REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.11.e1650

ISSN 2007-3380

Review / Artículo de revisión

Micronuclei and nuclear abnormalities in oral epithelial cells: effective and simple tool in early detection of individuals highly susceptible to genomic instability

Micronúcleos y anormalidades nucleares en células epiteliales orales: herramienta eficaz y sencilla en la detección temprana de individuos altamente susceptibles a la inestabilidad genómica

Torres Bugarín, O.[1*], *Santiago Martínez, R.* [1], *González Barajas, A. J.* [1], *Ríos Esquivel, P. E.* [1], *Ramos Ibarra, M.L.*[2] *Arellano García, M.E.* [3*]

[1] *Laboratorio de Evaluación de Genotóxicos. Departamento de Medicina Interna II, Facultad de Medicina. Unidad Académica de Ciencias de la Salud, Universidad Autónoma de Guadalajara. Zapopan, Jalisco, México.*
[2]*Laboratorio de Toxicología Genética. Departamento de Salud Pública, División Ciencias Veterinarias, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, Jalisco, México.*
[3] *Laboratorio de Genotoxicología Ambiental, Facultad de Ciencias, Universidad Autónoma de Baja California, Ensenada, Baja California, 22860, México.*

Please cite this article as/Como citar este artículo: Torres Bugarín, O., Santiago Martínez, R., González Barajas, A. J., Ríos Esquivel, P. E, Ramos Ibarra, M.L., Arellano García, M.E. (2024). Micronuclei and nuclear abnormalities in oral epithelial cells: effective and simple tool in early detection of individuals highly susceptible to genomic instability. *Revista Bio Ciencias*, 11, e1650. https://doi.org/10.15741/revbio.11.e1650

Article Info/Información del artículo
Received/Recibido: February 26th 2024.
Accepted/Aceptado: May 21th 2024.
Available on line/Publicado: June 12th 2024.

ABSTRACT

Micronuclei can form in any dividing cell. These are small cytoplasmic bodies containing DNA that cause major pleiotropic problems. They are one of the most studied biomarkers of genotoxicity, instability, and genetic chaos. The micronuclear membrane is easily ruptured and when DNA is released into the cytoplasm, it then chronically stimulates the innate immune system, senescence, and apoptosis. DNA has the potential for massive rearrangements and, during mitosis or meiosis, to reincorporate into the nucleus and thus in a single event introduce multiple mutations to daughter cells, generating genomic instability and chaos; thus allowing them to rapidly become malignant. Specifically, the micronucleated cells of the oral epithelium are an early “internal sensor” for these types of impairment. If 90 % of all cancers are of epithelial origin, then the oral mucosa offers a unique, highly efficient, painless, easy-to-perform, and inexpensive opportunity to monitor at-risk individuals; it even allows the evaluation of genotoxicity and cytotoxicity biomarkers. Therefore, this review aims to highlight the causes and consequences of micronuclei and their applicability in oral mucosa for the timely detection of individuals highly susceptible to genomic instability.

KEY WORDS: Micronuclei, genotoxicity, genomic instability, susceptibility, oral epithelium.

***Corresponding Author:**

Olivia Torres-Bugarín. Laboratorio de Evaluación de Genotóxicos. Departamento de Medicina Interna II, Facultad de Medicina. Universidad Autónoma de Guadalajara. Montevideo Avenue, 3035, Zapopan. 44100, Zapopan, Jalisco, México. Teléfono: (333) 808 5766. E-mail: oliviatorres@hotmail.com. | **María Evarista Arellano-García**. Laboratorio de Genotoxicología Ambiental, Facultad de Ciencias. Universidad Autónoma de Baja California. Carretera Transpeninsular, 3916, Playitas. 22860, Ensenada, Baja California, México. Teléfono: [01 (686) 551-9497] E-mail: evarista.arellano@uabc.edu.mx.

Revista Bio ciencias
ISSN 2007-3380

RESUMEN

Los micronúcleos pueden formarse en cualquier célula en división. Estos son pequeños cuerpos citoplasmáticos que contienen DNA que causan grandes problemas pleiotrópicos. Son uno de los biomarcadores más estudiados de genotoxicidad, inestabilidad y caos genético. La membrana micronuclear se rompe con facilidad y al liberar al DNA al citoplasma, entonces este estimula crónicamente al sistema inmunitario innato, la senescencia y la apoptosis. El DNA tiene el potencial de reorganizaciones masivas y durante la mitosis o meiosis re-incorporarse al núcleo y así en un solo evento introducir múltiples mutaciones a las células hijas, generar inestabilidad y caos genómico; propiciando entonces que estas rápidamente se puedan malignizar. Específicamente, las células micronucleadas del epitelio oral son un “sensor interno” temprano a estos tipos de daño y si el 90 % de todos los cánceres son de origen epitelial, entonces la mucosa bucal ofrece una oportunidad única altamente eficiente, indolora, de fácil ejecución y económica para monitorear a individuos en riesgo; incluso permite evaluar otros biomarcadores de genotoxicidad y citotoxicidad. Por ello, el objetivo de esta revisión-opinión es destacar causas y consecuencias de los micronúcleos, y su aplicabilidad en mucosa oral en la detección oportuna de individuos altamente susceptibles a la inestabilidad genómica.

PALABRAS CLAVE: Micronúcleos, genotoxicidad, inestabilidad genómica, susceptibilidad, epitelio oral.

Introduction

DNA carries and transmits genetic information from generation to generation, and damage to this biomolecule causes cell degeneration that accompanies pathological processes of aging and apoptosis. Therefore, its imbalance has great consequences for both the functionality and the preservation of the cell and organisms.

Genomic instability is characterized by an increase in the normal rate of mutations, random, permanent, and heritable DNA changes in somatic or germ cells, which usually have deleterious effects on cellular functions (López-Gil *et al.,* 2023). It is a distinctive feature of some hereditary, chronic degenerative diseases such as autoimmune diseases and cancer, in which it defines their genomic composition and determines their behavior, aggressiveness, and treatment response (Chen *et al.,* 2022; López-Gil *et al.,* 2023; Di Bona & Bakhoum, 2024). The genomic instability causes can be genomic and epigenetic alterations, repair defects, replication stress, spindle disassembly, and micronucleus formation. While the association between genomic instability, cancer, and micronuclei has long been recognized, it is nowadays that the mechanisms leading

Revista Bio ciencias
ISSN 2007-3380

to micronuclei formation and their role in tumor progression are better understood (Di Bona & Bakhoum, 2024).

A biomarker is defined as "a characteristic at the cellular or molecular level that objectively measures and evaluates normal biological processes, pathogenic processes, or responses to an exposure or even therapeutic interventions". This is interpreted as an indicator of health status, life expectancy, or disease risk and is classified as exposure, effect, and susceptibility (Sauer *et al.,* 2018; Strimbu & Tavel, 2010).

- Biomarkers of exposure; allow the assessment of the presence of xenobiotics, metabolites, or by-products in an organism (Sauer *et al.,* 2018; Strimbu & Tavel, 2010).

- Effect biomarkers; assess the biochemical, physiological, or behavioral alterations produced in the organism as a result of xenobiotic exposure and are associated with pathological processes (Sauer *et al.,* 2018; Strimbu & Tavel, 2010).

- Susceptibility biomarkers; indicate individual sensitivity, which is the ability of an organism to respond to exposure to a foreign agent (Sauer *et al*., 2018; Strimbu & Tavel, 2010).

In this context, micronuclei, since they are derived from cytogenetic damage, are traditionally used as biomarkers of "*genotoxic effect*" and are perhaps the most widely used. However, due to their causes and consequences, they are also used as exposure and susceptibility biomarkers.

Micronuclei: characteristics

Among the most studied biomarkers of DNA damage and chromosomal instability are micronuclei, which are derived from chromosomal missegregation and therefore are small masses of nuclear DNA that are erroneously found in the cytoplasm, without connection to the nucleus. Through microscopy, they are observed as tiny bodies similar to the nucleus but smaller, hence their designation. Historically, they have been used as biomarkers of cytogenetic damage. However, it was recently discovered that they are an active structure that causes major pleiotropic complications [*pleio* (many) and *trepein* (influences)], as they activate chronic inflammatory signaling sensors and induce premature senescence, apoptosis, and genetic chaos (Lopez-Gil *et al.,* 2023; Chen *et al.,* 2022; Guo *et al.,* 2019; Krupina *et al.,* 2021). Therefore, assessing the micronucleated cells frequency in vulnerable populations to genotoxicants is a preventive, low-cost alternative, with many technical facilities to be applied to both individuals and populations at risk (Figure 1) (Fenech *et al.,* 2020; Torres-Bugarín & Arias-Ruiz, 2023).

Although the particular characteristics of micronuclei are very diverse, such as size, origin, chromatin condensation, presence of nuclear lamina, or gene expression, some general characteristics can define them (Shimizu, 2011; Schmid, 1975).

Definition: a micronucleus, or Howell-Jolly body, is a small chromatin body with a high probability of genomic rearrangement, erroneously located in the cytoplasm and enveloped in

Revista Bio ciencias
ISSN 2007-3380

an imperfect membrane. It has a similar texture, focal plane, and staining to the main nucleus, but as a rule, it is smaller and unlinked to the main nucleus; therefore, it is called a micronucleus (Schmid, 1975).

Size: less than one-third of the size of the main nucleus (Schmid, 1975).

