A systematic review and meta-analysis of the genotoxic and cytotoxic effects on oral epithelium induced by cone beam computed tomography

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Objective. To evaluate the occurrence of genotoxic and cytotoxic effects in oral epithelium after exposure of patients to cone beam computed tomography (CBCT).

Methods. A systematic review (SR) was conducted following the PECO (Population, Exposure, Comparison, Outcome) criteria. The study was registered in the International Prospective Register of Systematic Reviews (PROSPERO). A search was performed on the PubMed, Scopus, ScienceDirect, and Google Scholar databases. Effect size and heterogeneity of data were evaluated statistically. The Joanna Briggs Institute questionnaire for observational studies was utilized to assess the risk of bias. The GRADE tool was applied for the assessment of the quality of evidence. Begg's funnel plot was used to evaluate publication bias.

Results. In total, 10 full-text articles were included in the SR, with 6 of them in the meta-analysis. The SR showed a significant increase in micronuclei after exposure, with a large effect size of 1.03. For genotoxicity, the tau^2 for heterogeneity was 0.96, the chi-squared test for heterogeneity $P < .00001$, the I^2 statistics for random effects was 91%, and the overall effect for Z value was 2.46 ($P = .01$). The risk of bias was low, the quality of evidence was strong, and publication bias was absent.

Conclusion. CBCT can cause genotoxicity in the oral epithelium with a large effect size. The measure of cytotoxicity after CBCT exposure was not possible due to the lack of homogeneity of the included articles. (Oral Surg Oral Med Oral Pathol Oral Radiol 2024;138:324−334)

Cone beam computed tomography (CBCT) provides 3-dimensional imaging of dental and maxillofacial structures with considerable definition of mineralized tissues.[1-3] CBCT is widely used for implant planning, complicated endodontic cases, the location and evaluation of impacted teeth, and the examination of other maxillofacial abnormalities.[1] Thus, its benefits are evident in diagnosis and treatment planning.

X-radiation, like other forms of ionizing radiation, is capable of causing DNA damage.[4] It can lead to genotoxicity, which includes several types of DNA damage such as micronucleus formation, single-strand break, double-strand break, and base alteration. The micronucleus consists of chromosome fragments or whole chromosomes lagging behind at anaphase during nuclear division that are not incorporated into the resulting daughter cell nuclei.[5] Single-strand break is the breakage of 1 of the 2 strands of the DNA double helix, double-strand break is the damage to both strands, and base alteration is the change occurring within the sequences of bases in DNA.[6] When repaired inaccurately, this damage can result in mutations, chromosome rearrangements, chromosome aberrations, and the loss of genetic information.[7]

A systematic review (SR) of the genotoxic effects on the oral epithelium of panoramic radiography (PR) concluded that this technique can induce genotoxic damage to the oral epithelium with a small effect size.[4] The ubiquitous presence of CBCT, however, is cause for concern because the radiation dose to patients is higher compared to conventional dental radiography. The effective dose of radiation produced by a CBCT scan is 5-16 times higher than that from PR.[8] A meta-analysis reported that the effective doses for adults in any CBCT protocol ranged from 46 to 1073 mSv for large fields of view (FOVs), 9-560 mSv for medium FOVs, and 5-652 mSv for small FOVs.[9]

Ionizing radiation is also responsible for cytotoxicity, which is estimated by the nuclear alterations of pyknosis, karyolysis, karyorrhexis, nuclear buds, and broken eggs. Pyknosis is the process of chromatin and nucleus shrinkage, karyolysis is the fragmentation of the entire nucleus and the cleavage of chromatin, karyorrhexis is the process of chromatin degradation, nuclear buds are attached to the nucleus by a stalk, and the broken egg is a stalkless nuclear bud.[10]

Statement of Clinical Relevance

Studies have assessed genotoxicity and cytotoxicity after CBCT exposures. CBCT was found to cause genotoxicity in the oral epithelium with a large effect size. Knowledge of the effect size could deter unnecessary CBCT exposure.

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Biomonitoring studies are used to assess the mutagenic or carcinogenic effects of various environmental agents.[11] These studies include a variety of assays of the frequency of micronucleus formation, metaphase chromosomal aberrations, sister chromatid exchanges, DNA damage, and host cell reactivation. Of these investigations, the micronucleus test has been widely used, primarily to monitor genotoxicity in human blood peripheral lymphocytes.[12] The human buccal micronucleus cytome assay (BMCyt) is a well-known tool for detecting DNA damage and assessing genotoxicity and cytotoxicity.[11] It includes the micronucleus test that assesses genotoxicity, and it also measures pyknosis, karyolysis, karyorrhexis, nuclear buds, and broken eggs to evaluate cytotoxicity. BMCyt measures the frequency of these alterations in basal and fully differentiated cells in human buccal mucosa.[13] There is evidence that links the frequency of buccal micronuclei in healthy individuals with accelerated aging, the risk of cancer, cardiovascular diseases,[14] and neurodegenerative disorders.[15]

