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A Schematic Analytical Perspective of Immunohistochemical and Clinicopathological Analysis of p53 and Ki−67 as Biomarkers of Tumor Behaviour in Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is observed to account for most of the malignancies in the oral cavity. This systematic review and meta-analysis aimed to assess whether p53 and Ki-67 expression form a prognostic marker in oral squamous cell carcinoma (OSCC), and its relationships to the lymph node metastasis (LNM) and tumour aggressiveness.

Methods: PubMed, Scopus, Web of Science, and Google Scholar were searched up to the month November 2025. The studies that were included were observational cohort and cross-sectional studies that assessed p53 or Ki-67 expression in immunohistochemistry (IHC) and provided extractable counts of high/low expression and LNM. Titles, abstracts, and full texts were screened by two reviewers, and disagreements were settled by a third reviewer. The risk of bias was also determined based on the Newcastle–Ottawa Scale (NOS) and the certainty of evidence was determined based on a GRADE methodology.

Results: There was no statistically significant correlation between the p53 expression and LNM (OR = 6.56; 95% CI: 0.28-155.9; I^2 = 89%), indicating that the heterogeneity was high. On the contrary, Ki-67 overexpression was much related to LNM (OR = 5.67; 95% CI: 2.22-14.51; I^2 = 35%), and this effect was reported in all studies. The stability of the Ki-67 pooled estimate was validated by the sensitivity analysis, but the results of p53 were sensitive to the elimination of specific studies.

Conclusion: Ki-67 overexpression can serve as a predictable prognostic biomarker of metastasis to the lymph node, and p53 outcomes are inconclusive due to the heterogeneity of the methodology.

Keywords: Squamous Cell Carcinoma of Head and Neck, Tumor Suppressor Protein p53, Ki-67 Antigen, Biomarkers, Lymphatic Metastasis, Tumor Suppressor Proteins.

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is observed to account for most of the malignancies in the oral cavity [1]. It is a major health problem affecting the world as it demonstrates an aggressive nature, high recurrence rates, and high metastasis to cervical lymph nodes. Prognostication requires precision to be used in order to individualize treatment options, schedule operations, and prognosis[2]. Conventional clinicopathological parameters, e.g., tumor size, depth of invasion, and histological grade, offer some information, but fail to reflect the underlying molecular heterogeneity and are not always reliable as predictors of metastatic potential. This has resulted in the search for molecular biomarkers that can be used to stratify patients in relation to metastatic risk and prognosis [3,4].

The nuclear proliferation marker Ki-67 and the tumor suppressor protein p53 are among the most widely investigated biomarkers in OSCC, showing an important role in DNA damage response, apoptosis, and regulating cell cycle, and their mutation or overexpression has been linked to tumor progression, aggressive phenotype, and resistance to therapy [5]. Ki-67, which is expressed during active phases of the cell cycle, is commonly employed in measuring cellular proliferation, and overexpression of Ki-67 has been linked with poor differentiation, increased tumor invasiveness, and negative prognosis [6]. The two markers have been explored in several studies as being associated with lymph node metastasis, which is an independent predictor of recurrence and mortality in patients with OSCC [7].

The associations between the p53 or Ki-67 expression and lymph node metastasis reported are inconsistent, even after a substantial amount of research was conducted [8]. The strong correlation of a high p53 expression with a metastatic potential and poor survival has been shown in some studies, with no association being significant association in others [9]. On the same note, variable associations with tumor grade, nodal involvement, and prognosis have been observed with Ki-67 expression. The variability is because of the inconsistency of IHC scoring procedures, cutoff points, location of the samples in the tumor (invasive front or tumor margin), population of the study, and periods of follow-up. Such discrepancies make it difficult to interpret the results of individual studies and apply them in clinical decision-making [10,11].

