

Review

Does Panoramic X-ray Induce Cytogenetic Damage to Oral Cells? A Systematic Review With Meta-analysis

DANIEL VITOR DE SOUZA¹, MARIA ESTHER SUAREZ ALPIRE¹, INGRA TAIS MALACARNE¹,
GLAUCIA MONTEIRO DE CASTRO¹, MILENA DE BARROS VIANA¹, REGINA CLAUDIA BARBOSA DA SILVA¹,
GLENDA NICIOLI DA SILVA², ANA CLAUDIA MUNIZ RENNO¹ and DANIEL ARAKI RIBEIRO¹

¹Department of Biosciences, Institute of Health and Society,
Federal University of São Paulo, UNIFESP, Santos, SP, Brazil;

²Clinical Research Laboratory, Federal University of Ouro Preto, Ouro Preto, MG, Brazil

Abstract. *Aim: The aim of this review was to evaluate the scientific literature regarding the cytogenetic damage in oral exfoliated cells of adult patients submitted to panoramic X-ray. Materials and Methods: An extensive search of the literature was conducted on PubMed, Scopus and Web of Science databases for all studies published until April 2021 using combinations of the following keywords: “panoramic X-ray,” “DNA damage,” “genetic damage”, “genotoxicity”, “mutagenicity”, cytotoxicity”, “buccal cells”, “oral mucosa”, “tongue”, “gingiva”, “micronucleus assay”, according to the PRISMA guidelines. All clinical studies in English language were included in the study. A total of 10 studies were identified. Results: As expected, the results regarding the cytogenetic damage induced by panoramic X-ray are conflicting. Some authors have demonstrated that panoramic X-ray induces mutagenesis in oral cells, whereas others did not. After reviewing the 10 studies, two were classified as strong, four were considered moderate, and four were considered weak, according to the quality assessment components of the Effective Public Health Practice Project (EPHPP). Meta-analysis data revealed a negative response related to mutagenicity in oral cells by panoramic X-ray. Conclusion: Taken together, this review failed to demonstrate the association between micronucleus frequency and panoramic X-ray.*

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Correspondence to: Prof. Daniel A. Ribeiro, Department of Biosciences, Federal University of Sao Paulo, UNIFESP, Rua Silva Jardim, 136, Room 332, Vila Mathias, Santos – SP, 11050-020, Brazil. Tel: +55 1332290156, e-mail: daribeiro@unifesp.br

Key Words: Panoramic X ray, mutagenesis, micronucleus assay, oral mucosa.

Dental X-rays are widely employed by dentists because the technique is very useful for investigating potential abnormalities either in soft or mineralized tissues of the oral cavity (1). The most prominent advantages of the technique include speed, low cost, and high suitability of obtaining images. In particular, panoramic X-ray is a very common dental X-ray technique required in different conditions since a single image of the facial structures, such as of maxillary and mandibular dental arches as well as the supporting structures is achieved (2). It is therefore considered an excellent technique for obtaining an overview of the dentition, as well as to diagnose some pathologies regarding endodontics, periodontics, and stomatology (3).

Although dental X-rays have many benefits in clinical practice, it has been widely accepted that radiation induces injury to eukaryotic cells (4). This is because exposure to radiation generates reactive oxygen species in mammalian cells, which damage their genetic material in a dose-dependent fashion (5).

To date, there are many tools to assess genetic damage in eukaryotic cells. Among those, the application of the micronucleus test in epithelial exfoliated cells has gained interest (6). “Micronucleus” is the result of no incorporation of fragments or even whole chromosomes into the main nuclei during mitosis. It can be induced by substances that cause chromosome breakage (clastogens) as well as by agents that disrupt the spindle apparatus (aneugens) (7, 8). Therefore, the estimation of the micronucleus frequency is a suitable approach for assessing genetic damage. It is widely accepted that an increased frequency of micronuclei in oral exfoliated cells is useful for identifying the presence of several carcinogens in the environment (9). In fact, several authors have used the micronucleus assay to investigate genetic damage in individuals (adults and children) exposed to environmental conditions, such as dental radiographs, drugs, and chemical compounds (10-12).

It has been postulated that panoramic X-ray induces the formation of micronucleus in oral exfoliated cells (13). However, the literature in this field is yet very controversial, since other studies do not demonstrate positive findings (14, 15). Therefore, we performed this systematic review of the literature to clarify the following question: Does panoramic X-ray induce cytogenetic damage to oral cells?