Several studies have assessed genotoxicity and cytotoxicity after CBCT exposures, but the effect size of the global data is not clear. The difference in the effect size between PR and CBCT should indicate the likelihood of developing genotoxicity and cytotoxicity from CBCT when compared to PR. Given this background, it is important to assess the current literature for studies of genotoxicity and cytotoxicity following CBCT exposures. Thus, this systematic review was performed with a meta-analysis. Meta-analysis is a systematic, quantitative epidemiological study design that is used to methodically evaluate the findings of earlier studies in order to draw conclusions from that body of work.[16]

OBJECTIVE

This systematic review was conducted to answer the following 2 questions: 1) Does exposure to CBCT induce genotoxicity and cytotoxicity in oral epithelium? 2) What is the effect size of genotoxicity and cytotoxicity after CBCT exposure? Meta-analysis was performed to assess if exposure to CBCT can lead to genotoxicity and cytotoxicity of oral epithelial cells. The pre-exposure frequencies of micronucleus production and nuclear alteration were compared with post-exposure frequencies. We hypothesized that radiation exposure in CBCT is sufficient to cause genotoxic and cytotoxic damage.

MATERIALS AND METHODS

The systematic review was performed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis protocols (PRISMA).[17] It was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD 42022362512.

Study design and inclusion criteria

The Population, Exposure, Comparison, Outcome (PECO) framework was used to formulate criteria for the eligibility of studies for this review.

Population: Individuals undergoing CBCT examination for dental diagnostic evaluation.

Exposure: Absorbed dose of CBCT.

Comparison: Pre-exposure frequency of micronucleus formation and nuclear alteration compared to post-exposure frequency of these abnormalities in the same individuals.

Outcome: Genotoxicity was measured by the presence of micronuclei, and cytotoxicity was measured by the presence of pyknosis, karyolysis, karyorrhexis, nuclear buds, and broken eggs.

Inclusion criteria

Studies that assessed genotoxicity using the micronucleus test and cytotoxicity based on nuclear alterations in oral epithelium of healthy individuals after exposure to CBCT were included.

Exclusion criteria

The following publications were excluded: 1) studies with incomplete data, 2) conference paper abstracts, 3) letters to the editor, 4) narrative literature reviews, 5) pilot studies, 6) book chapters, 7) personal or short communications, and 8) editorials.

Information sources and search strategy

An electronic search was carried out in PubMed, Scopus, and ScienceDirect. A grey literature search was also performed in Google Scholar. All articles in English were searched with no restriction on the date of publication. A manual search of the cross-references of all original articles was conducted to include articles that were not located in the electronic database search.

The literature search was performed using MeSH (Medical Subject Headings) terms. The Boolean operators (AND and OR) were used to combine MeSH terms. The final search was conducted on July 27, 2023. The database search strategy is documented in Table I.

Study selection

A flowchart following the PRISMA protocol (Figure 1) shows the process of study selection, the number of studies identified, and inclusions and exclusions along with specific reasons. Independent data collection was performed by 2 reviewers (T.S.J. and K.P.S.) in 3 different phases. If consensus could not be achieved

Table I. Electronic database and search strategy

Database	*Search Strategy*
PubMed(https://www.ncbi.nlm.nih.gov/pubmed/)	((((Cytotoxicity) OR (Genotoxicity) AND (Cone-Beam Computed Tomography) AND (Oral Cavity)
ScienceDirect (http://www.sciencedirect.com/)	"Cytotoxicity" AND "Genotoxicity" AND "Cone-Beam Computed Tomography" AND "Oral Cavity"
Scopus (http://www.scopus.com/)	"Cytotoxicity" AND "Genotoxicity" AND "Cone-Beam Computed Tomography"
Google Scholar (https://scholar.google.com.br/)	(Cytotoxicity assays) OR (Genotoxicity assays) OR (Toxicity, Genetic) OR (Mutagen Screening) AND (Cone-Beam Computed Tomography) AND (Oral Cavity) OR (Oral Epithelium) OR (Mouth)

between the 2 reviewers in any phase, discrepancies were resolved by a third reviewer (V.S.), who was consulted for the final decision. Studies that were excluded and the reasons for their exclusion were recorded.

In the Identification phase, the titles of the articles discovered in the 4 databases were read carefully and those outside the area of interest of this review were excluded. The reviewers were not blinded to authorship and journal information. This resulted in a total of 251 articles.

In the Screening phase, 18 duplicate articles were excluded, which resulted in a total of 233 papers that were screened for title and abstract by the 2 reviewers independently. At this level, the abstracts of 223 studies did not meet the inclusion criteria, and these papers were excluded. One study identified in the cross-reference was added, resulting in a total of 11 articles.

