Systematic Review
Orthodontics

The use of micronucleus assay in exfoliated oral cells in patients undergoing fixed orthodontic therapy: a systematic review with meta-analysis

Daniel Vitor de SOUZA[a]
Wilton Mitsunari TAKESHITA[b]
Glaucia Monteiro de CASTRO[a]
Ana Claudia Muniz RENNO[a]
Jean Nunes dos SANTOS[c]
Daniel Araki RIBEIRO[a]

[a]Universidade Federal de São Paulo – Unifesp, Institute of Health and Society, Department of Biosciences, Santos, SP, Brazil.

[b]Universidade Federal de Sergipe – UFS, Department of Dentistry, Aracaju, SE, Brazil.

[c]Universidade Federal da Bahia – UFBA, School of Dentistry, Department of Oral Diagnosis and Therapeutics, Salvador, BA, Brazil.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
Daniel Araki Ribeiro
E-mail: daribeiro@unifesp.br

https://doi.org/10.1590/1807-3107bor-2023.vol37.0116

Submitted: February 21, 2022
Accepted for publication: June 15, 2023
Last revision: August 7, 2023

Abstract: The aim of this systematic review was to evaluate published papers regarding the micronucleus assay in oral mucosal cells of patients undergoing orthodontic therapy (OT). A search of the scientific literature was made in the PubMed, Scopus, and Web of Science databases for all data published until November, 2021 using the combination of the following keywords: "fixed orthodontic therapy," "genetic damage", "DNA damage," "genotoxicity", "mutagenicity", "buccal cells", "oral mucosa cells," and "micronucleus assay". The systematic review was designed according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines. Nine studies were retrieved. Some authors demonstrated that OT induces cytogenetic damage in oral mucosal cells. Out of the nine studies included, two were classified as strong, five as moderate, and two as weak, according to the quality assessment components of the Effective Public Health Practice Project (EPHPP). Meta-analysis data revealed no relationship between mutagenicity in oral cells and OT in different months of treatment. At one month, the SMD = 0.65 and p = 0.08; after three months of OT, the SMD = 1.21 and p = 0.07; and after six months of OT, the SMD = 0.56 and p = 0.11. In the analyzed months of OT, I^2 values were >75%, indicating high heterogeneity. In summary, this review was not able to demonstrate that OT induces genetic damage in oral cells. The study is important for the protection of patients undergoing fixed OT, given that mutagenesis participates in the multi-step process of carcinogenesis.

Keywords: Mutagenesis; Micronucleus Tests; Mouth Mucosa.

Introduction

The importance of investigating environmental health is well established, considering that it focuses on the presence of hazardous agents in the environment, as well as on the association with possible adverse health effects. Human biomonitoring studies play a pivotal role because they are able to estimate exposure based on environmental risk.[1] Particularly, human biomonitoring studies provide relevant information about the risk of carcinogenesis in that these hazardous compounds can induce mutations or cell cycle disruption.[2] As a result, several methodologies

have been proposed in the scientific literature to monitor and protect human populations against potential harm.

As usual, mutagenicity assays evaluate chromosome breakage or loss and sister chromatid exchange. A great deal of enthusiasm has been dedicated to the application of the micronucleus test to exfoliated epithelial cells.[3] Micronucleus finds its origin in acentric fragments or even in whole chromosomes that are not incorporated into the main nuclei of the daughter eukaryotic cells. It is induced by chemical compounds that cause chromosome breakage (clastogens), as well as by chemical agents that affect the spindle apparatus (aneugens).[4]

The micronucleus test is a reliable method for evaluating risk assessment because most tumors originate from epithelial cells.[5] It is widely accepted that a large number of micronuclei in oral exfoliated cells have been classically utilized as a biological parameter for mutagenicity based on the exposure to various carcinogens.[6] Recently, several research groups have successfully applied the assay to populations (adults and children) exposed to several environmental agents, such as drugs, dental radiographs, and chemicals.[7-9]