This systematic review and meta-analysis aimed to assess whether p53 and Ki-67 expression form a prognostic marker in oral squamous cell carcinoma (OSCC), as well as their relationships to the lymph node metastasis (LNM) and tumor aggressiveness. This review assessed the relationship between the p53 and Ki-67 expression and lymph node metastasis in OSCC, and then gave a pooled odds ratio. It is expected that the synthesized data will provide insights to clinicians, researchers, and pathologists regarding the prognostic value of such markers, which may help them to make a decision on treatment and counsel a patient in the management of OSCC.

METHODS

This systematic review and meta-analysis adhered to the PRISMA 2020 guidelines [12].

Searches in PubMed, Scopus, Web of Science, and Google Scholar were conducted in November 2025. Search terms consisted of both free-text words and MeSH terms, and Boolean operators (AND, OR) were used, as well as the reference lists of included studies were screened manually to get more eligible articles.

One of the search strings was:

(("p53" OR "TP53" OR "Ki-67" OR "MIB-1") AND ("oral squamous cell carcinoma" OR "OSCC") AND ("lymph node metastasis" OR "nodal metastasis" OR "cervical metastasis" OR "regional metastasis") AND ("prognosis" OR "outcome" OR "survival")).

The studies had to be observational cohort or cross-sectional studies that evaluated the relationship between p53 or Ki-67 expression (measured by immunohistochemistry, IHC) and lymph node metastasis (LNM) in OSCC patients. They only considered the studies that reported extractable 2x2 contingency information (marker high/low expression vs LNM present/absent) to compute odds ratios (ORs).

The exclusion criteria were non-human studies, in vitro studies, reviews, case reports, conference abstracts, editorials, non-English articles, and studies that lacked sufficient quantitative data to obtain ORs.

Two reviewers screened titles, abstracts, and full texts, and a third reviewer resolved any disagreements. The following variables were gathered using a standardized extraction form and included the following: author, year, study design, sample size, population characteristics, biomarker assessed, IHC scoring method and cut-offs, sampling location (invasive front, tumor margin, whole tumor), outcome definition (LNM), and follow-up duration.

The primary outcome of this meta-analysis was the lymph node metastasis regarding the expression of p53 or Ki-67. Studies that reported extractable high/low counts of expression and LNM outcomes were only included.

The Meta Analysis Online tool[13] was used to conduct meta-analysis (use of a random-effects model with inverse-variance approach) to adjust clinical and methodological diversity of studies (e.g., IHC cut-offs, sampling sites, cohort characteristics, follow-up periods). Standard formulas were used to

calculate odds ratios (ORs) with 95% confidence intervals (CIs) of dichotomous outcomes: *Odds Ratio (OR)= (a/c) / (b/d) = a x d / b x c.* In which a = high expression and LNM, b = high expression and no LNM, c = low expression and LNM, d = low expression and no LNM.

The I^2 statistics were used to measure heterogeneity, and an I^2 equal to 50% was defined as moderate-high heterogeneity. The robustness of pooled estimates was determined with subgroup analysis (e.g., sampling site, marker cut-off) and sensitivity analysis (leave-one-out).

The studies that were not meta-analyzed because of heterogeneity or incomparable results were summarized, describing them. Findings were given in forest plots and summary tables that contained the study characteristics, effect estimates, and 95% CIs.

Risk of bias was assessed using the Newcastle–Ottawa Scale (NOS), appropriate for observational study designs, whereas GRADE criteria were changed to measure the overall quality of evidence of prognostic associations.

Finally, twelve articles were included in the research and included observational cohort and cross-sectional studies that specifically assessed the relationship between p53 or Ki-67 expression and lymph node metastasis in OSCC.

RESULTS

Out of all four electronic databases and other sources searched, 222 research articles were initially selected. The duplicates were eliminated, and the number was narrowed down to 92 records. Screening of title and abstract also dropped 30 studies. Out of the rest of the 62 articles, 22 articles were disqualified because of a lack of access to the entire contents. An additional 28 articles were filtered away based on the inability to find stratified data and the presence of animals, in vitro, reviews, case reports, or non-English articles. In the end, this systematic review had twelve studies that met the inclusion criteria.