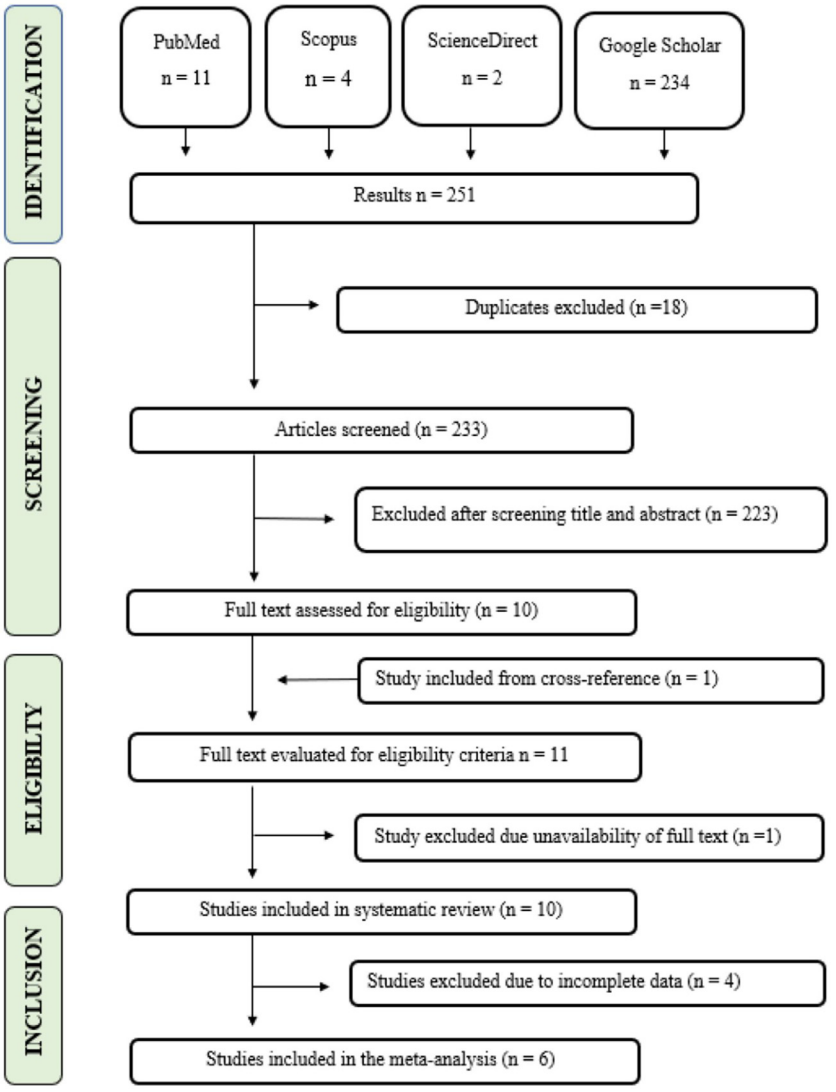

Fig. 1. Flowchart of the article selection process following the PRISMA protocol.

In the Eligibility phase, full texts and abstracts were obtained for 10 studies; we did not gain access to the full text of 1 article. The corresponding author of the article was contacted with a request to provide the full text, but we received no response.

In the Inclusion phase, the full texts of the remaining 10 articles were evaluated to ensure they fulfilled the eligibility criteria. All 10 studies were included in the systematic review.[3,11,13,19-25] However, 4 articles were excluded from the meta-analysis because 2 presented data in graphs and numerical values could not obtained,[3,19] 1 expressed results in percentage,[20] and 1 expressed a mean value but failed to mention the standard deviation.[22] As a result, 6 articles were included in the meta-analysis.[11,13,21,23-25]

Data collection process and data items

The 2 reviewers independently extracted data from the 10 included articles to allow comparison and summarize the available evidence. The following data were extracted: authors, country, study population, patient age range/mean age, days of cell collection, site of cell collection, staining method, methods to measure genotoxicity and cytotoxicity, exposure to other known genotoxins and/or cytotoxins aside from ionizing radiation, and results of genotoxicity and cytotoxicity measurements. Any differences in data collection were settled by mutual consensus. In cases of disagreement, the senior reviewer was consulted and the decision was finalized. All extracted data were then exported to Excel spreadsheets.

Data analysis

Meta-analysis using a random effects model was performed to estimate the genotoxic and cytotoxic effects of CBCT in the selected articles. Effect size was measured by the standardized mean difference (SMD), also known as the Cohen d statistic,[18] and was calculated by obtaining the means and standard deviations (SD) from each study group and outcome of interest. A value of 0.2 was considered a small effect, 0.5 a medium effect, and 0.8 or higher a large effect. Effect sizes were displayed graphically using forest plots with confidence intervals (CI) of 95%.

The heterogeneity among the included studies was calibrated using the tau^2, chi-squared, and I^2 statistics. Tau^2 measures the dispersion of true effect size among studies in terms of the scale of the effect size. The chi-squared statistic tests the null hypothesis that there are no differences in intervention effects across studies. The I^2 statistic measures the percentage of observed variance that represents actual variations in effect size. It is expressed as a value in the range of 0% to 100%. An I^2 value lower than 25% indicates low heterogeneity among the studies, between 25% and 75% moderate heterogeneity, and above 75% high heterogeneity. The Z-test of overall effect was applied, with the null hypothesis that there is no overall effect of the experimental intervention compared with the comparator on the outcome of interest.

