REVIEW

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Micronuclei in the Buccal Mucosal Cells are Genotoxicity Markers in Oral Potentially Malignant Disorders: A Systematic Review and Meta-Analysis

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Abstract

Objective: Micronuclei (MN) genotoxicity, linked to chromosomal anomalies, is a key biomarker for carcinogen exposure and cancer susceptibility, with higher frequencies observed in cancer patients. The micronuclei assay, using exfoliated buccal cells, offers a non-invasive method for diagnosing oral lesions caused by tobacco, betel nut, and alcohol. This review aims to systematically review micronuclei frequencies in buccal mucosal cells and assess their potential as genotoxicity markers in oral potentially malignant disorders (OPMD). **Methods:** A systematic search updated to 2024 was conducted using PubMed, Scopus, ProQuest, Cochrane, and Google Scholar for original studies analyzing micronuclei frequencies in buccal cells as genotoxicity markers for OPMD. Studies including leukoplakia, lichen planus, and OSMF, were selected. The assessment of risk bias was done using modified Newcastle-Ottawa Scale followed by meta- analysis. The review was registered in PROSPERO (Registration No. CRD42024536661). **Results:** Twenty-six articles encompassing a pooled sample of 1,078 healthy controls and 1,489 OPMD cases (417 leukoplakia, 180 oral lichen planus, 401 OSMF, and 491 unsegregated OPMDs) were included. A significant increase in micronuclei frequency was observed in OPMD patients compared to controls (meta-Cohen's d = 3.08, 95% CI: 1.80-4.35). Subgroup analysis revealed a gradual rise in MN frequency from healthy controls to leukoplakia (d = 2.75), oral lichen planus (d = 1.47), and the highest in oral submucous fibrosis (d = 5.55). Considerable heterogeneity was detected among studies (overall I^2 = 99.23%, OSMF I^2 = 99.85%, lichen planus I^2 = 49.59%). This variability highlights methodological and population differences across studies. **Conclusion:** Micronuclei genotoxicity is emerging as a valuable biomarker for the early detection of OPMD's. Due to its non-invasive and cost-efficient characteristics, examining micronuclei in exfoliated buccal cells could be incorporated into regular screenings for groups at high risk of OPMD and oral cancer. However, the considerable variability among studies necessitates careful interpretation and highlights the importance of establishing standardized protocols in future research.

Keywords: Micronuclei- Genotoxicity marker- Oral potentially malignant disorders- Leukoplakia- Lichen Planus

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Introduction

Oral potentially malignant disorders (OPMDs) are defined as "any oral mucosal abnormality that is associated with a statistically increased risk of developing oral cancer." They include leukoplakia, erythroplakia, oral lichen planus, and oral submucous fibrosis, each with variable clinical presentations and risk factors [1]. OPMDs are major precursors of oral squamous cell carcinoma, an aggressive malignancy with a 5-year survival rate of only 20–40% when diagnosed at advanced stages [2]. There is an urgent need for early and dependable biomarkers, as current diagnostic methods like biopsies are invasive, prone to variability among observers, and may not detect early molecular changes. In areas with high consumption of tobacco, alcohol, or areca nut, the incidence of oral cancer is increasing, highlighting the necessity for minimally invasive and cost-effective tools for the early detection and monitoring of OPMDs. Among the various techniques used to evaluate the risk and progression of these disorders, such as histopathological examination, cytology, biopsy, and salivary diagnostics, the identification of micronuclei in buccal mucosal cells has gained attention as a non-invasive and reliable biomarker for genotoxicity. This method shows promise for studying epithelial carcinogens [3]. Increased MN frequencies have been observed in people exposed to tobacco, alcohol, environmental carcinogens, and nutritional deficiencies [4]. Recent research indicates that MN frequency is associated with the presence and progression of OPMDs and oral cancer, underscoring its potential utility in clinical biomonitoring [3].

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Micronuclei are small, round to oval chromatin bodies arising from lagging chromosomes or fragments excluded from the main nucleus during mitosis [5]. These appear as round to oval cytoplasmic chromatin masses near the nuclei under the microscope [4]. Their frequency in oral exfoliated cells reflects early genotoxic events, with significant differences observed between normal mucosa, epithelial dysplasia, and carcinoma [6]. The progressive increase in MN counts across these stages supports its utility as a cost-effective tool for identifying high-risk individuals and enabling timely intervention [6].

The aim of this study is to systematically review and perform a meta-analysis of existing literature to assess the efficacy and reliability of micronuclei (MN) in buccal mucosal cells as genotoxicity markers for OPMDs. By synthesizing current evidence, this review seeks to determine their diagnostic and prognostic value in the early detection and prevention of oral cancers.

Materials and Methods

Design

The search followed the guidelines outlined in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and the Cochrane Handbook for Systematic Reviews. Systematic review was registered in PROSPERO, ID No. CRD42024536661.

Search Strategy

Systematic literature searches were conducted across multiple electronic databases, including PubMed/MEDLINE, Scopus, ProQuest, Cochrane, and Google Scholar, without restrictions on publication dates. The search strategy combined both Medical Subject Headings (MeSH) and free-text keywords using Boolean operators to optimize sensitivity and precision.

