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PROGNOSTIC ROLE OF ΓH2AX AND OTHER BIOMARKERS IN MALIGNANT TRANSFORMATION OF ORAL POTENTIALLY MALIGNANT DISORDERS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract
Oral potentially malignant disorders (OPMD) represent a heterogeneous group of lesions with an increased risk of progression to oral squamous cell carcinoma (OSCC), and conventional histopathological assessment has limited predictive accuracy, highlighting the need for reliable molecular biomarkers. Among these, γH2AX, a marker of DNA damage, along with other genomic, epigenetic, and protein biomarkers, has gained increasing attention. This study aimed to systematically evaluate and quantitatively synthesize the prognostic role of γH2AX and other biomarkers in predicting malignant transformation in OPMD. A comprehensive literature search was conducted across PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar, and observational cohort studies assessing biomarkers with reported malignant transformation outcomes were included. Data extraction and quality assessment using the Newcastle–Ottawa Scale were performed, and a random-effects meta-analysis was used to calculate pooled effect sizes, along with subgroup, sensitivity, and publication bias analyses. A total of 13 studies were included, and the pooled analysis demonstrated a significant association between biomarker expression and malignant transformation (HR/OR = 4.05, 95% CI: 2.71–6.06). Subgroup analysis indicated that genomic and epigenetic biomarkers showed stronger predictive value compared to protein biomarkers. Moderate heterogeneity was observed (I^2 = 48.8%), while sensitivity analysis confirmed the robustness of the findings, and no significant publication bias was detected. In conclusion, molecular biomarkers, particularly γH2AX and other genomic and epigenetic markers, are strong predictors of malignant transformation in OPMD, and their integration into clinical practice may enhance risk stratification, early detection, and personalized management strategies.

KEYWORDS: γH2AX; Oral potentially malignant disorders; Biomarkers; Malignant transformation; Meta-analysis

1. INTRODUCTION

Oral cancer continues to be a major public health concern with increasing incidence and mortality rates in many parts of the world. Global cancer statistics have estimated that oral and oropharyngeal cancers contribute to a significant burden of the global cancer burden, especially in low- and middle-income countries where the risk factors such as smoking, alcohol intake and betel quid chewing are common (Bray et al., 2018; Sung et al., 2021). Previous epidemiological studies have also reported the high prevalence and rising incidence of these cancers, along with the importance of prevention and early detection initiatives (Warnakulasuriya, 2009). Although there has been great improvement in treatment options, the survival rate of oral cancer has not changed substantially in the past few decades, primarily because of the late diagnosis of the disease (Jeihooni & Jafari, 2022).

Early detection and management of oral potentially malignant disorders (OPMDs) play a key role in oral cancer prevention, as they are a group of lesions with a higher risk of malignant transformation. These range from oral leukoplakia, oral epithelial dysplasia to other mucosal lesions (Warnakulasuriya et al., 2021; Fitzpatrick et al., 2014). The clinical presentation of OPMDs varies, including asymptomatic white or red lesions and more complex mixed lesions, making it difficult to detect them early (Warnakulasuriya, 2018). Prospective studies have shown that some of these lesions transform to oral squamous cell carcinoma (OSCC) underpinning their clinical importance (Arduino et al., 2009; Aguirre-Urizar et al., 2021).

The evaluation of the risk of malignant transformation in OPMDs has historically been based on the histopathological classification of epithelial dysplasia. But it has several drawbacks, primarily its subjectivity and subject to inter-observer differences (Kujan et al., 2007). Differences in interpretation can result in variable risk assessment, which can impact treatment strategies. In addition, histopathological examination fails to reflect the molecular alterations involved in carcinogenesis. These drawbacks highlight the need for more accurate and robust prediction models.