Materials and Methods

The present systematic review was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines (16). The focused question was: “Does panoramic X-ray induce cytogenetic damage to oral cells?”

Search strategy. An electronic search of databases (PubMed, Scopus, and Web of Science) was performed to identify all relevant articles using a combination of the following keywords: “panoramic X-ray,” “DNA damage”, “genetic damage”, “genotoxicity”, “mutagenicity”, “cytotoxicity”, “buccal cells”, “oral mucosa” “tongue”, “gingiva”, “micronucleus assay” for all studies published until April 2021. In addition, a manual search of the references in the retrieved articles was conducted to identify further studies. Two independent reviewers (DVS and DAR) screened abstracts as well as titles of all studies retrieved by the research strategy adopted in the review. Full articles were evaluated and reviewed by the two reviewers (DVS and DAR).

Eligibility criteria. Predefined eligibility criteria were adopted in order to screen all identified studies. Studies were included if they fulfilled the following criteria: i) Human adult subjects; ii) All types of clinical studies; iii) Studies reporting on panoramic X-ray and written in English language; iv) Experimental studies, *in vitro* studies, animal studies, case reports, review articles, editorials and letters to the editor were excluded from the analysis.

Data extraction. The data extracted were: authors and year of study, study design, country of study, number of patients, patient’s gender, patient’s age, DNA stain, control group, exclusion criteria, metanuclear changes, blind analysis and statistics, main results and conclusion.

Risk of bias in individual studies. The internal quality of included studies was assessed using the Effective Public Health Practice Project (EPHPP) Modified scale by two independent reviewers with minor modifications (17). The quality assessment instrument used contains the following components: (i) study design, (ii) identification of confounders, (iii) blind analysis and (iv) data analysis. The studies with no weak ratings and at least three strong ratings were considered as strong. Those with less than three strong ratings and one weak rating were considered moderate. Finally, those with two or more weak ratings were considered as weak (17).

In the item study design, the following parameters were considered: number of participants, gender and exclusion criteria. As confounding factors, the following parameters were taken into consideration: number of cells evaluated per volunteer, cytotoxicity, and stain used. If the article controlled all items, this study item was considered strong; if the study controlled two of these items, this study item was considered moderate; and if the article controlled one or none of these confounders, this study item was considered weak.

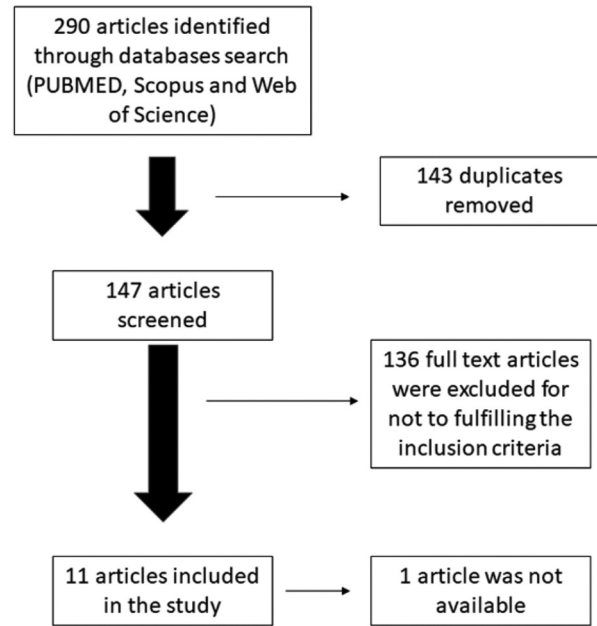


Figure 1. Flowchart of the study.

Meta-analysis. The meta-analysis was performed on all studies by means of JASPER statistics software, version 4.1 (Amsterdam, Netherlands). Only studies categorized as strong or moderate at the final rating in the quality assessment were included in the meta-analysis.

Results

Study selection. The initial online search yielded a total of 290 publications, 183 of which were duplicates and thus excluded. After screening the titles and abstracts, 96 studies were not found to be relevant and were therefore removed since they were reviews, case reports, *in vitro* studies, papers not written in English, editorials, proceedings of congress or letters to the Editor. Full-texts of the remaining 11 studies were sought and thoroughly read by the two authors (DVS and DAR). However, one study was also excluded because the full-length article was not available. The results of the search strategy are demonstrated in Figure 1.