Figure 1 below is a PRISMA flow diagram showing the selection process.

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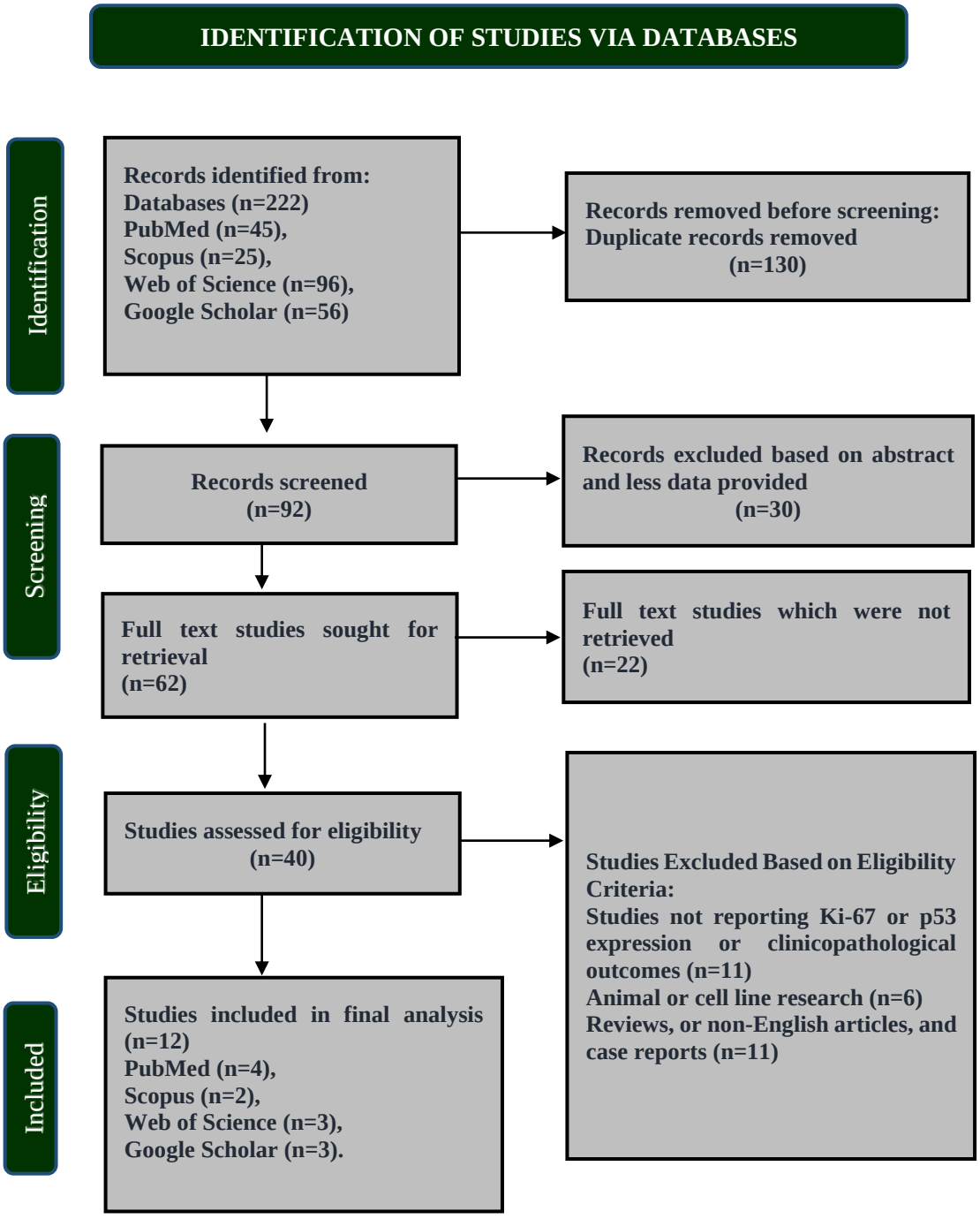

Figure 1: PRISMA Flow Diagram for Study Selection. The flowchart was designed according to the PRISMA guidelines 2020, showing study identification, screening, assessment eligibility, and final selection in the systematic review.

