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Applicability of micronucleus (MN) teste in health areas: what do we know?

Aplicabilidade do teste de micronucléo (MN) na área da saúde: o que nós sabemos?

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ABSTRACT
The Micronucleus (MN) test is done to assess the genotoxic effects of the environment to the cell. It consists in identifying an error in the cell division where there is separation of chromosomes from the cell nucleus, thus forming a small fragment called a micronucleus. This may arise as a result of exposure factors of the body such as smoking, index, physical trauma, a potential risk for cancer development. There are several methodologies to obtain MN at laboratory level, which can be analyzed in cells of the oral mucosa, blood cells, defense cells such as lymphocytes. In addition, the test has a high cost-benefit, since it has low values and requirements less the examiner's expertise when compared to other methods. It is believed that the test can be used to identify possible carcinogenic risks arising from tobacco, ethanol, pesticides, radiotherapy, environmental and physiological factors such as aging, and can be used as a prognosis for several therapies.

Keywords: tumor biomarkers, tests for micronuclei, public health.

RESUMO
O teste Micronucleus (MN) é feito para avaliar os efeitos genotóxicos do ambiente para a célula. Consiste em identificar um erro na divisão celular onde há separação de cromossomas do núcleo celular, formando assim um pequeno fragmento chamado micronúcleo. Isto pode surgir como resultado de factores de exposição do corpo, tais como fumar, índice, trauma físico, um risco potencial para o desenvolvimento de cancro. Existem várias metodologias para obter MN a nível laboratorial, que podem ser analisadas em células da mucosa oral, células sanguíneas, células de defesa, tais como linfócitos. Além disso, o teste tem um custo-benefício elevado, uma vez que tem valores e requisitos baixos menos a perícia do examinador quando comparado com outros métodos. Acredita-se que o teste pode ser usado para identificar possíveis riscos cancerígenos decorrentes do tabaco, etanol, pesticidas, radioterapia, factores ambientais e fisiológicos como o envelhecimento, e pode ser usado como prognóstico para várias terapias.

Palavras-chave: biomarcadores tumorais, testes para micronúcleos, saúde pública.

1 INTRODUCTION

The effects of genotoxic agents on health need to be investigated and become a priority. Because most of these agents cause damage to the individual's integrality, mainly by providing the development of cancers (FLORES; UEDA YAMAGUCHI, 2008). Micronucleus (MN) test is one of the tests capable of identifying the impact of the carcinogenic agent on the cell (ANDRADE et al., 2005). The test, besides being low cost, requires less expertise from the researcher when compared with other research methods (BALLESTRERI, 2017).

Based on the premises, this review article aims to clarify theoretical concepts for the understanding of the micronucleus technique, as well as its laboratory principles for the execution of the test, its feasibility and application in the health area. And, with that, expand the understanding of pathological processes and diagnosis of diseases.

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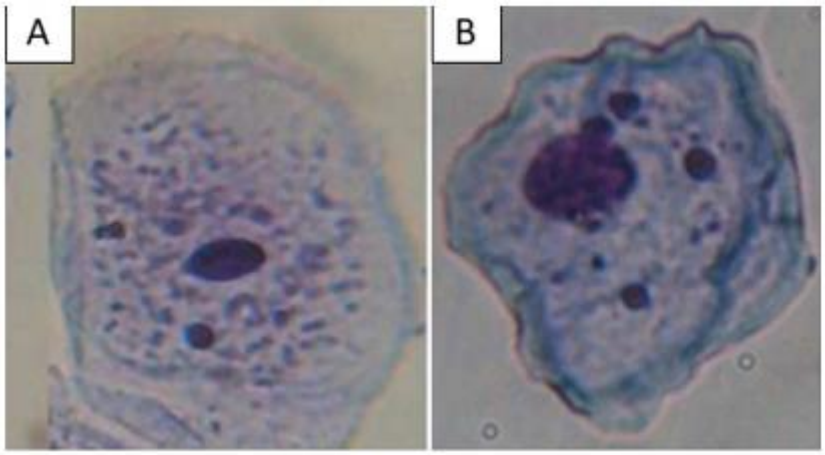
2 MICRONUCLEUS (MN)

For years several authors have proposed definitions about the micronucleus, some defining it as chromosome requests that were lost due to a delay in cell division (DECORDIER; KIRSCH-VOLDERS, 2006; HAYASHI, 2016; REIS et al., 2002). These pieces are found in the cytoplasm of interphasic cells where they present a connection to the main nucleus (HEDDLE et al., 1983).

