

Micronucleus Formation in Oral Mucosal Cells Following Dental X-ray Exposure: A Systematic Review and Meta-analysis

Yaxin Wang¹, Rozita Hassan¹, Liyana Ghazali¹, Shanshan Cai², Anani Aila Bt Mat Zin³,
Sanhui Yang⁴, Linxian Zeng⁵

¹Department of Orthodontics, School of Dental Science, USM Health Campus, Kubang Kerian, Kelantan

²Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster LA1 4YG, UK

³Department of Pathology, School of Medical Science, Health Campus, Kubang Kerian, Kota Bharu. Kelantan

⁴Exercise and Sports Science Program, School of Health Sciences, Health Campus, Universiti Sains Malaysia

⁵Familiy department, Medical health science, USM Health Campus, Kubang Kerian, Kelantan

Corresponding Author: Rozita Hassan, Orthodontic Unit, School of Dental Science, Health Campus, Universiti Sains Malaysia, Malaysia

Phone: 0199886161

Email: rozitakb@usm.my

Shanshan Cai, Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster LA1 4YG, UK

Phone: +447898954901

Email: s.cai6@lancaster.ac.uk

Micronucleus Formation in Oral Mucosal Cells Following Dental X-ray Exposure: A Systematic Review and Meta-analysis

Abstract

Background

Radiographic imaging is essential in dental diagnostics, yet concerns persist regarding its genotoxic impact. While digital advancements have reduced radiation doses, the effects of X-ray exposure on oral mucosal cells remain debated. This study systematically reviews and quantitatively analyzes the effects of X-ray exposure on micronucleus (MN) formation in oral epithelial cells, examining potential age-related variations.

Methods

A thorough literature review was performed across PubMed, Web of Science, EBSCO, and Scopus (updated to November 2024), adhering to PRISMA criteria. Eligible studies examined cytogenetic changes in oral epithelial cells after X-ray exposure in healthy individuals. Data extraction covered study design, imaging modality, radiation dose, micronucleus frequency, and statistical methods. Risk of bias was assessed using a modified EPHPP tool. A random-effects model synthesized micronucleus frequency changes, and Fisher's Z-transformation analyzed age correlations.

Results

Eighteen studies met the inclusion criteria, with 16 centered on panoramic imaging and two examining a combination of CBCT and lateral cephalometric X-rays. Meta-analysis confirmed a significant increase in micronucleus frequency post-exposure (SMD = 0.30, 95% CI: 0.07–0.52, $p = 0.01$), indicating genotoxic effects. However, age showed a weak correlation with micronucleus formation ($r = 0.149$, 95% CI: -0.009 to 0.3, $p = 0.065$).

Conclusions

X-ray exposure induces measurable genotoxic damage in oral epithelial cells, though age-related effects remain inconclusive. Adhering to the ALARA principle is crucial to minimize unnecessary radiation. Future studies should employ larger cohorts and refined biomarkers to enhance risk assessment.

Keywords: X-ray, oral mucosa, micronucleus assay, genotoxicity, cytotoxicity, radiation exposure, age

Introduction

With the growing need for dental diagnostics and treatment, radiographic imaging has become an integral part of clinical practice. Whether for routine evaluations or advanced aesthetic procedures, it is essential for precise diagnosis and effective treatment planning. Among the available imaging modalities, panoramic radiography

(orthopantomography, OPG) is widely utilized due to its comprehensive anatomical coverage and relatively lower radiation exposure compared to full-mouth periapical radiography. Radiation exposure from panoramic radiography typically falls between 10 and 30 Sv, whereas full-mouth periapical imaging can deliver up to 33 Sv, positioning panoramic scans as a comparatively lower-radiation option.¹ CBCT, a high-resolution volumetric imaging modality, delivers radiation doses ranging from 25 to 1025 Sv, contingent upon scanner parameters and imaging protocols. Concerns regarding cumulative exposure in dental radiography have emerged, particularly as repeated panoramic scans accumulate radiation doses akin to multiple individual exposures.² While digital imaging advancements have led to dose reductions, apprehensions remain about genotoxic and cytotoxic risks, especially in head and neck radiography, where CT scans administer approximately 46 mGy. Research indicates that even low-dose radiation can trigger cytogenetic changes in oral epithelial cells, leading to chromosomal instability, DNA damage, and apoptosis.³ Genomic instability, stemming from both single- and double-strand DNA breaks, has been linked to tumor formation and developmental abnormalities.⁴ Given the nuclear genome's heightened vulnerability to radiation-induced damage, continuous monitoring of radiological safety remains essential.⁵

To assess the biological impact of radiation, conventional techniques include metaphase aberration detection, sister chromatid exchange analysis, and chromosomal instability evaluations in peripheral lymphocytes (Ribeiro et al. 2011). However, these techniques are often labor-intensive, require specialized expertise, and involve prolonged processing times. The micronucleus (MN) assay is widely recognized as a reliable, minimally invasive method for detecting radiation-induced cellular damage.⁶ Micronuclei (MNs) are extranuclear bodies formed from chromosome fragments or whole chromosomes that fail to reintegrate during mitosis, serving as indicators of genotoxic stress.⁷ The European Network for Biological Dosimetry (RENEB) has endorsed this assay for large-scale radiation exposure assessments.⁸ Since oral mucosal cells come into direct contact with ionizing radiation during dental imaging, they serve as an optimal biological model for evaluating the formation of micronuclei. Compared to venipuncture-dependent cytogenetic tests, micronucleus analysis in exfoliated oral cells provides a cost-effective, swift, and non-invasive alternative.⁹ This approach is frequently employed in occupational and environmental radiation monitoring and presents a practical means of assessing genotoxic risks among dental patients.¹⁰

Age significantly influences radiation-induced genotoxic and cytotoxic effects.¹¹ Rapidly dividing tissues, especially in children, exhibit heightened sensitivity due to their diminished DNA repair capacity.¹² Early identification of radiation-induced genetic alterations in pediatric populations is critical to minimizing long-term health consequences.¹³ Despite extensive research on micronucleus formation following radiation exposure, findings on the correlation between age and X-ray-induced genotoxicity remain inconsistent.¹⁴ While certain studies¹⁵ establish a clear relationship between micronucleus frequency and age, others¹⁶ report no significant link. These

discrepancies likely stem from differences in sample demographics, radiation protocols, staining methods, and analytical techniques.¹⁷

Material and methods

Search strategy, eligibility criteria, and study selection

Following PRISMA guidelines, this study conducted a systematic review of cytogenetic changes in exfoliated oral mucosal cells due to radiation exposure.¹⁶ This study, structured using the PICOS framework, investigated whether age affects the cytotoxic and genotoxic impact of X-ray exposure on oral epithelial cells. A comprehensive literature search was performed across PubMed, Web of Science, EBSCO, and Scopus to identify studies published up to November 26, 2024, examining the correlation between radiation exposure and cytogenetic alterations in oral epithelial cells within the general population. The search strategy incorporated Boolean operators: (X-rays OR "radiation exposure") AND ("oral mucosa" OR "buccal mucosal cells" OR "epithelial cells") AND ("toxicity" OR "cytotoxicity" OR "genotoxicity" OR "DNA damage"). Database-specific filters were applied, restricting searches in Scopus to the dentistry field, considering only original research in Web of Science, applying no limitations in PubMed, and including only full-text, English-language articles in EBSCO.

Endnote software was used for reference management and study selection. The screening process followed a two-stage approach: an initial title and abstract review was conducted by author Yaxin Wang, followed by full-text evaluation, where two independent reviewers (authors Yaxin Wang and Sanhui Yang) assessed each study for eligibility. Any selection disagreements were settled through discussion, with Linxian Zeng acting as an arbitrator when needed. To ensure thorough coverage, the reference lists of chosen articles were manually reviewed for further relevant studies.

Only peer-reviewed English-language studies that analyzed X-ray-induced cytotoxicity and genotoxicity in exfoliated oral epithelial cells from healthy individuals were considered. Studies were excluded if they involved patients with oral mucosal diseases (such as stomatitis, oral ulcers, candidiasis, mucosal inflammation, or burning mouth syndrome), individuals with systemic conditions (such as nasopharyngeal carcinoma, oral cancer, head and neck malignancies, pneumonia, or AIDS), in vitro or animal studies, research assessing alternative genetic endpoints, studies examining the effects of non-ionizing genotoxic agents, investigations of non-oral epithelial cells (such as nasal or urinary tract epithelia), or articles classified as reviews, editorials, letters to the editor, or conference abstracts. The final review incorporated only research that adhered to strict methodologies and clearly established inclusion criteria.

Data extraction

1 A structured data extraction protocol was implemented to ensure accuracy and
2 consistency. Extracted variables included study authors, publication year, research
3 objectives, methodology, geographic location, sample size, gender distribution,
4 participant age, DNA staining procedures, oral cell sampling sites, imaging techniques,
5 radiation dose, exposure duration, control group characteristics, observed cytogenetic
6 alterations, micronucleus frequency correlations pre- and post-radiation, statistical
7 analyses, and key study conclusions.¹¹ Two independent reviewers conducted data
8 extraction, with discrepancies resolved through discussion.
9

10 11 12 **Risk of bias in individual studies**

13
14
15 Methodological rigor and bias were evaluated using a modified Effective Public Health
16 Practice Project (EPHPP) tool.¹⁷ Studies were appraised based on critical parameters
17 for micronucleus assays in exfoliated oral cells, including the use of validated nucleic
18 acid staining techniques, the assessment of a minimum of 2000 exfoliated cells per
19 participant, cytotoxicity evaluation, blinded analysis, and appropriate statistical
20 reporting. The final quality classification was based on methodological rigor: studies
21 were rated "strong" if all confounders were controlled, "moderate" if one confounding
22 factor was unaccounted for, and "weak" if two or more methodological limitations were
23 present. Quality assessment disagreements were resolved through reviewer discussions,
24 with unresolved cases arbitrated by a third evaluator.¹¹
25
26
27
28
29

30 **Meta-analysis**

31
32
33 To analyze variations in micronucleus frequency pre- and post-radiation exposure in
34 the general population, RevMan version 5.4.1 (The Cochrane Collaboration, Oxford,
35 UK) was employed using a random-effects model.¹⁸ Pearson correlation coefficients
36 were calculated using data from studies such as those by Popova, L,¹⁹ Basha, S,²⁰ Li,
37 G¹⁸ to examine the relationship between age and micronucleus frequency. Statistical
38 analyses were executed in SPSS, employing Pearson's correlation coefficient as the
39 effect size metric for this investigation. To ensure consistency in correlation
40 coefficients across studies, Fisher's Z-transformation was applied (Equation 1). Sample
41 size and inverse variance of correlation coefficients determined the weighting of
42 computed values, following Equation (2). The aggregated estimate was then converted
43 back into correlation coefficients (Equation 3) to refine the evaluation of age-related
44 changes in micronucleus frequency before and after X-ray exposure. Cohen's
45 classification (1992)²¹ defines r values of 0.1, 0.3, and above 0.5 as indicators of weak,
46 moderate, and strong correlations, respectively.
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

$$\text{Fisher's } Z = 0.5 \ln \frac{1+r}{1-r} \quad (1)$$

$$S_{EZ} = \sqrt{1/(n-3)} \quad (2)$$

$$\text{Summary } r = \frac{e^{2z}-1}{e^{2z}+1} \quad (3)$$

To assess heterogeneity across studies, Cochran's Q test and the I² statistic were applied, with I² values below 50% suggesting low heterogeneity, thereby justifying the application of a fixed-effect model. When heterogeneity exceeded the set threshold, a random-effects model was selected to account for population variability.²² Funnel plot analysis, along with Begg's test²³ and Egger's regression analysis²⁴, was conducted to detect potential publication bias. A symmetrical funnel plot was anticipated if no publication bias was present, with Begg's and Egger's tests yielding non-significant p-values (p > 0.05). To assess the robustness of results, sensitivity analysis was carried out by systematically excluding individual studies and evaluating their impact on overall findings.²⁵ Comprehensive Meta-Analysis (CMA) version 3.7 was utilized for all meta-analytical procedures to maintain statistical accuracy and methodological rigor.

Certainty of Evidence

GRADEpro GDT software (<http://gdt.guidelinedevelopment.org>) was employed to assess evidence certainty, ensuring a systematic evaluation of the reliability of the findings. The evaluation process incorporated multiple factors, including study design, potential methodological biases, inconsistency of results, indirectness of evidence, and imprecision in effect estimates. Each study underwent systematic appraisal to confirm the robustness of reported outcomes, accounting for methodological disparities. Evidence quality was classified as high, moderate, low, or very low based on cumulative assessment.¹¹ By implementing this classification approach, the findings were objectively interpreted, strengthening their reliability and relevance for both research and clinical applications.

Results

Literature Search

After duplicate removal, 678 unique records underwent title and abstract screening, with 637 excluded, including three unavailable through open-access sources. After a thorough full-text review of the 41 remaining publications, 15 studies were deemed directly relevant to the research question. Additionally, screening reference lists identified three more studies, bringing the total to 18 for qualitative synthesis and meta-analysis.

General Characteristics of Included Studies

Of the 18 included studies, 16 focused on the impact of panoramic X-ray exposure on oral mucosal cells. Two additional studies investigated the combined effects of panoramic X-ray with lateral/posteroanterior X-ray, comparing these modalities with cone-beam computed tomography (CBCT), as well as the toxicity effects of panoramic X-ray combined with lateral X-ray projection. Three studies examined CBCT-related effects on oral epithelial cells, with one²⁶ comparing genetic and cytotoxic alterations induced by CBCT and multi-detector computed tomography (MDCT).

India had the highest number of relevant studies, followed by Iran,^{26–29} Brazil,^{6,28,30} Bulgaria,¹⁹ China,¹⁸ Egypt,²⁰ and Turkey.³¹ Gender distribution was not reported in one study³². Participant ages ranged from eight years¹⁸ to 73 years¹⁹. Two studies^{19,29} did not provide age information. Studies ranged from 2007¹⁹ to 2024²⁷. A summary of key findings and study characteristics is presented in Table 1.

Description of Study Variables (Confounders)

Core study variables comprised staining techniques, cell count per sample, overall sample size, cytotoxicity assessment, statistical rigor, blind implementation, exclusion criteria robustness, and radiation parameters. Each of these factors was carefully examined to determine their potential influence on study outcomes.

Regarding staining methods, Papanicolaou staining emerged as the most frequently used technique, reported in seven studies. One study²⁸ did not report the staining method, while five used Feulgen staining and four employed Giemsa staining, both of which, like Papanicolaou staining, lack DNA specificity. Acridine Orange and AgNORs staining were used in one study.³² Exfoliated cell counts ranged from 1,000 per sample in five studies to 2,000 in five others.^{18,19,33–35} Four studies^{27,28,36,37} did not specify cell counts. The sample size ranged widely, with the largest cohort consisting of 100 participants, while the smallest study included only 30 individuals.

Cytotoxicity assessment was performed in 11 studies, whereas 7 studies did not include any cytotoxic biomarker evaluations. Pai A et al. 2012³⁸ was the sole study lacking a clear statistical methodology description. While eight studies implemented blinded analysis, the remaining ten did not report such procedures. Exclusion criteria were detailed in all studies except Silva MB et al. 2018,²⁸ ensuring methodological rigor by restricting participants with recent imaging, systemic conditions, mucosal disorders, or medication use. A more detailed breakdown of these study characteristics is available in Table 2.

Regarding radiation parameters, exposure conditions varied significantly. The recorded X-ray voltage ranged from a minimum of 60 kV to a maximum of 110 kV. Exposure times ranged from 0.5 to 18 seconds, with the longest exposure duration for pediatric patients recorded at 18 seconds. Tube currents ranged from 7.1 mA to 35 mA; however, seven studies did not specify exposure duration or radiation dose details. A comprehensive summary of radiation parameters is available in Table 3.

Study Design and Main Findings

Among 18 included studies, ten reported significant changes in cytotoxic markers, while the rest observed no major nuclear alterations. Notably, all studies employed a self-controlled design, allowing for more reliable biomarker comparisons. Table 4 presents a summarized overview of these findings.

The potential cytotoxic and genotoxic effects of panoramic radiography were investigated in sixteen studies. Micronucleus frequency was assessed ten days post-exposure by Torabinia N et al. 2024²⁷, revealing no significant increase, whereas Sreeshyla H et al. 2023³⁴ reported a substantial elevation (Table 5). In addition, three studies explored the impact of cone-beam computed tomography (CBCT). Li G et al. 2018¹⁸ examined CBCT-related cytotoxic and genotoxic effects in buccal epithelial cells, comparing them with those induced by panoramic X-rays and lateral/posteroanterior imaging. Kaur, I et al. 2020³⁹ and Malik S et al. 2022³² analyzed cytological alterations over various time points. Both studies observed a notable rise in micronucleus frequency ten days following panoramic radiography, though results differed for longer time frames. One study observed a substantial increase in micronucleus frequency at 40 days post-exposure, whereas another recorded a decrease between 10 and 21 days post-exposure. Kaur, I et al. 2020³⁹ reported no significant changes in micronucleus frequency among individuals aged 40–50 years at 10 and 21 days post-exposure compared to baseline levels.

