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Comparison of micronuclei frequency between smokers and non-smokers: a systematic review

Comparação da frequência de micronúcleos entre indivíduos fumantes e não fumantes: uma revisão sistemática

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ABSTRACT

Introduction: Cancer results from the accumulation of several modifications in the genetic material and, therefore, it is possible to carry out its cytogenetic detection using biomarkers. Micronuclei (MN) have been addressed in the literature as biomarkers of genotoxic damage. These markers provide information on biological or biochemical changes in a target tissue at early stage, enabling a favorable prognosis. **Objective:** To compare whether the frequency of MN in active smokers is higher than that in non-smokers. **Material and method:** The search was performed in the Medical Literature Analysis and Retrieval System (PubMeb), in the Latin American and Caribbean Literature in Health Sciences (LILACS), and in the Scientific Electronic Electronic Library Online (SciELO), for articles published in the last ten years. Randomized clinical trials, with a cross-sectional design, which compared the MN frequency in the oral mucosa of adult smokers and non-smokers, were selected. **Results:** A total of 52 articles were identified, four of them were removed due to duplicity. From the remaining 48 studies, after reading their titles and abstracts, 14 remained, which had their full texts read. Finally, eight articles remained for the qualitative analysis. **Discussion and conclusion:** Individuals who use tobacco present genotoxic and cytotoxic damages that interfere in the mitosis process, which leads to MN formation. The hypothesis of smoking as the cause of this genetic alteration is corroborated by the authors when comparing the data obtained in their studies between risk groups and control groups.

Key words: lung cancer; micronucleus; smoking.

RESUMO

Introdução: *O câncer é o resultado do acúmulo de diversas modificações no material genético, portanto, é possível realizar sua detecção citogenética utilizando biomarcadores. Micronúcleos (MN) têm sido abordados pela literatura como biomarcadores de danos genotóxicos. Esses marcadores fornecem informações sobre alterações biológicas ou bioquímicas em um tecido-alvo ainda de forma precoce, possibilitando um prognóstico favorável.* ***Objetivo:*** *Comparar se a frequência de MN em indivíduos fumantes ativos é superior à dos não fumantes.* ***Material e método:*** *Realizou-se a busca no Medical Literature Analysis and Retrieval System (PubMeb), no Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) e no Scientific Eletronic Library On-line (SciELO) de artigos publicados nos últimos dez anos. Foram selecionados ensaios clínicos randomizados, com delineamento transversal, que compararam a frequência de MN na mucosa bucal de indivíduos adultos fumantes e não fumantes.* ***Resultados:*** *Cinquenta e dois artigos foram identificados; quatro deles foram removidos devido à duplicidade. Dos 48 estudos restantes, após a leitura dos seus títulos e resumos, restaram 14, os quais tiveram seus textos completos lidos. Por fim, oito artigos permaneceram para a análise qualitativa.* ***Discussão e conclusão:*** *Indivíduos que utilizam tabaco apresentam danos genotóxicos e citotóxicos que interferem no processo de mitose, o que acarreta a formação de MN. A hipótese do tabagismo como causa dessa alteração genética é corroborada pelos autores ao confrontar os dados obtidos em seus estudos entre grupos de risco e grupos-controle.*

Unitermos: *câncer de pulmão; micronúcleo; tabagismo.*

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RESUMEN

Introducción: *El cáncer es el resultado de la acumulación de diversas modificaciones en el material genético, por lo tanto, es posible realizar su detección citogenética usando biomarcadores. Micronúcleos (MN) se han abordado por la literatura como biomarcadores de daño genotóxico. Estos marcadores ofrecen información sobre cambios biológicos o bioquímicos en un tejido diana todavía en forma precoz, permitiendo un pronóstico bueno.* ***Objetivo:*** *Determinar si la frecuencia de MN en fumadores activos es superior a la de los no fumadores.* ***Material y método:*** *Hicimos una búsqueda en Medical Literature Analysis and Retrieval System (PubMeb), Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS) y Scientific Eletronic Library On-line (SciELO) de artículos publicados en los últimos diez años. Hemos seleccionado ensayos clínicos aleatorizados, de diseño transversal, que compararon la frecuencia de MN en la mucosa bucal de personas adultas fumadoras y no fumadoras.* ***Resultados:*** *Se identificaron 52 artículos; cuatro de ellos fueron eliminados debido a duplicidad. Entre los 48 estudios restantes, tras la lectura de sus títulos y resúmenes, quedaron 14, que tuvieron sus textos completos leídos. Por último, ocho artículos permanecieron para el análisis cualitativo.* ***Discusión y conclusión:*** *Personas que utilizan tabaco presentan daños genotóxicos y citotóxicos que interfieren con el proceso de mitosis, lo que acarrea la formación de MN. La hipótesis del tabaquismo como causa de ese cambio genético es corroborada por los autores al cotejar los datos obtenidos en sus estudios entre grupos de riesgo y grupos de control.*