Over the past few years, there has been an increased focus on molecular biomarkers to enhance risk stratification for malignant transformation of OPMDs. Biomarkers have a key advantage of capturing biological processes, such as genetic, epigenetic and proteomic changes related to cancer development (Santosh et al., 2016). Modern cancer biology has shown

that the development of cancer is a multistep process that includes the alteration of important cellular pathways (Hallmarks of cancer, Hanahan & Weinberg, 2011). These changes include the ability to sustain proliferation, evasion of cell death, genomic instability and epigenetic changes, all of which lead to tumour initiation and progression (Johnson, 2020; Leemans et al., 2011). Genomic instability is one of the key processes in carcinogenesis, as it contributes to the accumulation of genetic mutations during tumourigenesis (Negrini et al., 2010). The DNA damage response (DDR) pathway plays a crucial role in preserving genomic stability and its disruption can result in cell proliferation and transformation (Jackson & Bartek, 2009). One of the most sensitive markers of DNA damage and repair, the phosphorylated form of H2AX (γH2AX), is a promising candidate for predicting malignant transformation in OPMDs (Mah et al., 2010). Recent studies have also revealed that high levels of H2AX/γ-H2AX are linked to specific pathway changes and poor prognosis in patients with oropharyngeal squamous cell carcinoma, thus adding further significance to H2AX/γ-H2AX in oral carcinogenesis (Lyu et al., 2025). Its presence is indicative of early molecular events in cancer development, and may serve as a valuable biomarker to predict the risk of malignant transformation in OPMDs. Besides genetic changes, epigenetic modifications have been implicated in cancer formation. Epigenetic changes, including DNA methylation and post-translational modifications of histones, are reversible changes that control gene expression without changing the DNA sequence (Baylin & Jones, 2016). Altered DNA methylation, especially of tumor suppressor genes, has been frequently reported in oral cancer and its precursor lesions (Esteller, 2008). These changes are reversible and occur early during the carcinogenic process, and may serve as important markers for early diagnosis and risk stratification. In addition, protein biomarkers that reflect changes in signaling pathways and cellular functions have also been studied. These involve proteins associated with cell growth, death, new blood vessel formation and invasion. Proteomic studies, including salivary biomarkers, have also been used to gain further understanding of the biology of the tumors, and have the potential for non-invasive diagnosis (Riccardi et al., 2022). Experimental and translational research has also shown the involvement of pathways, such as the Akt pathway, in tumor growth and survival, further highlighting the importance of molecular markers in the development of cancer (Mandal et al., 2006).

Although there is an increasing amount of research on biomarkers, their use in predicting malignant transformation of benign tumors is still limited. While numerous studies have examined single biomarkers or small sets, the results are often conflicting and hard to reconcile. Furthermore, there is no standard method to assess biomarkers, and the sensitivity and specificity of individual biomarkers has shown considerable variability. Recent studies using transcriptomic and molecular profiling have demonstrated the potential to enhance risk prediction, but more research is needed (Sathasivam et al., 2021). A further key area for future research is the integration of various types of biomarkers. Although genomic, epigenetic, and protein biomarkers have been investigated separately, there is growing recognition of the need to integrate different classes of biomarkers in order to better understand the process of malignant transformation. Oral carcinogenesis is a complex process that may involve several molecular pathways, and a single biomarker may not be able to characterise this process. Consequently, a quantitative approach is needed to synthesise existing evidence to assess the overall prognostic significance of biomarkers in OPMDs. For example, the utility of γH2AX, as an indicator of DNA damage, and other biomarkers that represent distinct molecular pathways, should be explored. Meta-analysis enables the pooling of information from several studies to reach more accurate and general conclusions.

The aim of the study was to systematically review and quantitatively synthesize the existing evidence on the prognostic value of γH2AX and other biomarkers in malignant transformation prediction in oral potentially malignant disorders. In particular, the purpose of this meta-analysis was to assess the overall impact of expression of biomarkers on the risk of oral squamous cell carcinoma development and to compare the predictive value of various types of biomarkers, such as genomic, epigenetic, and protein-based biomarkers.

2. MATERIALS AND METHODS

2.1 Study Design and Reporting Guidelines

This systematic review and meta-analysis aimed to determine the prognostic value of γH2AX and other biomarkers in oral potentially malignant disorders (OPMD) in relation to malignant transformation. The study design adhered to the methodological standards and was performed in compliance with Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) guidelines to guarantee the transparency, reproducibility, and methodological rigor.