Cytotoxic biomarker assessments across all studies identified at least one form of cellular damage, supporting the idea that X-ray exposure affects nuclear stability. However, when evaluating the correlation between age and radiation-induced cytotoxicity and genotoxicity, the results were inconclusive. In the 18 studies included, qualitative results on the impact of age on genotoxicity and cytotoxicity induced by X-ray exposure are presented in Table 7. All studies address the relationship between age and micronucleus frequency, yet findings are inconsistent. Only four studies^{19,28,32,37} report a statistically significant correlation between X-ray exposure and micronucleus frequency. Conversely, 13 studies do not find such a correlation, and one study³⁹ reports age-dependent effects, with a significant relationship observed in the 15-25 age group, but not in the 40-50 age group. Regarding **cytotoxicity**, eight studies^{18,20,26,29,30,33,36,38} explored the correlation between age and cytotoxicity effects, but none found a significant relationship. Consequently, it is imperative to conduct a meta-analysis to further investigate the relationship between age and micronucleus frequency.

Detailed descriptions of the study groups, participant ages, changes in micronucleus frequency, and the statistical significance of age-micronucleus frequency relationships are outlined in Table 8. Studies varied in their correlation measurements, including correlations between age and pre-exposure micronucleus frequency, age and post-exposure micronucleus frequency, and age and the difference in micronucleus frequency before and after X-ray exposure, as detailed in Table 9. Standard errors for

each study are calculated. Among the six studies^{18–20,27,37,40} that designed age and micronucleus frequency correlation coefficients, all provided coefficients for age in relation to pre- and post-X-ray exposure micronucleus frequencies, derived either directly from the studies or calculated using SPSS based on participant age and micronucleus frequency data. Only three studies^{18–20} calculated coefficients for changes in micronucleus frequency before and after X-ray exposure related to age. Meta-analyses were conducted separately for different types of correlation coefficients, as depicted in Figures 4, 7, and 10. Distinct funnel plots and sensitivity analyses were employed to assess publication bias and the robustness of the results across these different categories of meta-analyses.

Assessment of the Risk of Bias

The Effective Public Health Practice Project (EPHPP) framework was utilized to evaluate study quality, with the results detailed in Table 6. Following a comprehensive evaluation, seven studies were categorized as moderate to strong, whereas the rest were deemed weak due to methodological constraints.

Data synthesis

The meta-analysis incorporated data from four studies^{20,33,36,38} focused on panoramic X-ray exposure or CBCT, while two studies^{18,30} were excluded due to missing standard deviation (SD) values. The research of Jahanshahiafshar Z et al. was omitted as it lacked distinct comparisons for different radiographic modalities.

The meta-analysis demonstrated a statistically significant shift in micronucleus frequency before and after radiation exposure, yielding a standardized mean difference (SMD) of 0.30 (95% CI: 0.07–0.52, $p = 0.24$). Heterogeneity analysis indicated low variability, with $\text{Tau}^2 = 0.02$, $\text{chi}^2 = 4.21$, $\text{df} = 3$ ($P = 0.24$), and $I^2 = 29\%$ (Figure 2). The forest plot indicated a rightward shift of the summary effect estimate (diamond) beyond the zero line, confirming a significant elevation in micronucleus frequency following exposure ($Z = 2.54$, $P = 0.01$). The data consolidate evidence connecting X-ray exposure to cytogenetic modifications in oral mucosal cells, stressing the need for in-depth exploration of its chronic effects.

Certainty of Evidence

The certainty of evidence regarding genetic toxicity before and after radiographic exposure was rated as very low (Figure 3), given the inherent limitations of observational studies, including bias risk, imprecision, heterogeneity, and publication

bias.¹¹ These findings suggest that multiple variables could impact the outcomes, necessitating more rigorous research in the future to validate the potential genotoxicity of X-ray radiation on oral epithelial cells.

Effect Size and Heterogeneity

Six studies involving 286 participants were analyzed (Table 9). Heterogeneity assessments were conducted across three groups to examine the relationship between age and micronucleus frequency. Before X-ray exposure, the first group examined the Pearson correlation to determine the relationship between micronucleus frequency and age. The second group, however, assessed the correlation coefficient linking micronucleus frequency and age following radiation exposure. Meanwhile, the third group concentrated on evaluating how age influenced variations in micronucleus frequency across pre- and post-exposure conditions. Table 9 provides a comprehensive breakdown of the Pearson correlation coefficients across the three groups, including individual study sample sizes and their corresponding standard errors.

Substantial heterogeneity was detected in Group 1 ($I^2 = 60.79\%$, $Q = 12.75$, $P = 0.026$) and Group 2 ($I^2 = 66.18\%$, $Q = 14.78$, $P = 0.011$), justifying the application of a random-effects model.

Meta-analysis employing both random-effects and fixed-effects models exhibited a moderate to low correlation across all three groups. The correlation coefficients were as follows: Group 1 ($r = 0.285$, 95% CI: $-0.065, 0.572$; see Figure 4), Group 2 ($r = 0.193$, 95% CI: $-0.021, 0.390$; see Figure 7), and Group 3 ($r = 0.149$, 95% CI: $-0.009, 0.3$; see Figure 10). These results suggest that while a weak correlation exists between micronucleus frequency and age, its clinical significance remains marginal, necessitating further investigation to address potential confounders and methodological inconsistencies.

Publication Bias and Sensitivity Analysis

Funnel plots, Begg's test, and Egger's linear regression were utilized within the meta-analysis to evaluate publication bias. Funnel plots for all three groups demonstrated overall symmetry, with most data points positioned within the expected range. However, minor asymmetries were observed, suggesting a low probability of publication bias. Statistical testing confirmed this, as Begg's test ($p > 0.05$) and Egger's regression ($p > 0.05$) indicated no significant bias.

To evaluate the reliability of the results, a sensitivity analysis was performed. In Group 1, excluding individual studies had little effect on the overall effect size and confidence intervals. Most confidence intervals encompassed zero, confirming the findings'

stability under the random-effects model. Waingade M et al. 2012⁴⁰ significantly influenced results, necessitating further scrutiny of its data reliability. Nevertheless, its exclusion had no substantial impact on the conclusions, as the random-effects model effect size remained 0.285, with a 95% CI encompassing zero ($P = 0.108$), indicating statistical insignificance.

In Group 2, findings remained stable, with no notable alterations in effect size or direction after individual study exclusion,²⁰ was also a key contributing factor, requiring further examination. The random-effects model yielded an effect size of 0.193 (95% CI: -0.021, 0.390; $P = 0.077$), suggesting a weak correlation between post-exposure micronucleus frequency and age, without reaching statistical significance.

Similarly, the Group 3 meta-analysis demonstrated stable findings, with minimal variations in effect size after excluding individual studies. The fixed-effects model effect size was 0.149 (95% CI: -0.009, 0.300; $P = 0.065$), with results approaching significance. Given the limited number of studies, the observed uncertainty is likely attributable to sample size constraints rather than inherent methodological deficiencies. Collectively, these findings suggest that despite the weak correlation between micronucleus frequency and age, analytical robustness is maintained across models, reinforcing the need for large-scale investigations to validate these associations.

Discussion

Micronucleus (MN) formation and cytotoxic alterations function as essential biomarkers in assessing the genotoxic and cytotoxic impact of ionizing radiation, especially X-rays. Micronuclei formation is a hallmark of DNA or chromosomal damage, arising from acentric chromosome fragments or lagging chromosomes lacking centromeres. These formations arise during metaphase-to-anaphase transition due to mitotic dysfunction or DNA damage.^{18,41–43} Radiation-induced cytotoxicity manifests as nuclear changes such as chromatin condensation, nuclear fragmentation, and dissolution, indicative of cell death or necrosis.⁴³

The standard for micronucleus analysis, as established by Tolbert et al. and Sarto et al.,^{44,45} highlights that nuclear abnormalities increase significantly in individuals exposed to carcinogens, reflecting cellular responses to genetic damage. Angelieri F et al. 2017⁴ and TOLBERT et al. 1992⁴⁵ propose that cytotoxic effects serve as indicators of cytogenetic damage while also augmenting sensitivity in biomonitoring assessments. This is especially pertinent, as cytotoxicity may confound mutagenicity evaluations—heightened cytotoxicity can induce apoptosis in micronucleated cells before detection, potentially underestimating genotoxic effects.

This meta-analysis primarily utilized buccal epithelial cells due to their increased susceptibility to chromosomal damage and lower DNA repair efficiency relative to lymphocytes.⁵ However, research has also explored other oral locations, including the keratinized gingiva, the lateral border of the tongue, and the upper dental arch.^{30,35,38,39}

Given that epithelial cells account for over 90% of human malignancies and are directly exposed to dental radiography, they serve as relevant targets for micronucleus assessment.⁸

Most clinical studies evaluated subjects before and after radiation exposure, typically over a 10-day period, corresponding to the rapid regeneration of epithelial tissue.^{4,30,46} Micronucleus formation initiates in the basal layer during epithelial cell mitosis, with exfoliated cells shedding over a 7–16-day period.⁴⁴ Some studies extended follow-up to 21–40 days to assess long-term genotoxic effects.⁴⁷ The results of this meta-analysis demonstrate a notable increase in micronucleus frequency in oral epithelial cells after exposure to panoramic X-rays, confirming its detectable genotoxic effects, aligning with the findings of Cerqueira et al.³⁰ Variability in radiation doses, participant demographics, biopsy methods, fixation techniques, staining protocols, and micronucleus scoring criteria may account for inconsistencies across studies.

While extensive evidence links X-ray exposure to genotoxicity, some studies reported no significant genetic alterations post-exposure.^{19,27,33,35–38} A plausible interpretation is the simultaneous effect of cytotoxicity, which induces cellular apoptosis, thereby diminishing the pool of micronucleated cells available for examination. This suggests that although X-rays exhibit cytotoxic properties, their mutagenic potential may not be consistent, reinforcing the necessity for further investigations with expanded sample sizes. All moderate-to-high-quality studies confirmed post-radiation cytotoxic effects in oral mucosal cells, underscoring the importance of cytotoxicity evaluation in assessing long-term impacts on cellular viability and DNA repair.^{48,49} As malignancies frequently arise from accumulated genetic mutations, persistent cytotoxic damage may contribute to tumor progression via non-genotoxic mechanisms.⁵⁰

A distinct meta-analysis investigating the relationship between micronucleus frequency and age, incorporating six studies, identified a weak correlation across all datasets. While the robustness of the findings remained intact, the correlation between micronucleus frequency variation and age was notably weak, likely attributable to limited sample sizes and the inclusion of lower-quality studies. Ribeiro DA et al. 2008³³ and Pai A et al. 2014³⁸ reported no statistically significant age-related effects, whereas other studies^{19,28,32,37} indicated a potential association between aging and elevated micronucleus formation, likely attributable to diminished DNA repair efficiency and progressive chromosomal instability.⁵¹ Notably, children may exhibit greater susceptibility to X-ray-induced genotoxicity due to their rapidly proliferating tissues, longer life expectancy (allowing radiation-induced mutations to accumulate over time), and higher absorbed organ doses, particularly in the salivary glands and thyroid.^{52–55} Without tailored exposure protocols, children may receive radiation doses exceeding those of adults, increasing their lifetime cancer risk.^{56–58} However, due to statistical homogeneity limitations, no definitive correlation between age and micronucleus frequency could be established.

Sensitivity analyses (Figures 6, 9, 12) identified Waingade M et al.⁴⁰ and Basha S et al.²⁰ as key influencing studies.⁴⁰ employed a more detailed age stratification, potentially capturing finer micronucleus frequency variations that broader age groupings obscured. Meanwhile, focused on CBCT-related genotoxicity, which involves radiation doses significantly higher than panoramic X-rays.²⁰ The effective dose of CBCT can range from 25 to 1025 μ Sv, equivalent to approximately 12 panoramic X-rays.⁵⁹ This variability likely influenced correlation coefficient discrepancies, emphasizing the importance of accounting for scanner type, imaging protocols, and radiation dose in genotoxic outcome interpretations. Notably, only three studies examined the association between age and changes in micronucleus frequency, limiting the statistical strength of this analysis and emphasizing the need for broader epidemiological research.

Among the methodological confounding factors, the choice of staining technique significantly influenced micronucleus detection. Papanicolaou staining, commonly used in most studies, offers clear nuclear visualization but lacks DNA specificity, leading to potential false positives from cytoplasmic artifacts like keratin granules, bacteria, and leukocytes.^{1,39} Although Feulgen staining offers DNA specificity and reduces false positives,^{60,61} its labor-intensive protocol and sensitivity to technical variations can lead to underestimation of micronucleus frequency.⁴⁰ The inconsistent use of staining methods across studies complicates direct comparisons, and future research should consider automated systems for staining and micronucleus counting to improve accuracy and reproducibility.⁶²

Additionally, the number of cells analyzed per participant significantly impacts MN frequency results. While most studies assessed 1,000 cells per individual, others analyzed 2,000 cells.^{19,33} The International Expert Group on Micronucleus Assays recommends a minimum of 2,000 cells per sample to ensure statistical reliability.¹¹ Given that sample size directly affects genotoxicity assessments, standardization in cell quantification is crucial for future research.

The collective evidence from the included studies strengthens the hypothesis that panoramic X-ray exposure induces notable genotoxic effects in oral mucosal cells. However, the variability observed across studies may be influenced by differences in radiation dose, levels of cytotoxicity, and methodological approaches. The absence of a consistent link between age and micronucleus (MN) frequency further reflects the multifaceted nature of genomic instability triggered by radiation. It is essential to acknowledge that heterogeneity in staining protocols, radiation types, sampled cell populations, and the number of cells assessed likely contributed to variations seen in the meta-analysis. For instance, while some studies employed panoramic radiography, others utilized cone-beam computed tomography (CBCT), with substantial differences in radiation dosage and exposure times. Regarding sample collection, Pai et al.³⁸ was distinctive in sampling cells from the upper dental arch, whereas others focused on exfoliated buccal mucosal cells. Inconsistencies were also noted in the number of cells analyzed per subject and the staining techniques applied. These methodological

variations highlight the pressing need for standardized study designs, consistent staining methods, and harmonized cell quantification practices to improve reproducibility across future investigations. Moreover, advancing our understanding of X-ray-induced genotoxicity and cytotoxicity—especially within vulnerable populations such as children—will require comprehensive, rigorously conducted studies.

Despite inherent limitations, such as sample size restrictions and follow-up duration, the findings provide crucial insights into the biological consequences of X-ray exposure, particularly the lack of a definitive age-related trend in genotoxic response. This study highlights the critical need for standardized methodologies, optimized study frameworks, and expansive prospective research integrating exposure parameters, lifestyle determinants, and pre-existing health conditions. Further investigations should explore additional chromosomal abnormalities to refine the assessment of radiation-induced genetic damage. Expanding the analytical approach to include diverse imaging techniques and advanced genotoxic assays—such as DNA adduct detection, strand break analysis, and point mutation assessment—could enhance the understanding of dental radiography’s biological effects.^{4,19,30,33} Future studies with expanded cohorts and extended follow-up periods will be essential for clarifying long-term risks and optimizing diagnostic frameworks.

The meta-analysis conducted in this review demonstrates that even exposure to low-dose dental X-rays results in quantifiable genotoxic alterations in oral epithelial cells, as reflected by elevated micronucleus frequencies.^{4,43} Despite the weak association with age, the results highlight the critical importance of implementing stringent radiation protection protocols across all age groups, with heightened attention to pediatric and adolescent populations.¹¹

In contemporary dental practice, micronucleus (MN) formation is increasingly recognized as a sensitive cytogenetic biomarker for detecting radiation-induced genotoxicity, with emerging potential for clinical application. Evidence suggests that MN assessment may be particularly informative in evaluating radiation-related risks among pediatric orthodontic patients. Lorenzoni et al. identified a marked rise in MN frequency in oral mucosal cells of children subjected to comprehensive orthodontic imaging—including panoramic and lateral cephalometric radiographs—indicating a greater radiosensitivity in developing tissues.⁶ These findings underscore the importance of minimizing repeated radiographic exposures in children and warrant careful justification of each imaging procedure to limit the cumulative biological burden of low-dose radiation.

MN frequency has also been proposed as an early indicator of radiation-induced genetic instability in adult patients undergoing multiple cone-beam computed tomography (CBCT) scans, such as those preparing for dental implant placement or prosthodontic interventions. Li et al. demonstrated that buccal epithelial cells exhibited significantly

higher DNA damage following CBCT compared to panoramic imaging, suggesting that increased dose intensity and examination frequency may result in persistent genotoxic effects on the oral epithelium.¹⁸ Consequently, for high-risk populations—including individuals with prior radiotherapy, immunosuppression, or oral neoplasia—preoperative MN evaluation may assist in risk stratification and customization of imaging protocols to ensure safe and targeted diagnostic workflows.