Boller *et al.* (BOLLER; SCHMID, 1970) characterized micronucleus as chromatic bodies produced along the anaphase as a consequence of some defect in the centromere that resulted in loss of pieces or whole chromosomes. However, Heddle and Fenech (FENECH, 2000) proposed that the micronuclei have no connection with the main nucleus, because they have positive DNA- feulgen.

MN can also be defined as a nucleus separated from the main cell nucleus during cell division that results from chromosomal structural changes that may or may not be induced, with a cell presenting more than one micronucleus (ARALDI et al., 2013) (Figure 1).

Figure 1: Cells containing a micronucleus: A) Only one MN (black arrow); B) More than one micronucleus (black arrows)



SOURCE: (SOUZA et al., 2014)

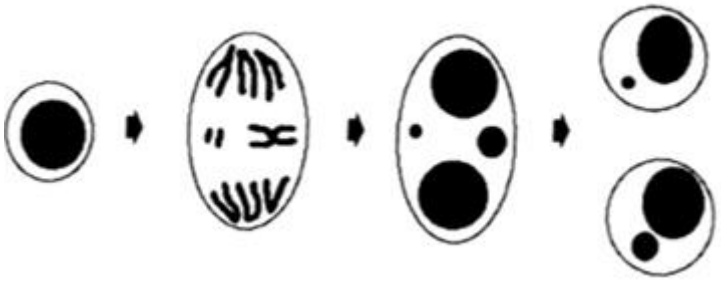
The micronucleus has gained several denominations, among them "fragments of nuclear material" or "intraglobular corpuscles", being demominated as “Corpuscle of Howell-Jolly” (HAYASHI, 2016).

2 .1 CLASSIFICATION AND CHARACTERISTICS OF THE MN

The criteria for classifying a cell with micronucleus are: 1) the structure of the chromatin must be similar or weaker than the nucleus; 2) rounded and in the same focal plane as the cellular nucleus, besides having a diameter of 1/5 smaller than the main nucleus (MOTGI et al., 2014). The micronuclei are reports in the literature as being 1/3 smaller than the cell nucleus (SCHMID, 1975).

Thus, based on the articles discussed, the MN tests become valid to identify changes in the cell cycle, in view of the loss of chromosomes during division and being characterized as a confidence test (FENECH, 1993).

Figure 2: MN formed from an error in the cell division process



SOURCE: (FENECH, 1993).

2.2 TEST DEVELOPMENT AND EVOLUTION

The applicability of the MN test in the health area gained relevance around 1970 through the Schmidt study that analyzed erythrocytes from the bone marrow of mice (SCHMID, 1975). The same author suggested the term "micronucleus test".

The technique gained notoriety and recognition from the 1980s through the description of cytogenetic damage in human epithelial cells that was a consequence of the growth of laboratories that made use of the test (FENECH, 2000). However, this technique was improved in 1985 by blocking cytokinesis, using the chemical agent cytokinesis B; this reagent has the ability to inhibit cytokinesis, but does not prevent cell division. Thus, the MN test could cause more than one cell nucleus, even though the cell undergoes only one division (LIRA; MARIA; FÉLIX, 2015).

2.3 PRACTICAL AND METHODOLOGICAL CONSIDERATIONS

Several methodologies are used to obtain and analyze the micronucleus, differing mainly in the mode of fixation and staining of cells (MENEGUETTI et al., 2012; REIS et al., 2002). According to the same authors, factors such as the type of cell to be analyzed and the number of cells that should be fixed in the slide should be taken into account when choosing the technique.