Specifically, terms related to genotoxicity and oral potentially malignant disorders were grouped using the OR operator to capture synonyms and variant expressions within each concept, then combined with AND to ensure conceptual intersection. The primary concepts and combined search string structure included:

Micronuclei terms: "micronuclei" OR "micronucleus"

Buccal mucosa terms: "buccal mucosa" OR "buccal cells"

Oral potentially malignant disorder terms: "oral precancer" OR "oral potentially malignant lesions" OR "leukoplakia" OR "oral lichen planus" OR "oral submucous fibrosis"

Example search string

(micronuclei OR micronucleus) AND (buccal mucosa OR buccal cells) AND (oral precancer OR oral potentially malignant lesions OR leukoplakia OR oral lichen planus OR oral submucous fibrosis)

Both electronic and manual searches, including grey literature, were performed. Two independent reviewers screened titles, abstracts, and full texts to identify eligible studies. Discrepancies were resolved by consensus. Full-text versions of selected articles were retrieved for detailed analysis and data extraction.

Article Selection

The relevance of each study was independently assessed by two evaluators (AC & PA) in two stages: first, screening of titles and abstracts, followed by full-text review. Inclusion criteria focused on studies involving human subjects with OPMD, assessing micronuclei in buccal cells, and including control groups. Only peer-reviewed, full-text, original research articles published in English were considered. Exclusion criteria included animal or in vitro studies, reviews, case reports, studies lacking controls or clear OPMD diagnostic criteria, and non-English or duplicate publications.

Disagreements on inclusion were resolved by consensus. One evaluator (AC) conducted a quality assessment, verified by the second (PA). A total of 5,140 articles were identified, and after title screening and removal of duplicates, 41 articles were selected for full-text review. Of these, 15 were excluded for not meeting inclusion criteria, resulting in 26 articles shortlisted for evaluation, with 25 included in the meta-analysis. Figure 1 shows the PRISMA flowchart used.

Quality Assessment

The risk of bias assessment for each article was done by two authors (AC & PA) independently using the Modified Newcastle- Ottawa Scale (NOS) used for evaluating the quality of the non-randomised studies in meta-analysis and systematic reviews. The NOS uses a star system to judge studies based on three broad criteria: the selection of study groups, the comparability of the groups, and the ascertainment of either the exposure or outcome of interest for case-control or cohort studies, respectively. Intra and Inter-reviewer agreement was assessed using kappa statistics. In cases of disagreement, consensus was reached through discussion with a third reviewer.

Data Extraction

Two authors (AC & PA) reviewed the 26 included articles, collecting data on the following: first author, publication year, article title, objectives, journal, study population, habit history, associated lesions, micronuclei levels in individuals with habits and lesions, potentially malignant disorders studied, healthy controls, sample size, study duration, age and gender, sample collection method, methodology, staining techniques, micronuclei count in OPMD, assessment method, cut-off values, statistical analysis, outcomes, and risk of bias assessment.

Statistical Analysis

The meta-analysis was performed using STATA statistical software, version 16.0, employing the random effects model due to heterogeneity (0% to 100%). Forest plots were generated to illustrate the effect size, the weight of each study, and to derive the pooled analysis with 95% confidence intervals. Funnel plots were generated to evaluate the heterogeneity and publication bias.

Results

Study Characteristics

This review included 26 studies, summarized in

Supplementary Table 1, to evaluate micronuclei (MN) as a biomarker for genotoxic damage in oral mucosal cells. Most studies focused on the Indian population, with two on Caucasian [8] and Turkish populations [9]. The review examined three oral potentially malignant disorders (OPMDs): oral leukoplakia (OLP), lichen planus, and oral submucous fibrosis (OSMF). Nine studies addressed OPMDs collectively, while others focused on specific conditions (11 on OLP, 6 on lichen planus, and 11 on OSMF). Participants ranged from 16 to 90 years, with a predominance of males and tobacco or alcohol use.

The total sample included 1,078 healthy controls and 1,489 OPMD cases (417 leukoplakia, 180 lichen planus, 401 OSMF, and 491 unsegregated OPMDs). Exfoliated buccal cells were collected using a scraping method, primarily stained with Papanicolaou (PAP) stain, and examined under light microscopy. The zigzag method was used for screening, and Tolbert et al.'s criteria for counting MN [7]. Most studies reported a higher prevalence of MN in OPMD patients compared to healthy controls.

Quality Assessment

The risk of bias was assessed using the modified Newcastle-Ottawa Scale (NOS) across three domains: selection (max 4 stars), comparability (max 2 stars), and outcome (max 3 stars). Most studies scored 3 or 4 stars in selection, indicating a low risk of bias, except for four studies, i.e., Sanchez-Siles et al. 2011 [8], Vidyalakshmi et al. 2016 [10], Kamboj et al. 2006 [11], Neha Gupta et al. 2019 [12], which scored 2, suggesting moderate bias. Two studies i.e., Saran et al. 2008 [13] and Sangle et al. 2016 [14] scored 1 due to incomplete confounding factor reporting. For outcomes, most studies scored 2 or 3 stars, indicating low bias with comprehensive statistical and outcome reporting. Overall, the 26 studies showed a low risk of bias, with only four presenting moderate risk, unlikely to affect the review's conclusions (Figure 2). The kappa value was 0.81 for intraobserver agreement and 0.80 for interobserver agreement, suggestive of good agreement.