Beyond individual-level risk assessment, MN analysis offers promise for system-level radiation quality assurance (QA) and monitoring of radiographic procedural standards. As noted by Angelieri et al., increased MN frequencies following routine dental radiography may reflect suboptimal radiological practices, particularly in settings with limited technical oversight.⁴ Informed by our findings, we propose the inclusion of MN cytogenetic monitoring for patients subjected to repeated imaging, especially pediatric and orthodontic populations. The assay's non-invasive sampling, cost-effectiveness, and methodological reproducibility make it suitable for broader integration into clinical workflows, including enhanced patient education, informed consent processes, and long-term radiation risk monitoring.

From a radiological operations standpoint, strict compliance with the ALARA (As Low As Reasonably Achievable) principle remains critical in safeguarding against unnecessary radiation exposure.⁵⁹ Radiographic examinations should be performed only after rigorous clinical justification, and—when feasible—non-ionizing or low-dose alternatives, such as digital optical scanning or ultrasound, should be considered.⁶³ Effective radiation mitigation strategies include field size limitation, employment of high-sensitivity digital detectors, anatomy-adapted exposure adjustments, and preferential use of rectangular collimation, which significantly reduces scatter dose compared to circular systems.⁶⁴ Moreover, developing longitudinal exposure records—particularly for patients undergoing serial imaging, such as those in orthodontic or implant therapy—could enhance institutional radiation stewardship efforts.

In summary, the incorporation of MN assay into routine dental radiographic protocols may serve not only to quantify subclinical genotoxic effects but also to support the implementation of individualized radioprotection strategies. Such integration has the potential to reduce genomic instability risks associated with diagnostic imaging, without compromising diagnostic efficacy, thereby promoting higher standards of patient safety and radiological care.⁴³

Conclusion

This review validates those different types of X-ray exposure exerts notable genotoxic and cytotoxic effects on oral mucosal cells, radiation ranges from Panoramic radiography to MDCT. However, a weak association was detected between age and micronucleus frequency. Considering the established risks of ionizing radiation,

adhering strictly to the "As Low As Reasonably Achievable (ALARA)" principle remains essential in clinical settings. Comprehensive clinical assessment is essential before diagnostic imaging to limit unnecessary radiation exposure.²⁷

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

1. Mounika G, Sridevi K, Krishnaveni B, et al. Evaluation of genomic damage from buccal epithelial cells in patients subjected to cone beam computed tomography. *J Indian Acad Oral Med Radiol.* 2021;33(4):372-378. doi:10.4103/jiaomr.jiaomr_83_21
2. Angelieri F, Carlin V, Saez DM, Pozzi R, Ribeiro DA. Mutagenicity and cytotoxicity assessment in patients undergoing orthodontic radiographs. *Dentomaxillofac Radiol.* 2010;39(7):437-440. doi:10.1259/dmfr/24791952
3. Cerqueira EM, Gomes-Filho IS, Trindade SC, Lopes MA, Passos JS, Santelli GM. Genetic damage in exfoliated cells from oral mucosa of individuals exposed to X-rays during panoramic dental radiographies. *Mutat Res.* 2004;562(1-2):111-117. doi:10.1016/j.mrgentox.2004.05.008
4. Angelieri F, Yamasaki YV, Oshima CTF, Ribeiro DA. Do dental X-rays induce genotoxicity and cytotoxicity in oral mucosa cells? A critical review. *Anticancer Res.* 2017;37(10):5383-5388. doi:10.21873/anticancer.11964
5. Holland N, Bolognesi C, Kirsch-Volders M, et al. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. *Mutat Res Rev Mutat Res.* 2008;659(1-2):93-108. doi:10.1016/j.mrrev.2008.03.007
6. Lorenzoni DC, Ferreira FV, Carlin V, Ribeiro DA, Ervolino EF. Cytogenetic biomonitoring in children submitting to a complete set of radiographs for orthodontic planning. *Angle Orthod.* 2012;82(4):585-590. doi:10.2319/072311-468.1
7. Turkmen C, Ozen S, Unal SN, et al. Monitoring the genotoxic effects of radiosynovectomy with Re-186 in paediatric age group undergoing therapy for haemophilic synovitis. *Haemophilia.* 2007;13(1):57-64. doi:10.1111/j.1365-2516.2006.01406.x
8. Pastor S, Serra C, Creus A, Xamena N. Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells. *Mutagenesis.* 2001;16(6):539-545. doi:10.1093/mutage/16.6.539
9. Julu Y, Chatterjee N. Biomonitoring of genotoxic effect in children exposed to dental radiographs during pulpectomy procedure—BMCyt assay. *Int J Clin Pediatr Dent.* 2022;15(suppl 1):S63-S70. doi:10.5005/jp-journals-10005-2135
10. De Souza DV, Malacarne SAM, Malacarne IT, et al. Does panoramic X-ray induce cytogenetic damage to oral cells? A systematic review with meta-analysis. *Anticancer Res.* 2021;41(9):4203-4210. doi:10.21873/anticancer.15224
11. Malacarne IT, Tsubone W, Viana MB, Renno ACM, Ribeiro DA. Is micronucleus assay a suitable method for biomonitoring children exposed to X-ray? A systematic review with meta-analysis. *Int J Radiat Biol.* 2023;99(10):1522-1530. doi:10.1080/09553002.2023.2194405
12. Bonassi S, Znaor A, Ceppi M, et al. An increased micronucleus frequency in peripheral blood

- lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*. 2006;28(3):625-631. doi:10.1093/carcin/bgl177
13. Iarmarcovai G, Chaspoul F, Botta A, Orsiere T, Bonassi S. Micronuclei frequency in peripheral blood lymphocytes of cancer patients: a meta-analysis. *Mutat Res Rev Mutat Res*. 2008;659(3):274-283. doi:10.1016/j.mrrev.2008.05.006
14. Mischczyk J, Rybka K, Panek A, et al. Assessment of the nuclear medicine personnel occupational exposure to radioiodine. *Eur J Radiol*. 2019;121:108712. doi:10.1016/j.ejrad.2019.108712
15. Depuydt J, Beaton A, Barnard S, et al. RENEB intercomparison exercises analyzing micronuclei (cytokinesis-block micronucleus assay). *Int J Radiat Biol*. 2017;93(1):36-47. doi:10.1080/09553002.2016.1206231
16. Takkouche B, Neira-Gutierrez A. PRISMA statement. *Epidemiology*. 2011;22(1):128. doi:10.1097/EDE.0b013e3181fe7999.
17. Thomas BH, Ciliska D, Dobbins M, Micucci S. A process for systematically reviewing the literature: providing the research evidence for public health nursing interventions. In: DiCenso A, Guyatt G, Ciliska D, eds. *Evidence-Based Nursing: A Guide to Clinical Practice*. Elsevier Mosby; 2005:63-82.
18. Li G, Yang P, Hao S, et al. Buccal mucosa cell damage in individuals following dental X-ray examinations. *Sci Rep*. 2018;8(1):2509. doi:10.1038/s41598-018-20964-3
19. Popova L, Kishkilova D, Hadjidekova VB, et al. Micronucleus test in buccal epithelium cells from patients subjected to panoramic radiography. *Dentomaxillofac Radiol*. 2007;36(3):168-171. doi:10.1259/dmfr/29193561
20. Basha S, Essawy M. Genotoxic and cytotoxic effects of cone beam computed tomography on exfoliated buccal epithelial cells. *IOSR J Dent Med Sci*. 2018;17(3):66-70. doi:10.9790/0853-1703056670
21. Cohen J. Statistical power analysis. *Curr Dir Psychol Sci*. 1992;1(3):98-101. doi:10.1111/1467-8721.ep10768783
22. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327(7414):557-560. doi:10.1136/bmj.327.7414.557
23. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics*. 1994;50(4):1088-1094. doi:10.2307/2533446
24. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315(7109):629-634. doi:10.1136/bmj.315.7109.629
25. Zhou X, Yan F, Chen Y, Gao Y. The correlation between mobile phone addiction and procrastination in students: a meta-analysis. *J Affect Disord*. 2024;346:317-328. doi:10.1016/j.jad.2023.11.020
26. Jahanshahiafshar Z, Ghapanchi J, Seyedmajidi M, et al. Genotoxic and cytotoxic effects of cone beam computed tomography and multidetector computed tomography on exfoliated buccal epithelial cells. *Iran J Med Sci*. 2023;48(6):572-581. doi:10.30476/IJMS.2023.96522.2807
27. Torabinia N, Moghadam M, Keshani F, Mehdizadeh M, Soltani P, Spagnuolo G. Genotoxicity and micronucleus formation as a result of panoramic radiography in epithelial cells of the buccal mucosa: a cross-sectional study in adults. *Clin Exp Dent Res*. 2024;10(4):e915. doi:10.1002/cre2.915
28. Silva MB, De Abreu DA, Martinez EF, et al. Peroxiredoxin I expression in epithelial cells of

- buccal mucosa from patients exposed to panoramic X-rays: influence of the age. *Clin Oral Investig*. 2018;22(3):1587-1592. doi:10.1007/s00784-017-2254-4
29. Haghighi R, Sedaghat R, Ahmadvand M, Ogaghi Z. Cytological changes of oral mucosa following lateral cephalometry and panoramic radiograph. *Int J Dent Clin*. 2014;6(3):1-3.
30. Cerqueira EM, Meireles JR, Lopes MA, et al. Genotoxic effects of X-rays on keratinized mucosa cells during panoramic dental radiography. *Dentomaxillofac Radiol*. 2008;37(7):398-403. doi:10.1259/dmfr/56848097
31. Karabas HC, Ozkan I, Sener LT, Guler SD, Albeniz I, Erdem TL. Evaluation of cell and DNA damage induced by panoramic radiography. *Niger J Clin Pract*. 2019;22(8):1041-1048. doi:10.4103/njcp.njcp_675_18
32. Malik S, Uppal M, Pillai J, Sharma S, Singh M, Lehri S. Comparison between micronuclei and AgNORs in assessing the short-term genotoxic effects of panoramic radiography on oral mucosa: a cross-sectional study. *J Indian Acad Oral Med Radiol*. 2022;34(2):188-192. doi:10.4103/jiaomr.jiaomr_264_21
33. Ribeiro DA, De Oliveira G, De Castro GM, Angelieri F. Cytogenetic biomonitoring in patients exposed to dental X-rays: comparison between adults and children. *Dentomaxillofac Radiol*. 2008;37(7):404-407. doi:10.1259/dmfr/58548698
34. Sreeshyla H, Arun R, Hegde U, et al. A study on micronuclei in exfoliated buccal epithelial smears to detect epithelial changes in patients undergoing panoramic radiography. *Biomed Biotechnol Res J*. 2023;7(3):387-390. doi:10.4103/bbrj.bbrj_127_23
35. Arora P, Devi P, Wazir SS. Evaluation of genotoxicity in patients subjected to panoramic radiography by micronucleus assay on epithelial cells of the oral mucosa. *J Dent (Tehran)*. 2014;11(1):47-55.
36. Anbumeena S, Kumar A, Krithika CL, Vasanthi V. Genotoxic and cytotoxic biomonitoring in patients exposed to panoramic dental radiography: comparison between five different age groups. *J Indian Acad Oral Med Radiol*. 2021;33(1):16-21.
37. Manzoor S, Santhosh K, Sushanth A, Seralathan S, Rajasekar V, Jacob A. A cross-sectional study to evaluate nuclear changes in buccal mucosa following panoramic radiography. *J Contemp Dent Pract*. 2021;21(11):1258-1261. doi:10.5005/jp-journals-10024-2921
38. Pai A, Shetty R, Naik R, Guruprasad Y. Biomonitoring of genotoxic and cytotoxic effects of gingival epithelial cells exposed to digital panoramic radiography. *J Orofac Sci*. 2012;4(2):124. doi:10.4103/0975-8844.106207
39. Aggarwal A, Singh R, Mago J, et al. Evaluation of genotoxic effects of panoramic dental radiography on cells of oral mucosa by micronucleus assay and evaluation of time period required by cells of oral mucosa to recover from the genotoxic effects. *World J Dent*. 2020;11(1):17-23. doi:10.5005/jp-journals-10015-1703
40. Waingade M, Rathi M. Analysis of micronuclei in buccal epithelial cells in patients subjected to panoramic radiography. *Indian J Dent Res*. 2012;23(5):574. doi:10.4103/0970-9290.107329
41. Agarwal P, Varma D, Haranal S, et al. Genotoxic and cytotoxic effects of X-ray on buccal epithelial cells following panoramic radiography: a pediatric study. *J Cytol*. 2015;32(2):102-104. doi:10.4103/0970-9371.160559
42. Altoukhi D, AlSanea A, El Ashiry E, Nassif O, Sabbahi D. Genotoxicity and cytotoxicity of cone beam computed tomography in children. *BMC Oral Health*. 2021;21(1):427. doi:10.1186/s12903-021-01792-w

43. Belmans N, Oenning AC, Salmon B, et al. Radiobiological risks following dentomaxillofacial imaging: should we be concerned? *Dentomaxillofac Radiol.* 2021;50(6):20210153. doi:10.1259/dmfr.20210153
44. Sarto F, Finotto S, Giacomelli L, Mazzotti D, Tomanin R, Levis AG. The micronucleus assay in exfoliated cells of the human buccal mucosa. *Mutagenesis.* 1987;2(1):11-17. doi:10.1093/mutage/2.1.11
45. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat Res.* 1992;271(1):69-77. doi:10.1016/0165-1161(92)90033-1
46. Majer B. Use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention trials. *Mutat Res Rev Mutat Res.* 2001;489(2-3):147-172. doi:10.1016/S1383-5742(01)00068-0
47. Ait-Ali L, Andreassi MG, Foffa I, Spadoni I, Vano E, Picano E. Cumulative patient effective dose and acute radiation-induced chromosomal DNA damage in children with congenital heart disease. *Heart.* 2010;96(4):269-274. doi:10.1136/hrt.2008.160309
48. Coates PJ, Lorimore SA, Wright EG. Damaging and protective cell signalling in the untargeted effects of ionizing radiation. *Mutat Res.* 2004;568(1):5-20. doi:10.1016/j.mrfmmm.2004.06.042
49. Ceppi M, Biasotti B, Fenech M, Bonassi S. Human population studies with the exfoliated buccal micronucleus assay: statistical and epidemiological issues. *Mutat Res Rev Mutat Res.* 2010;705(1):11-19. doi:10.1016/j.mrrev.2009.11.001
50. Mally A, Chipman JK. Non-genotoxic carcinogens: early effects on gap junctions, cell proliferation and apoptosis in the rat. *Toxicology.* 2002;180(3):233-248. doi:10.1016/S0300-483X(02)00393-1
51. Maffei F. Micronuclei frequencies in hospital workers occupationally exposed to low levels of ionizing radiation: influence of smoking status and other factors. *Mutagenesis.* 2002;17(5):405-409. doi:10.1093/mutage/17.5.405
52. Myers DR, Shoaf HK, Wege WR, Carlton WH, Gilbert MA. Radiation exposure during panoramic radiography in children. *Oral Surg Oral Med Oral Pathol.* 1978;46(4):588-593. doi:10.1016/0030-4220(78)90390-0
53. Kleinerman RA. Cancer risks following diagnostic and therapeutic radiation exposure in children. *Pediatr Radiol.* 2006;36(suppl 2):121-125. doi:10.1007/s00247-006-0191-5
54. Theodorakou C, Walker A, Horner K, et al. Estimation of paediatric organ and effective doses from dental cone beam CT using anthropomorphic phantoms. *Br J Radiol.* 2012;85(1010):153-160. doi:10.1259/bjr/19389412
55. De Oliveira FM, Campanella AC, Ladeira C. Is mobile phone radiation genotoxic? An analysis of micronucleus frequency in exfoliated buccal cells. *Mutat Res Genet Toxicol Environ Mutagen.* 2017;822:41-46. doi:10.1016/j.mrgentox.2017.08.001
56. American Academy of Oral and Maxillofacial Radiology. Clinical recommendations regarding use of cone beam computed tomography in orthodontics. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2013;116(2):238-257. doi:10.1016/j.oooo.2013.06.002
57. Neri M, Fucic A, Knudsen LE, Lando C, Merlo F, Bonassi S. Micronuclei frequency in children exposed to environmental mutagens: a review. *Mutat Res Rev Mutat Res.* 2003;544(2-3):243-254. doi:10.1016/j.mrrev.2003.06.009
58. Smith-Bindman R. Radiation dose associated with common computed tomography examinations and the associated lifetime attributable risk of cancer. *Arch Intern Med.*

2009;169(22):2078-2086. doi:10.1001/archinternmed.2009.427

59. White SC, Pharoah MJ, eds. Oral Radiology: Principles and Interpretation. 7th ed. St Louis, MO: Elsevier; 2014.

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

61. Bolognesi C, Baan R, Knasmueller S, et al. Clinical application of micronucleus test in exfoliated buccal cells: a systematic review and meta-analysis. *Mutat Res Rev Mutat Res.* 2015;766:20-31. doi:10.1016/j.mrrev.2015.07.002

62. Preethi N, Chandrappa NS, Bethur SS. Genotoxic effects of X-rays in buccal mucosal cells in children subjected to dental radiographs. *BDJ Open.* 2016;2(1):16001. doi:10.1038/bdjopen.2016.1

63. The University of Manchester. Cone beam CT for dental and maxillofacial radiology (evidence-based guidelines). The University of Manchester Research Explorer website. Published unknown. Accessed April 27, 2025. <https://research.manchester.ac.uk/en/publications/cone-beam-ct-for-dental-and-maxillofacial-radiology-evidence-base>

64. American Dental Association Council on Scientific Affairs. The use of dental radiographs: update and recommendations. *J Am Dent Assoc.* 2006;137(9):1304-1312. doi:10.14219/jada.archive.2006.0393

Author Contributions

Study design: Yaxin Wang, Shanshan Cai, Rozita Hassan, Sanhui Yang

Data search: Yaxin Wang, Shanshan Cai, Sanhui Yang

Data analysis: Yaxin Wang, Rozita Hassan, Shanshan Cai, Sanhui Yang, Liyana Ghazali, Anani Aila Bt Mat Zin, Linxian Zeng

Writing the paper: all authors.