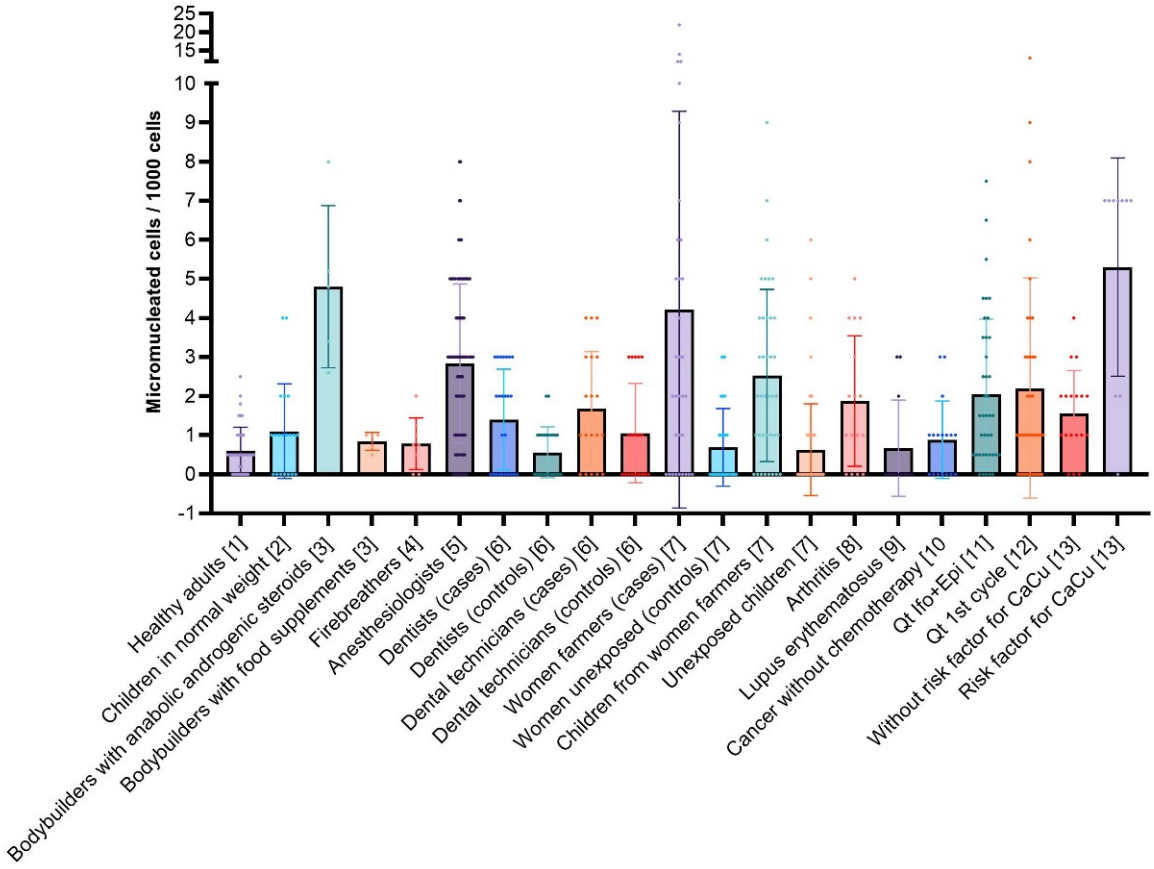

Figure 1. Frequency of micronucleated cells in different study groups.

Attention to values outside the mean, these are the cases of interest, as they are highly vulnerable individuals to genomic instability (QT-Chemotherapy, Ifo-Ifosfamide, Epi-Epirubicin, CaCu-Cervical Cancer). The number in square brackets is the data source. (Jara-Ettinger *et al*., 2015 [1]; Vizcarra, 2005 [2]; Torres-Bugarín *et al*., 2007 [3]; Torres-Bugarín *et al*.,1998 [4]; Torres-Bugarín *et al*., 2016 [5]; Molina-Noyola *et al*., 2019 [6]; Castañeda-Yslas *et al*., 2016 [7]; Ramos-Remus *et al*., 2002 [8]; Rodríguez-Vázquez *et al*., 2000 [9]; Torres-Bugarín *et al*., 2000 [10]; Torres-Bugarín *et al*., 2003 [11]; Torres-Bugarín *et al*., 2013c [12], Flores-García *et al*., 2018 [13]).

Origin: they can originate at any cell cycle stage, during mitosis or in pre-mitosis, post-mitosis, and even interphase (Guo *et al.,* 2019; Guo *et al*., 2020). The causes are various aneugenic or clastogenic agents, and the effects of these can be differentiated by the size of

Revista Bio ciencias
ISSN 2007-3380

the micronucleus and the centromere or kinetochore presence (Migliore *et al.,* 1996; Afshari *et al.,* 1994). Aneugenic agents cause anaphase delay of whole chromosomes, either by damage to the mitotic spindle, defect in sister chromatid cohesion, inadequate microtubule-kinetochore bonding, chromosome condensation defect, or abnormal cytokinesis. Clastogens cause the loss of acentric chromatin fragments by double-strand breaks (Schmid, 1975; Guo *et al.,* 2019). Other mechanisms described are repair errors, chromatin bridging during anaphase resulting in dicentric chromosomes (Shimizu, 2011), fragile sites that promote the formation of double-strand breaks and bridges (Li & Wu, 2020; Umnreit *et al.,* 2020), and gene amplification in interphase cells termed double-minutes. These circular extrachromosomal bodies lack centromeres and telomeres, can attach to daughter cell chromosomes, stably segregate, and are prevalent in cancer cells (Shimizu, 2011; Maass *et al.,* 2018).

Fate: once the micronucleus is formed, the genetic material it contains can be reorganized, and in subsequent cell cycles, it can be excluded from the cell or incorporated into the genome of daughter cells during the next cell division; even these phenomena could be intertwined (Kirsch-Volders *et al.,* 2020).

Chromatin: some micronuclei exhibit highly condensed chromatin, making expression or replication improbable (Maass *et al.,* 2018). These can form from chromatin bridges (Shimizu, 2011). Where chromatin is highly relaxed, transcription and replication could occur (Hintzsche *et al.,* 2017; Shimizu, 2011).

Membrane: the function of the nuclear membrane is to protect the genome; in contrast, the micronuclear membrane does not fulfill this function since it is very fragile and is destined to break without the possibility of being repaired. By its nature, it is highly unstable as it is not connected to the nucleus or the endoplasmic reticulum and presents major deficiencies of structural proteins and pores; these are generally disarticulated (Maass *et al.,* 2018). Due to its obvious fragility, DNA leakage into the cytoplasm is inexorable, a fact that plays a crucial role in pathological processes, senescence, and apoptosis (Guo *et al.,* 2019, 2020; Maass *et al.,* 2018).

Transport: since the pores do not function properly, transport cannot be regulated (Hintzsche *et al.,* 2017).

Proteasome: they are present only in some micronuclei but are not active due to the lack of processing subunits and ubiquitination factors (Maass *et al.,* 2018).

Gene expression and repair: in the micronucleus, gene expression and DNA repair are reduced, defective, and asynchronous. This is due in part to the significant decrease or absence of replication initiation factors and enzymes required for replication. Although there is no replication in micronuclei lacking lamins (Hintzsche *et al.,* 2017). Chromosomes that underwent genomic rearrangement are unevenly distributed among daughter cells, causing copy asymmetry (Hintzsche *et al.,* 2017; Zhang, 2015). Transcription has been observed only in micronuclei containing complete or double-minute-derived chromosomes (Shimizu, 2000).

Revista Bio ciencias
ISSN 2007-3380

Micronucleus test: methodologically, its evaluation is simple, and its application is very versatile. It allows cross-sectional as well as longitudinal studies, and its results are highly reliable and obtained in short periods. Another advantage is that, compared to other cytogenetic studies, the costs are relatively inexpensive (Torres-Bugarín *et al*., 2014a; Torres-Bugarín *et al*., 2015). It is used *in vitro* and *in vivo* in the laboratory or field to reveal genotoxic, mutagenic, and teratogenic effects. It is even a test protocol established by the Organization for Economic Cooperation and Development (OECD) (OECD, 2016).

Micronuclei: biological consequences

Although some of the mechanisms underlying the micronuclei formation are understood, and the elimination processes are being unraveled, their functional organization and consequences are still difficult to understand. The main problem lies in knowing the fate of the micronuclei, as their biological consequences. In general, these biomarkers disturb different cellular functions, which can trigger apoptosis, senescence, alterations in cell cycle checkpoints and repair systems, massive and chaotic DNA rearrangement, as well as cytoplasmic changes and overexpression of free radicals. They can also turn on and chronically activate the innate immune response and inflammatory processes. All this highlights the role of micronuclei in pathological processes (Guo *et al.,* 2019; Maass *et al.,* 2018; Terradas *et al.,* 2010).

Micronucleated cells may not represent a risk if the cell does not survive, but everything will be different if cell division continues. In any case, their fate differs according to the micronucleogenic inducer (clastogenic or aneugenic), their lineage (primary or tumor), the extent of genomic damage, the kinetochore functionality, or the micronuclear membrane. Thus, a micronucleus can be removed by extrusion or eliminated "*in situ*" by lysosomal or apoptotic-specific mechanisms. However, if it persists in the next division, two cells without or with micronucleus could originate (Maass, 2018; Shimizu, 2011).

On the other hand, if the micronucleus remains in the cytoplasm, it may prevent or delay the next mitosis. If the membrane is ruptured, then DNA escapes into the cytoplasm and triggers warning signals, such as chronic innate immune response and inflammation, or it may induce early autophagy, senescence, or apoptosis. Thus, the micronucleus itself could become a direct inducer of the process of controlling and eliminating micronucleated cells (Sommer *et al.,* 2020; Mohr *et al.,* 2021).

Another point is that micronuclear DNA can undergo massive rearrangements and significant genomic losses through chromoanagenesis (chromoplexy, chromothripsis, and chromosynthesis). During the next cell division, it could be reintegrated into metaphase and, at the end of mitosis, be included in the nucleus of the daughter cells, which would not be genetically identical. They would suffer chaos and genetic instability and have a high risk of malignancy. On the other hand, and no less important, is what happens with the sister cells that have lost genetic material given the micronuclei formation. If these cells are not eliminated, they will perpetuate cell lines with incomplete genetic material (Hintzsche *et al.,* 2017; Kirsch-Volders *et al.,* 2020; Yasui *et al.,* 2010).

Revista Bio ciencias
ISSN 2007-3380

Micronuclei: cytoplasmic DNA and innate immune response

The genetic material of eukaryotes is primarily located in the nucleus, mitochondria, or chloroplasts. Its cytosolic presence can occur due to damage in any of these organelles, rupture of the micronucleus membrane, or pathogenic invasion. Thus, DNA presence in the cytoplasm serves as a danger signal and is detected and eliminated by the innate immune system, particularly by the cGAS-STING (cyclic GMP-AMP synthase - STimulator of INterferon Genes; STING or INF) axis. This axis identifies invading pathogens or both foreign and self-double-stranded DNA (dsDNA). Specifically, the cGAS enzyme identifies and binds to cytosolic DNA, initiating the cell signaling process for immune activation that specifically targets and destroys this DNA. However, activation of the cGAS-STING signaling pathway leads to a proinflammatory immune response and prolonged activation of cGAS. This process induces the expression of a group of genes and increases interferons, interleukins IL-6, IL-8, and other chemokines associated with senescence and a high risk of autoimmune disorders (Hintzsche *et al.,* 2017; Kirsch-Volders *et al.,* 2020). If these cells are not detected and chemokine production is not resolved, the proinflammatory state persists, generating a vicious cycle of inflammation and oxidative damage that eventually affects DNA. Such damage induces micronuclei formation and activates chromoanagenesis. These events tend to increase with age, are gender-dependent, and contribute to autoimmune diseases, likely due to the inability of the immune system to distinguish self-released DNA and nuclear proteins associated with foreign antigens (Kirsch-Volders *et al.,* 2020).