Intraoral fixed orthodontic appliances comprise brackets, bands, archwires, and cements made of alloys and containing different concentrations of cobalt, chromium, and nickel. Currently, dentistry offers a wide range of orthodontic brackets, which are associated with a growing number of protocols for orthodontic therapy (OT). Some authors have claimed that OT induces the occurrence of micronuclei in oral mucosal cells.[10] Nevertheless, the literature in the field is contradictory, as other authors have not confirmed the positive findings as yet.[11,12]

Considering the lack of scientific consensus, the aim of this systematic review was to answer the following question: Is micronucleus assay in exfoliated oral mucosal cells a useful tool for biomonitoring patients undergoing fixed orthodontic therapy?

Methodology

This systematic review was designed according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines.[13] The following question was proposed: "Is micronucleus assay in exfoliated oral mucosal cells a useful tool for biomonitoring patients undergoing fixed orthodontic therapy?"

Search strategy

Scientific databases (PubMed, Scopus, and Web of Science) were searched to identify all published papers with the following keywords: "fixed orthodontic therapy," "DNA damage," "genetic damage," "mutagenicity," "genotoxicity," "buccal cells," "oral mucosa," and "micronucleus assay" for all data published until November 2021. In addition, a manual search of the references was made to identify additional studies. Two independent authors (DVS and DAR) evaluated the titles and abstracts of all studies retrieved by means of the search strategy used.

Eligibility criteria

Only those studies that met the following criteria were included: a) Human subjects; b) Clinical studies; c) Studies reporting on OT and micronucleus assay; d) Studies reporting on OT with oral mucosal cells; e) Studies written in English; f) In vitro studies, review articles, commentaries, case reports, and letters to the editor were excluded from the analysis.

Data extraction

The following data were selected from all studies: authors, year of publication, study design, country, number of patients evaluated, sex, age, staining method, control group, exclusion criteria, metanuclear changes, blinded review, statistical approach, main results, and conclusion.

Risk of bias in individual studies

The internal quality of included studies was evaluated by two independent authors using the EPHPP (Effective Public Health Practice Project) modified scale, with some modifications.[14]If the article controlled all items, the study was classified as strong; if the investigation controlled two items, the study was classified as moderate; and, finally, if the article controlled one or none of the items, the study was considered weak.

Meta-analysis

A meta-analysis using a random-effects model was conducted to estimate cytogenetic damage to oral exfoliated cells in patients subjected to OT. The random-effects model with the Der Simonian-Laird (DS-L) method was performed in this setting and the standard mean difference (SMD) was used as an effect measure. The effect size was evaluated by Cohen's d statistic.[15] The heterogeneity among the studies was evaluated by using Cochran's Q test[15] and quantified byI^2 statistics.[16] The analyses were performed using the Cochrane Collaboration Review Manager software (RevMan v5.4.1, The Cochrane Collaboration, Oxford, UK).

Results

Study selection

The initial online search yielded 107 publications, 39 of which were redundant and were thus excluded. After careful evaluation of the titles and abstracts, 68 studies were irrelevant and were excluded from the study as they were literature reviews, case reports, letters to the editor, commentaries, or papers not written in English. The full texts of nine studies were carefully reviewed by two authors (DVS and DAR). The search strategy employed is presented in Figure 1.

General characteristics of the included studies and treated Patients

As previously mentioned, nine studies were included (Table 1). Five studies had been conducted in Brazil,[11,12,17-19] one study in Italy,[20] one in India,[10] one in Turkey,[21] and one in Iran.[22] The age of patients at the beginning of the orthodontic therapy ranged between 6 and 35 years. Regarding sex, four studies did not report the total number of females and males. On the other hand, five studies revealed the ratio between females and males, which ranged between 10–14 for males and 12–15 for females (Table 1).