All authors have read and approved the final manuscript, and they are accountable for all aspects of the work, ensuring that any issues related to the accuracy or integrity of any part of the work are properly investigated and addressed.

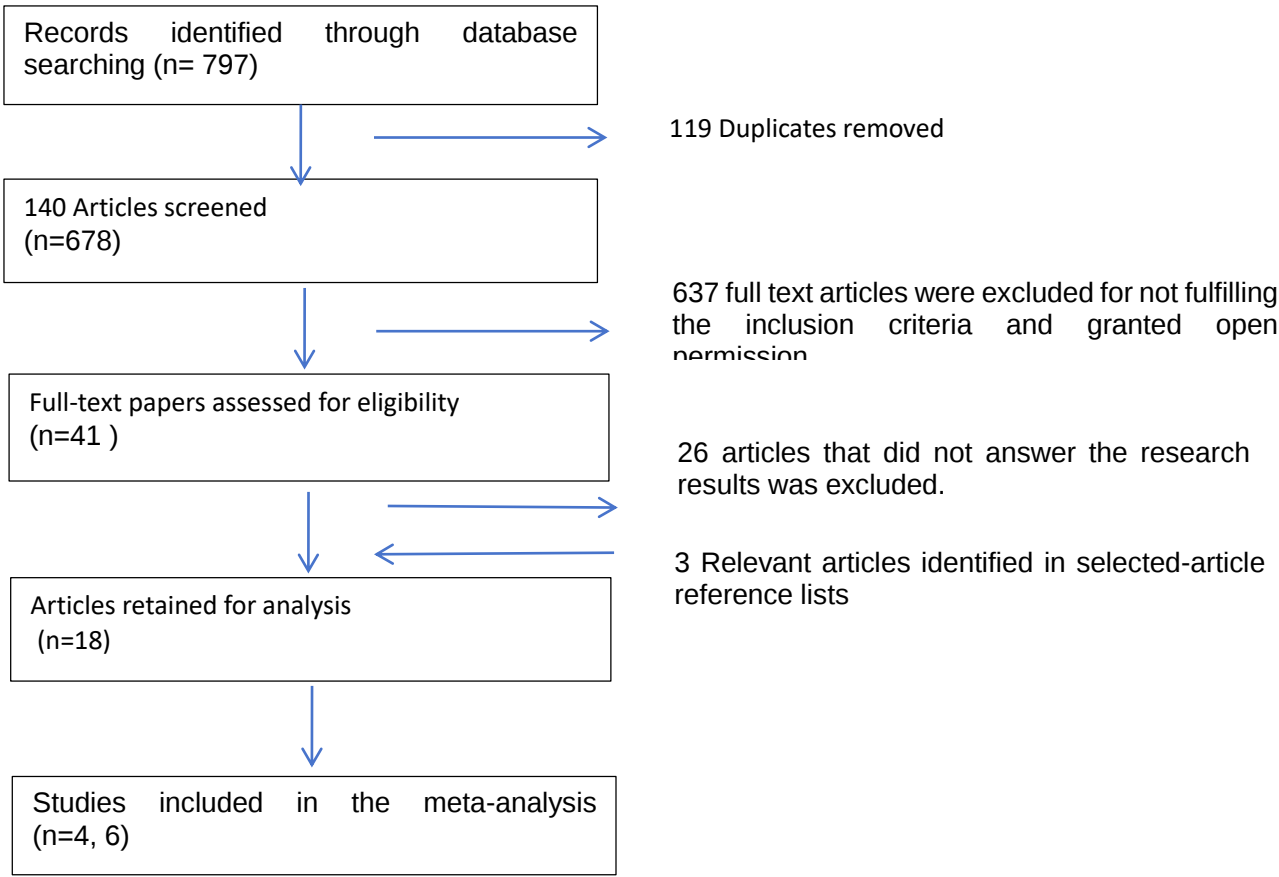


Figure 1 Study selection flow char

Table 1 The main important characteristics of the papers included in this study

Authors	Country	Age of patients/years old	N/Gender	Radiograph exposure
Popova et al. (2007)	Bulgaria	24-73	32/12 males and 20 females	Panoramic radiography
Cerqueira et al. (2008)	Brazil	26±9.18	40/9 males and 31 females	Panoramic radiography
Ribeiro et al. (2008)	Brazil	7.7±1.5/39.6±5.4	17 adults/11 males and 6 females, 17 children/8 males and 9 females	Panoramic radiography
Pai et al. (2012)	India	20-30,23 mean age	50/14 males and 36 females	Panoramic radiography
Waingade et al. (2012)	India	27.63±10.93 mean age	60/19 males and 41 females	Panoramic radiography
Arora et al. (2014)	India	25.21±12.67mean age	53/21 males and 32 females	Panoramic radiography
Haghgoo et al. (2014)	Iran	Not informed	15 adults and 15 children	Panoramic and lateral cephalometric x-ray
Basha et al. (2018)	Egypt	27-43,34.27±3.83mean age	30 males	CBCT
Li, G. et al. (2018)	China	8-42,23.63±6.64mean age	98/28 males and 70 females	Panoramic + lateral/posteroanterior radiographs, CBCT
Silva et al. (2018)	Brazil	20-50	50/21 males and 32 females	Panoramic radiography
Karabas et al. (2019)	Turkey	20-46, 23 mean age	30/21 males and 9 females	Panoramic radiography
Kaur et al. (2020)	India	(G1)15-25,21.06 mean age (G2)40-50,45.14 mean age	100/44males and 56 females	Panoramic radiography
Santhosh et al. (2020)	India	24-65, 28.96 mean age	30/15 males and 15 females	Panoramic radiography
Anbumeena et al. (2021)	India	6-65	60/ Not informed	Panoramic radiography
Malik et al. (2022)	India	15-25, 40-50	100/Not informed	Panoramic radiography
Jahanshahiafshar et al (2023)	Iran	34.37±7.72、 34.97±6.83	60/26 males and 34 females	CBCT、 MDCT
Sreesshyla et al (2023)	India	11-40	60/22 males and 38 females	Panoramic radiography
Torabinia et al (2024)	Iran	27.36±8.19 mean age	36/ 24 males and 12 females	Panoramic radiography

Author	Exclusion criteria	Collection time	Site of smear	Stain	Total number of cells	Analysis of cytotoxicity	Number of individuals	Blind analysis	Proper statistics description
Popova et al. (2007)	Yes	Day 0-10±2 days	Right/left buccal mucosa	Giemsa	2000 cells	No	32	No	Yes
Cerqueira et al. (2008)	Yes	Day 0-10 days	Upper dental arch	Feulgen-Rossenbeck	1000 cells	Yes	40	Yes	Yes
Ribeiro et al. (2008)	Yes	Day 0-10 days	Right/left buccal mucosa	Feulgen	2000 cells	Yes	34	No	Yes
Pai et al. (2012)	Yes	Day 0-10 days	Upper dental arch	Feulgen	1000 cells	Yes	60	Yes	No
Waingade et al. (2012)	Yes	Day 0-10 days	Right/left buccal mucosa	Giemsa	1000 cells	No	60	Yes	Yes
Arora et al. (2014)	Yes	Day 0-10 days	Right/left buccal mucosa and Gingival mucosa	Giemsa	1000 cells	No	53	No	Yes
Haghgoo et al. (2014)	Yes	Day 0-10 days	Right/left buccal mucosa	Papanicolaou	600 cells	Yes	30	No	Yes
Basha et al. (2018)	Yes	Day 0-10 days	Right/left buccal mucosa	Papanicolaou	1000 cells	Yes	30	Yes	Yes
Li, G. et al. (2018)	Yes	Day 0-10 days	Right/left buccal mucosa	Feulgen	2000cells 1000 cells	Yes	98	No	Yes
Silva et al. (2018)	No	0-1 hour	left buccal mucosa	Not informed	Not informed	No	50	No	Yes
Karabas et al. (2019)	Yes	Day 0-14 days	Right/left buccal mucosa	Papanicolaou	1000 cells	Yes	30	No	Yes
Kaur et al. (2020)	Yes	Day 0-10 days and 21 days	Right/left buccal and Gingival mucosa	Papanicolaou	1000 cells	No	100	Yes	Yes
Santhosh et al. (2020)	Yes	Day 0-12 days	Right/left buccal mucosa	Feulgen	Not informed	No	30	No	No
Anbumeena et al. (2021)	Yes	Within 7-10 days	Right/left buccal mucosa	Papanicolaou/PAS	Not informed	Yes	60	Yes	Yes
Malik et al. (2022)	Yes	Day 0-10 days and 40 days	Right/left buccal mucosa	Acridine orange/AgNORs	100 cells	No	100	No	Yes
Jahanshahiafshar et al (2023)	Yes	Day 0-12 days	Right/left buccal mucosa	Papanicolaou	2000 cells	Yes	60	Yes	Yes
Sreeshyla et al (2023)	Yes	Day 0-10 days	Right/left buccal mucosa	Giemsa	2000 cells	No	60	No	Yes
Torabinia et al (2024)	Yes	Day 0-10 days	Right buccal mucosa	Papanicolaou	Not informed	No	36	Yes	Yes

Table 2 Description of study

Table 3 Radiation parameters of various types of radiology

Author	Radiograph exposure	Current radiation level	Time of irradiation
Popova et al. (2007)	Panoramic radiography	Not informed	Not informed
Cerqueira et al. (2008)	Panoramic radiography	65–90 kV, 15 mA	14s
Ribeiro et al. (2008)	Panoramic radiography	60–80 kV、10 mA	14 s
Pai et al. (2012)	Panoramic radiography	64-70 Kvp 12 mA	18s
Waingade et al. (2012)	Panoramic radiography	60-80 kV、10 mA	12 s
Arora et al. (2014)	Panoramic radiography	Not informed	Not informed
Haghgoo et al. (2014)	Panoramic and lateral cephalometric x-ray	Not informed	Not informed
Basha et al. (2018)	CBCT	84 kVp,9–14 mA	6 s
Li, G. et al. (2018)	Panoramic + lateral/posteroanterior radiographs, CBCT	Not informed	17.6 s/ 0.5-1 s/ 0.8-1.2 s/ 24 s
Silva et al. (2018)	Panoramic radiography	60–80 kV、10 mA	14 s
Karabas et al. (2019)	Panoramic radiography	66-74 kV, 5-8 mA	13.1-13.9 s
Kaur et al. (2020)	Panoramic radiography	Not informed	Not informed
Santhosh et al. (2020)	Panoramic radiography	70–74kV、10 mA	18 s
Anbumeena et al. (2021)	Panoramic radiography	65–79 kV、8 mA	12 s
Malik et al. (2022)	Panoramic radiography	Not informed	Not informed
Jahanshahiafshar et al (2023)	CBCT、MDCT	CBCT: male:90 kVp、8 mA; female: 85 kVp 、8 mA MDCT: 110 kVp、35 mA、	Not informed
Sreesshyla et al. (2023)	Panoramic radiography	Not informed	Not informed
Torabinia et al. (2024)	Panoramic radiography	66 kVp、7.1 mA	15.8 s

Table 4 The cytotoxicity and the genotoxicity of micronucleus in oral mucosa cells following X-ray exposure.

Authors	Radiograph exposure	Main findings	
		Evidence of Cytotoxicity	Evidence of genotoxicity
Popova et al. (2007)	Panoramic radiography	Not informed	No statistical differences
Cerqueira et al. (2008)	Panoramic radiography	↑Karyorrhexis, condensed chromatin	↑Micronucleus
Ribeiro et al. (2008)	Panoramic radiography	↑Karyolysis, karyorrhectic, pyknosis	No statistical differences
Pai et al. (2012)	Panoramic radiography	↑pyknosis, karyorrhexis, karyolysis and condensed chromatin	No statistical differences
Waingade et al. (2012)	Panoramic radiography	Not informed	↑Micronucleus
Arora et al. (2014)	Panoramic radiography	Not informed	↑Micronucleus
Haghgoo et al. (2014)	Panoramic and lateral cephalometric x-ray	↑Karyolysis, karyorrhectic, pyknosis	No statistical differences
Basha et al. (2018)	CBCT	↑Karyolysis, karyorrhectic, condensed chromatin cells	↑Micronucleus
Li, G. et al. (2018)	Panoramic + lateral/posteroanterior radiographs, CBCT	↑Karyolysis, pyknosis	↑Micronucleus
Silva et al. (2018)	Panoramic radiography	(<50) ↑PRDX1 mRNA(>50)↓PRDX1 mRNA	Not informed
Karabas et al. (2019)	Panoramic radiography	↑Karyolysis, karyorrhectic	↑Micronucleus
Kaur et al. (2020)	Panoramic radiography	Not informed	(G1:10 days) ↑(21 days)↓Micronucleus (G2)No statistical differences
Santhosh et al. (2020)	Panoramic radiography	Not informed	No statistical differences
Anbumeena et al. (2021)	Panoramic radiography	↑Karyolysis, karyorrhectic, pyknosis,	No statistical differences
Malik et al. (2022)	Panoramic radiography	Not informed	↑Micronucleus
Jahanshahiafshar et al (2023)	CBCT、MDCT	↑Karyolysis, karyorrhectic, pyknosis	↑Micronucleus
Sreeshyla et al (2023)	Panoramic radiography	Not informed	↑Micronucleus
Torabinia et al (2024)	Panoramic radiography	Not informed	No statistical differences

Table 5 Micronucleus among pre-exposure and post-exposure.

	Micronucleus or MN/1.000 cells	
Author	Pre-exposure Mean (SE)	Postexposure Mean (SE)
Popova et al. (2007)	2.34 ± 1.49%	2.81 ± 1.64%
Cerqueira et al. (2008)	Not informed	Not informed
Ribeiro et al. (2008)	0.04±0.06/ 0.04±0.04	0.05±0.06/ 0.05±0.07
Pai et al. (2012)	(<25)0.00044±0.000558/ (≥25)0.00050±0.000760	(<25)0.00061±0.000728/ (≥25)0.00064±0.000929
Waingade et al. (2012)	0.56±0.25	0.61±0.23
Arora et al. (2014)	0.10% ± 0.0899	0.1264% ± 0.0812
Haghighi et al. (2014)	Not informed	Not informed
Basha et al. (2018)	0.026 ± 0.0062	0.030 ± 0.0068
Li, G. et al. (2018)	Not informed	Not informed
Silva et al. (2018)	Not informed	Not informed
Karabas et al. (2019)	30.2 ± 18.12	57.53 ± 17.16
Kaur et al. (2020)	1.30 ± 0.839/1.32 ± 0.868	(10 days)1.68 ± 0.957/1.56 ± 0.957 (21 days)1.48 ± 0.953/1.34 ± 0.772
Santhosh et al. (2020)	1.03 ± 0.80	2.20 ± 0.84
Anbumeena et al. (2021)	(PAP) 9.73 ± 1.092 (PAS) 5.42 ± 7.278	(PAP) 9.85 ± 1.095/ (PAS) 6.60 ± 7.072
Malik et al. (2022)	6.96 ± 3.03/11.0 ± 4.14	(10)8.96±3.09/11.72±4.55;(40)11.1±4.09/14.86 ±5.72
Jahanshahiafshar et al (2023)	CBCT: 34.17 ± 9.17 MDCT: 32.90 ± 5.33	CBCT: 42.7 ± 11.46 MDCT: 46.70 ± 6.10
Sreeshyla et al (2023)	2.97 ± 1.02	3.97 ± 1.09
Torabinia et al (2024)	1.65 ± 1.41	1.88 ± 1.74

Table 6 Quality assessment and final rating of the studies.