Palabras clave: *cáncer de pulmón; micronúcleo; tabaquismo.*

INTRODUCTION

In 2017, at just over 10% of the Brazilian population was a smoker; the male gender had the highest prevalence, with 13.2%, and the female, 7.5% of the individuals[1]. Chronic non-communicable diseases are responsible for 60% of deaths worldwide, while in Brazil, they account for 72%[2]. In this bias, studies show that the product of tobacco burning is capable of generating chromosomal aberrations that may form micronuclei (MN) in cultured mammalian cells[3, 4].

MN consist of nuclear structures originating from events capable of altering the stability of the deoxyribonucleic acid (DNA), such as the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), shortening of telomeres, and inhibition of the action of the telomerase enzyme[3, 5, 6]. By expressing the genetic modification, the frequency of MN has been addressed in the literature as a possible bioindicator of the extent of chromosomal damage in individuals exposed to genotoxic agents or in those with a genetic profile susceptible to the development of cancer[7]. Once it is a minimally invasive technique, the MN test is adequate to monitor the individual and population risk of individuals to the pathologies triggered by tobacco consumption, as it presents high reliability and low cost[3, 6, 7].

OBJECTIVE

A systematic review of clinical studies in order to compare whether the frequency of MN in active smokers is higher than that of non-smokers was performed.

MATERIAL AND METHOD

The study was conducted based on the methodology suggested by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[8]. The searches for the studies were carried out in scientific databases (online platforms), among them, Medical Literature Analysis and Retrieval System (PubMed), Scientific Eletronic Library Online (SciELO), and Latin American and Caribbean Literature in Health Sciences (LILACS). Clinical trials with designs of the following types were considered: randomized clinical trial, cohort, cross-sectional, case series, and case report.

After selecting the studies, the analysis of the data obtained by the authors was carried out. For each study, variables such as sample number, study groups, age range of the population studied, number of cells analyzed, sample staining methodology, and MN mean for each type of methodology were considered.

Initially, the data obtained for each group were compared with each other, in order to validate the alternative hypothesis, that is, that smokers have a higher MN mean when compared to non-smokers. Shortly after, the data were compared between the different studies, in order to compare the effectiveness of the methodologies used, as well as to determine the agreement of the studies or to identify potential reasons for disagreement.

RESULTS

During the search for articles, 52 texts were obtained, based on the keywords and filters already described in the materials and

methods section. From this total, four studies were repeated and, therefore, one of its copies was excluded, remaining 48 studies that would have their titles and abstracts read. From the previous reading, only 14 articles moved on to the next step, which consisted of the complete reading of the text. Six articles were declassified due to information incompatible with the purpose of the review, with only eight articles remaining for this research, as shown in **Figure 1**.

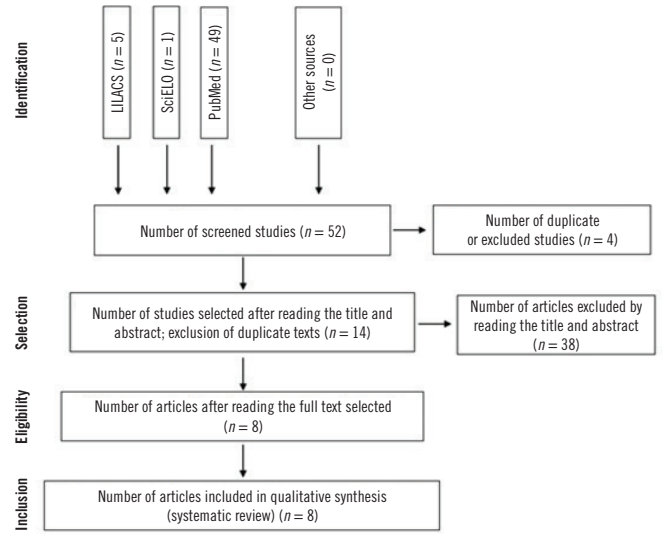

FIGURE 1 – *Flowchart of the selection steps of the elected studies for the systematic review*

Characteristics of the elected studies

The studies selected for this review were numbered and had their titles, authors, year of publication and objectives tabled for better knowledge of each one (**Table 1**).