2.2 Literature Search Strategy

The literature search was done in a systematic and inclusive manner across various electronic databases such as PubMed, Scopus, Web of Science, Science Direct, and Google Scholar. A mixture of keywords and controlled vocabulary terms concerning γH2AX, oral potentially malignant disorders, oral epithelial dysplasia, oral leukoplakia, biomarkers, malignant transformation and oral squamous cell carcinoma was used as the search strategy. Spreadsheets were used to optimize search sensitivity and specificity with the use of operators (AND, OR). Besides database search, reference lists of relevant studies were manually screened in order to realize other potentially eligible articles that might not have been picked during the first search.

2.3 Eligibility Criteria

The inclusion criteria were that the studies were observational cohort studies (prospective or retrospective) that examined the role of biomarkers, such as γH2AX, in patients with oral potentially malignant disorders, oral epithelial dysplasia, or oral leukoplakia. The studies were to be eligible and to have reported malignant transformation outcomes, which is the occurrence of progression to oral squamous cell carcinoma, and to provide effect size estimates (e.g., hazard ratios [HRs], odds ratios [ORs]) or adequate data to estimate these effect sizes. Only those studies that were in the English language were taken into account. The studies were filtered out as preclinical and non-clinical trials, trials that did not determine

the outcome of malignant transformation, could not provide adequate data to estimate the effect size, were conference abstracts, editorials, or review articles, had duplicate populations or overlapping populations and lacked access to the full text.

2.4 Study Selection Process

All the records identified were made in a reference management system and duplicates were deleted before screening. The rest of the studies were subjected to a two-step screening process involving title screening, abstract screening and eligibility screening that involved full-text screening. Those studies that failed to satisfy the inclusion criteria in the first screening were eliminated. Articles were then independently screened at the full-text level to ensure that they were eligible as per the predetermined criteria. The process of identifying a total of 260 records with the results of both database and manual search were first identified and after elimination of duplicates and screening, 13 studies were finally incorporated into qualitative and quantitative synthesis. Figure 1 (PRISMA flow diagram) shows the entire process of study selection.

2.5 Data Extraction

The standardized and structured method was used in data extraction so that the process is consistent and accurate. Each included study provided the following information: author and year of publication, country of study, study design, population characteristics (including OPMD, oral epithelial dysplasia, or leukoplakia), sample size, type of biomarker evaluated, method of detection (immunohistochemistry, polymerase chain reaction, methylation-specific PCR or fluorescence in situ hybridization), definition of malignant transformation Where the estimates of the effects were not directly provided, the odds ratios were determined using the available event data with the use of standard statistical techniques.

2.6 Quality Assessment

The methodological quality of the studies included in the study was measured using the Newcastle-Ottawa Scale (NOS) of cohort studies, which measures three areas of studies: selection of participants, study groups comparability and outcome measurement. The score of each of the studies was out of a possible nine points. The studies that had a score of 7 to 8 were regarded as of high quality. The evaluation showed that all the studies considered had a high level of methodological rigor with clearly defined populations and suitable outcome measurement, and overall, sufficient control of confounding factors.

2.7 Statistical Analysis

To take into consideration both within-study and between-study variability, statistical analysis was performed using the random-effects meta-analysis model, assuming the DerSimonian-Laird approach. The effect sizes, such as the hazard ratios and odds ratios, were transformed into the log form before analysis, and the standard errors were estimated using the confidence intervals reported. The pooled effect estimate was estimated by weighting the individual studies by the inverse of their variance and the end results were inverted to interpret. The results were reported as pooled effect sizes together with their respective 95% confidence intervals and represented in the form of forest plots (Figure 2).