Statistical significance was established at $P < .05$. A P value is the standard result of a statistical test and is the probability of obtaining the observed effect (or larger) under a null hypothesis. The analyses were performed using RevMan 5.4.1 software.

Risk of bias in individual studies

The risk of bias in the selected studies was investigated through the Joanna Briggs Institute (JBI) Critical Appraisal Tool for systematic reviews involving diagnostic accuracy (http://joannabriggs.org/research/critical-appraisal-tools.html). The following questions were used for the evaluation: Q1.) Does the study clarify the 'cause' and the 'effect'? Q2.) Were the participants included in any similar comparison? Q3.) Were the participants included in any comparisons receiving similar treatment/care other than the exposure or intervention of interest? Q4). Was there a control group? Q5) Were there multiple outcomes measurements both before and after the intervention/exposure? Q6.) Was follow-up complete and, if not, were the differences among groups regarding their follow-up adequately described and analyzed? Q7.) Were the outcomes of participants included in any comparison measured in the same way? Q8.) Were outcomes measured reliably? Q9.) Was appropriate statistical analysis used?

According to the tool, the risk of bias is rated high when the study had a less than 50% score of "yes," moderate when it reached 50% to 69% score of "yes," and low when it reached more than 70% score of "yes."

Quality of evidence

The quality of evidence and the strength of recommendations were assessed using the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) criteria, which apply to study design, methodological limitations, inconsistency, indirectness, imprecision, and other considerations. They were classified as high, moderate, low, or very low, as defined in Table II.

Publication bias

Begg's funnel plot was used to assess publication bias in the articles included in the meta-analysis. It is a scatter plot of the effect estimate from individual studies against each study's size measure or precision. The standard error of effect estimate is often chosen as the measure of study precision and plotted on the vertical axis with a reversed scale that places the larger, most

Table II. GRADE categories for quality of evidence

Grade	*Definition*
High	True effect lies close to that of the estimate of the effect.
Moderate	True effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low	True effect may be substantially different from the estimate of the effect.
Very low	True effect is likely to be substantially different from the estimate of the effect

GRADE, Grades of Recommendation, Assessment, Development, and Evaluation criteria.

powerful articles toward the top. Funnel plot asymmetry suggests the possibility of publication bias.

RESULTS

Study selection

As stated above, 10 articles were included in the systematic review[3,11,13,19-25] and 6 of these articles in the meta-analysis.[11,13,21,23-25] Corresponding authors of all included articles were contacted for the details of the manufacturer and model of the CBCT units, FOVs, and exposure parameters. However, we received a response from only 1 author.[24] Thus, these parameters were not included in the subgroup analysis.

Study characteristics

The characteristics of the individual studies are presented in Table III. All 10 articles[3,11,13,19-25] included in the systematic review and meta-analysis were published between 2010 to 2022. These investigations were conducted in 7 different countries, with 3 from Brazil[3,11,13] 2 from China,[19,22] and 1 each from Iran,[20] Egypt,[21] India,[23] United Arab Emirates,[24] and Saudi Arabia.[25]

The study populations ranged from 16 to 98 participants. All selected studies included a healthy population undergoing dental treatment that required CBCT for radiographic investigation. Five[11,13,21,24,25] of the 6 articles included in the meta-analysis reported the mean age of the participants. Mounika et al.[23] provided age range and but failed to mention mean age. The mean age of participants ranged from 11 $\pm$ 1.0 years to 34.27 $\pm$ 3.83 years. Subgroup analysis to evaluate correlation between genotoxicity and age could not be performed due to variation in the age of the patients in the included articles.

Regarding days of cell collection, most of the investigations performed postexposure exfoliative cytology of respective sites immediately before and 10 days after exposure for radiographic examination. However, Altoukhi et al.[25] performed the collection 10 $\pm$ 2 days after and 1 month after exposure. Mounika et al.[23] collected cells at 15 and 30 days after exposure. Luke et al.[24] collected cells immediately after exposure and 8 and 14 days after exposure. Farhadi et al.[20] did not clearly state the time frame for cell collection; therefore, this article was not considered for further analysis. For uniformity, the first exfoliative cytology after the exposure to CBCT was considered for analysis in the research of Altoukhi et al.[25] and Mounika et al.,[23] while cell collection for Luke et al.[23] was considered after 8 days.

All 10 studies[3,11,13,19-25] reported the site for performing exfoliative cytology in the oral epithelium as the buccal mucosa. Yang et al.[19] also collected cells from the lateral border of each side of the tongue and gingival cells from the keratinized mucosa of the maxillary and mandibular dental arches. Interestingly, there was no statistically significant difference in genotoxicity or cytotoxicity between the 3 sites. Genotoxicity and cytotoxicity data from the buccal mucosa were considered for our analysis. Regarding the staining technique, 5 papers[3,20,21,24,23] reported the use of the Papanicolaou staining method, 4 studies[11,13,22,25] used the Feulgen/fast green staining method, and one[19] used Schiff's reagent counterstained with the fast green staining technique.