The micronuclei of the oral and nasal mucosa are obtained by an exfoliation of the cells through a disposable *Pap smear* (BRUSCHWEILER et al., 2014). In this case, according to Souza *et al.* (SOUZA et al., 2014) ten rotations are performed with the brush over the oral mucosa, increasing the diameter of the rotation to achieve a greater number of cells. Other materials for obtaining oral mucosa cells can be used as *plastic spatula* and *toothbrush* (JYOTI et al., 2015; PRADEEP et al., 2014). However, according to Landgraf et al. (LANDGRAF et al., 2017) it is necessary to sanitize the site before performing the collection to avoid sampling errors and loss of results of the MN technique.

The transport of the sample for analysis must be done in buffer solution with pH near 7.0 (MENEGUETTI et al., 2012). Other authors point to the use of Saline (0.9%) and a mixture of EDTA (0.1 M), Tris HCL (0.001 M), NaCl (0.02 M) can also be used as transport solution (PRADEEP et al., 2014).

The MN test can also be done on blood cells, such as lymphocytes, for this purpose it is done via venous puncture, and it can be or not with bone marrow analysis (ARALDI et al., 2013). The collected blood is always arranged in a heparinized tube and the amount of collection may vary between 4-5 ml, depending on the extent of analysis (FENECH, 2000; GASHI et al., 2018).

One of the ways of staining the cell is through the technique of *Feulgen* that stains the DNA of the pink color, being able to be associated to the counter-coloration Fast Green and being indicated when there is the intention of quantifying the DNA (KESIMCI; KARAHALIL, 2017). In addition, cells can be stained using the *May-Grünwald/Giemsa* technique (ARALDI et al., 2013) . This last method of staining is used mainly in blood cell tests, being low cost compared to the *Feulgen* technique, but not specifically staining the DNA (NERSESYAN et al., 2006).

Another method of staining is with the use of Triarylmethane (0.1%), Xanthenes (0.1%) and Thiazines (0.1%) (MENEGUETTI et al., 2012). According to the same authors, these dyes have the advantage of speed and low cost, since they are found on the market in quick kits. These dyes also allow good visualization of the anaphase-telophase, micronucleus and mitotic index bridges, as demonstrated in the works of Meneguetti & Poletto (MENEGUETTI; POLETTO, 2011). Some techniques using different dyes and fixing agents can be used (Table 1). Silva *et al.* (SILVA et al., 2020) pointed out that the technique using triarylmethane, xanthenes and thiazines reagents is the best way to obtain the exact fixation and staining of cells.

Table 1: Scheme representing three techniques with different types of coloring and fixing agents.

ANALYSIS	**ARALDI ET AL, 2013**	**MENEGUETTI ET AL, 2012**	**FEKI-TOUNSI ET AL, 2014**
	Bone marrow cells.	Exfoliated cell of the oral mucosa.	Exfoliated cell of the oral mucosa.
CENTRIFUGATION	1500 rpm for five minutes..	1000 rpm por dez minutos.	—
FIXATION	100% ethanol for five minutes.	2ml 0.1% Triarylmethane and 2ml 0.1% Xanthenes.	Methanol-glacial acid (3:1) for three minutes.
DYE	Methylene blue-eosin (Giemsa).	0.1% Triarylmethane, 0.1% Xanthenes and 0.1% Thiazines.	Methylene blue-eosin (Giemsa).
COLORATION	Three minutes in absolute Giemsa, washing in distilled water, one minute in 1: 5 solution Giemsa-phosphate buffer pH 6.0 and washing in xylol.	As lâminas devem ser inseridas dez vezes em cada recipiente seguindo a ordem: Triarilmetano 0,1%, Xantenos 0,1% e Tiazina 0,1%. Depois disso, as lâminas devem ser lavadas em água destilada e secadas a temperatura ambiente por 30 minutos.	4% Giemsa solution for five minutes.

Source: (ARALDI et al., 2013; FEKI-TOUNSI et al., 2014; MENEGUETTI et al., 2012).