2.8 Subgroup Analysis

Subgroup analyses were conducted to test the differences in the effect estimates within the categories of biomarkers. The biomarkers included could be categorized into genomic/DNA damage markers (including γH2AX, DNA ploidy, and loss of heterozygosity), epigenetic markers (including P16 methylation and ZNF582 methylation), and protein biomarkers (including EGFR, Notch1, SMAD4, S100A7, COX-2, Individual pooled estimates were developed on each subgroup to assess their comparative ability to predict malignant transformation and results were reported in subgroup forest plots (Figure 3).

2.9 Heterogeneity Assessment

The Cochran Q-test was used to test statistical heterogeneity of the included studies and the value of I2 was used to quantify them. The I2 was reported based on the traditional thresholds, whereby a value less than 25% showed a low degree of heterogeneity, between 25% and 50% showed moderate heterogeneity, and above 50 percent showed high heterogeneity. Heterogeneity as a phenomenon informed the selection of the random-effects model to use in the meta-analysis.

2.10 Sensitivity Analysis

The sensitivity analysis based on leave-one-out was conducted to determine how robust the estimate of the pooled effect is. In this strategy, one study was eliminated one by one in the analysis and the pooled effect size was re-computed to check whether a single study exerted disproportionate impact on the entire outcomes. The results of the sensitivity analysis were in the form of graphs (Figure 4).

2.11 Publication Bias

Visual and statistical methods were used to determine the presence of publication bias. A funnel plot was created to assess the symmetry of the frequency distribution of the effect sizes against their standard errors, and asymmetry indicated the possibility of publication bias. Secondly, Egger, ran regression test to statistically determine funnel plot asymmetry, and a p-value lower than 0.05 was taken as a sign of significant publication bias. Figure 5 shows the funnel plot of this evaluation.

3. RESULT

3.1 Study Selection

There was a thorough literature search in various electronic databases, such as Scopus, PubMed, Web of Science, ScienceDirect, and Google Scholar. It was first found that there were 260 records with 230 records in databases and 30 records in manual reference screening (Figure 1). Upon elimination of 60 duplicate entries, 200 studies were left to be screened in terms of their titles and abstracts. Among them, 100 records were filtered through based on their irrelevance to the study objectives and this shortlisted 100 articles to a full-text eligibility assessment. In the eligibility phase, 87 full-text articles were eliminated due to the following reasons: preclinical or non-clinical trials (n = 18), no malignant transformation outcomes (n = 22), no data to estimate the effect size (n = 20), conference abstracts, editorial or review articles (n = 15), or duplicate or overlapping populations (n = 7). Lastly, 13 studies were included into the qualitative and quantitative synthesis (meta-analysis) meeting the inclusion criteria. A stepwise selection of the studies is presented in Figure 1 (PRISMA flow diagram) and the features of the included studies are summarized in Table 1.