Variables related to orthodontic treatment and cytogenetic damage

Table 2 outlines the variables related to OT and genetic damage. All included studies had a control group, thus ensuring proper comparison. Most studies established exclusion criteria, such

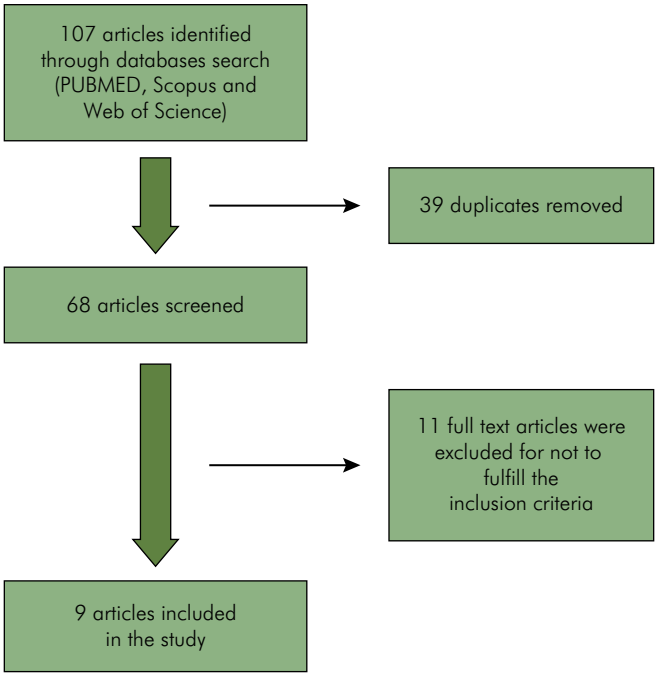

Figure 1. Flowchart of the selected studies.

Table 1. Main characteristics of the papers included in the systematic review, listed in alphabetical order of authors

Authors	Country	Age of patients (years)	Sex	Treatment	Study design
Angelieri et al.[11]	Brazil	18.5 + 7	10 males; 13 females	Brackets were composed ofiron(71%), nickel(8%), and chromium(19%). The archwires were composed ofnickel(50.8%), titanium(49.2%) during the treatment or stainless steel nickel (8.6%), iron(72.6%), chromium(20%) at the end of orthodontic therapy.	0, 6, and 12 months
Faccioni et al.[20]	Italy	10.3 + 1.2	14 males; 12 females	Andresen activator (AA): acrylic resin and two Adam's clasps and a buccalstainless steel arch	0, 7, 15, 30, 60, and 90 days
Flores-Bracho et al.[17]	Brazil	nov/35	Not informed	Brackets were bonded with composite Transbond XT and followed the Edgewise standard system: 0.022" × 0.028" slot and are composed of stainless steel (17.0 to 20.0% chromium, 8.0 to 10.5% nickel, molybdenum 0.60% max) with stainless steel wires (0.016", 0.018", 0.020", or 0.019" × 0.025").	1-12, 13-24, 25-48, and >48 months
Cunha et al.[12]	Brazil	06/dez	Not informed	Haas appliances were formed with stainless steel bands. They were madeof silver welded onto 0.9-mm-diameter stainless steel wire. Wires and bands were welded with silver solder, and metallic structures were bondedwith self-curing acrylic resin	0,1, and 3 months
Gonçalves et al.[18]	Brazil	jul/14	Not informed	Metallic extensions of the Hyrax expansion screw were silver-soldered (eight silver-soldered joints in each appliance). The bands, according to the manufacturer's information, were composed of: Cr, 17–20%; Ni, 8–10%; Mo, max. 0.60%, and Fe; the silver-soldered alloy wascomposed of Ag, 55–57%; Cu, 21–23%; Zn, 15–19%; and Sn, 4–6%	-7,0, and 28 days, 6 and 12 months
Heravi et al.[22]	Iran	dez/20	10 males; 15 females;	Brackets (standard edge-wise, 0.018-in slot). The bands and brackets were made of stainless steel.The archwires used over the course of this study included 0.014-in nickel-titanium (NiTi;), 0.016-in stainless steel and 0.016×0.022-in stainless steel	0 and 9 months
Toy et al.[21]	Turkey	14 + 1.79	12 males; 18 females	Transbond XT, Kurasper F	0, 1, 3, and 6 months
Natarajan et al.[10]	India	14-24	Not informed	Orthodontic appliances were composed of0.07% carbon,0.70% manganese, 1% silicon, 1%-17.5% chromium,3.0%-5.0% nickel, 3.0%-5.0% copper, 0.04% phosphorus, 0.04% sulfur, and 0.15%-0.45% tantalum and niobium	0 and 18 months
Westphalen et al.[19]	Brazil	16 + 2.54	6 males; 14 females	Orthodontic appliances were made of stainless steel (0.07% carbon, 1.0% manganese, 1.0% silicon, 15.5-17.5% chromium, 3-5% nickel, 3-5% copper, 0.15-0.45% niobium + tantalum)	0 and 30 days