Micronuclei: genomic instability

Genomic instability involves increased punctual, numerical, and structural changes and is characteristic of early tumorigenesis. It facilitates uncontrolled cell growth, cancer progression, and genetic heterogeneity, and is associated with antineoplastic resistance (Siri *et al.,* 2021). Cancer development occurs through mutation accumulation, which could occur progressively or all at once (Siri *et al.,* 2021; Levine & Holland, 2018). Micronuclei were once considered passive biomarkers of DNA damage, but today it is known that they are also promoters of chromosomal instability. These structures are prone to rearrangement and massive loss of genetic material. During cell division, they can incorporate into daughter nuclei, causing two cells to acquire multiple mutations simultaneously (Terradas *et al.,* 2016). Such events can occur spontaneously or after exposure to xenobiotics or stressors such as genetic, metabolic, psychosocial, environmental, or lifestyle factors (Figure 1) (Torres-Bugarín *et al.,* 2015; Ramos-Ibarra *et al.,* 2020). For example, micronucleated cells have been detected in day laborers and their children exposed to high pesticide concentrations (Castañeda-Yslas *et al.,* 2016), fire-eaters exposed to hydrocarbons (Torres-Bugarín *et al.,* 1998), welders exposed to heavy metal vapors (Jara-Ettinger *et al.,* 2015), dentists and dental technicians (Molina-Noyola *et al.,* 2019), anesthesiologists (Torres-Bugarín *et al.,* 2016), and bodybuilders who use anabolics (Torres-Bugarín *et al.,* 2007). Additionally, micronuclei have been identified in people with eating disorders such as bulimia and anorexia (Torres-Bugarín *et al.,* 2009; Torres-Bugarín *et al.,* 2014b). Similarly, they have been found in individuals suffering from various chronic degenerative diseases, including genomic instability syndromes such as Bloom syndrome (Gratia *et al.,* 2019), spinocerebellar ataxia type 2 (Cuello-Almaraes *et al.,* 2017), cancer (Wu & Lu, 2012; Flores-García *et al.,* 2014; Flores-García *et al.,*

Revista Bio ciencias
ISSN 2007-3380

2018), autoimmune diseases (Torres-Bugarín *et al.,* 2015; Mihaljevic *et al.,* 2018), renal diseases (Pastor *et al.,* 2018), neurodegenerative diseases (including Ataxia telangiectasia, Parkinson's disease, Alzheimer's, as well as Down, Cockayne, and Werner syndromes) (Petrozzi *et al.,* 2002; Migliore *et al.,* 2011; Thomas & Fenech, 2015), sickle cell disease (Naga *et al.,* 2016), cardiovascular diseases (Andreassi *et al.,* 2011), periodontitis (Borba *et al.,* 2019; Tadin *et al.,* 2019), Hunter-type mucopolysaccharidoses (Diaz-Jacques *et al.,* 2018), and polycystic ovary syndrome (Karataylı *et al.,* 2017), among many others.

Micronucleus testing: use of models, organs and tissues

For the application of the micronucleus test, there are multiple models and a wide range of organs and tissues, among which the following are prominent:

Erythrocytes from bone marrow and peripheral blood: the micronucleus technique was initially developed for use in bone marrow erythrocytes but was later modified to be applied in peripheral blood and other tissues. The use of bone marrow erythrocytes implies sacrificing the organism, limiting the test to laboratory species, or using samples obtained for diagnostic purposes. In contrast, the technique performed in peripheral blood can be applied without risking the life of an organism, as only a few drops of blood are required. This technique is easily reproducible and allows for cross-sectional and longitudinal studies in any vertebrate that meets the characteristics of a good biomarker. Both young erythrocytes (polychromatic) are counted to evaluate acute genotoxicity and cytotoxicity (myelosuppression), as well as normochromic erythrocytes, for chronic exposure (Fenech *et al.,* 2020; Kasamoto *et al.,* 2013).

L*ymphocyte culture with cytokinesis block (CBMN - cytokinesis-block micronucleus)*: this is a validated test used to evaluate mutagenicity in binucleated cells. It measures chromosomal breakage or loss, nucleoplasmic bridges, and nuclear buds (Fenech, 2020).

*Exfoliated epithelial cells: o*btaining samples from exfoliated epithelial cells is easily accessible, minimally invasive, and relatively low in cost. This method is applicable in non-keratinized stratified epithelia such as oral mucosa (cheeks, lips, gums, tongue), nasal passages, bladder, and cervix. Specifically, in oral mucosal tissue, micronuclei are formed in the dividing cells of the basal layer, which migrate to the surface over a period of 4 to 14 days. This migration timeline should be considered when interpreting results (Torres-Bugarín & Arias-Ruíz, 2023).

Other less commonly used organs and tissues

Skin: this technique allows for the assessment of *in vivo* and *in vitro* photogenotoxicity risks associated with exposure to chemical and physical agents. The EpiDerm™ model involves a human epidermis reconstructed from normal human keratinocytes cultured on a collagen matrix at the air-liquid interface. This model is histologically similar to the human epidermis *in vivo*. In this model, cultures are exposed to the agent being tested, and the replication rate is calculated in 500 cells to assess cytotoxicity and in 1000 binucleated cells to assess genotoxicity. For this purpose,

Revista Bio ciencias
ISSN 2007-3380

cells are harvested from the basal cell layer, resuspended, centrifuged, and smears are prepared with the cellular button for analysis (Kidd *et al.,* 2021).

*Colon and intestine: t*hese organs are exposed to numerous carcinogens through substances entering the organism via the food chain, such as pesticides, additives, preservatives, and drugs. Evaluation of associated risks with these substances is crucial. In this case, the micronucleus assay is applied to histological sections stained with hematoxylin and eosin. Columnar epithelial cells from the colon lumen are counted. Alternatively, the colon can be introduced into a hypotonic solution to separate epithelial cells from the smooth muscle through washes. The isolated cells are then suspended, centrifuged, and smears are prepared from the cell button. The frequency of micronucleated cells is set to at least 1,000 cells (Morita *et al.,* 2011; Ohyama, 2019; Fenech *et al.,* 1993).

Liver: the liver is highly responsive to carcinogens, but in adult animals, liver cells replicate very slowly. Therefore, the micronucleus assay is applied in organisms with partial hepatectomy or with hepatotoxic drugs to stimulate cell division. Alternatively, the liver of young rodents (5-6 weeks of age) with active cell division can be used. Hepatocytes are isolated, suspended, and centrifuged. The button is resuspended with buffer, and then cell spreads are made, fixing at least 1,000 hepatocytes, staining, and analyzing them through microscopy (Morita *et al.,* 2011; Uno *et al.,* 2015).

Spleen (reticulocytes - splenocytes): the spleen, being a lymphoid organ, is excellent for studying reticulocytes. The spleen is washed with phosphate-buffered saline with 10 % fetal bovine serum, and the cells are dissociated with this serum, then centrifuged at 800 rpm/5 min. For splenocytes, the spleen is removed, macerated, centrifuged, and cultured for subsequent analysis (Moore *et al.,* 1995; Shindo *et al.,* 1983).

Lung: It is one of the target organs of inhaled carcinogens, for its study the procedures are similar to those used in hepatocytes (Xi *et al*., 2021).

Micronuclei and nuclear abnormalities in exfoliated cells

Stich *et al* (1982) developed the protocol for the micronucleus assay in exfoliated oral mucosal cells to assess exposure to cellular stressors related to work and lifestyles (). Since then, this test has been widely used with great success and has become one of the most commonly employed tests. Its applications have even been extended to other mucosal tissues, albeit with varying degrees of success, including nasal, esophageal, bronchial, cervical, and bladder mucosa (Nersesyan *et al.,* 2006; Sommer *et al.,* 2020).

The advantages include, in parallel with the micronucleated cell count, that other complementary toxicological biomarkers can be evaluated (other nuclear abnormalities, figures 2 and 3). Besides, the collection process is minimally invasive and painless, minimizing bioethical implications. The methodology is relatively simple, not requiring cell cultures or specialized facilities. Sample storage is comparatively straightforward, and the analysis of cell spreads does

Revista Bio ciencias
ISSN 2007-3380

not necessitate extensive training time. Moreover, the results are highly reliable and can be obtained within short time frames. Furthermore, it is a relatively inexpensive methodology (Torres-Bugarín *et al.,* 2014; Sommer *et al.,* 2020). While this assay can be applied to all vertebrates, the vast majority of published articles focus on humans, with only a small percentage performed on other organisms (Benvindo-Souza *et al.,* 2017). Thus, the application of micronucleus testing and other nuclear abnormalities in exfoliated cells serves as a frequently used tool to assess damage to genetic material and thereby monitor populations at high risk. Consequently, the frequency of micronuclei and other nuclear abnormalities in exfoliated cells functions as an "internal dosimeter" to estimate chromosomal damage, identify genomic instability, genotoxicity, cytotoxicity, proliferation, and cell death (Benvindo-Souza *et al.,* 2017).

It's important to consider that the oral cavity serves as a vulnerable point or target for many exogenous agents due to direct contact, inhalation, or ingestion. It also reflects changes indicative of health or disease and can reveal systemic conditions or nutritional deficiencies. Additionally, it shows the side effects of certain drugs or drug addiction, some of which are significant public health problems. Specifically, the oral mucosal epithelium, being of ectodermal origin, presents unique advantages for the application of the micronucleus test. The oral mucosal epithelium is stratified and non-keratinized, with characteristics that include high proliferation and a low DNA repair rate (Hovhannisyan *et al.,* 2018; Holland *et al.,* 2008; Tolbert *et al.,*1991). Moreover, the epithelial cells are large enough to easily identify micronuclei and nuclear abnormalities, and upon desquamation, these cells retain the nucleus and are easily stained (Squier & Kremer, 2001).