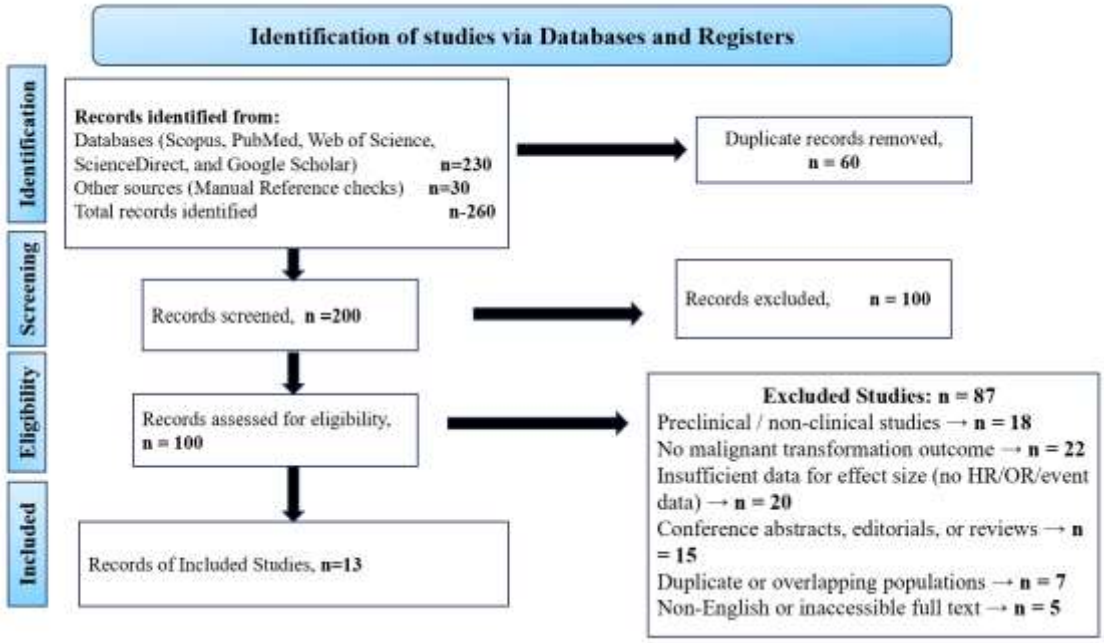

Figure 1. PRISMA Flow Diagram

3.2 Study Characteristics

The meta-analysis involved a total of 13 studies, all of which were observational cohort studies. The research was done in various countries, such as the United Kingdom, Netherlands, Canada, China, the United States, Spain, Taiwan and Germany, which reflects a diverse study population. The studies included were related to patients with oral potentially malignant disorders (OPMD), oral epithelial dysplasia (OED) and oral leukoplakia (OL). The sizes of the samples were between 43 and 296 people, which reflects a moderate level of variation in studies.

A wide range of biomarkers were investigated. These included:

- DNA damage and genomic instability markers such as γH2AX, DNA ploidy, and loss of heterozygosity (LOH) with TP53 alterations
- Epigenetic biomarkers including P16 methylation and ZNF582 methylation
- Protein and signaling biomarkers such as EGFR, Notch1, SMAD4, S100A7, COX-2, CD9, and podoplanin

The main outcome of the studies was malignant transformation, which is characterized by the development of the OPMD/OED/OL into oral squamous cell carcinoma (OSCC) or histopathological progression to malignancy. In general, the majority of the studies gave the hazard ratios (HRs) or odds ratios (ORs), whereas some had to calculate the effect sizes based on the available data. Table 1 shows the detailed characteristics of the studies included.

Table 1. Characteristics of Included Studies (n = 13)

Author (Year)	Country	Study Design	Population	Sample Size (n)	Biomarker	Detection Method	Outcome Definition	Effect Measure	Effect Size (95% CI)	NOS Score
Leung et al., 2017	UK	Retrospective cohort	OED	86	γH2AX	IHC	Progression to OSCC	HR	3.15 (1.41–7.39)	7
Bremmer et al., 2011	Netherlands	Retrospective cohort	Leukoplakia	62	DNA ploidy	Flow cytometry	OSCC development	HR	3.7 (1.1–13.0)	7
Kaur et al., 2013	Canada	Retrospective	Oral dysplasi	110	S100A7	IHC	Malignant transform	HR	2.36 (NR)	7

		cohort	a				ation			
Nankivell et al., 2013	UK	Multicenter cohort	OED	148	COX-2 / CD9	IHC	Malignant progression	HR	1.64 (1.12–2.40)	8
Liu et al., 2015	China	Prospective cohort	OED	152	P16 methylation	MSP	Cancer development	OR	4.6 (NR)	8
Xia et al., 2013	China/USA	Retrospective cohort	Leukoplakia	88	SMAD4	IHC	Malignant transformation	OR*	5.23 (calculated)	7
Ding et al., 2018	China	Retrospective cohort	Leukoplakia	78	Notch1	IHC	OSCC development	OR*	4.87 (calculated)	7
Zhang et al., 2012	Canada	Prospective cohort	OPMD	296	LOH (3p/9p)	PCR-based	Malignant progression	HR	22.6 (3.1–164.5)	8
Taoudi Benchekroun et al., 2010	USA	Prospective cohort	OPMD	145	EGFR (FISH)	FISH	OSCC development	HR	3.62 (1.44–9.10)	8
Graveland et al., 2013	Netherlands	Prospective cohort	OPMD	43	LOH / TP53	PCR / sequencing	Malignant transformation	OR*	7.33 (1.03–52.1)	7
Juan et al., 2021	Taiwan	Prospective cohort	OPMD	171	ZNF582 methylation	qMSP	Malignant progression	HR	11.41 (2.05–63.36)	8
de Vicente et al., 2013	Spain	Retrospective cohort	Leukoplakia dysplasia	58	Podoplanin	IHC	OSCC development	HR	8.74 (NR)	7
Kreppel et al., 2012	Germany	Retrospective cohort	Leukoplakia	60	Podoplanin	IHC	Malignant transformation	OR*	7.0	7