Author	Number of confounders	Details	Final rating
Popova et al. (2007)	3	Not specific stain for nucleic acids; absence of blind analysis; no evaluation of cytotoxicity	Weak
Cerqueira et al. (2008)	1	1000 cells evaluated per volunteer:	Strong
Ribeiro et al. (2008)	1	absence of blind analysis;	Strong
Pai et al. (2012)	2	1000 cells evaluated per volunteer: absence of proper statistics description	Moderate
Waingade et al. (2012)	3	Not specific stain for nucleic acids;1000 cells evaluated per volunteer; no evaluation of cytotoxicity	Weak
Arora et al. (2014)	4	Not specific stain for nucleic acids;1000 cells evaluated per volunteer; absence of blind analysis; no evaluation of cytotoxicity	Weak
Haghgoo et al. (2014)	3	Not specific stain for nucleic acids;600 cells evaluated per volunteer; absence of blind analysis	Weak
Basha et al. (2018)	2	Not specific stain for nucleic acids;1000 cells evaluated per volunteer	Moderate
Li, G. et al. (2018)	1	Not specific stain for nucleic acids;	Strong
Silva et al. (2018)	4	absence of blind analysis; absence of blind analysis; no evaluation of cytotoxicity, absence of cells evaluated per volunteer	Weak
Karabas et al. (2019)	3	Not specific stain for nucleic acids;1000 cells evaluated per volunteer and absence of blind analysis	Weak
Kaur et al. (2020)	3	Not specific stain for nucleic acids;1000 cells evaluated per volunteer; no evaluation of cytotoxicity;	Weak
Santhosh et al. (2020)	4	absence of total number of cells; no evaluation of cytotoxicity; absence of blind analysis and improper statistics description	Weak
Anbumeena et al. (2021)	2	Not specific stain for nucleic acids; absence of total number of cells	Moderate
Malik et al. (2022)	4	Not specific stain for nucleic acids;100 cells evaluated per volunteer; absence of blind analysis; no evaluation of cytotoxicity	Weak
Jahanshahiafshar et al (2023)	1	Not specific stain for nucleic acids;	Strong
Sreeshyla et al (2023)	3	Not specific stain for nucleic acids; no evaluation of cytotoxicity; absence of blind analysis	Weak
Torabinia et al (2024)	3	Not specific stain for nucleic acids; absence of total number of cells; no evaluation of cytotoxicity	Weak

	Whether age affects cytotoxicity or mutagenicity	
Authors	Cytotoxicity	Mutagenicity
Popova et al. (2007)	Not informed	Relative
Cerqueira et al. (2008)	Not relative	Not relative
Ribeiro et al. (2008)	Not relative	Not relative
Pai et al. (2012)	Not relative	Not relative
Waingade et al. (2012)	Not informed	Not relative
Arora et al. (2014)	Not informed	Not relative
Haghgoo et al. (2014)	Not relative	Not relative
Basha et al. (2018)	Not relative	Not relative
Li, G. et al. (2018)	Not relative	Not relative
Silva et al. (2018)	Not informed	Relative
Karabas et al. (2019)	Not informed	Not relative
Kaur et al. (2020)	Not informed	(15-25) Not relative/ (40-50) Relative
Santhosh et al. (2020)	Not informed	Relative
Anbumeena et al. (2021)	Not relative	Not relative
Malik et al. (2022)	Not informed	Relative
Jahanshahiafshar et al (2023)	Not relative	Not relative
Sreeshyala et al (2023)	Not informed	Not relative
Torabinia et al (2024)	Not informed	Not relative

Table 7 Correlation between age and genotoxicity and cytotoxicity of x-ray

Authors	Research grouping	Age range	Mean age	Difference of micronucleus change	Correlation between age and micronucleus frequency	statistical significance
Popova et al. (2007)	Not informed	24-73 years	Not informed	+0.47%, P > 0.05	Before:0.602, After:0.448 Difference: -0.111	P < 0.01
Cerqueira et al. (2008)	≤22.5y,>22.5y	Not informed	26±9.18	P < 0.05	Not informed	P > 0.05
Ribeiro et al. (2008)	Children VS adults	Not informed	7.7±1.5/39.6±5.4	+0.01%, P > 0.05	Not informed	P > 0.05
Pai et al. (2012)	<25y, ≥25y	20-30 years	23	+0.00017%, P = 0.777/ +0.00014%, P = 0.899	Not informed	P > 0.05
Waingade et al. (2012)	11–20y,21–30y,31–40y, ≥41y	Not informed	27.63±10.93	+0.03%, P = 0.02 +0.05%, P = 0.047	MCF: Before: -0.13, After: -0.07 MN/1000Before:-0.11,After:0.08	P > 0.05
Arora et al. (2014)	<25y, ≥25y cell types	Not informed	25.21±12.67	+0.0264%, P = 0.0038 P=0.2249	Not informed	P=0.5020 P=0.0209/0.0203
Haghgoo et al. (2014)	Children VS adults	Not informed	Not informed	P=0.548	Not informed	P=0.841
Basha et al. (2018)	≤35years old,>35years old	27-43years	34.27±3.83	+0.004, P < 0.001	Before: -0.193, after: -0.104, Difference:0.061,	P=0.306/0.584/0.749
Li, G. et al. (2018)	≤1 mGy,>1 mGy <18y, ≥18y	8-42years	23.63±6.64	+0.22, P = 0.008	Before:0.602, after:0.048, Difference:0.250	P = 0.0118
Silva et al. (2018)	<20y、20-50y、>50y	20-50 years	Not informed	PRDX1 : P=0.0293/ 0.0447/ 0.0120	Not informed	P < 0.05
Karabas et al. (2019)	Not informed	20-46 years	23	+27.33, P < 0.001	Not informed	P<0.001
Kaur et al. (2020)	15-25y,40-50y cell types	(G1)15-25 years (G2)40-50 years	(G1)21.06 (G2)45.14	P = 0.020/P > 0.05/ P = 0.002/P = 0.001	Not informed	Not informed
Santhosh et al. (2020)	Not informed	24-65 years	28.96	+1.17, P > 0.05	Before:0.569, After: 0.608	P = 0.001/P = 0.000
Anbumeena et al. (2021)	6-11y,12-18y,19-39y, 40-60y,>60y	6-65 years	Not informed	+1.18, P=0.192	Not informed	P = 0.432
Malik et al. (2022)	15-25y,40-50y	15-25/40-50years		+4.14, +3.86	Not informed	Not informed

Jahanshahiafshar et al (2023)	Not informed	Not informed	34.37±7.72、 34.97±6.83	+0.90, P < 0.05	Not informed	Not informed
Sreeshyla et al (2023)	11-20y,21-30y,31-40y	11-40 years	Not informed	+0.70, +1.00, +1.30, P < 0.05	Not informed	P > 0.05
Torabinia et al (2024)	whole	Not informed	27.36±8.19	+0.24%, P = 0.468	Before: 0.012, After: 0.065	P = 0.946/P = 0.707

Table 8 Parameters of age group, micronucleus change and correlation between age and micronucleus frequency

Table 9 Correlation coefficient between age and micronucleus frequency and SE

Authors	Number of individuals	Correlation between age and micronucleus frequency			SE
		Before	After	Difference	
Popova et al. (2007)	32	0.602	0.448	-0.111	0.146
Basha et al. (2018)	30	-0.193	-0.104	0.061	0.189
Li, G. et al. (2018)	98	0.602	0.048	0.25	0.099
Santhosh et al. (2020)	30	0.569	0.608	Not informed	0.155 0.150
Torabinia et al (2024)	36	0.012	0.065	Not informed	0.171 0.065
Waingade et al. (2012)	60	-0.11	0.08	Not informed	0.13

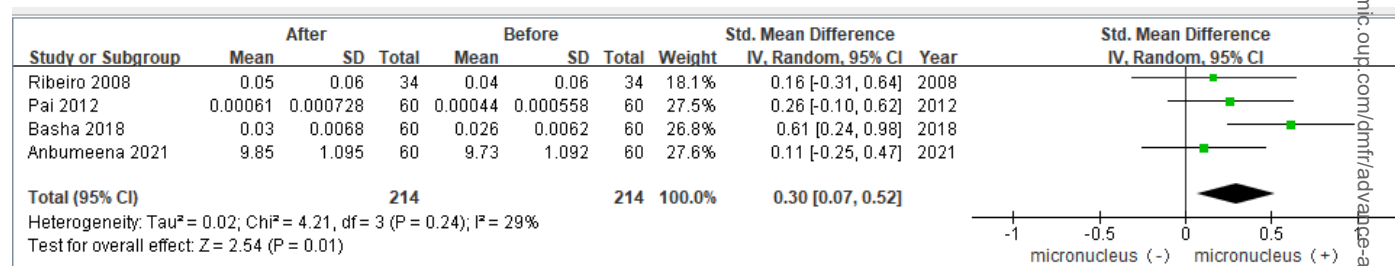


Figure 2 Meta-analysis data regarding micronucleus assay on oral cells of patients exposed to X-ray.

Certainty assessment							N: of patients		Effect		Certainty
N: of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	patients exposed to X-rays before	patients exposed to X-rays after	Relative (95% CI)	Absolute (95% CI)	
New Outcome											
4	non-randomised studies	serious ^a	not serious	not serious	serious ^a	strong association	214	214	-	SMD 0.3 higher (0.07 higher to 0.52 higher)	 Very low ^a

CI: confidence interval; SMD: standardised mean difference

Figure 3 GRADE analysis

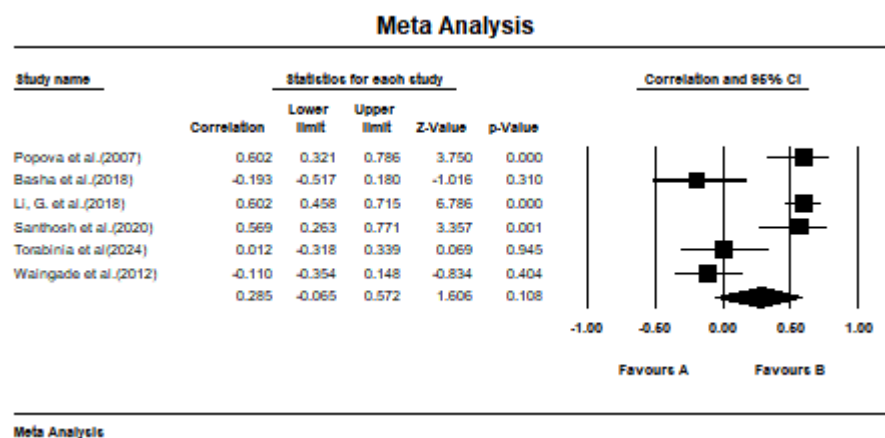


Figure 4 Forest plot of the correlation between micronucleus frequency before x-ray exposure and age

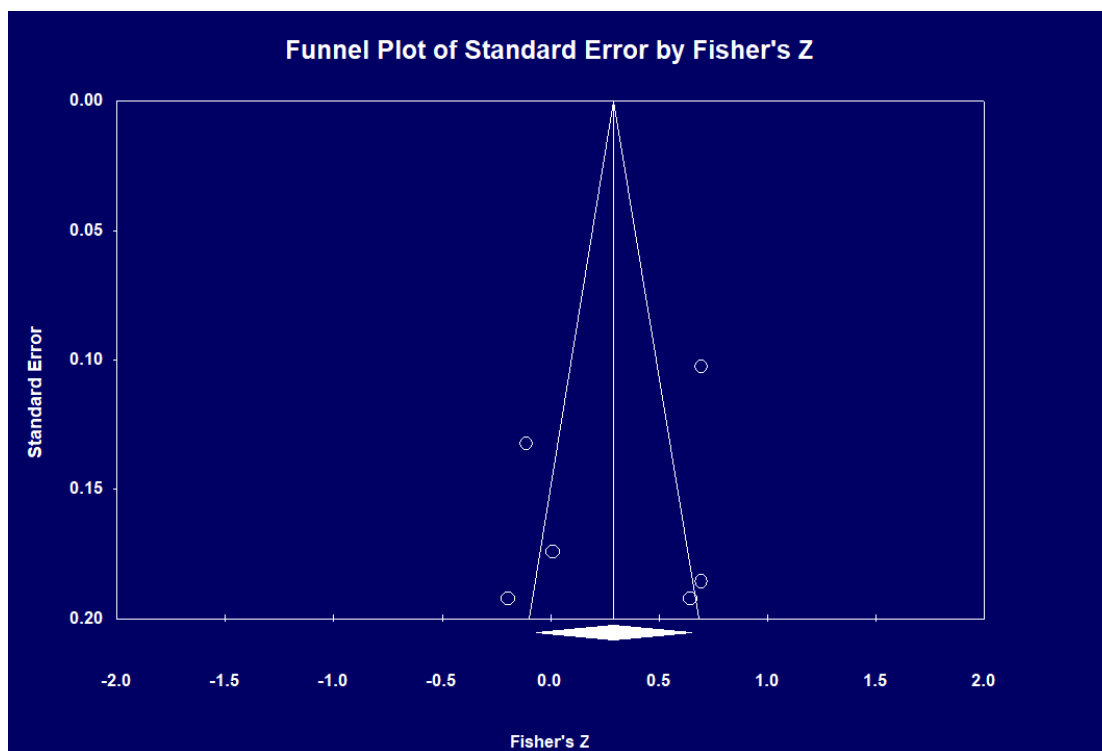
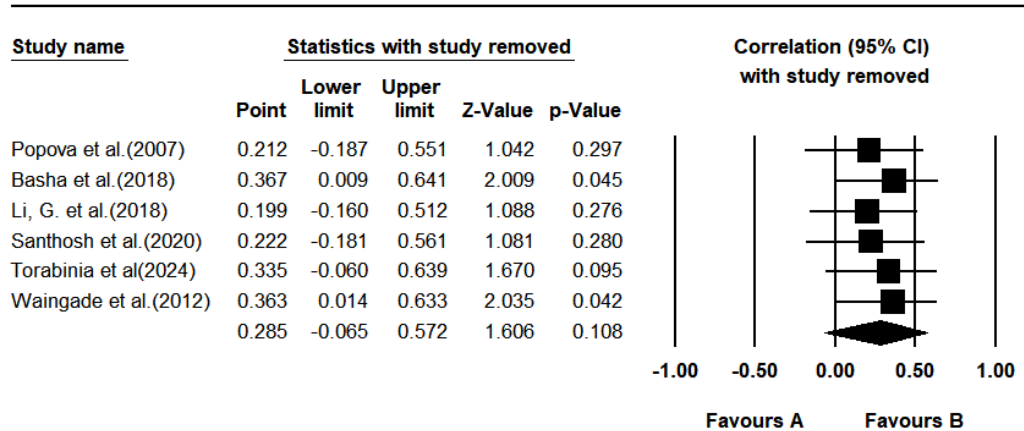


Figure 5 Funnel plot of the correlation between micronucleus frequency before x-ray exposure and age

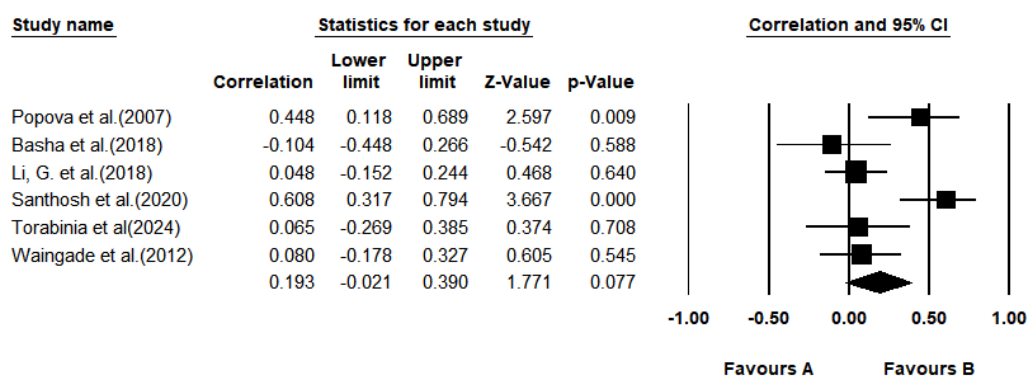
Meta Analysis



Meta Analysis

Figure 6 Sensitivity analysis of the correlation between micronucleus frequency before x-ray exposure and age

Meta Analysis



Meta Analysis

Figure 7 Forest plot of the correlation between micronucleus frequency after x-ray exposure and age