Meta-analysis

The meta-analysis included studies reporting mean ± standard deviation (SD) of micronuclei (MN) counts in healthy controls and OPMDs. Of the 26 articles reviewed, 25 were included (Table 1). Four meta-analyses compared MN counts between healthy controls and individuals with OPMDs in general, and others of healthy controls with leukoplakia, lichen planus, and OSMF individually.

Overall Micronuclei Count in OPMD vs. Healthy Controls

Nine studies i.e Saran et al. [13], Paravathi Devi et al. [15], Grover et al. [16], Khalida Begum et al. [17], Sangle et al. [14], Juneja et al. [18]; Dave et al. [19]; Gupta et al. [20] and Sudha et al. [21] were included in the forest plot analysis, providing mean MN counts. The meta-analysis revealed a significantly higher mean MN count in the OPMD group compared to healthy controls, with a standardized mean difference (SMD) of 2.14 (95% CI: 0.13-4.15, p=0.04) (Figure 3). High heterogeneity was noted (I^2 = 99.23%), potentially due to sample size discrepancies, with significant variation observed in studies by Sudha et al. [21] and Sangle et al. [14] (Figure 3). Figure 5A shows the funnel plot for this meta-analysis, and majority of the studies were outside the funnel suggesting large variation in the methodology and sample size which may account for the heterogeneity.

Micronuclei Count in Leukoplakia vs. Healthy Controls

Eleven studies i.e (Kamboj et al. [11], Mahimkar et al. [22], Khanna et al. [23], Katarkar et al. [24], Grover et al. [16]. Pushpanjali et al. [25], Kohli et al. [26], Wagh et al. [27], Singam et al. [28], Neha et al. [12] and Gupta et al. [20] contributed data on MN counts in leukoplakia. The analysis indicated a higher MN count in the leukoplakia group as compared to healthy controls, with an SMD of 2.75 (95% CI: 1.76-3.74, p=0.00) (Figure 4 A). The funnel plot shows that majority of the studies were outside the funnel suggesting large variation in the methodology and sample size which may account for the heterogeneity and suggests potential publication bias.The spread of effect sizes and standard error indicates variability among included studies, which might be due to study design, population, or methodologies. (Figure 5B)

Micronuclei Count in Lichen Planus vs. Healthy Controls

Six studies i.e Sanchez-Siles et al. [8], Sarahanglu et al. [19], Katarkar et al. [24], Grover et al. [16],

Table 1. List of Articles Included in Meta-Analysis.

Sl. No	Author	Year
1.	M.Kamboj et. al.	2006
2.	Saran R. et. al.	2007
3.	Joshi et. al.	2010
4.	M.B. Mahimkar et. al.	2010
5.	Koneru. A et. al.	2011
6.	M. Sanchez-Siles et. al.	2011
7.	Paravathi Devi et. al.	2011
8.	S.Jyoti et. al.	2012
9.	A. Sarahanglu et. al.	2013
10.	Khanna. et. al.	2014
11.	A. Katarkar et. al.	2014
12.	Grover et. al.	2014
13.	Khalida Begum et. al.	2016
14.	Sangle et. al.	2016
15.	S. Vidyalakshmi et. al.	2016
16.	Pushapanjali et. al.	2017
17.	Kohli. M et. al.	2017
18.	Wagh et. al.	2018
19.	Juneja. et. al.	2019
20.	Dave et. al.	2019
21.	Singam et. al.	2019
22.	Neha Gupta et. al.	2019
23.	Gupta. et. al.	2019
24.	Jaiswal and Sharma	2019
25.	Sudha. et. al.	2023