as smoking, presence of dental restorations, and drinking habit, or presence of systemic diseases. Only the investigation made by Angelieri et al.[11] did not inform the exclusion criteria.

All studies collected samples from the buccal mucosa. In addition, the studies conducted by Natarajan et al.[10] and Westhphalen et al.[19] obtained oral mucosal cells from inside the lip. Another important aspect concerns the adopted staining technique. Most studies used DNA-specific staining, as follows: DAPI, Feulgen-Fast green, or acridine orange.[11,12,17,18,20,21] Three studies did not use specific DNA staining such as Papanicolaou or Giemsa.[10,19,22]

Taking into account the total number of evaluated cells, almost half of the studies evaluated 1,000

Table 2. Variables related to orthodontic treatment, listed in alphabetical order of authors

Authors	Exclusion criteria	Control group	Stain	Number of cells evaluated participant	Analysis of cytotoxicity	Number of patients	Blindedreview	Proper statistical description
Angelieri et al.[11]	No	Yes	Feulgen	2	Yes	23	No	Yes
Faccioni et al.[20]	Yes	Yes	DAPI	1	No	25	No	Yes
Flores-Bracho et al.[17]	Yes	Yes	Feulgen	2	Yes	95	Yes	Yes
Cunha et al.[12]	Yes	Yes	Feulgen	1	Yes	28	Yes	No
Gonçalves et al.[18]	Yes	Yes	Feulgen	2	Yes	20	Yes	Yes
Heravi et al.[22]	Yes	Yes	Giemsa	1	No	25	No	Yes
Toy et al.[21]	Yes	Yes	Acridine Orange	2	Yes	30	Yes	Yes
Natarajan et al.[10]	Yes	Yes	Papanicolaou	Not informed	No	40	No	Yes
Westphalen et al.[19]	Yes	Yes	Giemsa	Not informed	No	20	Yes	Yes

cells per patient, while four studies evaluated 2,000 cells per participant. The studies conducted by Flores Baracho et al.,[17] Cunha et al.,[12] Gonçalves et al.,[18] Toy et al.,[21] and Angelieri et al.[11] performed cytotoxicity assessments, while the studies conducted by Faccioni et al.,[20] Heravi et al.,[22] Natarajan et al.,[10] and Westphalen et al.[19] only evaluated the presence of micronucleated cells, binucleation, and cell buds.

Blinded review was adopted in five studies,[12,17-19,21] while four studies did not use blinded review.[10,11,20,22] Finally, all publications described the total number of patients enrolled in the study, but one study did not explain the statistical test used in the data analysis.[12] These findings are summarized in Table 2.

Main findings

Surprisingly, the data on cytogenetic damage induced by OT are conflicting. Faccioni et al.[20] demonstrated a significant increase in the total number of micronucleated cells, binucleation and cell buds in patients undergoing OT at 30, 60, and 90 post-treatment days. The same results were found by Cunha et al.[12] as the authors found a large number of binucleated cells and cell buds in oral exfoliated cells at 1 and 3 months after treatment. Toy et al.[21] also detected an increase in binucleated cells at 2, 4, and 6 months after treatment. Natarajan et al.[10] observed an increased number of micronucleated cells at the debonding of the fixed orthodontic devices. Likewise, Westphalen et al.[19] detected an increase in the total number of micronucleated cells at 30 days after the beginning of the treatment.