The MN test requires a minimum number of cells attached to the slides, which can be varied between 500, 1000, 1500 and 2000 cells (ARALDI et al., 2013; JYOTI et al., 2015; SARSHAR; NADERI; FARHADI, 2012). Sarshar *et al.* (SARSHAR; NADERI; FARHADI, 2012) proposed a formula to calculate the average number of MN per cell:

$$\frac{Total\ number\ of\ mn\ per\ cell}{number\ of\ cells\ with\ mn} \text{ X } 100$$

2.4 ADVANTAGES AND DISADVANTAGES OF THE TEST

The main advantage of the micronucleus test compared to other cytogenetic tests is that it is non-invasive, low cost and can be used for prophylaxis of several diseases, such as oral cancer (BALLESTRERI, 2017; FENECH, 2000; HAYASHI, 2016). In addition, the success of the technique is that it requires less training and expertise on the part of the analyzer in relation to other chromosomal aberration monitoring techniques (ARALDI et al., 2013; HAYASHI, 2016). The MN test, when compared to other techniques, provides easy statistical support for analysis (FENECH, 2000).

3 CONTRIBUTIONS OF MN TEST FOR HEALTH AREA

The MN test is able to identify the damage caused by the genotoxic agent to the cell since it analyzes the loss of pieces or entire chromosomes during cellular mitosis (HERNÁNDEZ RODRÍGUEZ; DOMÍNGUEZ; MENDOZA CHOQUETICLLA, 2018). This technique has been used in several welfare and quality of life analyses, such as the effect of cigarettes (SOUZA et al., 2014), alcoholic beverages (REIS et al., 2002), anabolic agents (ARALDI et al., 2013), as well as the damage that diseases can cause to the cell (LANDGRAF et al., 2017). Thus, we can state that several cells are used for analysis with emphasis on blood cells and oral mucosa.

The epithelium lining the oral mucosa due to its high absorption capacity and high degree of exposure to agents is one of the cells most susceptible to mutation (SOARES et al., 2015). The oral mucosa has a constant process of cell renewal that lasts approximately 25 days and may present a higher error of cell division (MENDES DE FREITAS et al., 2016).

Motgi *et al.* (MOTGI et al., 2014) analyzed oral mucosa cells from 100 individuals, which were divided into three groups: smokers, non-smokers and non-smokers. In their results, the total number of Mn was lower in the non-smoking group. The study concluded without smoke is associated with genotoxic and mutagenic effects of the cell. Tobacco and alcohol can be powerful agents causing cellular mutation and Mn formation, as demonstrated in the study by Barbon *et al.* (BARBON et al., 2014) where 100% of smokers and ethylists presented such aberration.

Eker *et al.* (EKER; KOYUNCU; DILER, 2016) studied 60 volunteers and analyzed the chromosomal aberrations through cells of the oral mucosa by the MN test in order to assess the genotoxic character of the hookah. The results showed that the group of hookah users presented a greater amount of MN.

The genotoxic effect of ethanol was analyzed by Reis *et al.* (REIS et al., 2002), in which 40 individuals participated, 20 of whom were alcohol and tobacco abstainers and 20 non-smoking alcoholics. As a result, it was evident that the frequency of MN was higher in the group of alcoholic subjects even though there was no statistical relevance ($p>0.05$). However, Dieckemann *et al.* (DICKEMANN et al., 2019), in their study concluded that the periodic use of alcohol does not present a risk for the development of MN. These results, in the meantime, do not exclude other pathological changes in the long run. (REGINA et al., 2006).

This technique was used to analyze oral mucosal cells from farmers exposed to organophosphorus pesticides. Rosales (PANIS et al., 2012) evaluated 59 workers exposed to genotoxic agents (pesticides) and 50 individuals without exposure to pesticides. In this study, workers presented a higher prevalence of MN indicating a potential factor for cancer development. A similar study was conducted by Brina *et al.* (BRINA et al., 2018), which sought to quantify the number of MN and nuclear anomalies in exfoliated oral mucosal cells of individuals who worked with solid waste recycling, as a result a greater amount of MN was revealed in the study group, in addition to binucleated and cariorrex cells.

The impact of radiotherapy on cells of patients with breast cancer was evaluated by the MN test in order to compare radiosensitivity in gamma irradiated G0-lymphocytes in patients with breast cancer and in normal individuals. At this point, the authors Nosrati *et al.* (NOSRATI et al., 2017) collected 4 ml of blood for analysis of patients in cancer treatment by radiotherapy. The authors concluded that lymphocyte irradiation resulted in a greater amount of MN in these cells. The same was observed by AHMED *et al.* (AHMED et al., 2018), in which ionizing irradiation induced a significant increase in Mn frequencies in blood cells. Dairot et al. (DAROIT et al., 2015), when analyzing the effect of electromagnetic radiation emitted by the cell phone, concluded that individuals who used the device for more than 60 minutes/week for more than 8 years, present alterations in oral mucosal cells, including binucleated cells demonstrated by the micronucleus test.