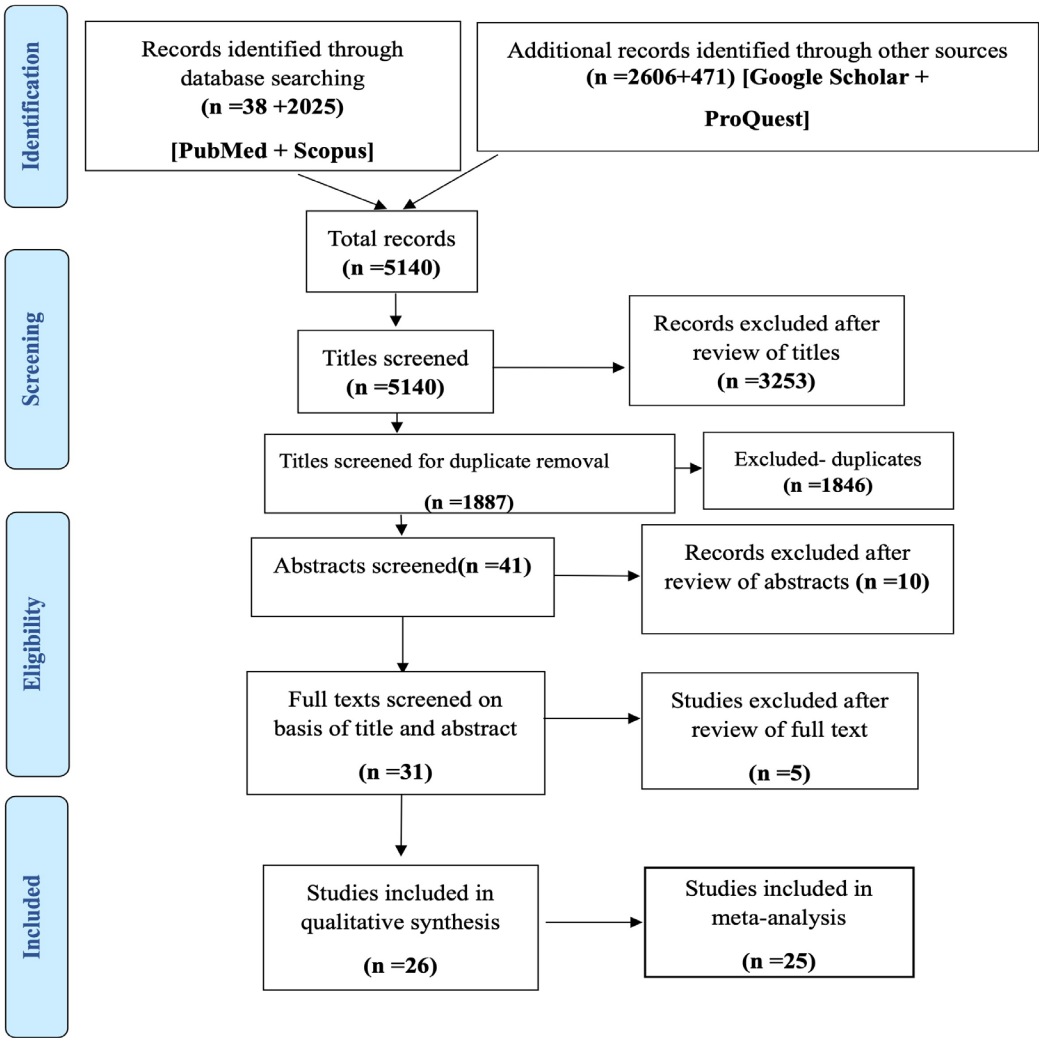

Figure 1. PRISMA Chart for the Review

Vidyalakshmi et al. [10] and Gupta et al. [20] provided data on MN counts in lichen planus. The meta-analysis showed that the MN count in the lichen planus group was significantly higher, with an SMD of 1.47 (95% CI: 1.12-1.82, p=0.00). This result was statistically significant, with moderate heterogeneity (I^2 = 49.59%). (Figure 4B) The funnel plot for this metanalysis shows no bias in publication among the data considered to compare the level of micronuclei count between Lichen planus and healthy controls as majority of the included studies were distributed symmetrically within the funnel (Figure 5C)

Micronuclei Count in OSMF vs. Healthy Controls

Eleven studies i.e. Joshi et al. [29], Koneru et al. [30], Jyoti et al. [31], Katarkar et al. [24], Grover et al. [16], Pushpanjali et al. [27], Kohli et al. [26], Wagh et al. [27], Neha et al. [12], Gupta et al. [20] and Sharma, [32] reported on MN counts in OSMF. The analysis demonstrated that the MN count in the OSMF group was significantly higher than in healthy controls, with an SMD of 5.55 (95% CI: 0.77-10.32, p=0.02) (Figure 4C). This result was statistically significant, albeit with high heterogeneity (I^2 = 99.85%) due to discrepancies in sample size and mean values. The funnel plot showed that most of the studies are clustered on the top around the point