Taking into account all negative data, several studies indicated that OT was not able to induce mutagenicity in oral mucosal cells. Flores-Bracho et al.[17] demonstrated no statistically significant differences in volunteers subjected to OT, as they did not find any differences in the number of micronucleated cells at 12 months of orthodontic therapy. Analogous results were observed by Heravi et al.[22] and Angelieri et al.[11]

As for cytotoxicity, some papers investigated if and to what extent OT induced cell death in oral cells. Considering the specific studies evaluating this biological parameter by means of the micronucleus assay in buccal cells, Flores-Bracho et al.[17] detected a decrease in karyolysis after 48 months of OT. Cunha et al.[12] pointed out an increased number of pyknosis and karyolysis in the oral exfoliated cells of patients undergoing OT after three months of treatment. On the other hand, the results obtained by Angelieri et al.[11] and Gonçalves et al.[18] failed to detect any statistically significant differences for all cytotoxicity parameters evaluated. All findings described above are shown in Table 3.

Risk of bias assessment

The quality assessment of all studies is shown in Table 4. After scrutinizing the nine studies, two were classified as strong,[17,21] five as moderate,[11,12,18-20] and two as weak.[10,22]

Table 3. Main findings of studies evaluating cytogenetic damage following orthodontic therapy, listed in alphabetical order of authors.

Authors	Main findings	
	Cytotoxicity	Mutagenicity
Angelieri et al.[11]	No significant changes	No significant changes
Faccioni et al.[20]	-	↑ MN, BN, and bud cells
Flores-Bracho et al.[17]	↑ Karyolysis	No significant changes
Cunha et al.[12]	↑Pyknosis and karyolysis	↑ BN and bud cells
Gonçalves et al.[18]	No significant differences	No significant differences
Heravi et al.[22]	-	No significant differences
Toy et al.[21]	↑ Karyolysis	↑ BN
Natarajan et al.[10]	-	↑ MN
Westphalen et al.[19]	-	↑ MN

MN: micronucleus; BN: binucleated cells; ↑: increase; -: not performed.

Table 4. Quality assessment and final rating of the studies, listed in alphabetical order of authors.

Author	Study design	Blinding	Data analysis	Confounders	Final rating
Angelieri et al.[11]	Moderate	Weak	Strong	Strong	Moderate
Faccioni et al.[20]	Strong	Strong	Strong	Weak	Moderate
Flores-Bracho et al.[17]	Moderate	Strong	Strong	Strong	Strong
Cunha et al.[12]	Moderate	Strong	Weak	Moderate	Moderate
Gonçalves et al.[18]	Weak	Strong	Strong	Strong	Moderate
Heravi et al.[22]	Strong	Weak	Strong	Weak	Weak
Toy et al.[21]	Strong	Strong	Strong	Strong	Strong
Natarajan et al.[10]	Strong	Weak	Strong	Weak	Weak
Westphalen et al.[19]	Strong	Strong	Strong	Weak	Moderate

Five studies were selected for the meta-analysis. The study by Westphalen et al.[19] was excluded, as the data presented were not mean and standard deviation (SD). In the study by Heravi et al.,[22] the period of comparison between the frequencies of micronuclei was different, hence the exclusion. Finally, the studies by Flores-Bracho et al.[17] and Natarajan et al.[10] were excluded because the control group was different from that of other research studies. It is recommended that the control group include the same patient so as to minimize bias in research into micronuclei .

Meta-analysis data revealed no relationship between mutagenicity in oral cells and OT in different months of treatment. After one month of treatment, $Tau^2 = 0.44$, $Chi^2 = 1\ 8.56\%$, $p = 0.0003$, and $I^2 = 84\%$, indicating high heterogeneity. The standard mean difference (SMD) value was 0.65, with a 95% confidence interval (CI) between -0.07 and 1.36 and p-value equal to 0.08, not showing statistically significant difference (Figure 2).

