been considered markers of genotoxic exposure since 1937 [18].

Etiology of Micronuclei

In normal healthy individuals due to exposure to environmental pollutants such as drugs, chemicals, food, and free radical injuries [14].

Exposures at work and in the environment (organic solvents, antineoplastics, paint solvents containing lead, and drinking water tainted with arsenic) [4,6,13]. Ionizing radiation, which is used to treat neoplasia but also damages genetic material [4,6]. Lifestyle factors, such as nutrition, vitamin deficits, alcohol use, and smoking [4,6]. A study on MN index: An early diagnosis of oral carcinoma was carried out by Gupta, Mhaske, et al. According to the data gathered, the premalignant cases linked to long-term tobacco use had the highest MN index in the stages of oral cancer and erythroplakia. Since tobacco users are among the risk categories, the scientists came to the conclusion that this score can be employed as a biomarker screening test [16]. Regular use of X-rays for diagnosis. X-rays are a powerful mutagenic agent that can cause chromosomal abnormalities and mutations [19].

Genotoxic factors

A range of things, including genotoxic agents like medical procedures (such as radiation and chemicals), carcinogenic components in tobacco, betel nut, and alcohol, and genetic factors such hereditary abnormalities in DNA metabolism and/or repair, can generate micronuclei in oral exfoliated cells [5].

Reactive oxygen species from areca nut extracts are believed to be promoted by a carcinogen produced from betel nuts. The DNA may then be damaged by these reactive oxygen species [8]. Vasudevan et al. used the MN test to examine the genotoxic effect linked to occupational exposure to chromium workers. He came to the conclusion that long-term occupational exposure might raise DNA damage levels, which would be reflected in a higher incidence of MN [20]. The proliferation of epithelial cells is very high [6]. Buccal cells can convert proximal carcinogens into reactive chemicals and serve as the initial barrier for the ingestion or inhalation route [1].

Over 90% of malignancies start in epithelial tissues, and it is simple to remove these cells from the mouth without making patients uncomfortable. The process is practical, affordable, and precise; results can be achieved more quickly [18]. Thus, it might be claimed that early genotoxic events caused by carcinogenic substances entering the body by food and inhalation preferentially target oral epithelial cells [6].

Micronucleus: Formation

The stem cells that may exhibit genetic damage (chromosome loss or breaking) as MN during nuclear division are found in the basal layer of the oral epithelium [5]. Extra-nuclear cytoplasmic structures are called micronuclei. Many genotoxic compounds harm the chromosomes in cells, causing them to develop. When central elements migrate toward the spindle poles during anaphase, the damaged chromosomes—represented by acentric chromatics or chromosomal fragments—lag behind. Both the whole chromosomes and the central pieces produce normal daughter nuclei following telophase. Although the daughter cells also include the lagging elements, a significant amount of them are moved into one or more subsidiary nuclei, which are often considerably smaller than the major nucleus and are referred to as micro nuclei.

This takes place in the basal layer of the epithelial tissue, where cells undergo mitosis. This rapid turnover of epithelial tissue brings the cells to the surface, where they exfoliate [21]. Bigger micro nuclei result from the exclusion of whole chromosome following damage to the spindle apparatus of the cell (aneugenic effect).

Whereas small micro nucleus result from structural aberrations causing chromosomal fragments (clastogenic effect) [8].

The oral epithelium maintains itself by continuous cell renewal whereby new cells produced in the basal layer by mitosis migrate to the surface replacing those that are shed [5].
Their frequency and the number of micronuclei are known to increase with carcinogenic stimuli, long before the development of clinical symptoms.
The optimal timing between 7 and 21 days after exposure is needed because peak expression may vary depending on the effects of the particular DNA damage or chromosomal exposure on the basal cell turnover rate [2]. It seems likely that cells with more chromosomal damage, and hence more micronuclei that leads to increased frequency of MN from precancer to cancer [22].

Collection of exfoliated oral mucosal cells

Exfoliated oral mucosa cells can be collected using a wooden tongue depressor, a metal spatula, toothpicks, toothbrush, and cytobrush. According to Ogden *et al.* the wooden spatula has been shown to be a satisfactory instrument for obtaining smears from the buccal mucosa [23]. Cytobrush sampling **(Figure 1)** is more frequently used which facilitates their uniform distribution onto the microscopic slide, thus probably improving sensitivity [24].

The instrument used for making smear should be easy to use in any location and provide an adequate number of epithelial cells [25]. MN frequency was higher when cells were collected by vigorous, rather than by light scraping [4].

The smear procedure can also be done after rinsing the mouth gently with water to remove food debris. Using a slightly moistened wooden spatula oral mucosal cells can be collected. The obtained cells are smeared on a sterile glass slide, fixed with a spray fixative and stained with papanicolaou stain. Smear can be observed under microscope with ×100 magnification.

Dr. George N. Papanicolaou was the first to introduce Pap smear. More Details in 1928 in cervical tissues and since then this technique has helped reduce cervical cancer incidence and mortality rates by 75%. This is an easy technique and can be replicated in the oral cavity for analysis of the changes caused by smoking [18]. Palaskar and Jindal have shown that Pap is better stain for counting MN due to the fact that MN were easily seen in clear cytoplasm in regard to other stains like Giemsa stain [24].