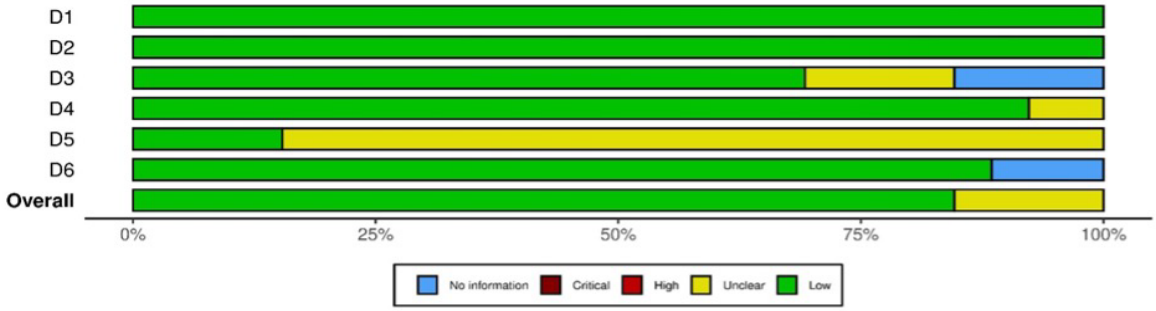

Figure 2. Risk of Bias Summary Plot

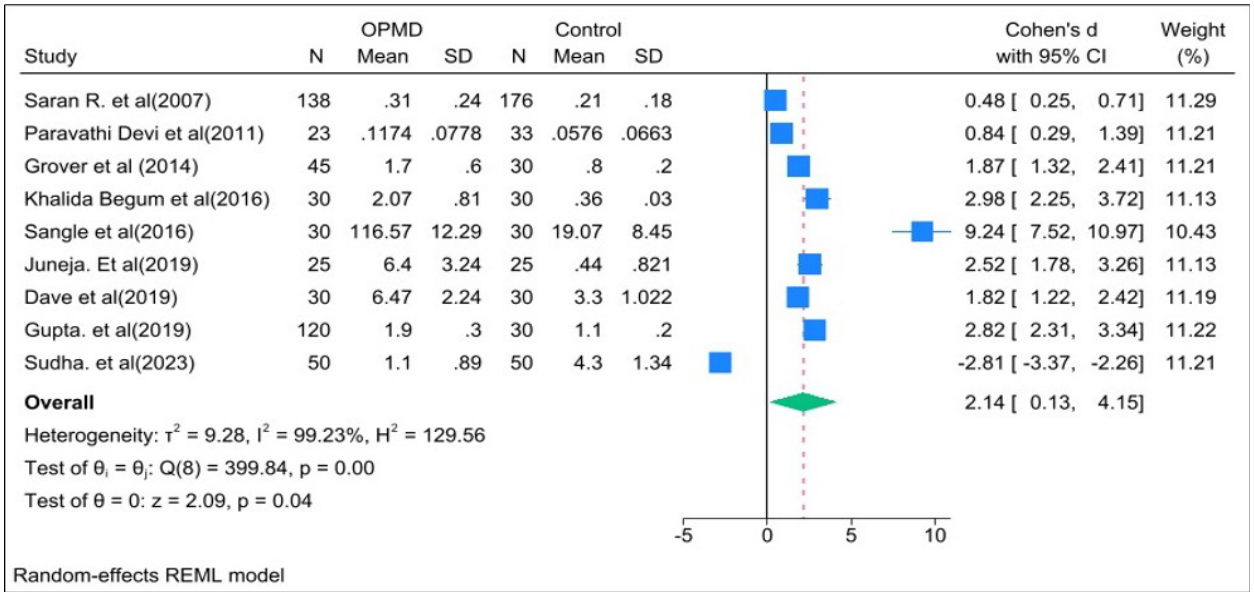

Figure 3. Forest Plot Depicting MN Count in OPMD and Healthy Control

estimate suggesting no publication bias. Since most are at the top of the funnel, they also suggest that these studies have greater precision (Figure 5D)

Discussion

Micronuclei (MN), initially identified as Howell–Jolly bodies in the late 19th century by William Howell and Justin Jolly, are residual nuclei of red blood cells found in pathological conditions [33]. MN have been observed in various cells, particularly lymphocytes, where studies show a linear relationship between radiation dose and MN frequency, establishing the micronucleus assay as a reliable method for detecting chromosomal damage from