Nine articles evaluated micronuclei along with nuclear alterations while one, Farhadi et al.,[20] evaluated only change in the frequency of micronucleus formation. Genotoxicity was measured by the change in the frequency of micronucleus formation. For cytotoxicity, the following nuclear alterations were investigated: pyknosis, karyolysis, karyorrhexis, nuclear buds, and broken eggs. However, not every article assessed all of these cytotoxic alterations. Thus, for the sake of homogeneity and analysis, only those articles that assessed at least pyknosis, karyolysis, and karyorrhexis were considered. None of the participants in any of the 10 studies were exposed to known genotoxins and/or cytotoxins apart from the X-ray exposure in CBCT examinations.

Yang et al.[9] Li et al.[22] and Farhadi et al.[20] mentioned dose area products in their articles, whereas Fonte et al.[3] and Altoukhi et al.[25] listed the average effective dose for partial and total scans and the approximate total effective dose, respectively. The absorbed, equivalent, or effective doses were not recorded in any other articles, and the effect of exposure dose could not be analyzed. The FOV mentioned in 7 articles ranged from 4 $\times$ 4 cm to 17 $\times$ 22 cm.[3,13,19,21,23-25] Three other papers did not report FOVs.[11,20,22] Therefore, the variation of results based on FOV could not be calculated for subgroup analysis.

Only 6 studies[11,13,21,23-25] provided pre- and postexposure data for genotoxicity and cytotoxicity. This was measured on the basis of mean and standard deviation.

Table III. Characteristics of the studies included in the systematic review and meta-analysis

Authors	*Country*	*Study population (n)*	*Patient age range/ Mean age*	*Days of cell collection*	*Site of cell collection*	*Staining method*	*Methods to measure genotoxicity and cytotoxicity*		*Exposure to other known genotoxins or/and cytotoxins*	*Results of genotoxicity and cytotoxicity measurements*	
							Genotoxicity	*Cytotoxicity*		*Genotoxicity*	*Cytotoxicity*
Fonte et al.[3]	Brazil	29	45.8 ± 12.5 years	10	Buccal mucosa	Papanicolaou	Micronuclei	Pyknosis, karyolysis, karyorrhexis, bud and broken eggs	None	Results are expressed in graph, numerical value cannot be derived.	Results are expressed in graph, numerical value cannot be derived.
Carlin et al.[11]	Brazil	19	26.8.±5 years	10	Buccal mucosa	Feulgen/ fast green	Micronuclei	Nuclear alterations were considered: pyknosis, karyolysis and karyorrhexis	None	BE: 0.04 ±0.05 AF: 0.05±0.06	BE:7.45±4.61 AE:17.84±5.47
Lorenzoni et al.[13]	Brazil	24	11±1.2 years 8 to 15 years	10	Buccal mucosa	Feulgen/ fast green	Micronuclei	Nuclear alterations were considered: pyknosis, karyolysis and karyorrhexis	None	BE:0.025±0.07 AE: 0.033± 0.08	BE:12.4±4.6 AE: 16.4 ±4.1
Yang et al.[19]	China	46	23-42 years	10	Buccal mucosa, tongue, and gingiva	Schiff's reagent counterstained fast green	Micronuclei	Nuclear buds, basal cells, binucleated cells, condensed chromatin cells, pyknosis cells, karyolysis cells, and karyorrhexis cells	None	Results are expressed in graph, numerical value cannot be derived	Results are expressed in graph, numerical value cannot be derived
Farhadi S et al.[20]	Iran	80	45.02±14.5years 19- 86 years	Not mentioned clearly	Buccal mucosa	Papanicolaou	Micronuclei	Not assessed	None	BE: 8.20% AE: 27.10%	Not assessed
Basha et al.[21]	Egypt	30	34.27± 3.83 (27 to 43 years)	10±2	Buccal mucosa	Papanicolaou	Micronuclei	Pyknosis, condensed chromatin, and karyorrhexis	None	BE:0.026 ± 0.0062 AE:0.030 ± 0.0068	BE:0.013 ± 0.0039 AE:0.027 ± 0.0072
Li et al.[22]	China	98	8 to 42 years 23.63± 6.64 years	10	Buccal mucosa	Feulgen/fast green	Micronuclei	Basal cells, binucleated cells, condensed chromatin cells, karyorrhectic cells pkynotic cells, karyolytic cells, and cells with nuclear buds	None	BE: 0.41 AE: 0.65	BE:16.46 AE: 20.80
Mounika et al.[23]	India	60 Group-1 with single exposure and Group-2 with double exposure with 30 subjects in each group	24- 50 years	15	Buccal mucosa	Papanicolaou	Micronuclei	Pyknosis	None	GROUP I: BE:11.27±2.612 AE:13.90±2.746 GROUP II: BE:9.30±2.246 AE:15.63±1.958	GROUP I BE:8.83±1.724 AE:11.03±2.042 GROUP II: BE:8.33±1.863 AE:13.03±2.125
Luke et al.[24]	UAE	35	31.8+ 13.28 years 6 to 75 years mean	8	Buccal mucosa	Papanicolaou	Micronuclei	Pyknosis, karyolysis, and karyorrhexis	None	BE: 0.89±1.88 AE: 15.37± 5.87	BE: 0.29 AE: 5.12
Altoukhi et al.[25]	Saudi Arabia	18	11±1.0 years	10	Buccal mucosa	Feulgen/ fast green	Micronuclei	Condensed chromatin, karyorrhexis, pyknosis, and karyolysis	None	BE: 0.3±0.3 AE: 2.2±2.3	BE: 3.46 AE: 6.73