Table 1: Characteristics of Included Studies

Author & Year	Design	Modeling / Intervention	Population Size	Key Findings
Motta et al., 2015 [14]	Retrospective	p53 & Ki-67 IHC expression vs lymph node metastasis	28 SCC patients	p53 expression showed no significant link with lymph node metastasis
Deshmukh et al., 2020 [15]	Retrospective observational	p53 IHC vs adverse outcomes	27 OSCC patients	p53 positivity is associated with adverse outcomes
Suresh et al., 2015 [16]	Retrospective	p53 IHC vs cervical LNM	105 OSCC patients	High p53 expression is strongly associated with lymph node metastasis
Coutinho-Camillo et al., 2010 [17]	Observational analysis	IHC assessment of NPM, Ki-67, p53; association with clinicopathological features	154 patients (HNSCC)	Ki-67 levels were significantly higher in tumors with lymph node metastasis.
Bhuyan et al., 2018 [18]	Cross-sectional observational	Ki-67 proliferative index measured at the invasive tumor front using IHC	Grade I: 40; Grade II: 32; Grade III: 30	Higher Ki-67 expression is strongly associated with lymph node metastasis and higher tumor grade.
Gawande et al., 2020 [19]	Observational cohort	p53 immunohistochemistry at invasive tumor front; survival follow-up 3 years	30 OSCC patients; 10 normal oral mucosa	Normal oral mucosa p53-negative. Higher p53 expression is associated with death.
Beovide Cortegoso et al., 2017 [20]	Retrospective cohort	Ki-67 and AgNOR expression at the tumor invasive front	40 OSCC patients	Ki-67 expression is not significantly associated with survival, clinical stage, or regional metastasis.
Biju Babu et al., 2020 [21]	Retrospective cohort	p53 (margin ≤10, ITF ≤10), Ki-67 (ITF ≤65.2, margin ≤24.2)	100 (Non-recurrent OSCC: 50; Recurrent OSCC: 50)	Higher p53 and Ki-67 LI in the margin and ITF associated with worse OS and DFS; Ki-67 margin in

				recurrent OSCC borderline significance
Dash et al., 2020 [22]	Cross-sectional	Ki-67 (mean LI across grades)	100 (Normal: 10; OED: 45; OSCC: 45)	Ki-67 expression increased with the severity of OED and OSCC; poorly differentiated OSCC showed the highest LI.
Chaudhari et al., 2018 [23]	Cross-sectional observational	Ki-67 IHC (MIB-1); proliferation categorized (<30%, 30–50%, >50%) and correlated with histologic grade	100 OSCC cases	High Ki-67 expression is associated with higher histologic grade.
Sharma et al., 2013 [24]	Cross-sectional observational	Ki-67 IHC in dysplastic and malignant oral lesions; expression categorized as low or high	65 patients (25 dysplasia, 40 OSCC)	High Ki-67 expression increases from dysplasia to carcinoma; it correlates with malignant transformation.
Yue Jing et al., 2019 [25]	Observational cohort	Ki-67 IHC in OSCC, dysplasia, and normal oral mucosa; correlation with clinicopathologic parameters and survival	298 OSCC cases, 62 dysplasia, 36 normal mucosa	Ki-67 expression is higher in OSCC. High Ki-67 is linked to poor differentiation, lymph node metastasis, worse OS, DFS, RFS, and MFS.

Low-risk studies featured clear methodology, standardized IHC protocols, and complete reporting of proliferative indices and clinical outcomes. Moderate-risk studies had minor limitations, such as small sample sizes, limited follow-up, or partial reporting of survival data. No study was deemed high risk for the primary outcomes, although methodological heterogeneity and lack of blinding were common.