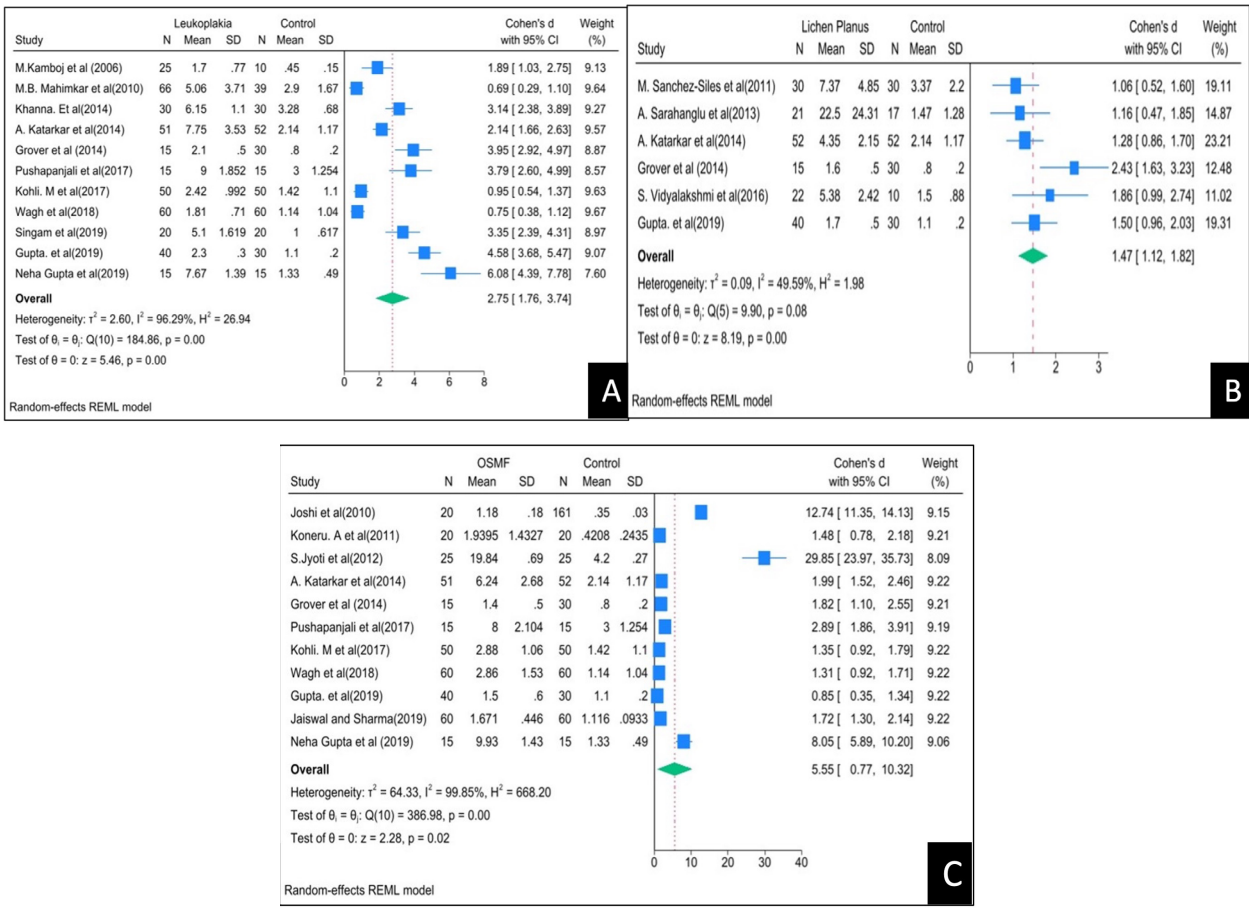

Figure 4. A: Forest plot depicting MN count in Leukoplakia and Healthy control. B: Forest plot depicting MN count in Lichen Planus and healthy control.B: Forest plot depicting MN count in OSMF and Healthy Control