BE, before exposure; *AE*, After exposure.

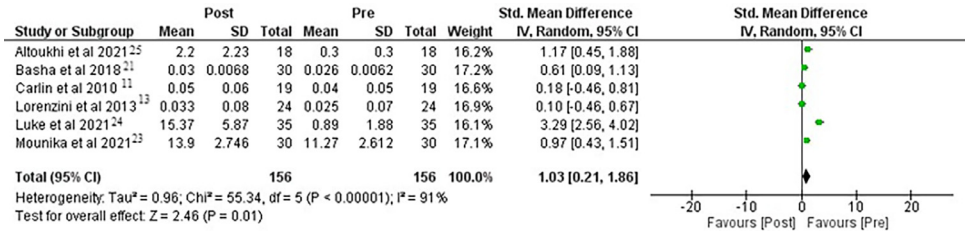

Fig. 2. Pooled impact of cone beam computed tomography exposure on genotoxicity in oral epithelium. The effect size measured by SMD for genotoxicity was 1.03 (CI 0.21, 1.86), representing a large effect. The tau^2 value was 0.96, and the chi-squared test for heterogeneity was 55.35 (df = 5), with $P < .00001$. The I^2 statistic for random effects was 91%, and the overall effect for Z value was 2.64 ($P = .01$). The forest plot "diamond" was displaced to the right side of the null line expressed in the graph for genotoxicity, indicating a significant increase in the frequency of micronucleus formation after exposure to CBCT.

Table IV. Risk of bias assessed by the Joanna Briggs Institute (JBI) critical assessment tool for systematic review of observational studies

Authors	*Q1*	*Q2*	*Q3*	*Q4*	*Q5*	*Q6*	*Q7*	*Q8*	*Q9*	*% Yes*	*Risk of bias*
Fonte et al.[3]	√	√	√	√	√	√	√	√	√	100	Low
Carlin et al.[11]	√	√	U	√	√	√	√	√	√	88.88	Low
Lorenzoni et al.[13]	√	√	√	√	√	√	√	√	√	100	Low
Yang et al.[19]	√	√	√	√	√	√	√	√	√	100	Low
Farhadi et al.[20]	√	√	√	√	√	√	√	√	√	100	Low
Basha et al.[21]	√	√	√	√	√	√	√	√	√	100	Low
Li et al.[22]	√	√	√	√	√	√	√	√	√	100	Low
Mounika et al.[23]	√	√	√	√	√	√	√	√	√	100	Low
Luke et al.[24]	√	√	√	√	√	√	√	√	√	100	Low
Altoukhi et al.[25]	√	√	U	√	√	√	√	√	√	88.88	Low

√) Yes; N) No; U) Unclear

From this data SMD was estimated for genotoxicity. The effect size measured by SMD for genotoxicity was 1.03 (CI 0.21, 1.86), representing a large effect (Figure 2). Thus, the hypothesis that radiation emitted by CBCT is sufficient to cause genotoxic damage was confirmed with a large effect size.

Regarding heterogeneity, the tau^2 value was 0.96, and the chi-squared test for heterogeneity was 55.34 (df = 5), with $P < .00001$. The I^2 statistic for random effects was 91%, and the overall effect for Z value of 2.46 ($P = .01$) was obtained for genotoxicity (Figure 2). Tau^2 is a measure of standard deviation of true effect sizes between the studies. The chi-squared distribution produces a probability that, when high, denotes greater variation between studies as opposed to within studies. I^2 reflects real difference in the effect size. A high level of heterogeneity resulting from tau^2, I^2, and a highly significant P value indicates heterogeneity not due to chance. The forest plot "diamond" was displaced to the right side of the null line expressed in the graph for genotoxicity (Figure 2). This indicated a significant increase in the frequency of micronucleus formation after exposure to CBCT.

Meta-analysis of cytotoxicity resulting from CBCT exposure was not conducted due to the wide range in the measurement of cytotoxic effects in the included studies. Of the 6 observational investigations, 3 provided combined values of pyknosis, karyolysis, and karyorrhexis as a measure of cytotoxicity,[11,13,21] 2 included separate values of pyknosis, karyolysis, and karyorrhexis,[23,24] and 1 considered only pyknosis.[25]

Risk of bias in individual studies

Two articles[11,25] had an unclear risk of bias regarding the participants included in any comparisons receiving similar treatment/care other than the exposure or intervention of interest. However, all studies included in the meta-analysis exceeded a 70% score of "yes," thereby exhibiting a low risk of bias (Table IV).