Twelve reviews and retrospective studies that have measured the Ki-67 and p53 expression in oral squamous cell carcinoma (OSCC) and in the premalignant lesions have been reviewed. Overall, an elevated index of Ki-67 labeling and p53 expression was related to a high tumor grade, metastasis to the lymph node, and poor survival. Others have found borderline or non-significant correlations, especially with Ki-67 in certain situations. All studies had a moderate risk of bias mainly because the sample sizes were small, the design used was retrospective, and the studies were not blinded.

The study design, interventions, populations, and outcomes are shown in **Table 1**.

Table 1 includes the studies that assessed the Ki-67 and p53 expression in oral squamous cell carcinoma and premalignant lesions. The study design, modeling/intervention, population size, highlighting findings, and the threat of bias are presented in the table.

Most of the studies were observational or retrospective, with immunohistochemistry (IHC) being applied to quantify the Ki-67 and/or p53 in tumor tissue or oral dysplasia, at the invasive tumor end or a random field. Higher Ki-67 labeling index and p53 expression were, on balance, always correlated with the increased tumor grade, lymph node metastasis, poor differentiation, and deteriorated survival results. Other studies have been found to indicate borderline or non-significant relationships between Ki-67 in given settings.

Table 2: Risk of Bias of Included Studies

Study	Selection (max 4)	Comparability (max 2)	Outcome (max 3)	Total Score (max 9)	Interpretation
Motta et al., 2015 [14]	★★★	★★	★★	6	Moderate
Deshmukh et al., 2020 [15]	★★★	★★	★★	6	Moderate
Suresh et al., 2015 [16]	★★★	★★	★★	6	Moderate
Coutinho-Camillo et al., 2010 [17]	★★★	★★	★★	6	Moderate
Bhuyan et al., 2018 [18]	★★★	★★	★★	5	Moderate
Gawande et al., 2020 [19]	★★★	★★	★★	6	Moderate
Beovide Cortegoso et al., 2017 [20]	★★★	★★	★★	6	Moderate
Biju Babu et al., 2020 [21]	★★★	★★	★★	4	Moderate
Dash et al., 2020 [22]	★★★	★★	★★	6	Moderate

Chaudhari et al., 2018 [23]	★★★	★★	★★	6	Moderate
Sharma et al., 2013 [24]	★★★	★★	★★	5	Moderate
Yue Jing et al., 2019 [25]	★★★	★★	★★	6	Moderate

Total Score (max 9): Higher scores indicate lower risk of bias. 7–9: Low risk, 4–6: Moderate risk, <4: High risk.

Risk of bias was assessed using the Newcastle–Ottawa Scale (NOS), appropriate for observational study designs; the majority of the studies were considered to be of moderate quality, which was primarily related to small sample sizes, retrospective design, or lack of blinding. In total, there was a moderate quality of evidence based on GRADE.

The risk of bias is given in **Table 2**.

META-ANALYSIS

The meta-analysis tested the correlation between levels of biomarkers and the presence of lymph node metastasis (LNM) in squamous cell carcinoma. There were two major outcomes studied: 1) LNM and p53, and 2) LNM and Ki-67. All the analyses were done in the MetaAnalysisOnline tool with the random-effects model and the inverse-variance methodology, which was predefined to consider anticipated clinical and methodological disparities among cohorts, IHC cut-offs, sampling sites, and outcome definitions.

In the case of p53, there were four studies that were used to create the pooled estimate. The summarized odds ratio (OR) was 6.56, and the 95% confidence interval was 0.28 -155.9 by using the random-effects inverse-variance method. The overall effect test failed to show a significant effect ($p > 0.05$). The heterogeneity was found to be significant ($p < 0.01$) with $I^2 = 89\%$ which implies that the major part of the variations among study estimates belongs to between-study differences and not to the sampling error.