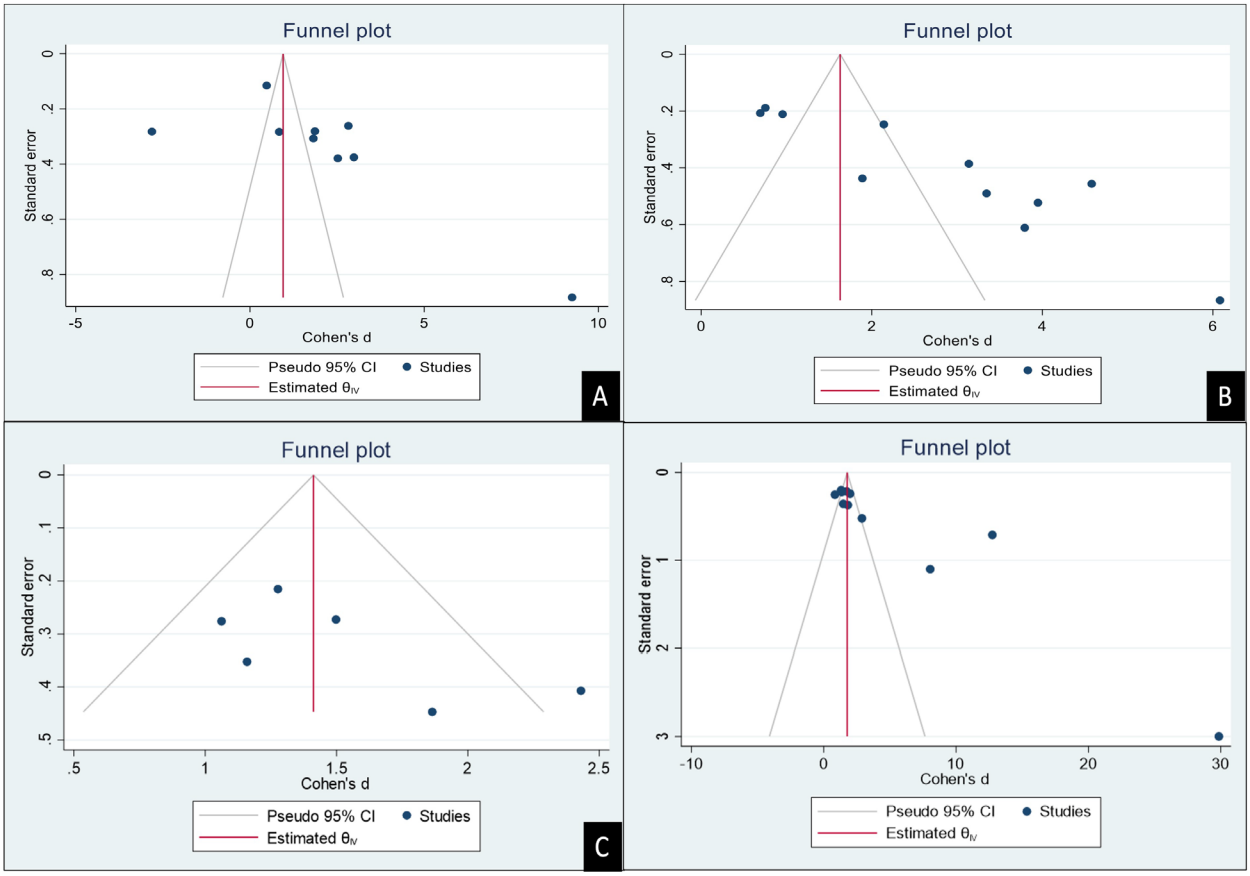

Figure 5. A: Funnel plot for MN count in OPMD and Healthy control. B: Funnel plot forMN count in Leukoplakia and Healthy control. C: Funnek plot depicting MN count in Lichen Planus and healthy control.D: Funnel plot for MN count in OSMF and Healthy Control

cytotoxic agents [34].

MN form when the nuclear envelope encloses lagging chromosomes or fragments that fail to integrate back into the primary nucleus after mitosis or meiosis. Defects in the nuclear envelope led to DNA damage due to interruptions in replication and exposure to the cytoplasm [35]. MN can contain acentric fragments from unrepaired DNA breaks or whole chromosomes resulting from issues such as hypomethylation of satellite sequences and defects in mitotic machinery [5].

Epigenetics influences chromatin organization and gene regulation, affecting genomic stability and cancer progression. Hypomethylation of centromeric regions is linked to chromosomal instability, while histone modifications and miRNA regulation also contribute to MN formation [36].

Genotoxic agents induce DNA damage and compromise nuclear integrity. They are classified as clastogens (causing chromosome breaks) or aneugens (affecting mitotic spindle function) [37]. MN formed from clastogenic treatments contain acentric fragments, while those from aneugenic treatments contain whole chromosomes [38].

MN accumulation serves as a biomarker for genotoxic stress and instability in various samples [39]. Extensive research links MN to genome rearrangements in aggressive cancers [34]. The micronucleus test, introduced in the 1970s, is now a non-invasive, cost-effective method for detecting genomic instability in buccal mucosal cells, with significant findings related to tobacco and betel quid exposure [38].

Genotoxic agents influence MN formation based on host factors (age, gender), habits (smoking, chewing tobacco, alcohol, diet), and disease susceptibility. Smokers and tobacco chewers show higher MN frequencies, with studies indicating that tobacco chewing may pose greater genotoxic risks. Elevated MN counts in tobacco chewers result from increased oxidative stress and DNA damage, while age and gender also affect MN frequency, with older individuals and females showing higher rates [39].

In oral epithelial cells, carcinogen exposure from tobacco, areca nut alkaloids, and alcohol metabolites leads to DNA double-strand breaks, spindle assembly defects, and chromosomal mis-segregation. Dysfunctional tumor suppressor pathways—particularly p53 inactivation—and impaired DNA repair mechanisms amplify this genomic instability, resulting in persistent MN formation. The accumulation of MN in exfoliated buccal cells reflects early chromosomal instability, a hallmark of oral carcinogenesis, and correlates with the malignant potential of OPMDs. Importantly, higher MN frequencies have been shown to parallel increasing grades of epithelial dysplasia and predict risk of malignant transformation, highlighting their clinical utility for early detection and risk stratification [38,39].

In recent years, there has been a notable increase in global research and meta-analyses focusing on the use

of micronuclei (MN) assays in buccal mucosal cells as indicators of genotoxicity in oral potentially malignant disorders (OPMDs). Several systematic reviews and extensive meta-analyses [40-42] conducted between 2022 and 2025 have assessed international cohorts, consistently revealing statistically significant increases in MN frequencies in OPMD patients compared to healthy individuals, thereby reinforcing the biomarker's global applicability. These studies cover a wide range of populations, including various international and multiethnic contexts, and underscore the diagnostic and prognostic significance of MN evaluation in conditions like leukoplakia, lichen planus, and oral submucous fibrosis corroborating our findings.

Micronuclei in Oral Potentially Malignant Disorders

As per this review, most studies on MN frequency in oral potentially malignant disorders (OPMDs) focus on buccal exfoliated cells, a minimally invasive collection method. Increased frequency of MN serves as a biomarker for genomic damage and instability in OPMDs like leukoplakia, lichen planus, and oral submucous fibrosis (OSMF), which are associated with a risk of progression to oral cancer.Thus, monitoring MN frequency in these disorders provides critical insights into genomic stability and the potential for malignant transformation, underscoring the importance of early detection in preventing oral cancer progression.