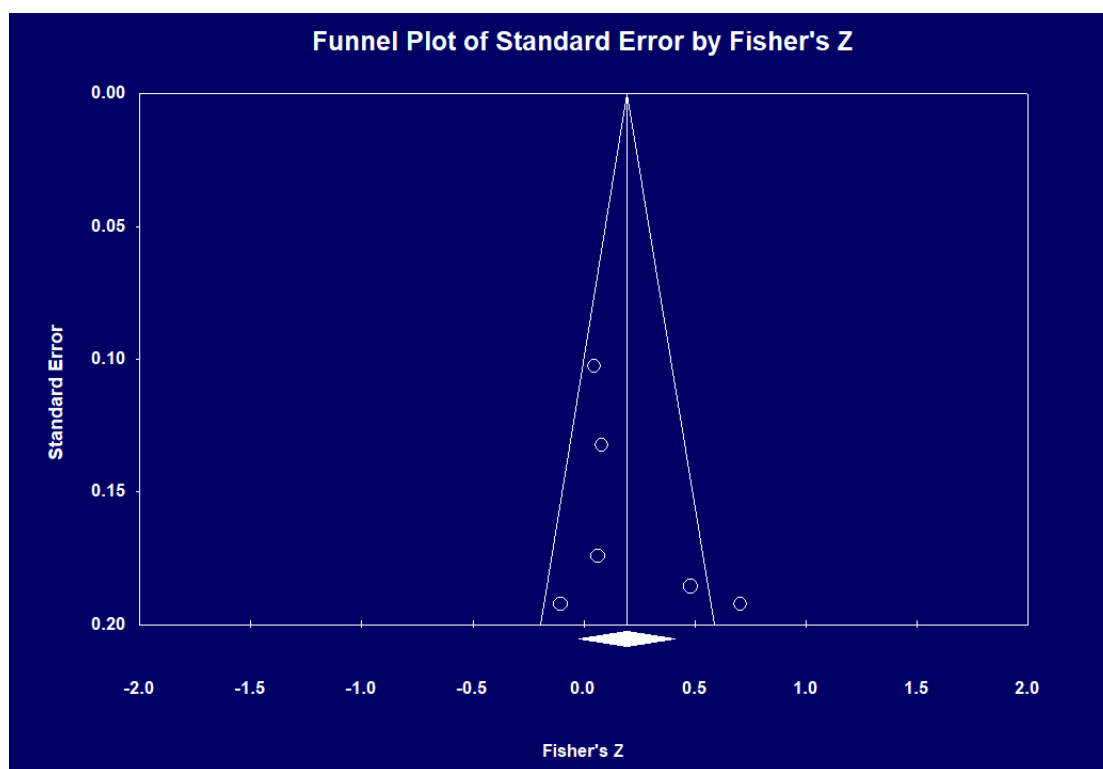
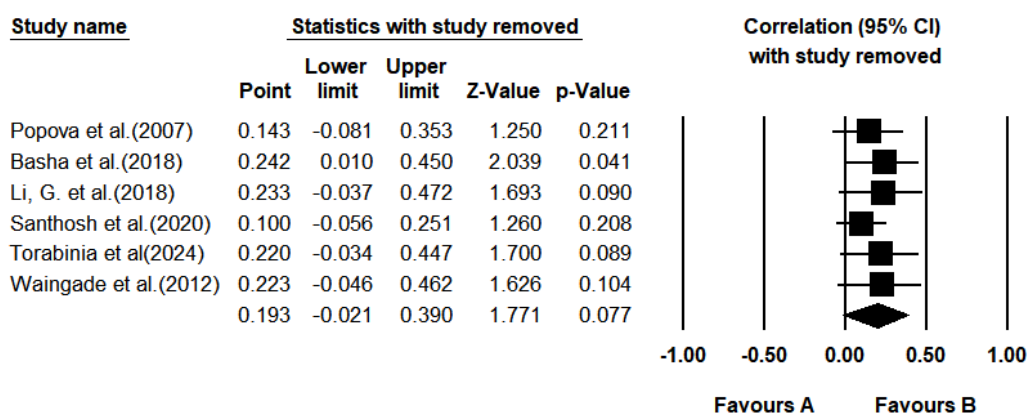


Figure 8 Funnel plot of the correlation between micronucleus frequency after x-ray exposure and age

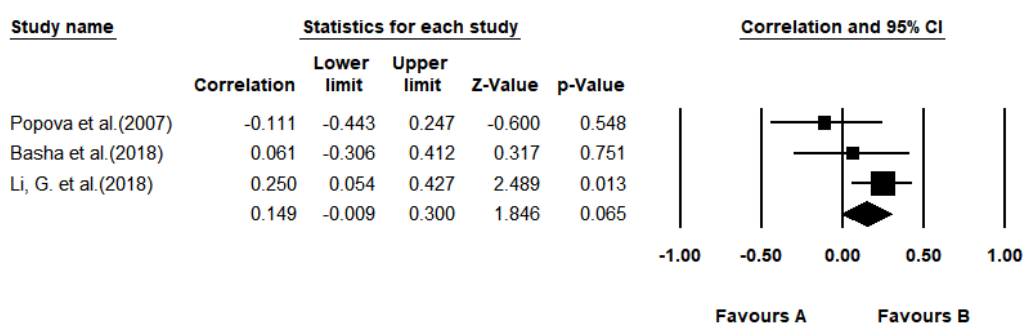
Meta Analysis



Meta Analysis

Figure 9 Sensitivity analysis of the correlation between micronucleus frequency after x-ray exposure and age

Meta Analysis



Meta Analysis

Figure 10 Forest plot of the correlation between difference of micronucleus frequency before and after X-ray exposure and age

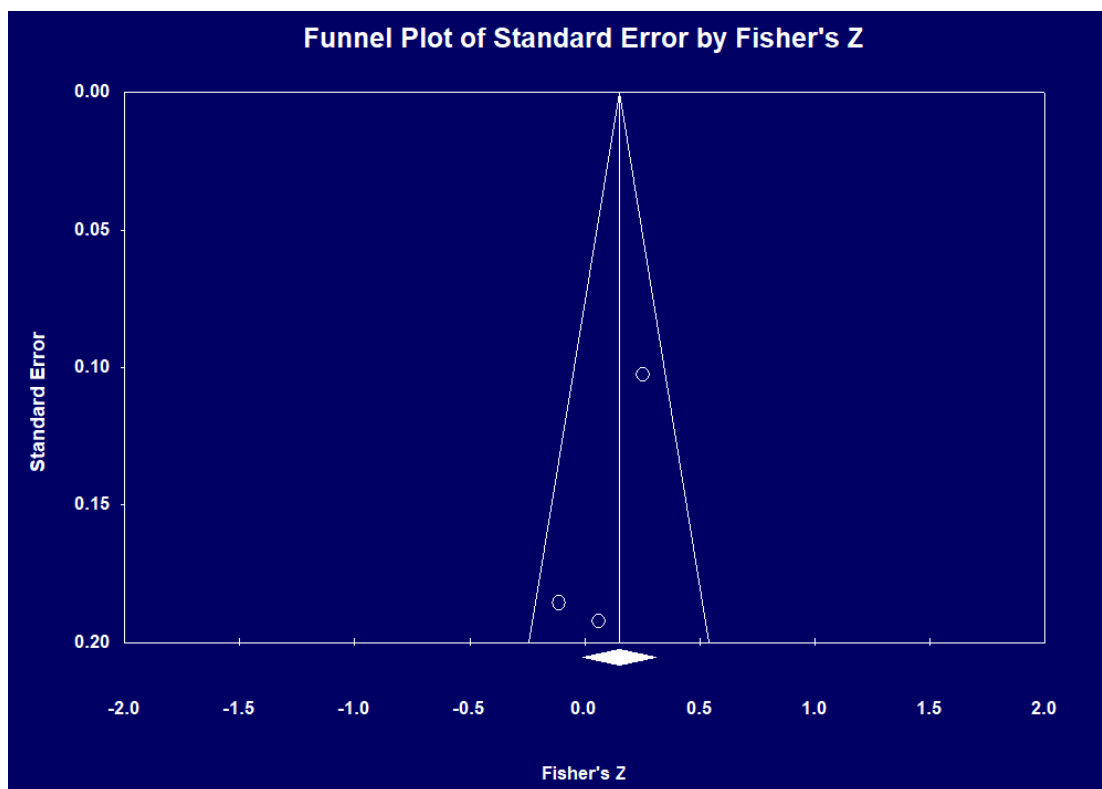
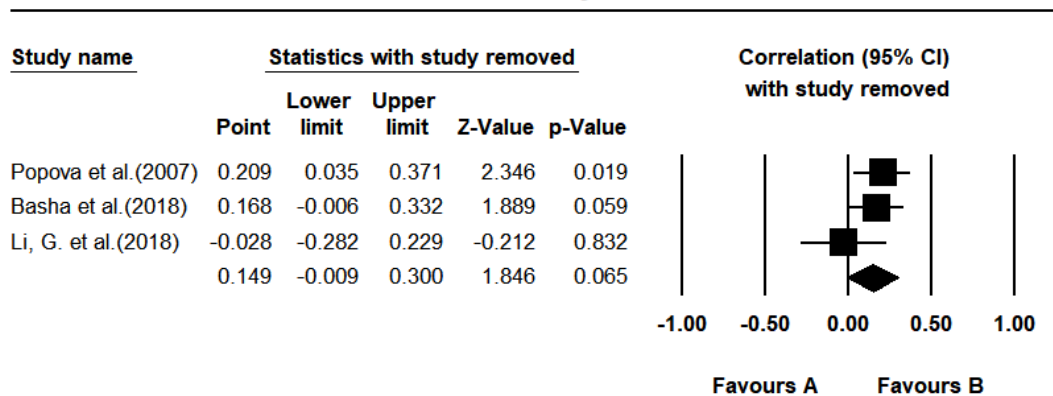


Figure 11 Funnel plot of the correlation between difference of micronucleus frequency before and after X-ray exposure and age

Meta Analysis



Meta Analysis

Figure 12 Sensitivity analysis of the correlation between difference of micronucleus frequency before and after X-ray exposure and age

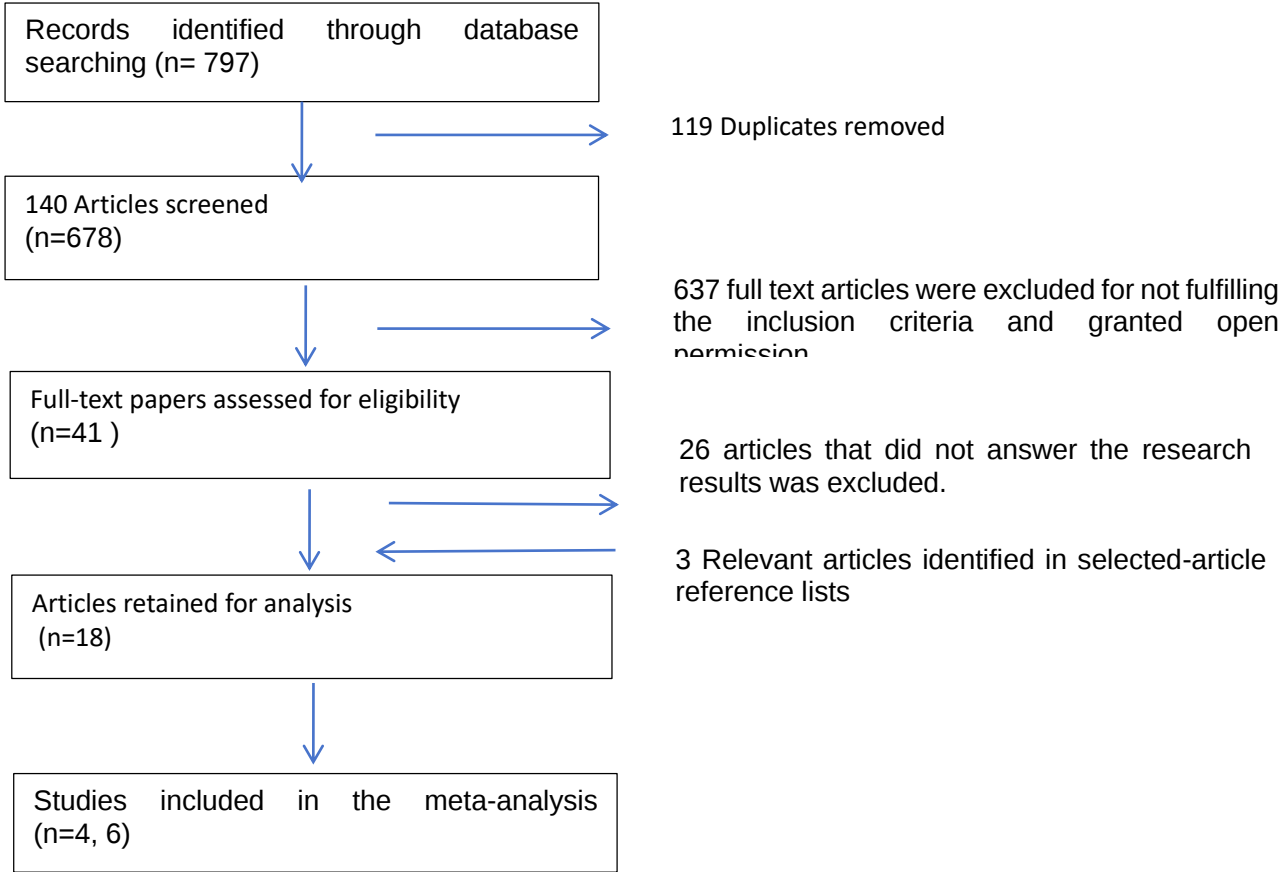


Figure 1 Study selection flow char

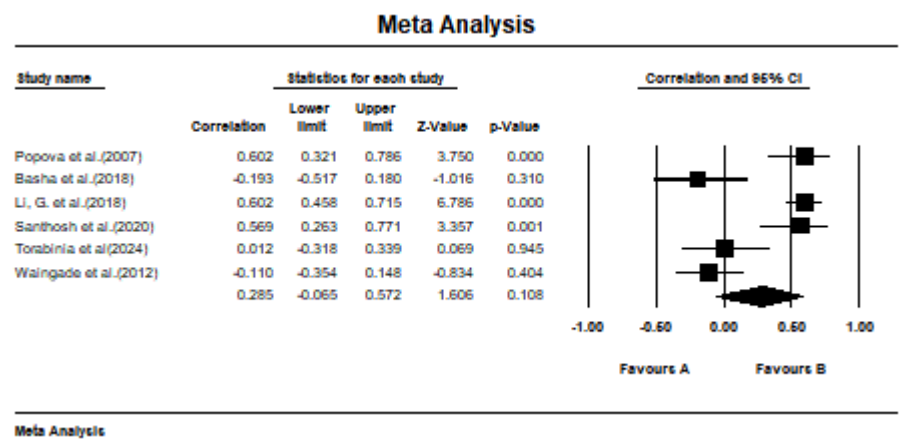


Figure 2 Meta-analysis data regarding micronucleus assay on oral cells of patients exposed to X-ray.

Certainty assessment							N: of patients		Effect		Certainty
N: of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	patients exposed to X-rays before	patients exposed to X-rays after	Relative (95% CI)	Absolute (95% CI)	
New Outcome											
4	non-randomised studies	serious ^a	not serious	not serious	serious ^a	strong association	214	214	-	SMD 0.3 higher (0.07 higher to 0.52 higher)	⊕○○○ Very low ^a

CI: confidence interval; SMD: standardised mean difference

Figure 3 GRADE analysis

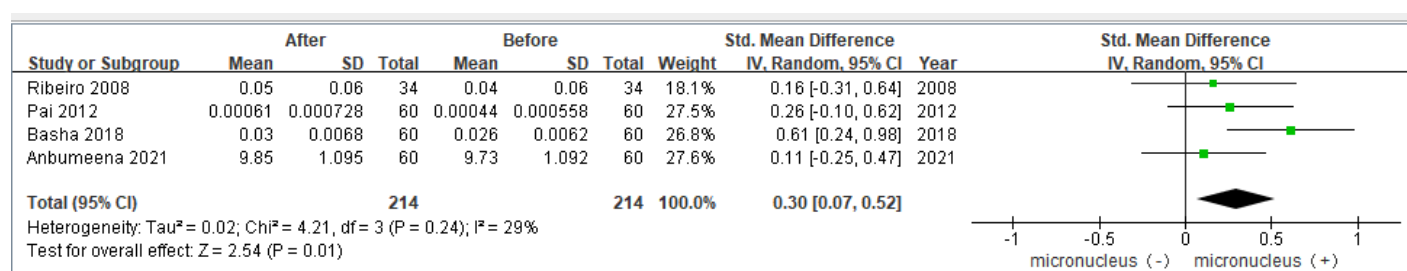


Figure 4 Forest plot of the correlation between micronucleus frequency before x-ray exposure and age