General characteristics of the included studies and treated patients. After evaluating the articles, a total of 10 studies were included (Table I). Among them, one in Turkey (18), three in India (13, 19, 20), and six studies were conducted in Brazil (21-26). The age of patients submitted to panoramic X-ray ranged between 20 and 65 years. Regarding sex, only one study did not report the number of

Table I. Main characteristics of the articles included in this study.

Authors	Country	Age of patients (Years)	Gender
Santosh <i>et al.</i> (13)	India	24-65	15 males; 12 females
Karabas <i>et al.</i> (18)	Turkey	20-46	21 males; 9 females
Arora <i>et al.</i> (19)	India	25.21±12.67	21 males; 31 females
Waingade and Mediken (20)	India	27.63±10.93	19 males; 41 females
Angelieri <i>et al.</i> (21)	Brazil	37.7±6.5	9 males; 6 females
Ribeiro <i>et al.</i> (22)	Brazil	36.6±5.4	11 males; 6 females
Cerqueira <i>et al.</i> (23)	Brazil	26±9.18	9 males; 31 females
Ribeiro and Angelieri (24)	Brazil	39.6±12	11 males; 28 females
Da Silva <i>et al.</i> (25)	Brazil	18-40	Not informed
Cerqueira <i>et al.</i> (26)	Brazil	24±1.0	17 males; 24 females

males and females (25). Thus, nine studies revealed the number of males and females, which ranged between 9-21 for males and 9-41 for females (13, 18-24, 26). Table I presents these findings.

Variables related to panoramic X ray and cytogenetic damage. Table II shows some variables related to the micronucleus assay in oral cells of patients submitted to panoramic X-ray. First, all studies included a control group for proper comparison. With regard to exclusion criteria, the vast majority of the studies had included such information, such as the presence of dental restorations, smoking, use of alcoholic beverages, or systemic diseases. Only the studies conducted by Angelieri *et al.* (21), Ribeiro and Angelieri (24) and Cerqueira *et al.* (26) did not describe any exclusion criteria for the selection of study participants.

A total of 8 studies collected samples of buccal mucosa (13, 18-22, 24, 26). However, Arora *et al.* (19) obtained and examined oral mucosa cells from buccal mucosa and keratinized gingiva as well. Moreover, Cerqueira *et al.* (23) also examined oral cells of keratinized gingiva from upper dental arch, whereas Angelieri *et al.* (21) and da Silva *et al.* (25) applied the micronucleus assay in cells from the lateral border of tongue.

Another important issue is the type of staining technique that was chosen. Most studies (seven studies) used DNA-specific staining, such as Feulgen-Fast green stain (13, 21-26). Nevertheless, a total of three studies did not use a specific DNA stain as, for example, Giemsa or Papanicolaou stain (18-20).

With regard to the number of cells evaluated per volunteer, only four studies (40% of total) evaluated 2,000 cells per volunteer (21, 22, 24, 25). A total of six studies evaluated 1,000 cells (19, 20, 23, 26). For cytotoxicity, the studies conducted by Karabas *et al.* (18), Angelieri *et al.* (21), Ribeiro *et al.* (22), Cerqueira *et al.* (23, 26), and Ribeiro and Angelieri (24) performed cytotoxicity

assessment, whereas the studies conducted by Santosh *et al.* (13), Arora *et al.* (19) and Waingard and Medikeri (20) did not evaluate cell death.

The description of blind analysis in the methodology was observed in three studies (23, 25, 26), and not in seven (13, 18-22, 24). In addition, all studies described the total number of patients used and one study did not properly describe the statistical test used in the data analysis (13). These findings are summarized in Table II.

Main findings. As expected, the results regarding the cytogenetic damage induced by panoramic X-ray are conflicting. Karabas *et al.* (18), Arora *et al.* (19), and Waingade and Mediken (20), have postulated a significant increase in the number of micronucleated oral cells in patients submitted to panoramic X-ray after ~10 days of exposure. The same results were found by Cerqueira *et al.* (23) since a high number of micronucleated oral cells in the upper dental arch was noticed. Da Silva *et al.* (25) also found a high number of cells presenting broken eggs, cell bud, and binucleation from the lateral border of the tongue after exposure to panoramic dental X ray. Nevertheless, Angelieri *et al.* (21), Ribeiro *et al.* (22) Ribeiro and Angelieri (24) and Cerqueira *et al.* (26) did not detect any remarkable changes in micronucleus frequency after exposure to dental X ray in buccal cells and cells of the lateral border of the tongue.