Quality of evidence

Table V shows that the quality of evidence regarding the effect of CBCT on oral epithelium was classified as high using the GRADE approach when the importance of the results was critical. The potential problems of risk of bias, inconsistency, indirectness, and imprecision were all rated as not serious. The high strength of evidence suggests the true effect lies close to that of the estimate of the effect.

Table V. Quality of evidence profile for studies included in the meta-analysis for genotoxicity

		Certainty assessment					№ of patients		Effect		Certainty	Importance
№ of studies	*Study design*	*Risk of bias*	*Inconsistency*	*Indirectness*	*Imprecision*	*Other considerations*	*Before CBCT*	*After CBCT*	*Relative (95% CI)*	*Absolute (95% CI)*		
Genotoxicity												
6	Non-randomized	Not serious	Not serious	Not serious	Not serious	Strong association all plausible residual confounding data would reduce the demonstrated effect	156	156	-	SMD **1.03 SD higher** (0.21 higher to 1.86 higher)	⊕⊕⊕⊕ High	CRITICAL

CI, confidence interval; *SMD*, standardized mean difference.

Publication bias

Begg's funnel plot with 95% confidence intervals demonstrated a symmetric distribution of the studies for genotoxicity with systematic heterogeneity of the individual study compared to the standard error, indicating the absence of publication bias (Figure 3).

DISCUSSION

Genotoxins are agents that are capable of inducing DNA or chromosomal damage, and cytotoxins induce cell damage. Currently, it is acknowledged that the accumulation of a number of mutations, reasonably 5 to 7, in critical genes in progenitor cells leads to the manifestation of the malignant phenotype. Human exposure to these agents at any level is considered genotoxic.[26] Although the overall medical exposure to X-rays was reduced by 15% to 20% between 2006 and 2016, CT exposure increased to 63% in 2016 from 50% in 2006.[28]

Natural background radiation accounts for approximately 50% of the radiation dose absorbed by human beings[27] It consists of cosmic, terrestrial, and inhalation or intake of radiation sources, and cannot always be controlled. A review of the possible effects of high natural background radiation on populations cited Brazil, India, Iran, and China as regions with high levels of terrestrial radiation.[29] Interestingly, 3 of the studies included in the present systematic review were from Brazil and 2 were from China.

Exposure to CBCT can cause genotoxic changes with a large effect size of 1.03. A large effect size implies a strong relation between the CBCT exposures and the genotoxic effect on oral epithelium. An earlier systematic review assessing the genotoxic effect of PR exposures reported that these radiographs can cause genotoxic damage in the oral epithelium but with a small effect size of 0.21.[4] Thus, the effect size of CBCT is 4.9 times greater than that of PR. Because of the lack of uniformity in the measurement of cytotoxicity in the included articles, meta-analysis on the cytotoxic effect of CBCT exposure on oral epithelium was not performed.

It should be noted that there is significant evidence of nontargeted (bystander) effects as a long-term side consequence of radiation exposure.[30,31] The radiation bystander effect is the induction of biological effects in cells that are not directly traversed by a charged particle but are in close proximity to cells that are exposed to radiation.[32,33] It could therefore be postulated that genotoxic and cytotoxic effects occur in the regions away from the field of exposure, such as the oropharynx and nasopharynx[2], which should be considered in future research.

Four articles[11,13,22,25] reported the use of the Feulgen/fast green reaction (DNA fluorescent dye) staining