Results of elected studies

The data analysis was based on the search for certain information, among them, the groups in which the participants were divided, their respective age groups, number of cells analyzed, types of stains used, mean MN found in each patient for each type staining, and whether or not there was a description of the presence of other nuclear changes.

The results of studies 1 to 7[9-15] are shown in **Table 2**, however, study 8[16], for having performed three types of stains (Feulgen, Giemsa and acridine orange), their results were distributed in a separate table for better interpretation (**Table 3**).

All studies presented a control group composed of non-smoking individuals and at least one risk group, composed of participants in tobacco consumption. Three articles considered, in addition to the group of smokers and the control, a third group with individuals who consumed chewing tobacco (1, 2 and 4)[9, 10, 12]. Two studies (7 and 8)[15, 16] evaluated the influence of the time of exposure to tobacco.

TABLE 1 – Studies comparing the frequency of MN between smokers and non-smokers included in the systematic review

Study number	Year	Authors	Country	Study	Focus
1	2018	Gopal KS, *et al*.	India	Evaluation of cytogenetic damage in the form of micronuclei in oral exfoliated buccal cells in tobacco users	To compare genotoxicity in cells of the oral mucosa and find the incidence of micronucleated cells according to the duration and frequency of tobacco use
2	2014	Dash KC, *et al*.	NI	Comparative study of micronuclei counts in patients with different tobacco-related habits using exfoliated buccal epithelial cells: a tool for assessment of genotoxicity	To identify and quantify MN in exfoliated cells of the oral mucosa in individuals with different tobacco-related habits and in the control group
3	2015	Silva VHP, *et al*.	Brazil	Cytogenetic biomonitoring in buccal mucosa cells from young smokers	Comparatively assess DNA damage (MN) and cell death (pyknosis, karyolysis, and karyorrhexis) in cells of the oral mucosa of smokers and non-smokers
4	2014	Chandirasekar R, *et al*.	India	Assessment of genotoxic and molecular mechanisms of cancer risk in smoking and smokeless tobacco users	Perform cytogenetic and genotoxic analysis in smokers and compare the data with healthy controls
5	2011	Zamani AG, *et al*.	India	Evaluation of smoking genotoxicity in Turkish young adults	To determine and evaluate the MN frequencies of young smokers and not in peripheral blood lymphocytes, oral mucosa, and exfoliative urothelial cells
6	2010	Haveric A, *et al*.	Bosnia	Micronuclei frequencies in peripheral blood and buccal exfoliated cells of young smokers and non-smokers	To evaluate the genotoxicity of cigarette smoking in young smokers and to correlate the results of the cytogenetic analysis in peripheral blood lymphocytes and exfoliated oral cells
7	2012	Naderi NJ, *et al*.	Iran	Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years	To evaluate the frequency of MN in oral mucosa cells of smokers with a smoking history less than and greater than 10 years and their comparison with non-smokers
8	2018	Metgud R, *et al*.	India	Effect of staining procedures on the results of micronucleus assay in the exfoliated buccal mucosal cells of smokers and nonsmokers: a pilot study	To evaluate the efficacy of specific DNA staining on non-specific DNA staining in exfoliated oral mucosa cells in smokers and non-smokers for the evaluation of MN and nuclear anomalies

MN: micronuclei; NI: not informed; DNA: deoxyribonucleic acid.

TABLE 2 – Methodological characteristics and results of selected studies from 1 to 7

Study number	*n*		Study groups	Age group	number of cells analyzed	Type of staining	MN average per group		Other changes
1	75	I II III	Smokers Chewing tobacco Controls	25-55	500	Feulgen	I II III	7.2 ± 7.08 8 ± 7.9 0.4 ± 1.2	NI
2	180	I II III IV	Smokers Betel quid consumption Chewing tobacco Controls	NI	2,000	Giemsa	I II III IV	3.11 ± 0.7 2.13 ± 1.2 1.67 ± 0.8 0.5 ± 0.8	NI
3	38	I II	Smokers Controls	21-33	2,000	Feulgen	I II	0.7 ± 0.8 0 ± 0.1	Pyknosis, karyorrhexis, and karyolysis
4	366	I II III V	Smokers Smokers and Chewing tobacco Chewing tobacco Controls	> 15	1,000	Feulgen	I II III IV	2.06 ± 1.28 2.38 ± 1.37 2.22 ± 1.52 1.43 ± 0.77	Chromosomal aberrations
5	30	I II	Smokers Controls	18-33	2,000	Feulgen	I II	1.2 ± 0.22 0.26 ± 0.1	NI
6	87	I II	Smokers Controls	20-37	1,000	Giemsa	I II	3.04 ± 3 2 ± 2.03	Apoptosis (karyorrhexis, karyolysis)
7	63	I II III	Smokers < 10 years Smokers > 10 years Controls	20-76	500	Feulgen	I II III	1.89 ± 0.62 2.01 ± 0.93 0.94 ± 0.94	NI

The groups designated as controls refer to those individuals who do not use tobacco in any form of consumption.