With regard to cytotoxicity, some studies have evaluated whether and to what extent panoramic X-ray induces death in oral mucosa cells. Karabas *et al.* (18), Angelieri *et al.* (21), Ribeiro *et al.* (22), Cerqueira *et al.* (23, 26), and da Silva *et al.* (25) have demonstrated an increased number of pyknosis, karyolysis and karyorrhexis, and condensed chromatin in the oral cells of patients submitted to dental X-ray. Conversely, Santosh *et al.* (13), Arora *et al.* (19) and Waingade and Mediken (20) did not investigate any cytotoxic parameters. All findings described above are summarized in Table III.

Table II. Variables related to micronucleus assay in oral cells of patients submitted to panoramic X-ray.

Authors	Exclusion criteria	Control group	Stain	Site of smear	Number of cells evaluated per volunteer	Analysis of cytotoxicity	Number of patients	Blind analysis description	Proper statistics	Radiation parameters
Santosh <i>et al.</i> (13)	Yes	Yes	Feulgen	Buccal mucosa	Not informed	No	30	No	No	70-74 kV and 10 mA for 18 s
Karabas <i>et al.</i> (18)	Yes	Yes	PAP	Buccal mucosa	1,000	Yes	30	No	Yes	66 74 kV, 5 8 mA, and 13.1 13.9 s
Arora <i>et al.</i> (19)	Yes	Yes	Giemsa	Buccal mucosa and keratinized gingiva of the upper dental arch	1,000	No	53	No	Yes	74 kV and 10 mA, 12 s, output dose rate of 0.325 m Gy/s at 70 kV, 10 mA.
Waingade and Mediken (20)	Yes	Yes	Giemsa	Buccal mucosa	1,000	No	60	No	Yes	60-80 kV, 10 mA, 12 s, output dose rate of 0.325 m Gy/s
Angelieri <i>et al.</i> (21)	No	Yes	Feulgen	Buccal mucosa and lateral border of tongue	2,000	Yes	15	No	Yes	250-71 kV/15 mA/14 s/110 mGy/cm ² , entrance dose 0.08R
Ribeiro <i>et al.</i> (22)	Yes	Yes	Feulgen	Buccal mucosa	2,000	Yes	17	No	Yes	60-80 kV, 10 mA, 14 s, 110 mGy/cm ² , entrance dose 0.08 R
Cerqueira <i>et al.</i> (23)	Yes	Yes	Feulgen	Keratinized mucosa from upper dental arch	1,000	Yes	40	Yes	Yes	65-90 kV, 15 mA, 14 s, 110 mGy/cm ² , effective dose 21.4 mSv
Ribeiro and Angelieri (24)	No	Yes	Feulgen	Buccal mucosa	2,000	Yes	39	No	Yes	71 kV, 15 mA, 14 s, 110 mGy/cm ² entrance dose was 0.08 R
Da Silva <i>et al.</i> (25)	Yes	Yes	Feulgen	Lateral border of tongue	2,000	Yes	22	Yes	Yes	60-85 kV, 14-17 s, 10 mA, 0.057 mSv dose
Cerqueira <i>et al.</i> (26)	No	Yes	Feulgen	Buccal mucosa	1,000	Yes	31	Yes	Yes	250-71 kV/15 mA/14 s/110 mGy cm ² , effective dose 21.4 Sv

Assessment of the risk of bias. The quality assessment of selected studies is shown in Table IV. After reviewing the 10 studies, two were classified as strong (23, 25), four were considered moderate (21, 22, 24, 26), and four were considered weak (13-18, 20), according to the quality assessment components of the Effective Public Health Practice Project (EPHPP).

Meta-analysis was performed in six studies, as four studies were classified as weak. In addition, the studies conducted by Cerqueira *et al.* (23, 26) did not present mean values and S.D. regarding the micronucleus data. For this reason, these articles were excluded from the analysis. Based on the remaining studies, a significant statistical difference ($p=0.035$) was identified. This is consistent with the notion that panoramic X-ray did not induce cytogenetic damage to oral cells (Figure 2). In addition, all data are homogeneous ($p=0.885$), assuming that all studies had similar effect size.