In addition to normal cells, biomarkers of genotoxicity and cytotoxicity that can be assessed include lobulated nuclei reflecting DNA damage and binucleated cells originating from damage to cytokinesis. These additional markers provide valuable insights into cellular responses to countless stressors (Figure 2a-d).

Also, some cell death markers such as karyorrhexis, condensed chromatin, pyknosis and karyolysis (Figure 3a-d) can be assessed (Torres-Bugarín *et al*., 2013a; Torres-Bugarín *et al*., 2013b; Torres-Bugarín *et al*., 2014a; Bonassi *et al.,* 2011; Nersesyan, 2005).

Revista Bio ciencias
ISSN 2007-3380

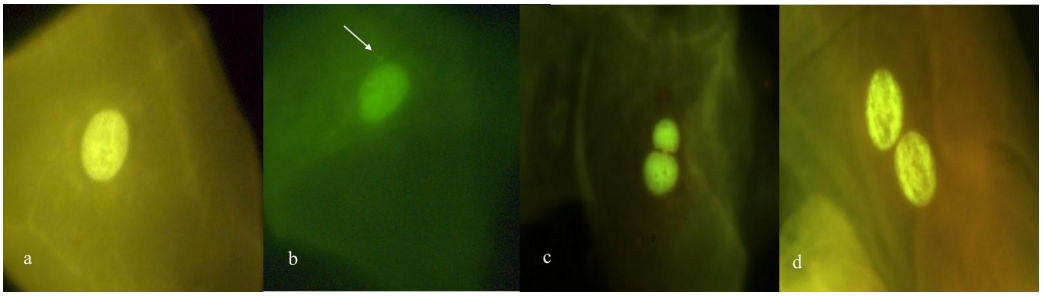

Figure 2. Exfoliated cells of oral mucosa.

a) Normal: These cells are completely differentiated, characterized by their large size, well-defined oval nucleus, and uniform staining. **b)** Micronucleated: These cells exhibit a main nucleus along with one or more smaller structures known as micronuclei. Micronuclei are typically between ⅓ and ⅙ the size of the main nucleus. They share similar staining intensity, texture, and focal plane with the main nucleus, without overlapping or bridging. **c)** Lobulated nucleus "broken egg": This anomaly is characterized by the presence of constrictions in the nucleus, with staining intensity between nuclear lobes being similar. **d)** Binucleate: These cells contain two nuclei of the same shape, staining, and focal plane, and they may be may be in contact. Due to alterations in cytokinesis. Stained with acridine orange, 100x optical magnification with Carl Zeiss IVFL Axiostar Plus microscope, 450-490 nm fluorescence filters. Microphotographs taken and donated by Olivia Torres Bugarín, PhD, from Laboratory of Evaluación de Genotóxicos. UAG.

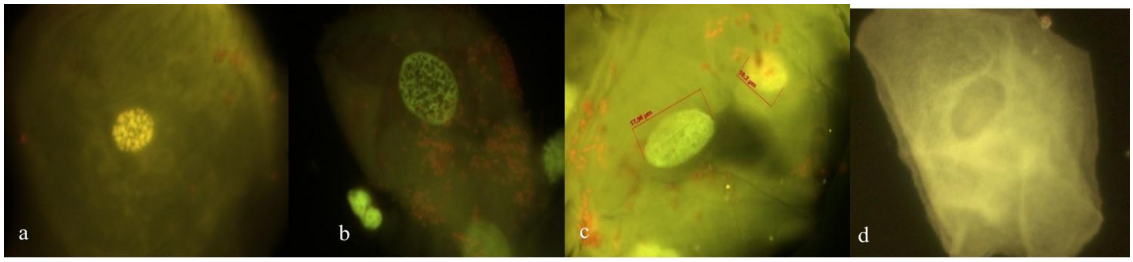

Figure 3. Exfoliated cells of oral mucosa.

a) Karyorrhexis: both the nucleus and the membrane are observed fragmented (like a puzzle whose pieces are dispersed), it is indicative of cell death. **b).** Condensed chromatin: the membrane is observed well defined, but the nucleus with agglutinated aggregates of chromatin and intensely stained. **c).** Pyknotic nucleus: nucleus smaller than a normal nucleus and more intensely stained. **d)** Karyolysis: cells with a nucleus devoid of genetic material (DNA), associated with cell death at a more advanced stage. Stained with acridine orange, 100x optical magnification with Carl Zeiss IVFL Axiostar Plus microscope, 450-490 nm fluorescence filters. Microphotographs taken and donated by Olivia Torres Bugarín, PhD, from Laboratory Genotoxics Evaluation. UAG.

Micronuclei: factors influencing their frequency

To study and validate the frequency of micronuclei in oral exfoliated cells as a biomarker for cancer risk in at-risk populations, the collaborative project "HUman MicroNucleus (HUMNXL)" was initiated. This project is an extension of the HUMN project, originally designed for the *in vitro* analysis

Revista Bio ciencias
ISSN 2007-3380

of binucleated lymphocytes. Among its objectives, the project aimed to identify confounding factors influencing the frequency of micronucleated cells, such as lifestyle, host factors, occupational exposures, and health status (Fenech *et al.,* 1999, Fenech *et al.,* 2011; Bonassi *et al.,* 2019). The results of this effort revealed that the spontaneous frequency of micronucleated cells (baseline values) in healthy individuals not exposed to genotoxic chemical agents or radiation is 0.74 % (95 % CI: 0.52-1.05), with a range between 0.3 % and 1.7 %. There were no spontaneous differences observed between sexes, and a notable trend showed that the frequency of micronucleated cells increased with age (*p*-trend test < *0.001*).

However, there is wide variability between populations, this fact must be considered. On the other hand, it has been observed that this biomarker is highly sensitive to occupational exposure. The frequency of micronucleated buccal cells is significantly higher in occupational groups exposed to solvents, polycyclic aromatic hydrocarbons (PAH), gasoline, arsenic, and antineoplastic drugs compared to the reference group. Regarding the effect of diseases, oncologic conditions, not necessarily confined to the head and neck area, have been significantly associated with a higher frequency of micronuclei. For oropharyngeal cancers, the frequency ratio (FR) was 1.72 (95 % CI: 1.19-2.49), for respiratory cancers it was 1.40 (95 % CI: 1.06-1.86), and for other oncologic diseases combined, it was 1.94 (95 % CI: 1.46-2.59). Smoking was found to induce significant genotoxicity when more than 40 cigarettes per day were consumed (FR = 1.37; 95 % CI 1.03-1.82). No association was detected between chewing tobacco or betel and the micronuclei frequency, although the univariate mean value of chewers was twice that of controls. Additionally, no association was found between alcohol consumption and the frequency of micronuclei. On the other hand, the consumption of fruits and green leafy vegetables has been shown to have cytoprotective effects. For consumers compared to non-consumers, the frequency of micronuclei was 1.17 % vs. 1.99 % for fruits and 1.80 % vs. 2.33 % for vegetables, respectively (Figure 1) (Fenech *et al.,* 1999, Fenech *et al.,* 1999, 2011; Bonassi *et al.,* 2019). Notwithstanding, the consequences of micronucleus formation generally remain uncertain (Terradas *et al.,* 2016).

Discussion

Genomic stability is crucial for the proper functioning and overall health of any organism. However, throughout life, we are continuously exposed to multiple genotoxicants, including xenobiotics or cellular metabolism byproducts. Often, these agents silently undermine genomic stability, and by the time the damage becomes evident, it may be irreversible. Moreover, the impact of genotoxicants varies between species and individuals, influenced by genetic, environmental, and lifestyle factors, among others (see Figure 1).

Identifying mutations that induce chromosomal instability or disease could serve as a diagnostic criterion for understanding pathological processes and guiding treatment decisions. The key question is how to promptly identify individuals who are more susceptible to genetic damage, thereby being at higher risk for developing oncological problems, complex diseases, or premature aging. Hence, there is an urgent need for easy-to-perform and relatively inexpensive biomarker-based techniques to identify those most vulnerable to genetic damage. The micronucleus test and

Revista Bio ciencias
ISSN 2007-3380

other nuclear abnormalities in buccal mucosa offer a promising solution. Widely used worldwide, this technique boasts numerous advantages, including methodological simplicity, minimal invasiveness, painlessness during sample collection, sensitivity, and cost-effectiveness. However, despite these benefits, it is important to highlight some considerations.

In situations where studies of at-risk populations are conducted to investigate genotoxic effects, statistical methods often struggle to specifically identify cases with micronucleus and nuclear abnormality values that exceed confidence intervals (CI = μ ± σ) or interquartile range (IR = m ± Q). Instead, descriptive measures such as means, standard deviations, or medians are typically calculated, along with measures of dispersion to illustrate the distribution variability. Relevant statistical tests like Student's t-test or Mann-Whitney U-test are then employed to determine if significant differences exist between study groups. Based on these tests, conclusions can be drawn regarding whether the object of study is genotoxic or has a cytoprotective effect, depending on the research objective.

However, a question arises concerning the fate of outlier cases, or "outliers," in each study group that occurs beyond the CI or IR, particularly those whose values exceed the mean plus one standard deviation or the third quartile. For instance, if no significant differences are detected between groups, can it be inferred that they have not experienced harm due to the absence of statistical effects ($p < 0.05$)?

Indeed, there is no doubt that "outliers" can significantly impact the results of a statistical analysis or may even indicate data errors, such as incorrect measurements or data entry. Therefore, identifying and addressing outliers can enhance data quality and prevent misleading conclusions. However, it is crucial to consider that the handling of outliers depends on the context and objectives of the statistical analysis. In some cases, eliminating or correcting them may be appropriate, while in other cases, they may be of interest and should be analyzed in detail. In many cases, outliers in the biomedical field may contain additional information or represent exceptional or unique situations in the data. Rather than eliminating them, their inclusion in the analysis may provide a more complete understanding of the phenomena studied. This underscores the importance of interpreting outliers with care and considering them in the appropriate clinical and scientific context. For instance, outliers may indicate the presence of rare genetic mutations or unique genetic variants associated with specific diseases or conditions in clinical trials and drug efficacy studies. They may also signal an unexpected or exceptional response to treatment, prompting further investigation into variability in patient response to certain treatments. Additionally, outliers in pharmacovigilance and adverse event detection may indicate unusual or serious reactions to drugs or therapies. By identifying these outliers, researchers can uncover rare but significant side effects and enhance the safety and efficacy of medical treatments (Guan & Tibshirani., 2022; Tibshirani, & Hastie, 2007).