The genotoxic damage caused by aging and its relation with gender and lifestyle was evaluated by the MN test in which the individuals were men and women over 60 years (experimental group) and between 19-29 years (control group). The frequency of NM was higher among the elderly showing that age can induce chromosomal changes (FERRAZ et al., 2016). Evaluation of the frequency of MN in oral mucosal cells to measure the genotoxicity of hormones used in hormone replacement in menopausal

women (OLIVEIRA; MORAES; CERQUEIRA, 2016). This study did not show that chromosomal damage is not induced by hormone therapy.

3.1 MN TEST IN THE USE OF DRUGS

The MN test was used to evaluate the carcinogenic damage of *Flinitrazepam*, a drug used for short-term insomnia treatment. Another variable analyzed by the MN test was the action of anabolic steroids through blood analysis. There was a higher occurrence of MN in bodybuilders who used anabolic agents (CUNHA; SOUZA; CRUZ, 2016) The occupation factor of these individuals was also analyzed, as demonstrated in the study by Annangi *et al.* (ANNANGI et al., 2016), in which cells from individuals exposed to Arsenio were analyzed by the MN test and presented a greater amount of binucleated cells. Another study suggests that the occupation of a frentist - due to exposure to benzene - can increase the amount of Mn, and this number is higher in frentists who ingest alcoholic beverages(MACIEL et al., 2019) .

In addition, the micronucleus test can also be used to assess the genotoxicity of the hydroxyurea (HU) drug, which is intended for the treatment of patients with sickle cell disease (DF). This drug shows positive results with a reduction in deaths and complications and an improvement in the quality of life of individuals, providing an increase in the concentration of fetal hemoglobin (HbF) in red blood cells and an improvement in nitric oxide metabolism, reducing endothelial interaction, episodes of pain, acute chest syndrome, hospitalizations and the need for blood transfusions. However, treatment with HU can lead to adverse reactions such as low neutrophil count, low platelet count, anemia, rash, headache, among others. Thus, for a better understanding of the genotoxicity of this drug, the monitoring of patients with DNA damage assessment methodologies is the most valid, which applies to the test, because through the assessment of DNA damage it can be investigated by the assay cytoma micronucleus blocking cytokinesis (CBMN-cit) in peripheral blood mononuclear cells, identifying genotoxicity (OLIVEIRA et al., 2019)

The micronucleus test proves to be of great value when it is desired to analyze a product exposure to a carcinogenic agent. The health professional can use this test to evaluate therapeutic measures, such as the use of drugs and devices, such as the impact of mechanics and the presence of endotracheal prosthesis in intubated patients. In addition, the test can be considered an indirect measure of prognosis.

In view of such practicality and competence of the micronucleus test, it is necessary to carry out further studies involving the most commonly used drugs at the moment, such as hydroxychloroquine, a drug considered as a supposed to alleviate the inflammatory process caused by the new coronavirus. due to scientific evidence that its use is ineffective and may cause adverse effects such as heart disease and retinopathy. Hydroxychloroquine being a medication directed to chronic diseases and also consumed

continuously by individuals with malaria and emphysematous lupus (GUERRA, 2018). Through a study with such patients, their genotoxicity in the organism would be highlighted and verified.

4 CONCLUSION

The micronucleus test is a safe form of assessment to quantify the impact of the genotoxic agent on cells, especially on exfoliated cells of the oral mucosa and blood cells. It is noticed that several techniques can be used in the preparation of the slides, being presented a variety of dyes and fixers, as well as materials for obtaining the cells. In addition, the MN test can be applied in several areas of health to analyze different carcinogens and thus contribute to cancer prevention. Through the micronucleus test it is possible to elucidate the harmful effects on human tissues and cells and to identify the genotoxicity of drugs that are used as a result by the population, such as hydroxychloroquine.

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