Discussion

Scientific evidence has supported the idea that the presence of potential mutagens in the environment is positively correlated with the frequency of cytogenetic damage in oral cells (27). For this reason, application of the micronucleus assay in oral cells is a good choice for screening some metanuclear changes, such as micronucleus, karyolysis, karyorrhexis, cell buds, pyknosis, and binucleated cells (27). Considering that epithelial tumors are dominant among cancers in the human body, the micronucleus assay is suitable for monitoring human populations against the risk for oral cancer (28). Of particular importance, an increased number of micronucleated cells serves as a biological parameter for detecting mutagenicity as cancer risk (29).

Some studies have validated the association between exposure to panoramic X-ray and cytogenetic damage in oral cells with conflicting results (2). The rationale is that

Table III. Main findings of studies evaluating cytogenetic damage in patients submitted to panoramic X-ray.

Authors	Main findings	
	Cytotoxicity	Mutagenicity
Santosh <i>et al.</i> (13)	-	↑ MN
Karabas <i>et al.</i> (18)	↑ Karyolysis, ↑ Karryorhexis	↑ MN
Arora <i>et al.</i> (19)	-	↑ MN
Waingade and Mediken (20)	-	↑ MN
Angelieri <i>et al.</i> (21)	↑ Karyolysis, Karryorhexis and Pyknosis	No significant differences
Ribeiro <i>et al.</i> (22)	↑ Karyolysis, Karryorhexis and Pyknosis	No significant differences
Cerqueira <i>et al.</i> (23)	↑ Karryorhexis, ↑ Condensed chromatin	↑ MN
Ribeiro and Angelieri (24)	↑ Karyolysis, Karryorhexis and Pyknosis	No significant differences
Da Silva <i>et al.</i> (25)	↑ Karryorhexis	↑ broken egg, ↑ cell bud, ↑ BN
Cerqueira <i>et al.</i> (26)	↑ Karryorhexis, ↑ Condensed chromatin	No significant changes

MN: Micronucleus; BN: binucleated cells; ↑: increase.

Table IV. Quality assessment and final rating of the studies.

Author	Study design	Blinding	Data analysis	Confounders	Final rating
Santosh <i>et al.</i> (13)	Strong	Weak	Weak	Weak	Weak
Karabas <i>et al.</i> (18)	Strong	Weak	Strong	Weak	Weak
Arora <i>et al.</i> (19)	Strong	Weak	Strong	Weak	Weak
Waingade and Mediken (20)	Strong	Weak	Strong	Weak	Weak
Angelieri <i>et al.</i> (21)	Moderate	Weak	Strong	Strong	Moderate
Ribeiro <i>et al.</i> (22)	Strong	Weak	Strong	Strong	Moderate
Cerqueira <i>et al.</i> (23)	Strong	Strong	Strong	Moderate	Strong
Ribeiro and Angelieri (24)	Moderate	Weak	Strong	Strong	Moderate
Da Silva <i>et al.</i> (25)	Strong	Strong	Strong	Strong	Strong
Cerqueira <i>et al.</i> (26)	Moderate	Strong	Strong	Moderate	Moderate

radiation induces genetic damage through single-, double-strand DNA breaks and DNA crosslinks (30).

The results of this study failed to demonstrate mutagenic effects of panoramic X-ray in oral cells of adult patients. This was confirmed by meta-analysis data. It is important to stress that, four studies were categorized as weak at the final rating of the quality assessment and they were not included in the meta-analysis.

Virtually, in all clinical trials scrutinized in this setting, the patients were evaluated before and roughly two weeks after X-ray exposure. The approach is coherent since it is well known that micronucleus is formed in the basal layer of the oral epithelium, and is only observed after epithelial differentiation. The turnover of the oral epithelium takes place between 7 and 16 days, and therefore micronucleus identification will be feasible only between 1 and 3 weeks after exposure to mutagenic agent (31).

Regarding the confounding factors, several staining methods were chosen when performing the micronucleus

assay in oral exfoliated cells. Most studies used specific DNA stains, such as Feulgen-Fast Green staining (13, 21-26). However, it was noticed that a high percentage of the studies, around 30%, used non-specific stains, such as Giemsa and Papanicolaou (PAP) (18-20). In light of the lack of DNA specificity of these stains, micronucleus identification is very hard due to the presence of some cell components in the cytoplasm of epithelial cells that are identical to micronucleus, such as keratin granules, bacteria, or even leukocytes. Karabas *et al.* (18) and Waingade and Medikeri (20), stained slides with PAP or Giemsa and the micronucleus data for the control group were 30.2 ± 18.12 and 0.48 ± 0.14 , respectively. These values are considered very high, when considering spontaneous micronucleus incidence in oral exfoliated cells established by the Micronucleus Assay Expert Group (32). Certainly, this was due to false positive results.