Hence, it is precisely these cases that now draw our attention (Figure 1). A question arises because, in this type of study, each point particularly represents a person in whom the damage indeed exists, and the genomic instability in these individuals is real; they are highly susceptible people who require special attention. Although the effects that the instability of the

Revista Bio ciencias
ISSN 2007-3380

hereditary material may generate in the future are unknown with precision, it is known that the risk of carcinogenesis is greater, as well as the development of chronic degenerative diseases. These individuals may not be given the importance that is required, as their data is missing in a statistical analysis, which expresses the general behavior of a population.

Therefore, these study subjects are the ones that should be well-thought-out as perfect candidates to take preventive measures since the number of micronuclei is a biomarker that can be modified through different preventive measures and healthy habits, such as supplementation with different antioxidants like folic acid (Gómez-Cabrera *et al.,* 2024; Zúñiga-González *et al.,* 2007). This finding becomes transcendent because of the application of the use of these biomarkers not only in research projects but also in clinical practice.

Thus, micronucleus frequency as a predictive biomarker of genomic instability and its pathological consequences is also useful for monitoring the efficacy and safety of interventions aimed at improving genomic health. For example, micronutrient deficiency or excess can have modifying effects on genomic integrity, since, micronutrients provide cofactors necessary for the proper function of enzymes involved in DNA repair, detoxification, or maintenance of genome methylation (Fenech *et al*., 2021; Bonassi & Fenech, 2021). Therefore, antioxidant supplementation, dietary and lifestyle interventions, and other epigenetic factors can contribute to reducing, protecting, or favorably modifying genome stability, and should even be a priority in public health since prevention is the best intervention. It is known that in some processes, the frequency of micronucleated cells is modifiable through different strategies such as the antioxidants use. For example, patients with Parkinson's disease characterized by high levels of oxidative stress who were given l-dopa and carbidopa showed potent antimicronucleogenic effects (Colamartino *et al.,* 2017). Similarly, folic acid supplementation (516 micrograms/day) in menopausal women significantly reduced micronucleus frequency (p = 0.010) (Titenko-Holland *et al.,* 1998), as did supplementation with 5 mg/3 times per day in patients with diabetes mellitus, where genotoxic damage was reduced by 50 % (Gómez-Meda *et al.,* 2016; Zúñiga-González *et al.,* 2007; Müllner *et al.,* 2014). Even under hemodialysis treatment, similar effects are observed with Brazil nut (*Bertholletia excelsa*) consumption (Macan *et al.,* 2024). This effect was also determined with the use of statins (5-10 mg/day rosuvastatin or 10-20 mg/day atorvastatin) in dyslipidemic patients (Donmez-Altuntas *et al.,* 2019). Additionally, in the case of patients with obesity who underwent bariatric surgery, the frequency of micronucleated lymphocytes decreased, along with weight, with the additional benefit of a decreased frequency of apoptosis and increased mitotic index (Bankoglu *et al.,* 2018). For patients infected with *Schistosoma haematobium* and treated with praziquantel (40 mg/kg/body weight), this drug was identified to decrease the frequency of urothelial exfoliated micronucleated cells 8-fold (Anwar & Rosin, 1993). On the other hand, the treatment of mitochondrial diseases with ubidecarenone, a coenzyme Q10 analog, decreased the frequency of micronucleated lymphocytes by 50 % (Migliore *et al.,* 2004). Similarly, in the case of chronic kidney disease, treatment with unfermented grape juice was observed to reduce genomic damage (Corredor *et al.,* 2016). Additionally, benfotiamine reduced genomic damage in peripheral lymphocytes of hemodialysis patients (Schupp *et al.,* 2008), while the use of alpha-tocopherol in the preventive treatment of oral leukoplakia showed efficacy (Benner *et al.,* 1994). Furthermore,

Revista Bio ciencias
ISSN 2007-3380

beta-carotene supplementation reduced micronuclei in patients with oral lichen planus (Buajeeb *et al.,* 2008).

In this context, it is crucial to highlight that early detection of vulnerable individuals is a fundamental element. Identifying these individuals not only represents a unique opportunity to apply preventive medicine but also offers the possibility of intervening proactively in their health. The evidence available so far suggests that it is feasible to modify micronucleated cell frequencies using a variety of agents. This finding underscores the importance of identifying susceptible individuals while opening the door to the implementation of personalized and strategic interventions that could mitigate or prevent adverse effects. Ultimately, these findings provide a deeper understanding of the underlying mechanisms and allow us to more effectively address challenges related to the health and well-being of the at-risk population.

Conclusions

Despite the diversity of factors contributing to the variability in the frequency of micronuclei and nuclear abnormalities, including genetic, health conditions, metabolic factors, environmental influences, lifestyle choices, and methodological considerations such as data collection and sample processing techniques, micronucleus testing in oral mucosa tissue remains an effective biomarker for identifying vulnerable individuals. This testing method holds promise for detecting individuals susceptible to genotoxicity and cytotoxicity, with significant potential for modification through interventions such as antioxidant supplementation and the adoption of healthier lifestyle habits.

Author contribution

Work conceptualization, drafting, and supervision of the manuscript, OTB; drafting, revising, and editing of the manuscript, MEAG; writing, manuscript preparation, and literature search, RSM, AJGB, PERE, MLRI.

All authors of this manuscript have read and accepted the published version of the manuscript.

Acknowledgments

To CONAHCYT - Programa Estancia posdoctoral por México 2023, Academic Modality (Registration: 19104). To the Autonomous University of Guadalajara (UAG) and the Autonomous University of Baja California (UABC) for all the facilities and support received for the postdoctoral stay.

Revista Bio ciencias
ISSN 2007-3380

Conflict of interest

The authors declare that they have no conflict of interest.

References

Afshari, A.J., McGregor, P.W., Allen, J.W., & Fuscoe, J.C. (1994). Centromere analysis of micronuclei induced by 2-aminoanthraquinone in cultured mouse splenocytes using both a gamma-satellite DNA probe and anti-kinetochore antibody. *Environmental and Molecular Mutagenesis*, *24* (2), 96-102. https://doi.org/10.1002/em.2850240204

Andreassi, M.G., Barale, R., Lozzo, P., & Picano, E. (2011). The association of micronucleus frequency with obesity, diabetes and cardiovascular disease. *Mutagenesis*, *26* (1), 77-83. https://doi.org/10.1093/mutage/geq077

Anwar, W.A., & Rosin, M.P. (1993). Reduction in chromosomal damage in schistosomiasis patients after treatment with praziquantel. *Mutation Research, 298* (3), 179-185. https://doi.org/10.1016/0165-1218(93)90039-G

Bankoglu, E.E., Arnold, C., Hering, I., Hankir, M., Seyfried, F., & Stopper, H (2018). Decreased chromosomal damage in lymphocytes of obese patients after bariatric surgery. *Scientific Reports*, 8 (1): 6–13. https://doi.org/10.1038/s41598-018-29581-6

Benner, S.E., Wargovich, M.J., Lippman, S.M., Fisher, R., Velasco, M., Winn, R.J., & Hong, W.K. (1994). Reduction in oral mucosa micronuclei frequency following alpha-tocopherol treatment of oral leukoplakia. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*, *3* (1), 73-76. PMID: 8118389.

Benvindo-Souza, M., Assis, R.A., Oliveira, E.A.S., Borges, R.E., & Santos, L.R.D.S. (2017). The micronucleus test for the oral mucosa: global trends and new questions. *Environmental Science and Pollution Research*, *24*, 27724-27730. https://doi.org/10.1007/s11356-017-0727-2

Bolologensi & Fenech, M. (2019). Micronucleus cytome assays in human lymphocytes and buccal cells. *Genotoxicity Assessment: Methods and Protocols*, 147-163. https://doi.org/10.1007/978-1-4939-9646-9_8.

Bonassi, S., & Fenech, M. (2021). Roadmap for translating results from the micronucleus assay into clinical practice: From observational studies to randomized controlled trials. *Mutation Research*, *788*, 108390. https://doi.org/10.1016/j.mrrev.2021.108390

Bonassi, S., Coskun, E., Ceppi, M., Lando, C., Bolognesi, C., Burgaz, S., Holland, N., Kirsh-Volders, M., Knasmueller, S., Zeiger, E., Carnesoltas, D., Cavallo, D., da Silva, J., de Andrade, V.M., Demircigil, G.C., Domínguez-Odio, A., Donmez-Altuntas, H., Gattas, G., Giri, A., Giri, S., *et al*., & Fenech, M (2011). The HUman MicroNucleus project on eXfoLiated buccal cells (HUMNXL): The role of life-style, host factors, occupational exposures, health status, and assay protocol. *Mutation Research*, 728 (3), 88-97. https://doi.org/10.1016/j.mrrev.2011.06.005

Borba, T.T., Molz, P., Schlickmann, D.S., Santos, C., Oliveira, C.F., Prá, D., Neto, L.K., & Franke, S.I.R. (2019) Periodontitis: Genomic instability implications and associated risk factors. Mutat Res Genet Toxicol Environ Mutagen. Apr;840:20-23. https://doi.org/10.1016/j.mrgentox.2019.01.005

Buajeeb, W., Kraivaphan, P., Amornchat, C., & Suthamajariya, K. (2008). Reduction of micronuclei in oral lichen planus supplemented with beta-carotene. Journal of oral science, 50(4), 461-467. https://doi.org/10.2334/josnusd.50.461

Castañeda-Yslas, I.J., Arellano-García, M.E., García-Zarate, M. A., Ruíz-Ruíz, B., Zavala-Cerna, M. G., & Torres-Bugarín, O. (2016). Biomonitoring with micronuclei test in buccal cells of female farmers and children exposed to pesticides of Maneadero Agricultural Valley, Baja California, México. *Journal of Toxicology*. 2016:2016:7934257. https://doi.org/10.1155/2016/7934257

Chen, M., Linstra, R., & van Vugt, M.A. (2022). Genomic instability, inflammatory signaling and response to cancer immunotherapy. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1877* (1), 188661. https://doi.org/10.1016/j.bbcan.2021.188661

Colamartino, M., Duranti, G., Ceci, R., Sabatini, S., Testa, A., & Cozzi, R. (2018). A multi-biomarker analysis of the antioxidant efficacy of Parkinson's disease therapy. *Toxicology, in vitro*, *47*, 1-7. https://doi.org/10.1016/j.tiv.2017.10.020