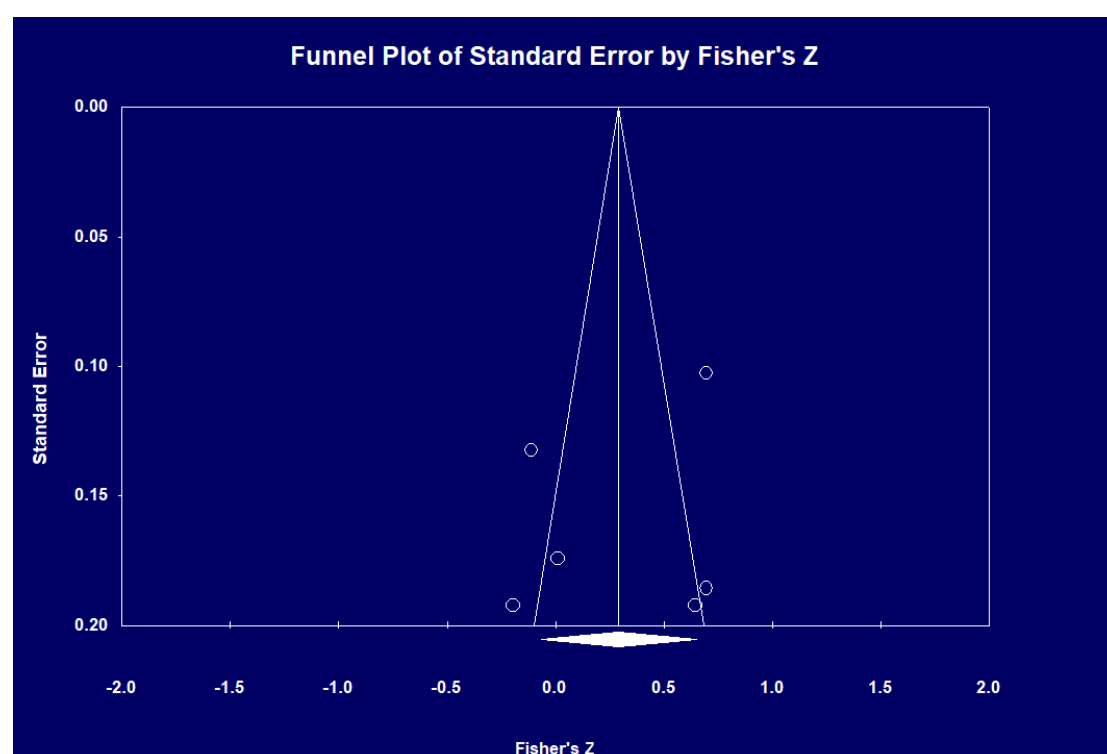
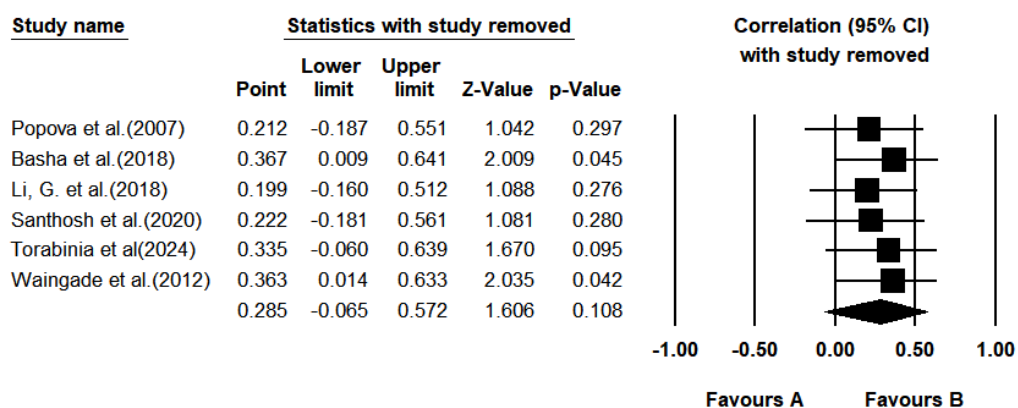


Figure 5 Funnel plot of the correlation between micronucleus frequency before x-ray exposure and age

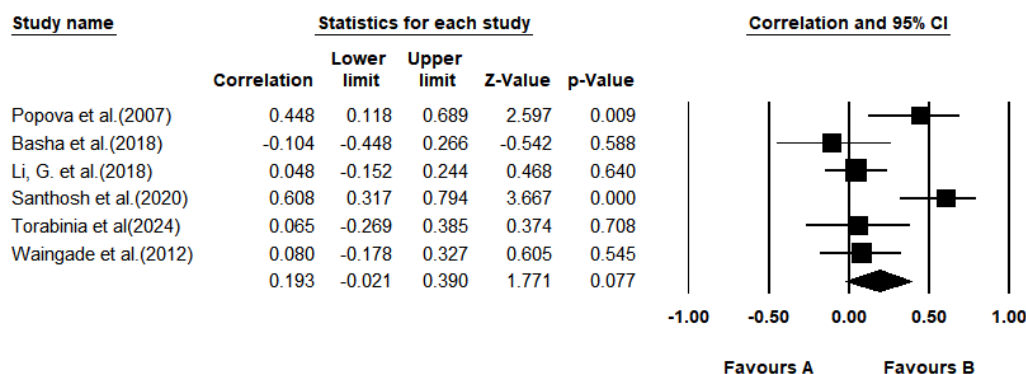
Meta Analysis



Meta Analysis

Figure 6 Sensitivity analysis of the correlation between micronucleus frequency before x-ray exposure and age

Meta Analysis



Meta Analysis

Figure 7 Forest plot of the correlation between micronucleus frequency after x-ray exposure and age

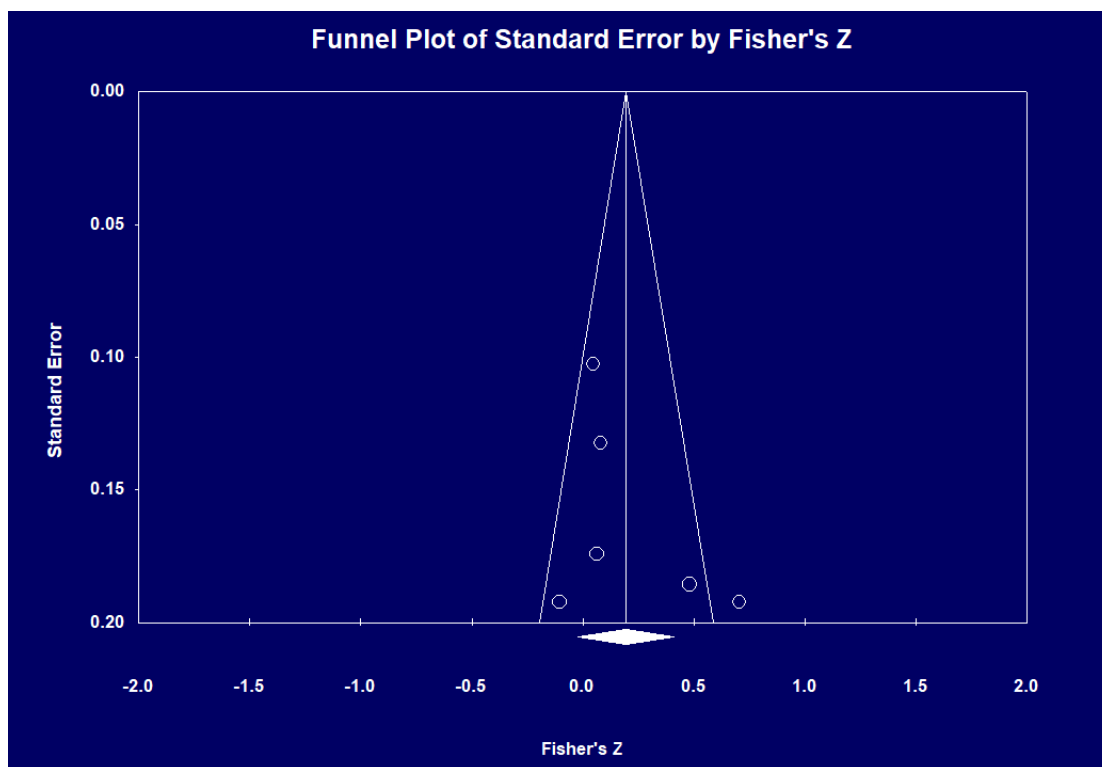
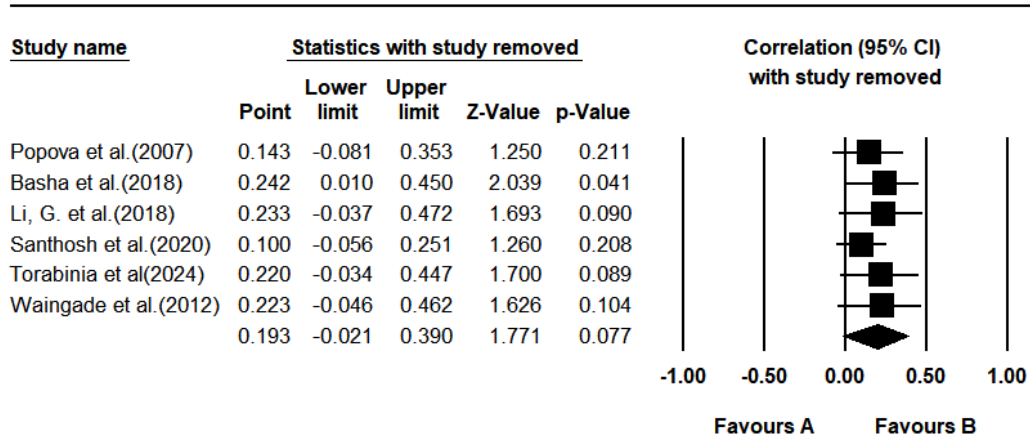


Figure 8 Funnel plot of the correlation between micronucleus frequency after x-ray exposure and age

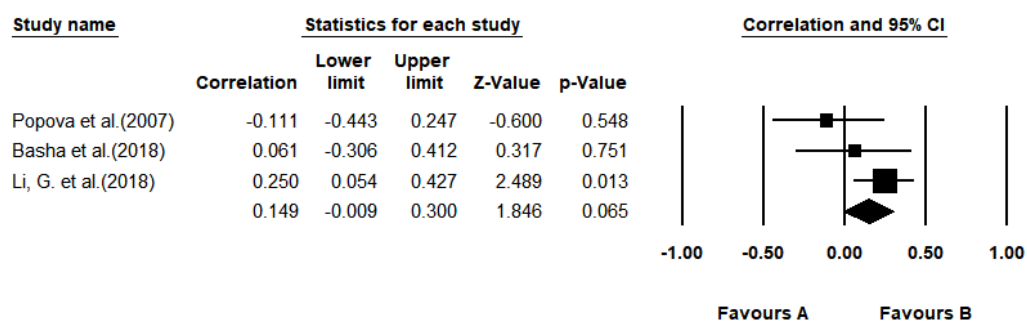
Meta Analysis



Meta Analysis

Figure 9 Sensitivity analysis of the correlation between micronucleus frequency after x-ray exposure and age

Meta Analysis



Meta Analysis

Figure 10 Forest plot of the correlation between difference of micronucleus frequency before and after X-ray exposure and age

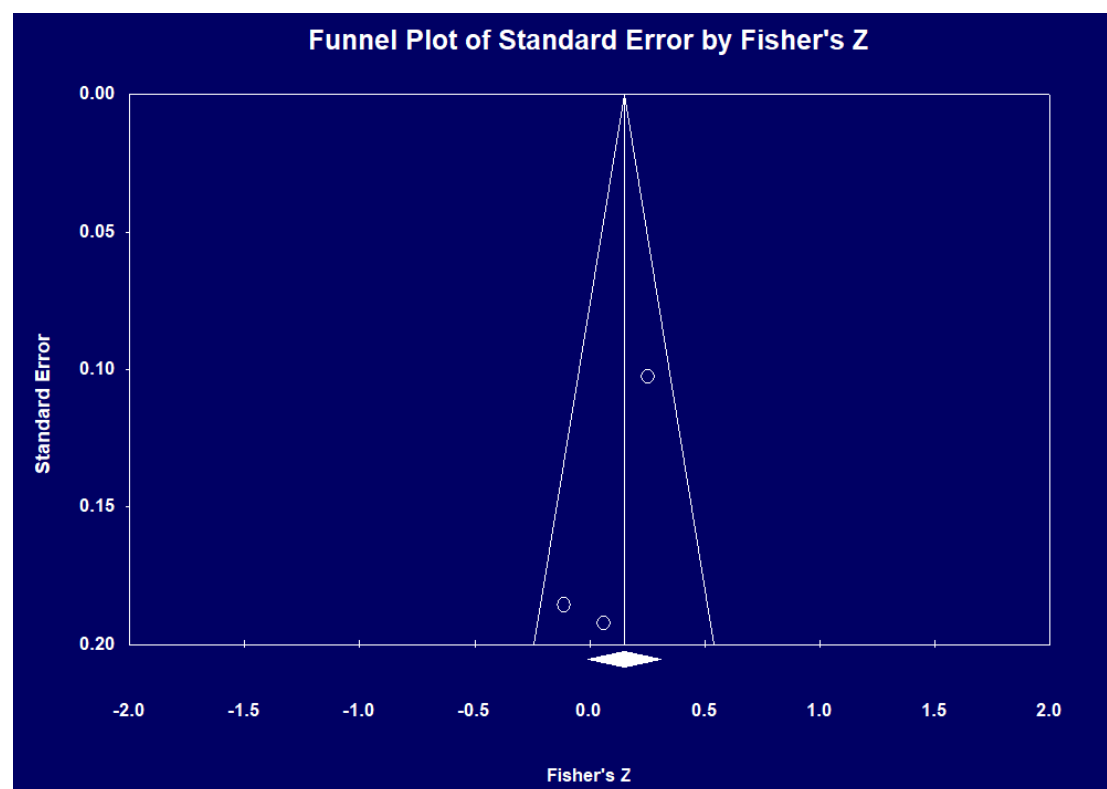


Figure 11 Funnel plot of the correlation between difference of micronucleus frequency before and after X-ray exposure and age