Pooled analysis: In our meta-analysis, we observed a progressive increase in micronuclei (MN) frequency from healthy controls to individuals with oral potentially malignant disorders (OPMDs). The most significant change was noted in oral submucous fibrosis (OSMF) (SMD = 5.55, 95% CI 0.77–10.32), followed by leukoplakia (SMD = 2.75, 95% CI 1.76–3.74) and oral lichen planus (SMD = 1.47, 95% CI 1.12–1.82). The higher MN count in OSMF can be attributed to several factors: chronic inflammation and fibrosis; areca nut chewing, which contains genotoxic alkaloids and reactive oxygen species (ROS); increased cell turnover due to epithelial atrophy and fibrosis; and tissue hypoxia from fibrotic changes, leading to reduced blood supply.

MN are robust indicators of genotoxicity in OPMDs, as they reflect chromosomal fragments or whole chromosomes failing to incorporate into the nucleus during cell division, signifying genetic damage. The quantifiable nature of MN frequency allows for precise assessment of genetic instability, facilitating early detection of genetic damage before malignant transformation [35]. Additionally, the non-invasive collection of exfoliated oral mucosal cells enhances the practicality of MN assessment as a diagnostic tool, making micronuclei valuable indicators of genotoxicity in OPMDs. This review highlights the potential of using micronuclei (MN) frequency in buccal epithelial cells as a simple, non-invasive tool for the early detection of changes and for assessing genotoxicity in oral potentially malignant disorders (OPMDs). Nevertheless, its clinical utility should be evaluated alongside other well-established techniques. Traditional cytology remains a cornerstone for detecting cellular abnormalities, while molecular methods such as HPV typing, DNA ploidy analysis, or biomarker panels like *p53* and *Ki-67* offer important prognostic information.[43] When used in conjunction with these supplementary tools, the micronucleus (MN) assay presents distinct benefits. Unlike conventional exfoliative cytology, which depends on subjective morphological assessment, MN scoring delivers an objective and quantifiable indicator of genomic instability. Although HPV typing and molecular assays provide high specificity, they are more resource-demanding and may not be practical for routine screening in low-resource environments. The MN assay is quick, cost-effective, and minimally invasive, making it an appealing option for point-of-care use. Its most significant contribution may be in augmenting cytology or molecular tests by acting as an early triage marker to identify patients who need more comprehensive diagnostic evaluation.

A key consideration in interpreting our findings is the variability in MN scoring methods and staining techniques across the included studies. Although such methodological heterogeneity could contribute to the high I^2 values, the consistent direction of effect observed across diverse protocols lends strength to the overall conclusion that MN frequency is elevated in OPMD and oral cancer patients.

Limitations: This meta-analysis has several limitations. Firstly, we found significant heterogeneity among the studies included, likely due to variations in study design, population characteristics, staining methods, and scoring criteria for assessing micronuclei. Although subgroup analysis or meta-regression might help identify the sources of this heterogeneity, the small number of studies in several OPMD categories and inconsistent reporting reduce the reliability of such analyses. Consequently, our pooled estimates should be interpreted with caution. Second, it is not possible to entirely dismiss the possibility of publication bias, as smaller studies with negative results might not be published. Finally, the absence of consistent reporting standards for MN assays underscores the necessity for future research to employ standardized protocols, which would enhance the comparability and reproducibility of studies. Moreover, the lack of longitudinal data restricts the evaluation of MN frequency in relation to disease outcomes. Developing standardized protocols for MN analysis and encouraging collaborative research are essential for effective public health strategies, improving early detection and management of OPMDs. Addressing these limitations in future research is vital for advancing the use of MN as a biomarker in OPMDs.

Future scope and recommendations: Future research should enhance the use of micronuclei (MN) frequency as a genotoxicity marker in OPMDs. Key areas include longitudinal studies to track MN frequency and understand disease progression, as well as investigating molecular mechanisms, particularly in oral submucous fibrosis (OSMF). Validating MN frequency alongside other diagnostic markers will improve accuracy.

This systematic review and meta-analysis gives a comphrehensive synthesis about micronuclei (MN) frequency in buccal mucosal cells for assessing genotoxic effect in OPMDs. Our findings reveal a considerable rise in MN prevalence across OPMDs in comparison to healthy controls, with highest MN counts observed in

oral submucous fibrosis (OSMF), followed by leukoplakia and oral lichen planus. Despite substantial heterogeneity, The consistent elevation of micronuclei frequency in OPMDs supports its role as a low-cost, minimally invasive biomarker that could be incorporated into routine screening of high-risk populations, such as tobacco and areca nut users. Our findings highlight the urgency for multicenter studies with standardized protocols to validate thresholds and integrate MN assays into routine screening strategies.

Author Contribution Statement

The conceptualization, investigation, validation, analysis, review, supervision and project administration were done by Dr. Punnya Angadi. The methodology, validation, investigation, analysis, collection of resources, writing and draft was done by Dr Anoushka Chauhan

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Ethical Approval and Declaration

Not Applicable as it is a systematic review and meta-analysis.

Study Registration

Systematic review was registered in PROSPERO, ID No. CRD42024536661.

Conflict of Interest

The authors declare no conflict of interest.

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