Corredor, Z., Rodríguez-Ribera, L., Coll, E., Montañés, R., Diaz, J. M., Ballarin, J., Marcos, R., & Pastor, S. (2016). Unfermented grape juice reduce genomic damage on patients undergoing hemodialysis. *Food and Chemical Toxicology*, *92*, 1-7. https://doi.org/10.1016/j.fct.2016.03.016

Cuello-Almarales, D.A., Almaguer-Mederos, L.E., Vázquez-Mojena, Y., Almaguer-Gotay, D., Zayas-Feria, P., Laffita-Mesa, J.M., Cuello-Almarales, D.A., Almaguer-Mederos, L.E., Vázquez-Mojena, Y., Almaguer-Gotay, D., Zayas-Feria, P., Laffita-Mesa, J.M., González-Zaldívar, Y., Aguilera-Rodríguez, R., Rodríguez-Estupiñán, A., Velázquez-Pérez, L. & (2017). Buccal cell micronucleus frequency is significantly elevated in patients with spinocerebellar ataxia type 2. *Archives of Medical Research*, 48 (3), 297-302. https://doi.org/10.1016/j.arcmed.2017.06.008

Di Bona, M. & Bakhoum, S.F. (2024). Micronuclei and Cancer. Cancer Discovery, 4 (2):214-226. https://doi.org/10.1158/2159-8290.CD-23-1073

Diaz-Jacques, C.E., de Souza, H.M., Sperotto, N.D.M., Veríssimo, R.M., da Rosa, H.T., Moura, D.J., Saffi, J., Giugliani, R., & Vargas, C.R. (2018). Hunter syndrome: Long-term idursulfase treatment does not protect patients against DNA oxidation and cytogenetic damage. Mutat Res Genet Toxicol Environ Mutagen. 835:21-24. https://doi.org/10.1016/j.mrgentox.2018.08.013

Donmez-Altuntas, H., Bayram, F., Coskun-Demirkalp, A.N., Baspınar, O., Kocer, D., & Toth, P.P. (2019). Therapeutic effects of statins on chromosomal DNA damage of dyslipidemic patients. *Experimental Biology and Medicine*, *244* (13), 1089-1095. https://doi.org/10.1177/1535370219871895

Fenech, M. (2020). Cytokinesis-block micronucleus cytome assay evolution into a more comprehensive method to measure chromosomal instability. *Genes*, *11*(10), 1203. https://doi.org/10.3390/genes11101203

Fenech, M., & Neville, S. (1993). Effect of cooked meat on micronucleus frequency. *Food and Chemical Toxicology*, *31* (5), 337-342. https://doi.org/10.1016/0278-6915(93)90188-5

Revista Bio ciencias
ISSN 2007-3380

Fenech, M., Holland, N., Chang, W. P., Zeiger, E., & Bonassi, S. (1999). The HUman MicroNucleus Project—an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 428(1-2), 271-283. https://doi.org/10.1016/S1383-5742(99)00053-8

Fenech, M., Holland, N., Zeiger, E., Chang, W.P., Burgaz, S., Thomas, P., Bolognesi, C., Knasmueller, S., Kirsch-Volders, M., & Bonassi, S. (2011). The HUMN and HUMNxL international collaboration projects on human micronucleus assays in lymphocytes and buccal cells—past, present and future. Mutagenesis, 26(1), 239-245. https://doi.org/10.1093/mutage/geq051

Fenech, M., Knasmueller, S., Bolognesi, C., Holland, N., Bonassi, S., & Kirsch-Volders, M. (2020). Micronuclei as biomarkers of DNA damage, aneuploidy, inducers of chromosomal hypermutation and as sources of pro-inflammatory DNA in humans. Mutation Research Reviews, 786:108342. https://doi.org/10.1016/j.mrrev.2020.108342

Fenech, M., Knasmueller, S., Knudsen, L.E., Kirsch-Volders, M., Deo, P., Franzke, B., Stopper, H., Andreassi, M.G., Bolognesi, C., Dhillon, V.S., Laffon, B., Wagner, K.H, & Bonassi, S. (2021). "Micronuclei and Disease" special issue: Aims, scope, and synthesis of outcomes. *Mutation Research, 788*, 108384. https://doi.org/10.1016/j.mrrev.2021.108384

Flores-García, A., Ruiz-Bernés, S., Aguiar-García, P., Benítez-Guerrero, V., Valle-Solís, M.O., Molina-Noyola, L.D., & Torres-Bugarín, O. (2018). Micronúcleos y anormalidades nucleares en células de la mucosa bucal de mujeres mexicanas con factores de riesgo para cáncer cervicouterino: estudio piloto. *El Residente, 13* (2), 56-61. https://www.medigraphic.com/pdfs/residente/rr-2018/rr182c.pdf

Flores-García, A., Torres-Bugarín, O., Velarde-Félix, J.S., Rangel-Villalobos, H., Zepeda-Carrillo, E.A., Rodríguez-Trejo, A. & Nersesyan, A. (2014). Micronuclei and other nuclear anomalies in exfoliated buccal mucosa cells of Mexican women with breast cancer. *Jounal of the Balkan Union of Oncology*, *19* (4), 895-9. https://www.jbuon.com/archive/19-4-895.pdf.

Gómez Cabrera A.S., González-Santiago A.E., Rodríguez-Mora J.F., Zúñiga-González G.M., Gómez-Meda B.C., Baptista Rosas R.C., Castañeda Arellano R., Roldan Mercado-Sesma A., Yareni Zúñiga L., & Sánchez-Parada M.G. (2024). Amelioration of cytogenotoxic damage in drug abusers supplemented with folic acid. Biomedicines, 12(2), 1-15. https://doi.org/10.3390/biomedicines12020352

Gómez-Meda, B.C., Zamora-Perez, A.L., Muñoz-Magallanes, T., Sánchez-Parada, M.G., Bañuelos, J.G., Guerrero-Velázquez, C., & Zúñiga-González, G.M. (2016). Nuclear abnormalities in buccal mucosa cells of patients with type I and II diabetes treated with folic acid. *Mutation Research, 797*, 1-8. https://doi.org/10.1016/j.mrgentox.2015.12.003

Gratia, M., Rodero, M. P., Conrad, C., Bou Samra, E., Maurin, M., Rice, G.I., Duffy, D., Revy, P., Petit, F., Dale, R.C., Crow, Y.J., Amor-Gueret, M., & Manel, N. (2019). Bloom syndrome protein restrains innate immune sensing of micronuclei by cGAS. *Journal of Experimental Medicine*, 216 (5), 1199-1213. https://doi.org/10.1084/jem.20181329

Guan, L., & Tibshirani, R. (2022). Prediction and outlier detection in classification problems. *Journal of the Royal Statistical Society Series B: Statistical Methodology*, 84(2), 524-546. https://doi.org/10.1111/rssb.12443

Guo, X., Dai, X., Wu, X., Zhou, T., Ni, J., Xue, J., & Wang, X. (2020). Understanding the birth

of rupture-prone and irreparable micronuclei. *Chromosoma*, *129*, 181-200. https://doi.org/10.1007/s00412-020-00741-w

Guo, X., Ni, J., Liang, Z., Xue, J., Fenech, M. F., & Wang, X. (2019). The molecular origins and pathophysiological consequences of micronuclei: New insights into an age-old problem. *Mutation Research, 779*, 1-35. https://doi.org/10.1016/j.mrrev.2018.11.001

Hintzsche, H., Hemmann, U., Poth, A., Utesch, D., Lott, J., & Stopper, H. (2017). Fate of micronuclei and micronucleated cells. *Mutation Research, 771*, 85-98. https://doi.org/10.1016/j.mrrev.2017.02.002

Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., Knasmueller, S., & Fenech, M. (2008). The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. *Mutation Research/Reviews in Mutation Research*, *659* (1-2), 93-108. https://doi.org/10.1016/j.mrrev.2008.03.007

Hovhannisyan, G., Harutyunyan, T., & Aroutiounian, R. (2018). Micronuclei and what they can tell us in cytogenetic diagnostics. *Current Genetic Medicine Reports*, *6*, 144-154. https://doi.org/10.1007/s40142-018-0149-6

Jara-Ettinger, A.C., López-Tavera, J.C., Zavala-Cerna, M.G., & Torres-Bugarín, O. (2015). Genotoxic evaluation of Mexican welders occupationally exposed to welding-fumes using the micronucleus test on exfoliated oral mucosa cells: a cross-sectional, case-control study. *PLoS One*, *10*(8), e0131548. https://doi.org/10.1371/journal.pone.0131548

Karataylı, R., Zamani, A.G., Gezginç, K., Tuncez, E., Soysal, S., Karanfil, F., Soysal, S., Karanfil, F., Acar, A., & Yıldırım, M.S. (2017). Micronuclei frequencies in lymphocytes and cervical cells of women with polycystic ovarian syndrome. *Turkish Journal of Obstetrics and Gynecology*, *14*(3), 151. https://doi.org/10.4274%2Ftjod.10734

Kasamoto, S., Masumori, S., & Hayashi, M. (2013). *In vivo* micronucleus assay in mouse bone marrow and peripheral blood. *Genotoxicity Assessment: Methods and Protocols*, 179-189. https://doi.org/10.1007/978-1-62703-529-3_9

Kidd, D., Phillips, S., Chirom, T., Mason, N., Smith, R., Saul, J., Whitwell, J., & Clements, J. (2021). The 3D reconstructed skin micronucleus assay: considerations for optimal protocol design. *Mutagenesis*, 36 (1), 37-49. https://doi.org/10.1093/mutage/gez037

Kirsch-Volders, M., Bolognesi, C., Ceppi, M., Bruzzone, M., & Fenech, M. (2020). Micronuclei, inflammation and auto-immune disease. *Mutation Research*, 786, 108335. https://doi.org/10.1016/j.mrrev.2020.108335

Krupina, K., Goginashvili, A., & Cleveland, D.W. (2021). Causes and consequences of micronuclei. *Current opinion in cell biology*, 70, 91-99. https://doi.org/10.1016/j.ceb.2021.01.004

Levine, M.S., & Holland, A.J. (2018). The impact of mitotic errors on cell proliferation and tumorigenesis. *Genes & development*, *32*(9-10), 620-638. http://www.genesdev.org/cgi/doi/10.1101/gad.314351.118