Meta Analysis

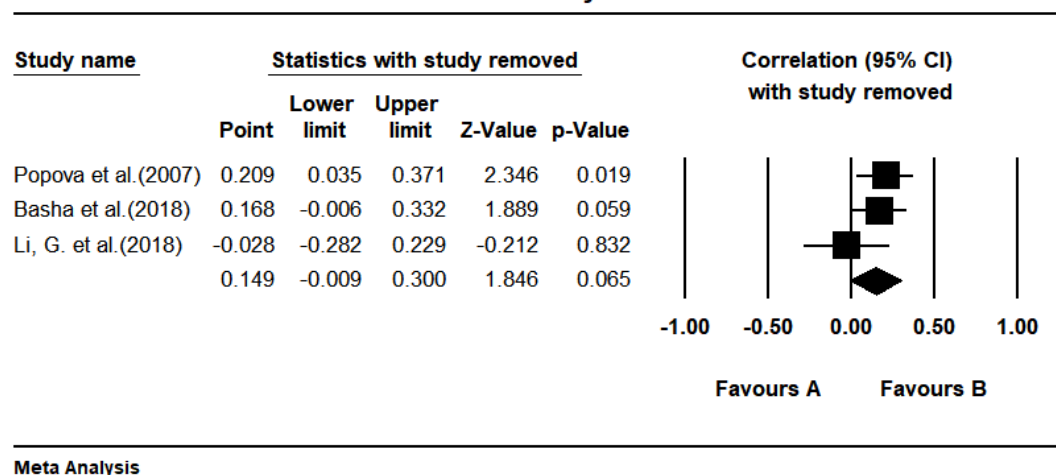
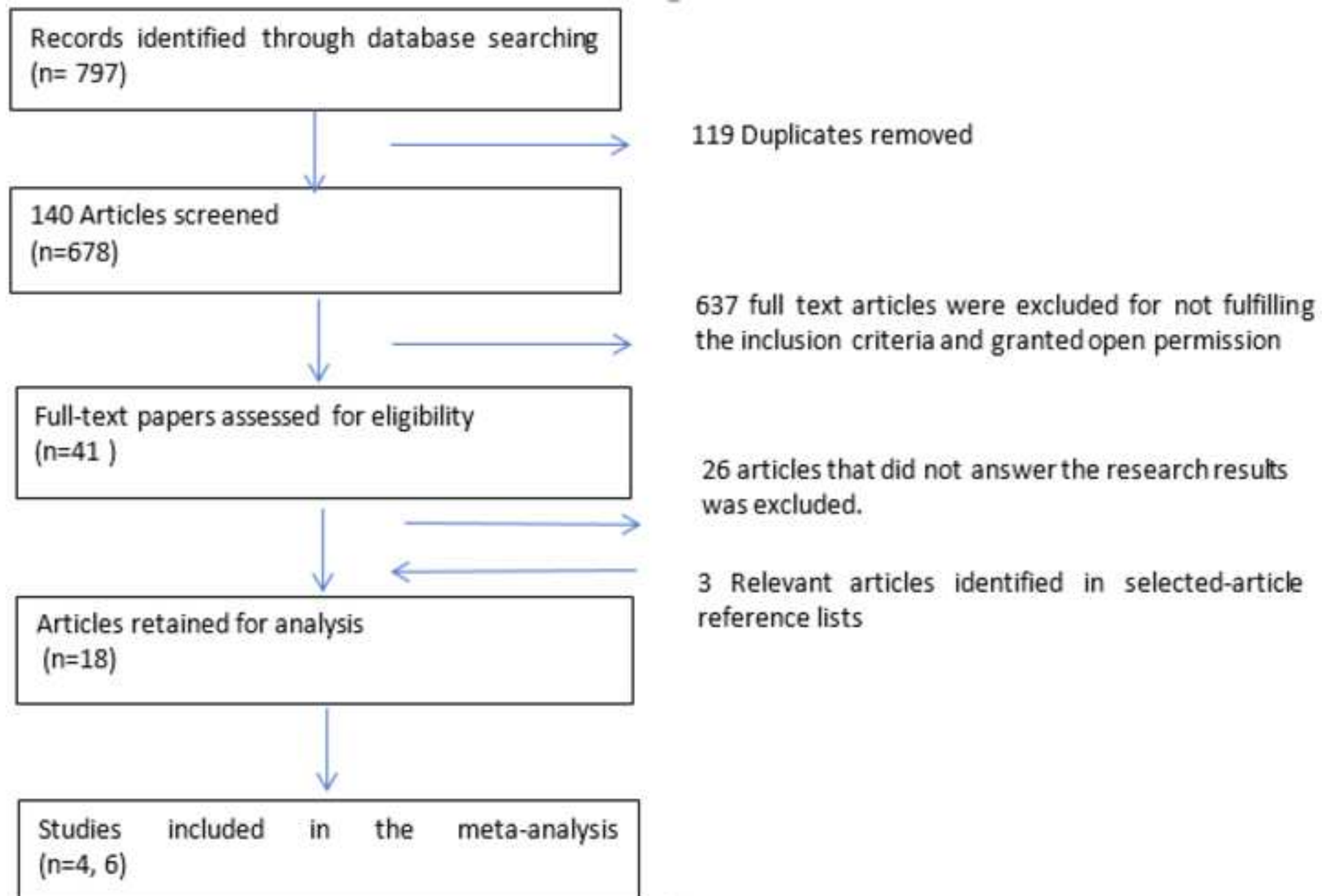
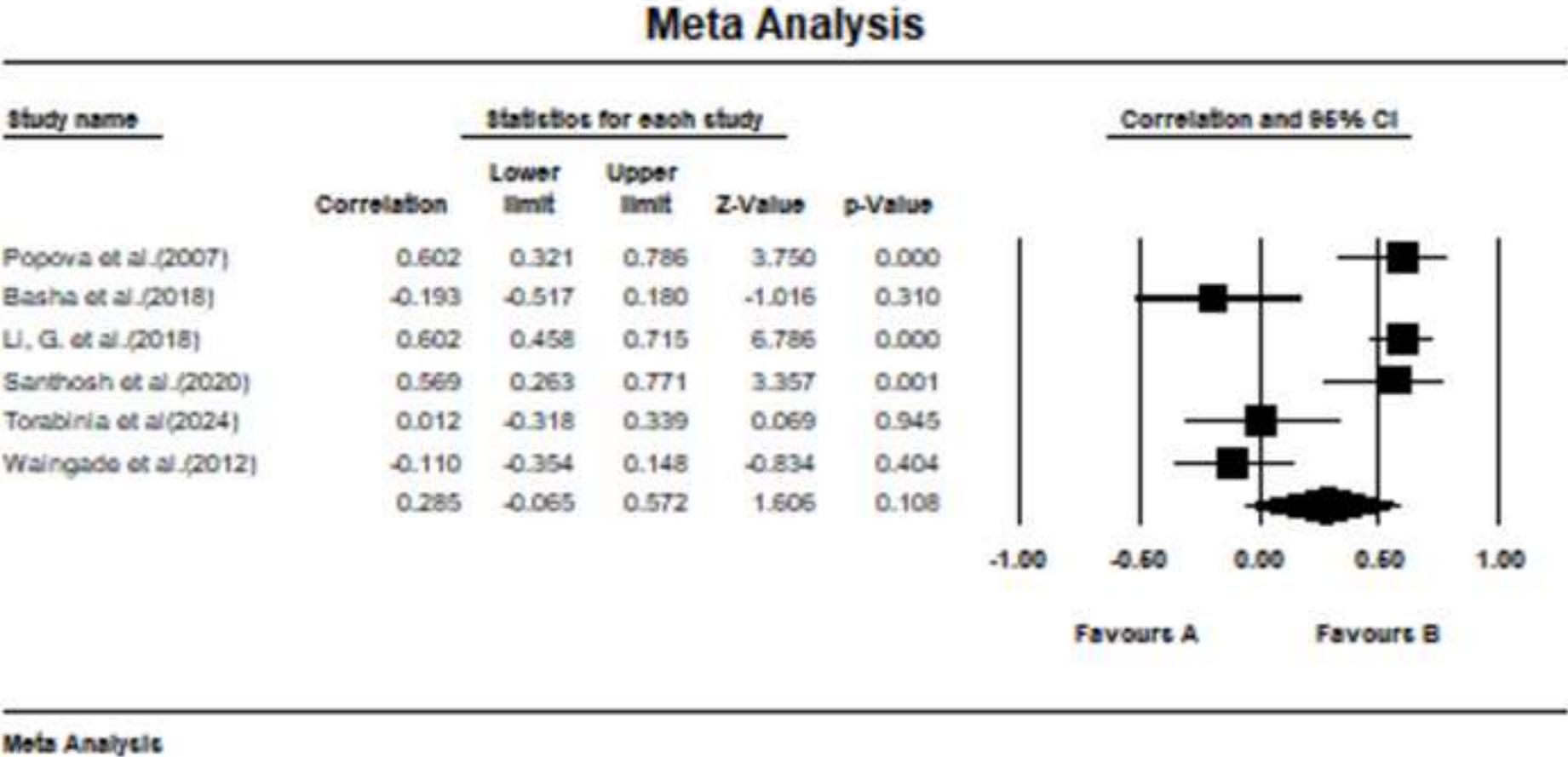


Figure 12 Sensitivity analysis of the correlation between difference of micronucleus frequency before and after X-ray exposure and age

Reviewers' comments	
First, The use of the term "age-related" in the title seems inappropriate, as the study was not methodologically designed to assess age as a primary factor. If the authors intended to evaluate the influence of age, the study design and analysis should have been structured accordingly.	Micronucleus Formation in Oral Mucosal Cells Following Dental X-ray Exposure: A Systematic Review and Meta-analysis
In addition, while the manuscript discusses the genotoxic implications of increased micronucleus frequency, its clinical relevance remains vague. The authors are encouraged to provide specific clinical examples or scenarios to illustrate how these findings could be applied in dental practice.	In contemporary dental practice, micronucleus (MN) formation is increasingly recognized as a sensitive cytogenetic biomarker for detecting radiation-induced genotoxicity, with emerging potential for clinical application. Evidence suggests that MN assessment may be particularly informative in evaluating radiation-related risks among pediatric orthodontic patients. Lorenzoni et al. identified a marked rise in MN frequency in oral mucosal cells of children subjected to comprehensive orthodontic imaging — including panoramic and lateral cephalometric radiographs — indicating a greater radiosensitivity in developing tissues. These findings underscore the importance of minimizing repeated radiographic exposures in children and warrant careful justification of each imaging procedure to limit the cumulative biological burden of low-dose radiation. MN frequency has also been proposed as an early indicator of radiation-induced genetic instability in adult patients undergoing multiple cone-beam computed tomography (CBCT) scans, such as those preparing for dental implant placement or prosthodontic interventions. Li et al. demonstrated that buccal epithelial cells exhibited significantly higher DNA damage following CBCT compared to panoramic imaging, suggesting that increased dose intensity and examination frequency may result in persistent genotoxic effects on the oral epithelium. Consequently, for high-risk populations—including individuals with prior radiotherapy, immunosuppression, or oral neoplasia—preoperative MN evaluation may assist in risk stratification and customization of imaging protocols to ensure safe and targeted diagnostic workflows. Beyond individual-level risk assessment, MN analysis offers promise for system-level radiation quality assurance (QA) and monitoring of radiographic procedural standards. As noted by Angelieri et al.,

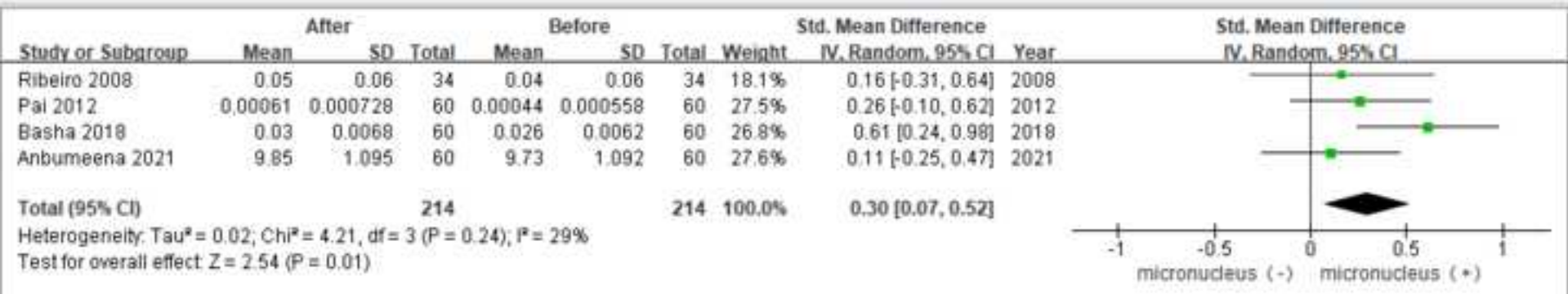
	increased MN frequencies following routine dental radiography may reflect suboptimal radiological practices, particularly in settings with limited technical oversight. Informed by our findings, we propose the inclusion of MN cytogenetic monitoring for patients subjected to repeated imaging, especially pediatric and orthodontic populations. The assay's non-invasive sampling, cost-effectiveness, and methodological reproducibility make it suitable for broader integration into clinical workflows, including enhanced patient education, informed consent processes, and long-term radiation risk monitoring. From a radiological operations standpoint, strict compliance with the ALARA (As Low As Reasonably Achievable) principle remains critical in safeguarding against unnecessary radiation exposure. Radiographic examinations should be performed only after rigorous clinical justification, and—when feasible—non-ionizing or low-dose alternatives, such as digital optical scanning or ultrasound, should be considered. Effective radiation mitigation strategies include field size limitation, employment of high-sensitivity digital detectors, anatomy-adapted exposure adjustments, and preferential use of rectangular collimation, which significantly reduces scatter dose compared to circular systems. Moreover, developing longitudinal exposure records—particularly for patients undergoing serial imaging, such as those in orthodontic or implant therapy—could enhance institutional radiation stewardship efforts. In summary, the incorporation of MN assay into routine dental radiographic protocols may serve not only to quantify subclinical genotoxic effects but also to support the implementation of individualized radioprotection strategies. Such integration has the potential to reduce genomic instability risks associated with diagnostic imaging, without compromising diagnostic efficacy, thereby promoting higher standards of patient safety and radiological care.
Lastly, the reference list contains formatting errors that should be corrected to comply with journal guidelines.	I have corrected all the formatting errors in the reference list based on journal guidelines.

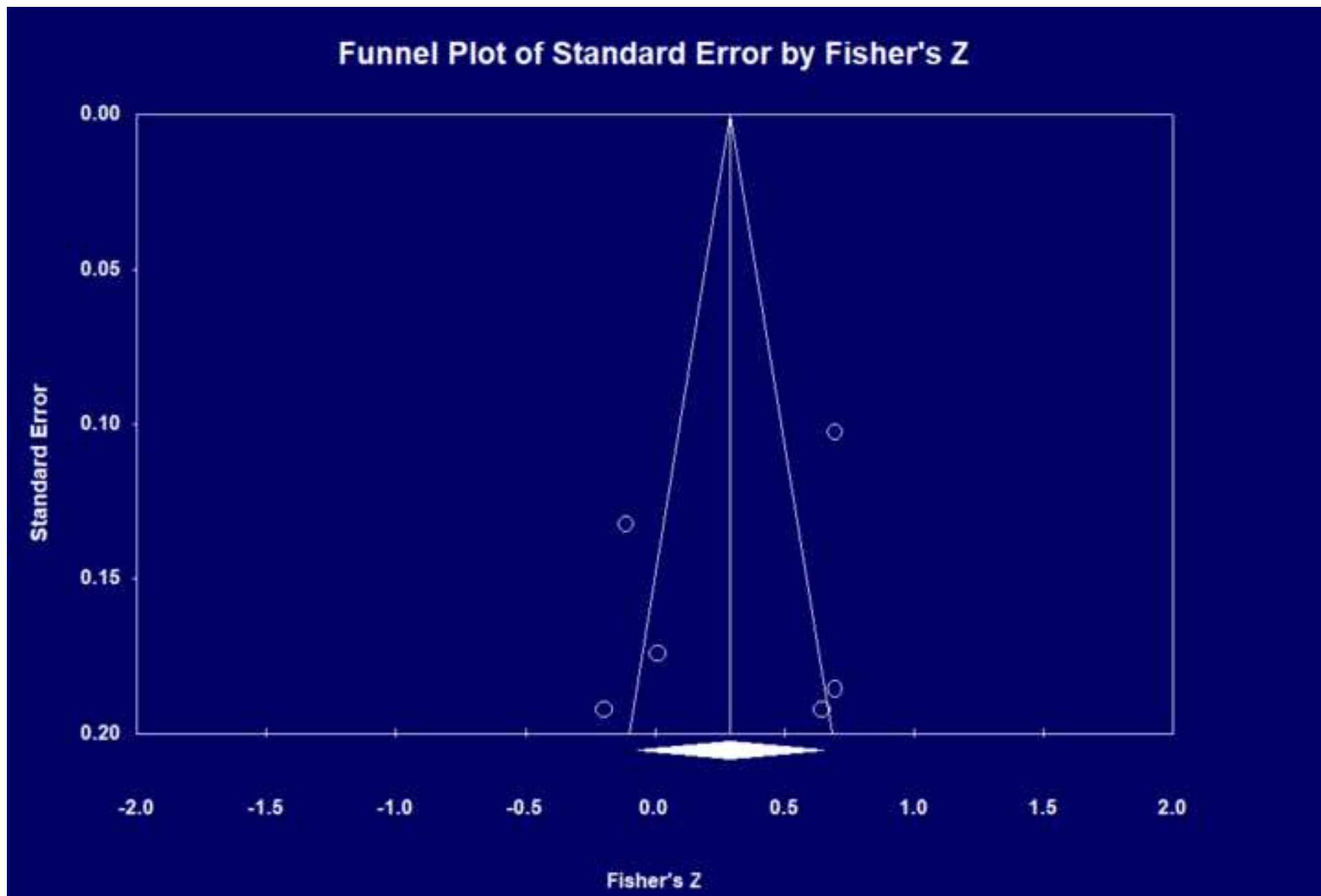




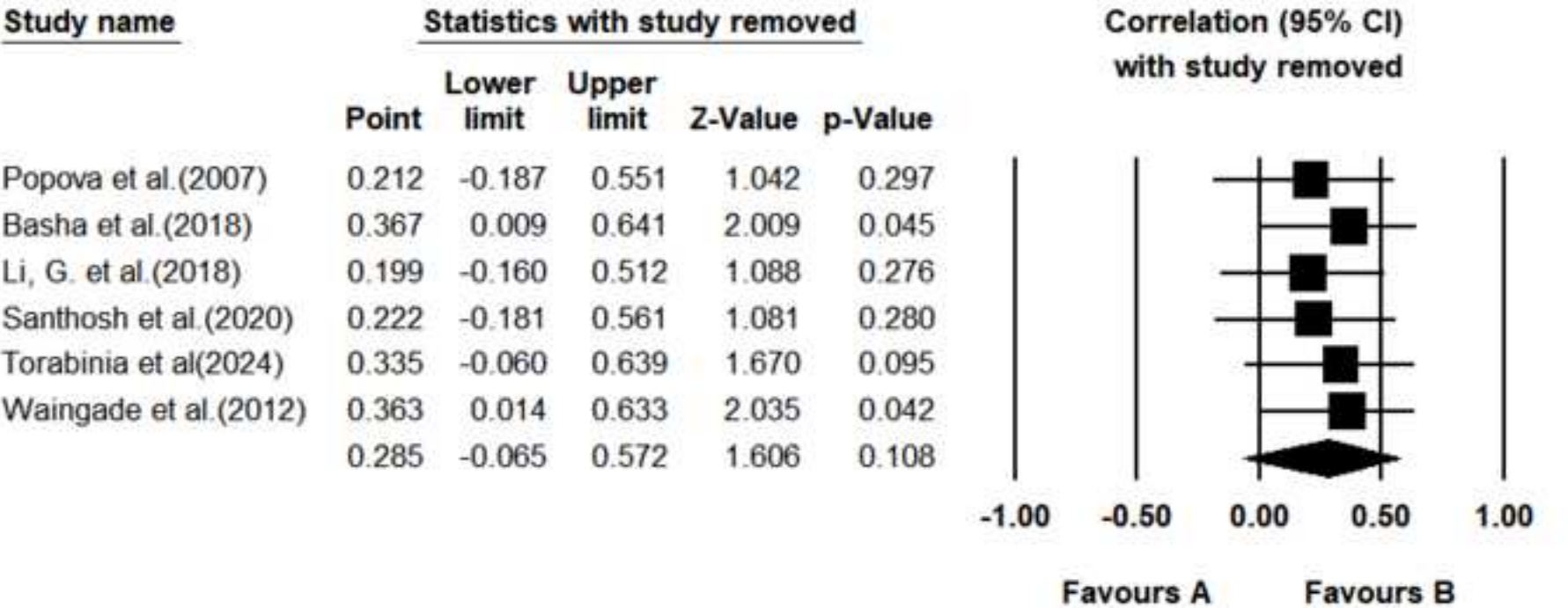
Certainty assessment							N: of patients		Effect		Certainty
N: of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	patients exposed to X-rays before	patients exposed to X-rays after	Relative (95% CI)	Absolute (95% CI)	
New Outcome											
4	non-randomised studies	serious ^a	not serious	not serious	serious ^a	strong association	214	214	-	SMD 0.3 higher (0.07 higher to 0.52 higher)	 Very low ^a

CI: confidence interval; SMD: standardised mean difference





Meta Analysis



Meta Analysis