NI: not informed.

TABLE 3 – Methodological characteristics and results of study number 8

Variables		Control	Smokers < 10 years	Smokers > 10 years
n		10	10	10
Average age range		± 38.3 years	± 39.8 years	± 44.4 years
Number of cells analyzed		10 fields per slide	10 fields per slide	10 fields per slide
Number of MN	Feulgen	1.6 ± 0.89	3.7 ± 0.67	5.5 ± 1.17
	Giemsa	2.8 ± 1	5.3 ± 1.25	11.1 ± 1.47
	Acridine orange	2.1 ± 1	4.4 ± 0.69	7.1 ± 0.99

The groups designated as controls refer to those individuals who do not use tobacco in any form of consumption.

MN: micronuclei.

Studies 3, 5 and 8[11, 13, 16] were those that obtained the smallest sample, while number 4[12] was the assay with the largest number of samples. Articles 2, 3, and 5[10, 11, 13] analyzed at least 2 thousand cells; 4 and 6[12, 14], one thousand cells; and 1 and 7[9, 15], only 500 cells. Only study 8[16] did not describe the exact number of cells analyzed, but cited having investigated at least 10 fields per slide.

All studies had volunteers aged over 18 years, except study number 4[12], which included individuals over 15 years old. Article 2[10] also did not inform the age of any of the individuals, and number 7[15] included participants in the research up to 76 years old. Seven of the eight articles used only one type of stain: 1, 3, 4, 5, and 7[9, 11-13, 15], used Feulgen stain; 2 and 6[10, 14], Giemsa staining; and only research 8[16] used both colors mentioned and also the acridine orange color.

Except for the control group of study 3[11], none of the other studies obtained a zero result for the number of MN in their groups. All articles obtained a higher number of MN for the groups that consumed tobacco, regardless of the form of consumption, when compared with the control groups. Fifty percent of the studies cited the presence of other nuclear alterations (3, 4, 6, and 8)[11, 12, 14, 16].

DISCUSSION

Several structures and molecules may be used as cytogenetic biomarkers[6]. As an example are the nucleoplasmic bridges, constituted by nuclear fragments attached to the main nucleus by an area of constriction; or the binucleated cells, with the presence of two main nuclei caused by a failure in the cytokinesis as a result of the failures in the formation of the microfilament ring or interruption of the cell cycle[6]. In addition, there are MN, which are formed during cell division, at the point of transition from metaphase to anaphase, due to the non-union of the acentric

chromosome fragments or whole chromosomes to the remainder of the genetic material[17].

The literature mentions that the higher incidence of MN in relation to age may be associated with the cumulative effects of mutations in DNA repair, inadequate division of chromosomes, and failure in the cell cycle checkpoints[3]. As a result of all these changes, there is the formation of MN, constituted through altered gene expression or aneuploidy, chromosomal rearrangements or effects associated with the chromosomal instability phenotype, which are observed in cancer[7]. Likewise, it is suggested that the increase in oxidative stress leads to the formation of this structure, whose production of free radicals can be increased by the influence of exogenous sources, such as exposure to air pollution, ultraviolet radiation and ionizing radiation, the consumption of certain foods and smoking, as illustrated in **Figure 2**[18, 19].

The purpose of this systematic review was to identify, select, analyze and synthesize the results obtained in relevant published clinical studies regarding the frequency of MN in active smokers and non-smokers individuals. In held of the selected articles and after reading them, it was observed that all studies concluded that tobacco is capable of inducing genetic damage regardless of its form of consumption. Gopal and Padma (2018)[9] suggest that the degree of damage varies according to the form of tobacco consumption, since chewing tobacco presented a higher amount of MN (8 ± 7.906). However, when analyzing the data, it is possible to notice a narrow range of difference between the results (7.2 ± 7.083). This situation differs when we compare the number of MN of these two groups with the control group (0.4 ± 1.2); since the difference in results is significant.