Li, S., & Wu, X. (2020). Common fragile sites: protection and repair. *Cell & Bioscience*, *10*, 1-9. https://doi.org/10.1186/s13578-020-00392-5

López-Gil, L., Pascual-Ahuir, A., & Proft, M. (2023). Genomic instability and epigenetic changes during aging. *International Journal of Molecular Sciences*, *24* (18), 14279. https://doi.org/10.3390/ijms241814279

Revista Bio ciencias
ISSN 2007-3380

Maass, K.K., Rosing, F., Ronchi, P., Willmund, K. V., Devens, F., Hergt, M., Herrmann, H., Lichter, P., & Ernst, A. (2018). Altered nuclear envelope structure and proteasome function of micronuclei. *Experimental Cell Research*, *371* (2), 353-363. https://doi.org/10.1016/j.yexcr.2018.08.029

Macan, T.P., Magenis, M.L., Damiani, A.P., de Oliveira Monteiro, I., Silveira, G.D.B., Zaccaron, R., Silveira, P.C.L., Teixeira, J.P.F., Gajski, G., & Andrade, V.M. (2024). Brazil nut consumption reduces DNA damage in overweight type 2 diabetes mellitus patients. *Mutation Research/ Genetic Toxicology and Environmental Mutagenesis*, 2024 Apr:895:50373. https://doi.org/10.1016/j.mrgentox.2024.503739

Migliore, L., Cocchi, L., & Scarpato, R. (1996). Detection of the centromere in micronuclei by fluorescence in situ hybridization: its application to the human lymphocyte micronucleus assay after treatment with four suspected aneugens. *Mutagenesis*, *11* (3), 285-290. https://doi.org/10.1093/mutage/11.3.285

Migliore, L., Coppede, F., Fenech, M., & Thomas, P. (2011). Association of micronucleus frequency with neurodegenerative diseases. *Mutagenesis*, *26* (1), 85-92. https://doi.org/10.1093/mutage/geq067

Migliore, L., Molinu, S., Naccarati, A., Mancuso, M., Rocchi, A., & Siciliano, G. (2004). Evaluation of cytogenetic and DNA damage in mitochondrial disease patients: effects of coenzyme Q10 therapy. *Mutagenesis*, *19* (1), 43-49. https://doi.org/10.1093/mutage/geg036

Mihaljevic, O., Zivancevic-Simonovic, S., Milosevic-Djordjevic, O., Djurdjevic, P., Jovanovic, D., Todorovic, Z., Grujicic, D., Radovic-Jakovljevic, M., Tubic, J., Markovic, A., Paunovic, M., Stanojevic-Pirkovic, M., & Markovic, S. (2018). Apoptosis and genome instability in children with autoimmune diseases. *Mutagenesis*, *33* (5-6), 351-357. https://doi.org/10.1093/mutage/gey037.

Mohr, L., Toufektchan, E., von Morgen, P., Chu, K., Kapoor, A., & Maciejowski, J. (2021). ER-directed TREX1 limits cGAS activation at micronuclei. Mol Cell. 18;81(4):724-738.e9. https://doi.org/10.1016/j.molcel.2020.12.037

Molina-Noyola, L., Coronado-Romo, M., Vázquez-Alcaraz, S., Izaguirre-Perez, M., Arellano-García, E., & Flores-García, A. (2019). Evaluation of genotoxicity and cytotoxicity amongst in dental surgeons and technicians by micronucleus assay. *Dental, Oral and Craniofacial Research*. 5: 1-5. https://doi.org/10.15761/docr.1000296.

Moore, F.R., Urda, G.A., Krishna, G., & Theiss, J.C. (1995). An *in vivo*/*in vitro* method for assessing micronucleus and chromosome aberration induction in rat bone marrow and spleen 1. Studies with cyclophosphamide. *Mutation Research, 335* (2), 191-199. https://doi.org/10.1016/0165-1161(95)90055-1

Morita, T., MacGregor, J.T., & Hayashi, M. (2011). Micronucleus assays in rodent tissues other than bone marrow. *Mutagenesis*, *26* (1), 223-230. https://doi.org/10.1093/mutage/geq066

Müllner, E., Brath, H., Nersesyan, A., Nitz, M., Petschnig, A., Wallner, M., Knasmüller, S., & Wagner, K.H. (2014). Nuclear anomalies in exfoliated buccal cells in healthy and diabetic individuals and the impact of a dietary intervention. *Mutagenesis*, 29 (1), 1-6. https://doi.org/10.1093/mutage/get056

Naga, M.B.S.S., Gour, S., Nallagutta, N., Ealla, K.K.R., Velidandla, S., & Manikya, S. (2016). Buccal micronucleus cytome assay in sickle cell disease. *Journal of Clinical and Diagnostic Research*, 10 (6), ZC62. https://doi.org/10.7860%2FJCDR%2F2016%2F19984.7998

Nersesyan, A., Kundi, M., Atefie, K., Schulte-Hermann, R., & Knasmuller, S. (2006). Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. *Cancer Epidemiology Biomarkers & Prevention*, *15* (10), 1835-1840. https://doi.org/10.1158/1055-9965.EPI-06-0248

Nersesyan, A.K. (2005). Nuclear buds in exfoliated human cells. *Mutation Research*, *588* (1), 64-68. https://doi.org/10.1016/j.mrgentox.2005.06.010

OECD. (2016), *Test No. 474: Mammalian erythrocyte micronucleus test*, OECD guidelines for the testing of chemicals, section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264762-en

Ohyama, W. (2019). Markedly enhanced micronucleus induction by 1, 2-dimethylhydrazine dihydrochloride in colonic cells of rats with bacterial colonization in the intestine. *Mutation Research*, *838*, 1-8. https://doi.org/10.1016/j.mrgentox.2018.11.007

Pastor, S., Rodríguez-Ribera, L., Corredor, Z., da Silva Filho, M.I., Hemminki, K., Coll, E., Försti, A., & Marcos, R. (2018). Levels of DNA damage (Micronuclei) in patients suffering from chronic kidney disease. Role of GST polymorphisms. *Mutation*, 836(Pt A):41-46. https://doi.org/10.1016/j.mrgentox.2018.05.008

Petrozzi, L., Lucetti, C., Scarpato, R., Gambaccini, G., Trippi, F., Bernardini, S., Del Dotto, P., Migliore, L., & Bonuccelli, U.(2002). Cytogenetic alterations in lymphocytes of Alzheimer's disease and Parkinson's disease patients. *Neurological Sciences*, 23, s97-s98. https://doi.org/10.1007/s100720200087

Ramos-Ibarra, M., Villa-Castellanos, J., Barba-León, J., Flores-Valdez, M., Zavala-Aguirre, L., & Torres Bugarín, O. (2020). Estudio exploratorio de la genotoxicidad de vacunas recombinantes para tuberculosis bovina. *Abanico Veterinario*, *10*. https://doi.org/10.21929/abavet2020.8

Ramos-Remus, C., Dorazco-Barragan, G., Aceves-Avila, F. J., Alcaraz-Lopez, F., Fuentes-Ramirez, F., Michel-Diaz, J., Torres-Bugarín, O., Ventura-Aguilar, A., & Zuñiga-Gonzállez, G. (2002). Genotoxicity assessment using micronuclei assay in rheumatoid arthritis patients. *Clinical and Experimental Rheumatology*, *20* (2), 208-212. PMID: 12051400

Rodríguez-Vázquez, M., Sánchez Ortiz, A., Ramos Remus, C., Zúñiga, G., & Torres Bugarín, O. (2000). Evaluación de la genotoxicidad de ciclofosfamida mediante prueba de micronúcleos en pacientes con lupus eritematoso sistémico. *Revista Mexicana de Reumatología*, 15(2): 41-45. https://pesquisa.bvsalud.org/portal/resource/pt/lil-292257

Sauer, J.M., Porter, A.C., & Biomarker Programs, Predictive Safety Testing Consortium. (2018). Preclinical biomarker qualification. *Experimental Biology and Medicine*, *243*(3), 222-227. https://doi.org/10.1177/1535370217743949

Schmid W. The micronucleus test. (1975). *Mutatation Research,* 31(1):9-15. https://doi.org/10.1016/0165-1161(75)90058-8

Schupp, N., Dette, E.M., Schmid, U., Bahner, U., Winkler, M., Heidland, A., & Stopper, H. (2008). Benfotiamine reduces genomic damage in peripheral lymphocytes of hemodialysis patients. *Naunyn-Schmiedeberg's archives of pharmacology*, *378*, 283-291. https://doi.org/10.1007/s00210-008-0310-y

Shimizu, N. (2011). Molecular mechanisms of the origin of micronuclei from extrachromosomal elements. *Mutagenesis*, *26* (1), 119-123. https://doi.org/10.1093/mutage/geq053

Revista Bio ciencias
ISSN 2007-3380

Shimizu, N., Shimura, T., & Tanaka, T. (2000). Selective elimination of acentric double minutes from cancer cells through the extrusion of micronuclei. *Mutation Research,* 448 (1):81-90. https://doi.org/10.1016/S0027-5107(00)00003-8

Shindo, Y., Hirano, F., Maeda, H., & Takeda, U. (1983). The micronucleus test with mouse spleen cells. *Mutation Research, 121* (1), 53-57. https://doi.org/10.1016/0165-7992(83)90086-6

Siri, S.O., Martino, J., & Gottifredi, V. (2021). Structural chromosome instability: types, origins, consequences, and therapeutic opportunities. *Cancers*, *13* (12), 3056. https://doi.org/10.3390/cancers13123056

Sommer, S., Buraczewska, I., & Kruszewski, M. (2020). Micronucleus assay: The state of art, and future directions. *International journal of molecular sciences*, *21* (4), 1534. https://doi.org/10.3390/ijms21041534

Squier, C.A., & Kremer, M.J. (2001). Biology of oral mucosa and esophagus. *JNCI Monographos.* 29):7-15. https://doi.org/10.1093/oxfordjournals.jncimonographs.a003443

Stich, H.F., Stich, W., & Parida, B.B. (1982). Elevated frequency of micronucleated cells in the buccal mucosa of individuals at high risk for oral cancer: betel quid chewers. *Cancer Letters*, *17* (2), 125-134. https://doi.org/10.1016/0304-3835(82)90024-6