Many studies have shown that a variety of different stains can be used in micronuclei studies. Among the DNA-specific stains, the ones which are most widely used are Feulgen, acridine orange, 4,6-diamidino-2-phenylindole (DAPI), and propidium iodide were also used. About 30% of the studies on epithelial cells were conducted using nonspecific stains (Giemsa, May-Grunwald's Giemsa, and less frequently, Orcein) [4,6,24].

Criteria to assess micronuclei

Tolbert *et al.* criteria [9] parameters for identifying micronucleus are as follows:

- Rounded smooth perimeter suggestive of a membrane
- Less than a third the diameter of associated nucleus, but large enough to discern shape and color
- Staining intensity similar to nucleus
- Texture similar to nucleus
- Same focal plane as nucleus
- Absence of overlap with or bridge to nucleus

In general, the most commonly used is, zigzag method for screening of the slides. Cells with intact nuclei and cell boundaries should be counted [26]. Tolbert *et al.* recommended the scoring of at least 1000 cells in a slide so that more precise results would be obtained with increasing number of cells [4].

Importance of micronuclei scoring in clinical pathology

Micronuclei have been used as a measurement and biomonitoring of genotoxicity of various carcinogens, heavy metal poisoning, antineoplastic drugs, pollutants, etc.

It projects as a biomarker of chromosomal damage.

This assay has been extensively used to evaluate the presence and the extent of chromosome damage in human populations exposed to genotoxic agents [3].

The frequency of MN can help to assess the therapeutic prognosis [9].

It represents as an "internal dosimeter" to estimate exposure to genotoxic and carcinogenic agents [3].

Their frequency of occurrence is a measure of chromosome breakage in early cell divisions [5].

It has been shown to have a sensitivity of 94%, specificity of 100%, and an accuracy of 95%[4].

Importance of early detection

- Early detection will help the clinician to plan the treatment thereby improving patient's survival rates. The prevention of PMD, oral cancer and its associated morbidity and mortality, hinges upon the early detection, allowing for histological evaluation and subsequent treatment depending on the stage of diagnosis [2].
- Early detection of MN in a cell represents an "internal dosimeter" to estimate exposure to genotoxic and carcinogenic agents [3].
- Screening of individuals who are at high risk of malignant transformation is more pivotal in preventing and reducing the costly and painful treatment later on.
- Micronuclei assay in exfoliated cells holds promise as, one of the biomarkers of exposure to genetic toxins and can provide as screening prognostic and educational tool in community center of PMD and cancer [9].
- The MN assay in exfoliated buccal cells is potentially an excellent candidate to serve as such a biomarker as one of the early diagnostic tool in oral pre cancer and cancer [4].

Kamboj and Mahajan conducted a study to define MN as an early diagnostic tool of leukoplakia and SCC. MN assay was performed on oral exfoliated cells of chosen subjects. They observed the frequency of mean

percentage occurrence of MN cells increased significantly in comparison to controls with leukoplakia and SCC. They concluded that MN can be stated as an early indicator and an upcoming marker for diagnosing oral pre cancer and cancer [27].

Buajeeb *et al.* conducted a study to determine the frequency of MN exfoliated cells in atrophic and erosive oral lichen planus (OLP). The authors revealed an increase in MN in OLP lesions. They concluded that the results of their study indicated genotoxic damage in atrophic and erosive OLP [28].

As a screening prognostic and instructional tool in the community center of PMD and cancer, the micronuclei assay in exfoliated cells shows potential as one of the biomarkers of exposure to genetic toxins.[9] Micronuclei scoring is quick and requires little skill, and it is a noninvasive, straightforward, uncomplicated, clinical chairside method that is risk-free and well-accepted by the patient with no contraindications [3,29].

Limitation of Micronuclei Scoring

- Micronuclei scoring can be interfered by the bacteria that are commonly found in the mouth which can be differentiated by their characteristic shape
- Small dye granules may sometime resemble MN, but usually have a slightly different refractility and color intensity
- Other cellular structures such as keratohyalin granules resembling MN can lead to false positive results [30].

Mahimkar *et al.* conducted a study to investigate the extent of chromosomal damage by analyzing micronucleated cell frequency in exfoliated buccal epithelial cells with oral leukoplakia. The authors concluded the chromosomal damage in target tissue was higher. MN frequency in combination with genetic polymorphism in DNA repair may serve as a better predicator of risk [31].

Jadav *et al.* conducted to observe micronuclei; an essential biomarker in oral exfoliated cells for grading of oral squamous cell carcinoma. The authors concluded the MN can be candidate to serve as a biomarker for prediction of the grade of oral squamous cell carcinoma [32].

CONCLUSION

An emerging area of study in the prevention and treatment of cancer is the detection of micronuclei and their assay. The presence and frequency of MN represent genomic damage. If correctly detected, these tiny nuclear offshoots may prove to be significant biomarkers with enormous potential for both screening and forecasting individuals with potentially malignant illnesses of the mouth and serving as risk assessors during the course of invasive cancer treatment. The frequency of MNCs rising from healthy mucosa to possibly cancerous conditions to oral cancer may indicate a connection between the development of malignant neoplasms and this biomarker.

Consequently, the MN assay in exfoliated cells has potential as a particular biomarker for exposure to different carcinogens and can be employed as a screening test in dental clinics. The simplicity of the micronuclei assay is its main asset because it scores MN quickly, practically, and with little skill.

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