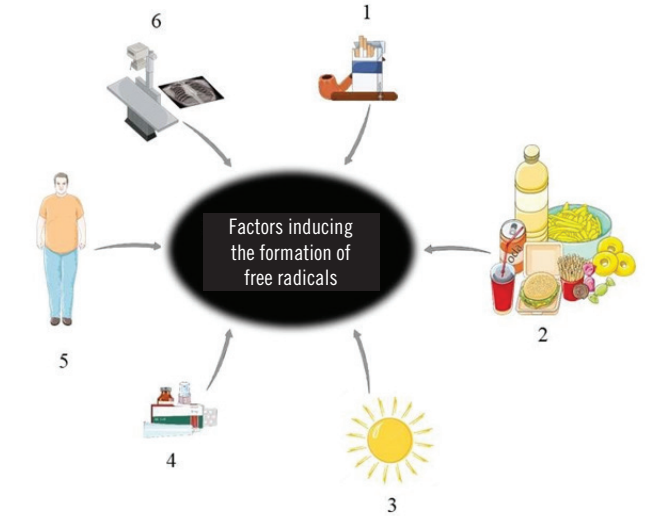

FIGURE 2 – *Factors that induce the formation of free radicals and, consequently, the genetic damage*

The studies by Gopal and Padma (2018)[9], Metgud and Neelesh (2018)[16] and Dash *et al*. (2018)[10] were the ones which obtained the highest results of MN for smokers, while the studies by Silva *et al*. (2015)[11], Zamani *et al*. (2011)[13] and Naderi *et al*. (2012)[15] demonstrated significantly lower results and the studies by Chandirasekar *et al*. (2014)[12] and Haveric *et al*. (2010)[14], intermediate results. All articles were in agreement regarding the use of tobacco in promoting the emergence of MN, however, the results showed significant differences, since the first study obtained more than twice the MN for each common variable studied. Several factors may be the cause of this difference in results, among them, we suggest the amount of cigarettes and the frequency of consumption/day for each individual, the contact with other smokers (passive smokers), the composition of the cigarette, the distinction in the number of samples and the form of tobacco consumption. In addition, the different staining procedures can cause interference in the analysis of the samples, as demonstrated in the research by Metgud and Neelesh (2018)[16], who presented around one to two standard deviations (SD) of difference from one technique to another among the same group. Therefore, there is a need to standardize the techniques used in the studies, so that they provide more specific, concordant results that are easy to apply and reproduce.

Study number 8[16] chose to analyze 10 fields per slide instead of establishing a minimum number of cells visualized per sample, as performed by the other studies, which may impact the results obtained. This interference can be a consequence of the number of cells: some fields present numerous cells, while others, rare cells. The exfoliated ones may present clusters, impairing the visualization of their limits and the MN.

In this regard, factors such as age, smoking time, and frequency are discordant in the literature, since some studies indicate that these factors lead to an MN increase[9], while others suggest they are irrelevant[14]. Naderi *et al*. (2012)[15] obtained a higher MN mean for individuals who had smoked for more than 10 years when compared to those who consumed cigarettes for less time; however, there was no statistically significant difference.

Half of the studies observed the presence of other nuclear alterations, such as pycnocytosis, karyorrhexis and karyolysis, which are indicative of apoptosis and chromosomal aberrations, showing that cells manifest their genotoxic damage not only by the formation of MN, but also by the manifestation of others nuclear structures[11, 12, 14]. Accordingly, the impact mechanism of smoking can be explained due to the high production of superoxide ions, which are capable of amplifying lipid peroxidation and DNA fragmentation, as well as triggering apoptosis, which generates an increase in the frequency of cellular divisions and, consequently, in the nuclear divisions. However, the process of separation of genetic material is impaired and leads to an increase in the number of MN[9].

CONCLUSION

The literature states that the MN assay can be used as a tool for cancer risk prediction, screening, diagnosis and prognosis, since all studies have shown that individuals who consume tobacco show a significant increase in the number of MN cells when compared to those who do not use it. In addition, this test consists of a simple, non-invasive, but reliable screening technique, through which it is possible to determine the impacts generated on the genetic material at an early stage, before the evidence of any clinical sign of histological changes resulting from cancer.

Regarding the results obtained in the selected articles, it is possible to conclude that individuals who use tobacco have genotoxic damages that interfere in the process of mitosis, during the anaphase stage, which causes formation of MN. The hypothesis of smoking as the cause of this genetic alteration is corroborated by the authors when comparing the data obtained in their studies between the risk groups (smokers) and the control groups (non-smokers).

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