Figure 2 demonstrates the forest plot of individual study ORs and the pooled OR.

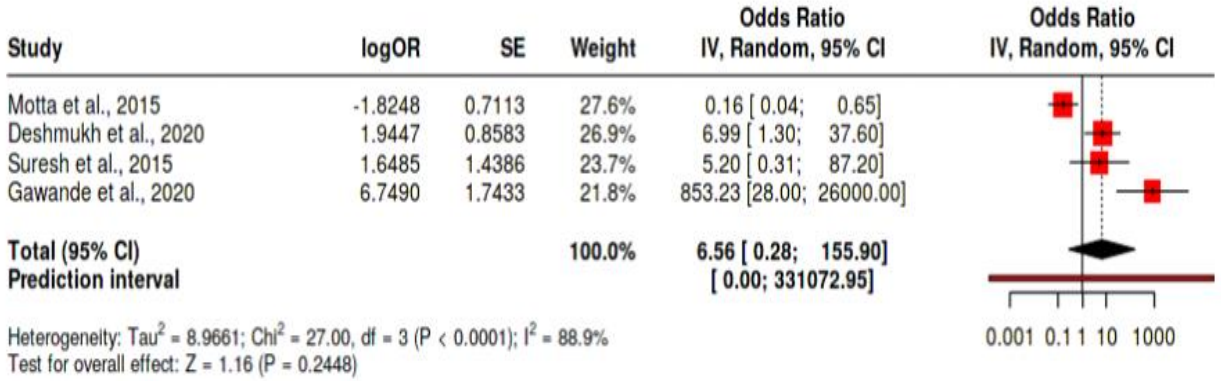

Figure 2: Forest plot of odds ratios (OR) and 95% confidence intervals of the relationship between p53 positivity and metastasis in lymph nodes (LNM). Squares = personal estimates of studies; horizontal lines = 95% CI; diamond = pooled OR (random effects). The values to the left of the line favor p53 positivity to be correlated with LNM and vice versa.

In the case of Ki-67, two studies were added to the pooled estimation. The summative odds ratio (OR) obtained by using the random-effects inverse-variance technique was 5.67, and the confidence interval was 2.22 - 14.51. The overall effect test was statistically significant ($p < 0.05$). The heterogeneity was not notable (I^2 was close to 35%, $p > 0.05$), which means that the effect sizes in the two cohorts were similar in their magnitude and direction.

Figure 3 is a forest plot of the individual study OR and the pooled OR of Ki-67 and LNM.

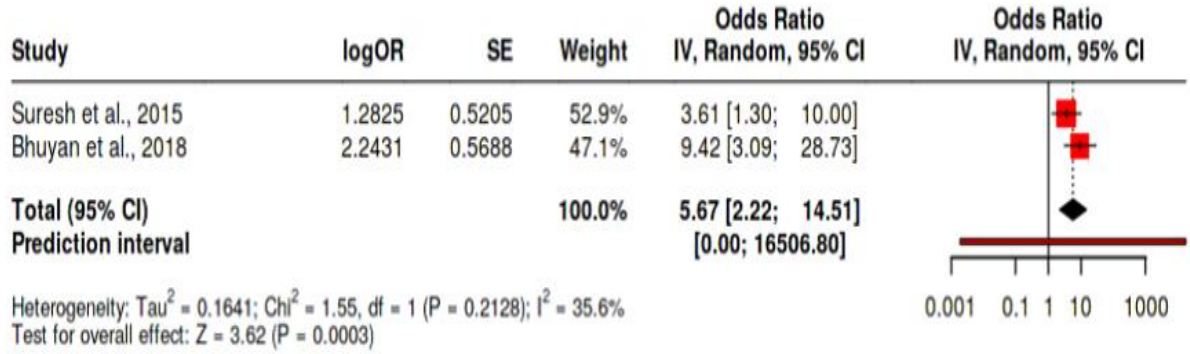

Figure 3: Forest plot of odds ratios (OR) and 95% confidence interval of the association between high Ki-67 expression and lymph node metastasis (LNM). Individual study estimates are squares; the 95% CI is represented by the horizontal lines; the pooled OR (random effects) is represented by the diamond. Higher odds of LNM with high Ki-67 expression are shown by the values on the left of the line.