After three months of OT, $Tau^2 = 1.21$, $Chi^2 = 28.34\%$, $p = 0.001$, and $I^2 = 94\%$, indicating high heterogeneity. The SMD value was 1.21, with a 95%CI between -0.08 and 2.51 and p value equal to 0.07, showing no statistically significant difference (Figure 3).

Finally, after six months of OT, $Tau^2 = 0.28$, $Chi^2 = 8.16\%$, $p = 0.02$, and $I^2 = 75\%$, indicating high heterogeneity. The SMD value was 0.56, with a 95 %CI between -0.13 and 1.25 and p value equal to 0.11, showing no statistically significant difference (Figure 4).

Based on these studies, OT was not able to induce cytogenetic damage in oral cells. In addition, the

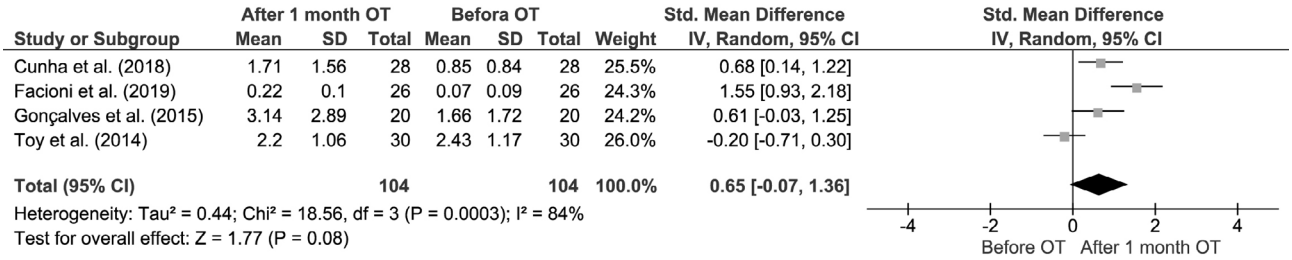

Figure 2. Meta-analysis of the frequency of micronuclei in patients undergoing fixed OT after one month.

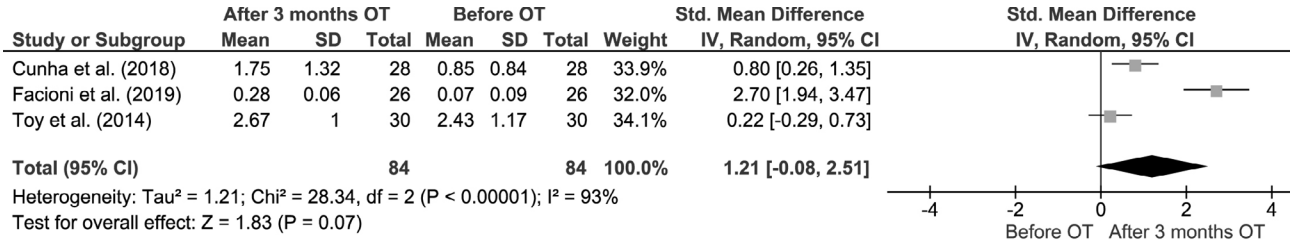

Figure 3. Meta-analysis of the frequency of micronuclei in patients undergoing fixed OT after three months.