It is important to mention that Tolbert *et al.* (33) have described metanuclear changes for cytotoxicity assessment

Fixed and Random Effects			
	Q	df	p
Omnibus test of Model Coefficients	4.424	1	0.035
Test of Residual Heterogeneity	0.649	3	0.885

Note. p-values are approximate.

Coefficients				
	Estimate	Standard Error	z	p
intercept	0.048	0.023	2.103	0.035

Note. Wald test.

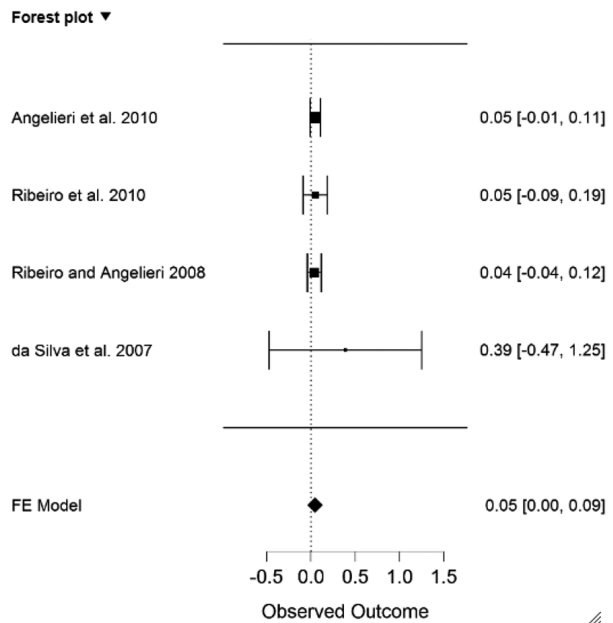


Figure 2. Meta-analysis data from studies investigating micronucleus frequency in patients submitted to panoramic X-ray.

using the micronucleus assay in exfoliated cells, such as karyorrhexis, pyknosis and karyolysis. This approach is very much necessary since cytotoxicity is a confounding factor in mutagenicity studies. If cytotoxicity is increased, the micronucleus frequency decreased because micronucleated cells are lost as a result of cellular death. The studies conducted by Santosh *et al.* (13), Arora *et al.* (19), Waingate and Medikeri *et al.* (20) did not evaluate cytotoxicity in oral cells after exposure to panoramic X ray.

An important concern under consideration is the site of smear. Our results revealed that the majority of studies collected oral cells from the buccal mucosa (13, 18-22, 24, 26). However, collection from other sites, such as keratinized gingiva, lateral border of tongue and upper dental arch was performed in four studies (19, 21, 23, 25). It is important to highlight, that buccal cells are more suitable for micronucleus assay since they have lower DNA repair capacity when compared to lymphocytes, reflecting chromosome damage more appropriately when compared to other sites (34). However, this reinforces, that divergent findings may occur depending on the region of the oral cavity the smear was collected when performing the micronucleus assay in oral exfoliated cells.

Finally, another biological parameter that merits discussion is the number of cells evaluated per volunteer. The studies conducted by Karabas *et al.* (18), Waingade and Medikeri (20), and Cerqueira *et al.* (23) evaluated

1,000 cells per volunteer, whereas the study conducted by Santosh *et al.* (13) did not reveal the number of cells evaluated. In the remaining studies, 2,000 cells were evaluated per patient. According to the Micronucleus Assay Expert Group, it is widely recommended to evaluate a minimum of 2,000 cells per individual (32). Certainly, the total number of cells evaluated interferes significantly with the quality of the data, especially for the experimental groups.

Conclusion

Taking into consideration the range of methods used in the interpretation of the data, this review failed to demonstrate an association between cytogenetic damage and exposure to panoramic X-ray.

Conflicts of Interest

All Authors declare that no conflicts of interest exist in relation to this study.

Authors' Contributions

Study design: DVS, ACMR and DAR. Data search: DVS, MESA, and RCBS. Data analysis: DVS, ITM, GMC, MBV, RCBS and ACMR and DAR. Writing the paper: all Authors.

Acknowledgements

The Authors acknowledge research grants received from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES. DVS thanks CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for scholarship grants. GNS, MBV, DAR and ACMR thank CNPq for productivity fellowships.

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Received June 9, 2021

Revised June 29, 2021

Accepted June 30, 2021