All these meta-analytic findings point to an arguably statistically significant and consistent relationship between high Ki-67 expression and LNM across the analyzed cohorts, but the data on p53 and LNM are inconclusive due to imprecision and a high level of heterogeneity. The large heterogeneity of p53 is probably due to clinical and methodological variation (diverse endpoints: LNM vs adverse outcome vs survival; heterogeneous p53 cut-offs and scoring; sampling site differences; low studies that used zero cell tables). The lack of uniformity in the number of dichotomized counts of marker status by outcome (retrospective study designs, small number of studies), limits the confidence in the evidence.

Primary research in the future must standardize IHC scoring, provide dichotomous numbers (high/low expression) versus a priori outcomes (LNM, OS, DFS), and include the full contingency tables to enable the robust pooling of meta-analytic outcomes.

Subgroup analysis

Preplanned subgroup analyses (such as by definition of outcome or site of sampling) were informative in the small populations of the studies and the heterogeneity of outcomes; they could not be estimated.

Sensitivity analysis

Leave-one-out sensitivity testing on pooled estimate and heterogeneity in p53 studies revealed that studies were sensitive, particularly with small cell counts or extreme/large effect estimates. For instance, when the study with the largest OR was removed, heterogeneity decreased from $I^2 = 89\%$ to 72%, and the pooled OR attenuated to 4.12 (95% CI: 0.15–111.6). Exclusion of other smaller studies reduced I^2 to between 80–85%, but the overall effect remained statistically non-significant. This highlights the frailty of the p53 pooled estimate as indicated by a few and varied inputs. Leave-one-out sensitivity analyses revealed the pooled effect to be directionally consistent and statistically significant following the removal of either of the studies, indicating that the pooled Ki-67 finding is not affected by a single outlier.

DISCUSSION

The difficulties with OSCC cervical lymph node metastasis, recurrence, and poor survival are still the main clinical concerns in head and neck cancer, and it is indisputable that proper prognostication is of paramount importance in this context [26]. Molecular biomarkers, like p53 and Ki-67, have been a subject of significant attention since they are tumor suppressor active and proliferative activity, respectively, and provide some insight into tumor aggressiveness and the potential to metastasize.

Their assessment by means of the use of immunohistochemistry enables the stratification of patients beyond the traditional clinicopathological parameters and assists in making the treatment decisions [27].

The overexpression or mutation of p53 causes abnormal cell-cycle regulation as well as resistance to apoptosis and increased genomic instability [27]. Equally, Ki-67 shows the percentage of proliferating tumor cells and is associated with increased histologic grading, invasive capacity, and poor clinical prognoses [28]. As implied in the evidence provided in the studies that have been included, the two markers are commonly tested at the invasive tumor front or the tumor margins, which highlights their applicability in the practice of predicting tumor behavior in areas that are highly prone to metastatic spread. It is worth noting that high Ki-67 expression was always a predictor of involvement of lymph nodes, whereas p53 results were less predictable because of variations in study populations, scoring, and cut-off values [29,30].

The addition of p53 and Ki-67 to a larger molecular context controls the OSCC aggressiveness, metastatic potential, and response to treatment in addition to their specific biological functions. OSCC development is not simply due to cellular growth or impaired tumor-suppressor pathways, but a concerted deregulation of various signaling networks, such as DNA-damage response pathways, epithelial-mesenchymal transition, aberrant cell-cycle progression and immune evasion of the microenvironment [31,32].