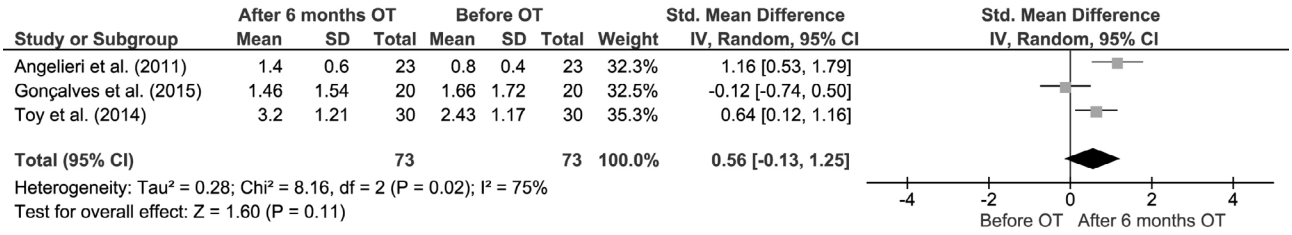

Figure 4. Meta-analysis of the frequency of micronuclei in patients undergoing fixed OT after six months.

data were heterogeneous, leading to the assumption that the evaluated studies did not share a common effect size.

Discussion

Several studies have been conducted to investigate the possible association between OT and the frequency of micronuclei in oral cells.[10,19,20] The underlying rationale behind these studies is based on the assumption that the continuous release of chemical agents from metals and cements used in OT can lead to their incorporation into the oral mucosa, resulting in genetic damage.[23]

The findings of this study do not indicate a relationship between the incidence of micronuclei in oral mucosal cells and patients undergoing OT. This was confirmed by the meta-analysis. In order to evaluate the quality assessment of all studies included in the review, the following aspects should be taken into consideration: a) study design, b) confounding factors, c) blinded review, and d) data evaluation. The final rating of these studies was categorized as weak, moderate, or strong.

In the scientific literature, various staining methods have been employed when using the micronucleus assay on oral cells, addressing potential confounding factors. In the review, most clinical studies used

specific DNA stains, such as Feulgen-Fast Green. However, it was noted that a considerable number of studies, around 30%, opted for non-specific stains (*e.g.*, Papanicolaou or Giemsa). Considering the absence of DNA specificity of stains such as Papanicolaou and Giemsa, the identification of micronuclei poses a challenge, as the components present in the cytoplasm, such as bacteria, cytoplasmic granules, or inflammatory cells, often resemble micronuclei in appearance. In the study conducted by Natarajan et al.,[10] the data from the control group showed a mean value of 53 micronuclei. This is an unusually high finding, considering that the index for spontaneous micronucleus origin in oral mucosal cells hovers around 0.3 to1.7‰.[24] This raises the possibility of false positive results.

It is important to emphasize that Tolbert et al.[25] proposed some changes in chromatin that might suggest cytotoxicity, such as karyorrhexis, pyknosis, and karyolysis. Cytotoxicity is a cause for concern because it interferes with micronucleus assay results. If cytotoxicity is high, the micronucleus is underestimated because micronucleated cells are not detectable due to cell death. In fact, the studies conducted by Cunha et al.,[12] Flores-Bracho et al.,[17] and Toy et al.[21] demonstrated an increase in the occurrence of pyknosis and karyolysis in oral mucosal cells following OT.

The total number of cells evaluated per person is another biological parameter that plays a pivotal role. The studies conducted by Faccioni et al.,[20] Cunha et al.,[12] and Heravi et al.[22] evaluated 1,000 cells per patient, while the studies by Natarajan et al.[10] and Westphalen et al.[19] did not report how many cells were evaluated. The remaining studies evaluated 2,000 cells per patient. In light of the micronucleus assay guidelines, it is necessary to evaluate at least 2,000 cells per individual.[25] Of note, the total number of cells scored interferes significantly with the quality of the results.

As previously described in the discussion, these three factors (type of staining method, number of cells evaluated, and site of smear collection) can significantly modulate the frequency of micronuclei in oral mucosal cells. Nevertheless, other conditions may also influence the quality of the results. For example, half of the studies included in this review did not report on the male to female ratio. This is a confounding factor, considering that some studies assumed that the frequency of micronuclei is higher in females than in males.[26]

This systematic review did not demonstrate that OT is able to induce DNA damage to oral cells.

Funding

The authors acknowledge research grants received from CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, Grant number #001) for productivity fellowships.

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