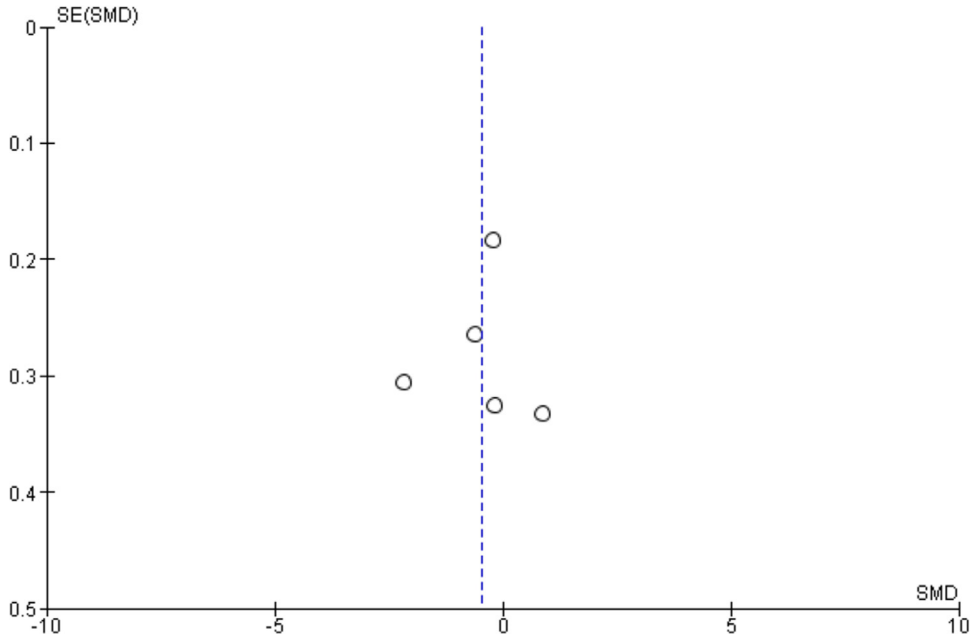

Fig. 3. Begg's funnel plot with 95% confidence intervals for genotoxicity. The plot revealed a symmetric distribution of the studies for genotoxicity with systematic heterogeneity of the individual study compared to the standard error, indicating the absence of publication bias. SE, standard error; SMD, standardized mean difference.

method. In the Human Micronucleus Project on exfoliated buccal cells ($HUMN_{XL}$), Bonassi et al.[12] described the protocol in which the use of Feulgen/fast green reaction was recommended for the BMCyt assay as it is a quantitative, DNA-specific cytochemical procedure. The Papanicolaou staining method was used in 4 articles.[3,20,21,22,24] This is also a preferred staining technique to detect micronuclei in exfoliated buccal mucosal cells when compared to Romanowsky-type stains (Giemsa stains).[34,35]

Only 3 articles [19,20,22] mentioned dose area product , while 2 papers[3,25] mentioned the mean and total effective dose respectively. None of the other articles mentioned either the absorbed, equivalent, or effective dose. It is, however, important for future studies to be consistent in reporting the type of dose measured in CBCT exposure. FOV was not mentioned in 3 articles.[11,20,22] The other 7 studies listed FOVs ranging from 4 × 4 cm to 17 × 22 cm.[3,13,19,21,23,24,25] Due to the wide variation in FOVs, subgroup analysis could not be performed. In addition, as the literature shows an association of age with accumulation of DNA damage,[7] age is considered a reliable indicator of micronucleus frequency. There was, however, a lack of correlation of genotoxicity and cytotoxicity with the age of the participants in the papers included in the SR.

The presence of heterogeneity as indicated by tau^2, chi-squared, and I^2, along with a highly significant *P* value, should be considered of interest. This heterogeneity may stem from variations such as staining method, measure of cytotoxicity, FOV, or radiation dose. Investigating the source of heterogeneity and following a homogenous methodology should be considered in future research. The GRADE tool indicated a high quality of evidence because the studies were rated as having non-serious problems in all 4 requirements of quality evaluation. The GRADE tool does not address the subjective assessment of individual studies with internal validity (methodological quality). It does, however, note that the body of evidence goes beyond the possibility of bias, relating confidence to the estimate of the observed effect and taking into account additional elements determined by it, which correlate to risk of bias, inconsistency, indirectness, and imprecision.

In 2014, the National Council on Radiation Protection and Measurements (NCRP) proposed the ALADA ("as low as diagnostically acceptable") principle to emphasize the importance of dose optimization in diagnostic imaging.[36] Currently, some CBCT units provide different exposure settings including ultra-low dose (ULD), low dose (LD), standard dose (STD), and high-resolution modes.[37,38] Charuakkra et al.[38] stated that the effective dose for small FOV CBCT in the ULD and LD modes is 5-5.8 μSv, which is lower than the minimum effective dose for the small FOV protocol in standard mode (11 μSv) and PR (7-11.4 μSv).[39] They proposed that ULD and LD CBCT protocols may be acquired as routine practice for diagnosis and treatment planning. The current systematic review was conducted on studies using standard CBCT protocols, and

therefore the genotoxic and cytotoxic effects might have been higher. It is important that future studies assess genotoxicity and cytotoxicity levels of the LD, ULD, and high-resolution modes.

CONCLUSIONS

Cone beam computed tomography can cause genotoxicity in the oral epithelium, as demonstrated in the current SR with a large effect size. Based on the view of the position paper of the American Academy of Oral and Maxillofacial Radiology[40], the risk versus benefit ratio of every prescribed radiograph needs to be monitored. However, the cumulative genotoxic and cytotoxic effects of radiation on humans are not known. Therefore, studies should assess this cumulative effect. On the basis of our findings, the following recommendations can be made.

1. Oral radiologists in clinical practice should exercise extra caution before exposing patients, especially children, to CBCT.
2. Future research should assess the impact of the site of cell collection on the effect size of genotoxicity and cytotoxic effects and evaluate the effect size for different FOVs and models of CBCT.
3. For accuracy and uniformity, the use of Feulgen/fast green reaction is recommended for future studies as it is a quantitative DNA-specific cytochemical procedure.
4. For evaluation of cytotoxicity, reporting nuclear alterations should be standardized.

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