Changes in p53 not only interfere with genomic surveillance but also stimulate clonal proliferation of non-malignant cells, as well as allow the growth of additional oncogenic mutations that enhance tumor adaptability in metastatic dissemination. On the same note, high levels of Ki-67 indicate high percentage of actively dividing malignant cells, which is associated with an increased rate of tumor turnover, increased metabolic activity, and greater susceptibility to micrometastatic spread [33]. Both of these markers have thus been suggested as major constituents of multiparametric prognostic models that combine histopathological grading, lymphovascular invasion, depth of invasion, perineural infiltration, and tumor-host immune interactions to enhance better risk stratification in OSCC [34].

Furthermore, molecular profiling research indicates that the prognostic capability of such biomarkers could be affected by anatomical subsites, HPV status and genetic heterogeneity in the tumor. All these reasons justify why methodological uniformity, standard immunohistochemical scoring, and harmonized cut-offs should be adopted in future investigations of p53 and Ki-67 use in precision-based prognostic algorithms in the management of OSCC [35,36].

The biomarker expression interacts with other factors, including tumor differentiation, depth of invasion, and patterns of infiltration, to determine the risk of metastasis. Evidence of integrated proliferative and tumor suppressor pathways has been proposed by studies assessing the joint markers or multi-parametric panels, which may be more useful as prognostic markers compared to isolated markers [37]. However, the general heterogeneity observed in the meta-analysis, especially with p53, highlights the importance of standard evaluation protocols and multicenter validation trials [38].

Clinically, such findings highlight the possible use of Ki-67 as a prognostic factor in the surgical planning, adjuvant therapy, and postoperative follow-up during OSCC [39]. The high level of Ki-67 might be used to signal patients who might respond better to more active treatment or closer monitoring. p53 evaluation, although biologically significant, needs further standardization, and then it may be adopted in clinical practice [40].

The shortcomings of the factored studies are that they have small sample sizes, different tumor locations, and not all studies have the same follow-up period.

Possible limitations of the review process, including English-language publication restrictions and failure to register protocols, can also serve as contributors to possible selection and reporting biases.

Future studies must seek to harmonize immunohistochemical assessment techniques, determine universal cut-off values, and determine the combined prognostic value of p53, Ki-67, and other molecular markers. These biomarkers can be improved with respect to predictive accuracy and clinical usability by multicenter prospective studies and by combining these with genomic or transcriptomic data. Molecular findings can be translated into clinical decision-making, which can enhance the risk stratification, plan therapeutic approaches, and eventually patient survival.

CONCLUSION

To sum up, Ki-67 overexpression is a reliable prognostic biomarker and is always correlated with lymph node metastasis and poor prognosis of OSCC, which needs further standardization to be used as a clinical tool. The evidence discussed in this paper shows that Ki-67 should be included in the regular examination of pathological processes to facilitate risk stratification and treatment decision-making. Further research on standardized assessment, integrated biomarker subsets, and incorporation with clinical variables would increase the level of prognosis and patient outcomes in OSCC treatment.

LIST OF ABBREVIATIONS

IHC – immunohistochemistry

LI – labeling index

ITF – invasive tumor front

OS – overall survival

DFS – disease-free survival

RFS – recurrence-free survival

MFS – metastasis-free survival

OED – oral epithelial dysplasia

AgNOR – argyrophilic nucleolar organizer region

MIB-1 – monoclonal antibody for Ki-67

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CONFLICT OF INTEREST

None.

AUTHORS' CONTRIBUTION

AS, SG: Conceptualization, protocol design, literature search strategy formulation, drafting the manuscript, and final approval. H**UR:** Study screening (title/abstract and full-text), data extraction, statistical analysis (meta-analysis execution), drafting the methodology, and final approval. **MH:** Study screening (title/abstract and full-text), data extraction, risk of bias/quality assessment, revising the manuscript critically, and final approval. **EA:** Risk of bias/quality assessment, acting as the third reviewer for screening disagreements, data interpretation, critical revision of the manuscript for intellectual content, and final approval.

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