Stopper, H., Treutlein, A.T., Bahner, U., Schupp, N., Schmid, U., Brink, A., Perna, A., & Heidland, A. (2008). Reduction of the genomic damage level in haemodialysis patients by folic acid and vitamin B12 supplementation. *Nephrology Dialysis Transplantation*, 23 (10), 3272-3279. https://doi.org/10.1093/ndt/gfn254

Strimbu, K., & Tavel, J.A. (2010). What are biomarkers? *Current Opinion in HIV and AIDS*, *5* (6), 463. https://doi.org/10.1097%2FCOH.0b013e32833ed177

Tadin, A., Gavic, L., Roguljic, M., Jerkovic, D., & Zeljezic, D. (2019). Nuclear morphological changes in gingival epithelial cells of patients with periodontitis. *Clinical Oral Investigations*, *23*, 3749-3757. https://doi.org/10.1007/s00784-019-02803-5

Terradas, M., Martín, M., & Genescà, A. (2016). Impaired nuclear functions in micronuclei results in genome instability and chromothripsis. *Archives of toxicology*, *90*, 2657-2667. https://doi.org/10.1007/s00204-016-1818-4

Terradas, M., Martín, M., Tusell, L., & Genescà, A. (2010). Genetic activities in micronuclei: is the DNA entrapped in micronuclei lost for the cell? *Mutation Research, 705* (1), 60-67. https://doi.org/10.1016/j.mrrev.2010.03.004

Thomas, P., & Fenech, M. (2015). Buccal cytome biomarkers and their association with plasma folate, vitamin B12 and homocysteine in Alzheimer's disease. *Lifestyle Genomics*, *8* (2), 57-69. https://doi.org/10.1159/000435784

Tibshirani, R., & Hastie, T. (2007). Outlier sums for differential gene expression analysis. *Biostatistics*, 8(1), 2-8. https://doi.org/10.1093/biostatistics/kxl005

Titenko-Holland, N., Jacob, R.A., Shang, N., Balaraman, A., & Smith, M.T. (1998). Micronuclei in lymphocytes and exfoliated buccal cells of postmenopausal women with dietary changes in folate. *Mutation Research, 417* (2-3), 101-114. https://doi.org/10.1016/S1383-5718(98)00104-1

Tolbert, P.E., Shy, C.M., & Allen, J.W. (1991). Micronuclei and other nuclear anomalies in buccal smears: a field test in snuff users. *American Journal of Epidemiology*, *134* (8), 840-850. https://doi.org/10.1093/oxfordjournals.aje.a116159

Revista Bio ciencias
ISSN 2007-3380

Torres-Bugarín, O. (2000). Evaluación de la genotoxicidad de las drogas antineoplásicas mediante el conteo de micronúcleos y otras anormalidades nucleares en mucosa bucal y micronúcleos en eritrocitos de sangre periférica. [Tesis de Doctorado en Genética Humana]. Universidad de Guadalajara, http://hdl.handle.net/20.500.12104/20824

Torres-Bugarín, O., & Arias-Ruiz, L.F. (2023). Micronúcleos: Actualización del papel en la inestabilidad genética, inflamación, envejecimiento y cáncer. Revisión panorámica. *Revista Biomédica*, *34* (2), 208-223. https://doi.org/10.32776/revbiomed.v34i2.1101

Torres-Bugarín, O., & Ramos Ibarra, M.L. (2013a). Micronúcleos y anormalidades nucleares en mucosa bucal para evaluar población en riesgo laboral por mutágenos. *Revista Costarricense de Salud Pública*, 22 (1), 01-0. https://www.scielo.sa.cr/pdf/rcsp/v22n1/art01v22n1.pdf

Torres-Bugarín, O., & Ramos-Ibarra, M.L. (2013b). Utilidad de la prueba de micronúcleos y anormalidades nucleares en células exfoliadas de mucosa oral en la evaluación de daño genotóxico y citotóxico. International Journal of Morphology, 31(2), 650-657. http://dx.doi.org/10.4067/S0717-95022013000200050

Torres-Bugarín, O., Covarrubias-Bugarín, R., Zamora-Perez, A.L., Torres-Mendoza, B., García-Ulloa, M., & Martínez-Sandoval, F. (2007). Anabolic-androgenic steroids induce micronuclei in muccal mucosa cells of body builders. *British Journal of Sports Medicine,* 1(9):592-596. https://doi.org/10.1136/bjsm.2006.032474

Torres-Bugarín, O., De Anda-Casillas, A., Ramírez-Muñoz, M.P., Sánchez-Corona, J., Cantú, J.M., & Zúñiga, G. (1998). Determination of diesel genotoxicity in firebreathers by micronuclei and nuclear abnormalities in buccal mucosa. *Mutation Research, 413* (3), 277-281. https://doi.org/10.1016/S1383-5718(98)00021-7

Torres-Bugarín, O., Fernández-García, A., Torres-Mendoza, B.M., Zavala-Aguirre, J.L., Nava-Zavala, A., & Zamora-Perez, A.L. (2009). Genetic profile of overweight and obese school-age children. *Toxicological & Environmental Chemistry*, *91* (4), 789-795. https://doi.org/10.1080/02772240802404966

Torres-Bugarín, O., Macriz Romero, N., Ramos Ibarra, M.L., Flores-García, A., Valdez Aburto, P., & Zavala-Cerna, M.G. (2015). Genotoxic effect in autoimmune diseases evaluated by the micronucleus test assay: our experience and literature review. *Biomed Research International*. 2015;2015:194031. https://doi.org/10.1155/2015/194031

Torres-Bugarín, O., Pacheco-Gutiérrez, A.G., Vázquez-Valls, E., Ramos-Ibarra, M.L., Torres-Mendoza. B.M. (2014b) Micronuclei and nuclear abnormalities in buccal mucosa cells in patients with anorexia and bulimia nervosa. *Mutagenesis*. 29(6):427-31. https://doi.org/10.1093/mutage/geu044.

Torres-Bugarín, O., Ramos-Ibarra, M.L., Carrillo-Gómez, C.S., & Zavala-Aguirre, J.L. (2016). Micronúcleos y otras anormalidades nucleares en células de mucosa bucal como biomarcadores de genotoxicidad y citotoxicidad en personal expuesto a gases anestésicos. *Revista Colombiana de Salud Ocupacional*, *6* (1), 3-9. https://doi.org/10.18041/2322-634X/rcso.1.2016.4877

Torres-Bugarín, O., Ramos-Ibarra, M.L., Morgan-Villela, G., Zúñiga-González, G. (2013c). Genotoxicidad de la quimioterapia antineoplásica evaluada por medio de la prueba de micronúcleos y anormalidades nucleares en células de mucosa bucal. *IX Encuentro Participación de la Mujer en la Ciencia". Instituto de Investigaciones en Óptica*. S5-MCS34. ISBN 978-607-95228-4-1.

Revista Bio ciencias
ISSN 2007-3380

Torres-Bugarín, O., Ventura-Aguilar, A., Zamora-Perez, A., Gómez-Meda, B.C., Ramos-Ibarra, M.L., Morgan-Villela, G., Gutiérrez-Franco, A., & Zúñiga-González, G. (2003). Evaluation of cisplatin+ 5-FU, carboplatin+ 5-FU, and ifosfamide+ epirubicine regimens using the micronuclei test and nuclear abnormalities in the buccal mucosa. *Mutation Research* 539(1-2), 177-186. https://doi.org/10.1016/S1383-5718(03)00163-3

Torres-Bugarín, O., Zavala-Cerna, M.G., Nava, A., Flores-García, A., & Ramos-Ibarra, M.L. (2014a). Potential uses, limitations, and basic procedures of micronuclei and nuclear abnormalities in buccal cells. *Disease Markers* 2014;2014:956835. https://doi.org/10.1155/2014/956835

Umbreit, N.T., Zhang, C.Z., Lynch, L.D., Blaine, L.J., Cheng, A.M., Tourdot, R., Sun, L., Almubarak, H.F., Judge, K., Mitchell, T.J., Spektor, A,. & Pellman, D. (2020). Mechanisms generating cancer genome complexity from a single cell division error. *Science*, *368*(6488), eaba0712. https://doi.org/10.1126/science.aba0712

Uno, Y., Morita, T., Luijten, M., Beevers, C., Hamada, S., Itoh, S., Ohyama, W., & Takasawa, H. (2015). Recommended protocols for the liver micronucleus test: report of the IWGT working group. *Mutation Research*, 783, 13-18. https://doi.org/10.1016/j.mrgentox.2014.10.010

Vizcarra, S. (2005). Micronúcleos y otras anormalidades nucleares en células epiteliales, de niños con normopeso, bajo peso y desnutrición.Tesis de Maestría en Nutrición Clínica. Universidad del Valle de Atemajac

Wu, X.Y., & Lu, L. (2012). Vitamin B6 deficiency, genome instability and cancer. *Asian Pacific Journal of Cancer Prevention,* 13 (11): 5333-5338. https://doi.org/10.7314/APJCP.2012.13.11.5333.

Xi, W.S., Li, J. B., Liu, Y.Y., Wu, H., Cao, A., & Wang, H. (2021). Cytotoxicity and genotoxicity of low-dose vanadium dioxide nanoparticles to lung cells following long-term exposure. *Toxicology*, *459*, 152859. https://doi.org/10.1016/j.tox.2021.152859

Yasui, M., Koyama, N., Koizumi, T., Senda-Murata, K., Takashima, Y., Hayashi, M., & Honma, M. (2010). Live cell imaging of micronucleus formation and development. *Mutation Research, 692* (1-2), 12-18. https://doi.org/10.1016/j.mrfmmm.2010.07.009

Zhang, C.Z., Spektor, A., Cornils, H., Francis, J.M., Jackson, E.K., Liu, S., Meyerson, M., & Pellman, D. (2015). Chromothripsis from DNA damage in micronuclei. *Nature*, *522* (7555), 179-184. https://doi.org/10.1038/nature14493

Zúñiga-González, G.M., Batista-González, C.M., Gómez-Meda, B.C., Ramos-Ibarra, M. L., Zamora-Perez, A.L., Muñoz-Magallanes, T., Ramos-Valdés, C., & Gallegos-Arreola, M.P. (2007). Micronuclei in diabetes: folate supplementation diminishes micronuclei in diabetic patients but not in an animal model. *Mutation Research*, 634 (1-2), 126-134. https://doi.org/10.1016/j.mrgentox.2007.06.006