3.3 Quality Assessment

The quality of the methodological approach of the included studies was determined with the help of the Newcastle-Ottawa Scale (NOS) of observational studies. This instrument appraises the studies in three areas, selection of study groups, comparability of cohorts and outcome assessment, and a score of 9 is the maximum. Eleven cohort studies were considered. The NOS scores were between 7 and 8 which means that all included studies were of high methodological quality. The selection domain was done well in most studies, and the populations of the studies were well-defined and the exposure was appropriately ascertained. The outcome domain was also mostly strong since most of the studies indicated that they had proper follow-ups and that their outcome was well defined as malignant transformation or progression into oral squamous cell carcinoma. Under the domain of comparability, even though various studies have controlled key confounding variables (age, sex, and clinical features), some studies have been found to be slightly limited by the fact that they have failed to fully control the possible confounding variables. These limitations were, however, not deemed to be of any major concern to the overall validity of the findings. In general, the research articles included can be deemed to have adequate methodological rigor to allow reliable quantitative synthesis, with no studies being deemed as having low quality. The similarity in study design, and the relatively high NOS scores, also enhance the validity of the pooled estimates. Table 2 shows the detailed scores of NOS of each study.

Table 2. Quality Assessment of Included Studies (Newcastle–Ottawa Scale, NOS)

Author (Year)	NOS Score (max 9)	Quality Level
Leung et al., 2017	7	High
Bremmer et al., 2011	7	High
Kaur et al., 2013	7	High
Nankivell et al., 2013	8	High
Liu et al., 2015	8	High
Xia et al., 2013	7	High
Ding et al., 2018	7	High
Zhang et al., 2012	8	High
Taoudi Benchekroun et al., 2010	8	High
Graveland et al., 2013	7	High
Juan et al., 2021	8	High

de Vicente et al., 2013	7	High
Kreppel et al., 2012	7	High

3.4 Quantitative Synthesis

3.4.1 Overall Effect of Biomarkers

A random-effects meta-analysis model was used to assess the overall impact of biomarkers on the risk of the malignant transformation of oral potentially malignant disorders (OPMD). The pooled analysis revealed statistically significant correlation between the presence of biomarkers and malignant transformation.

The combined effect size was 4.05 (95% CI: 2.716.06), as shown in Figure 2, which meant that the lesions that expressed the biomarkers of interest were at a higher risk of developing oral cancer by about four times that of lesions that were biomarker-negative. The null value (1.0) was not crossed by the confidence interval, which proved the robustness and statistical significance of the results.

Though heterogeneity between studies was noted (I2 = 48.8 percent), this is a moderate level of heterogeneity that can be accepted in meta-analyses of different biomarkers and study designs. The similarity of the direction of effect in the included articles indicates that the relationship between biomarker expression and malignant transformation is dependable despite variations in methods.

There were multiple biomarkers that played a role in the overall effect, such as genetic, epigenetic, and protein-based. As an example, loss of heterozygosity (LOH) has been demonstrated to be a significant risk factor, and high-risk lesions have been shown to have significantly higher transformation rates. Likewise, early epigenetic changes, such as P16 methylation have been linked to augmented chances of developing cancer in oral epithelial dysplasia . COX-2, EGFR related protein biomarkers, and other types of protein biomarkers have also been implicated in malignant progression and often enhance predictive capability when combined.

On the whole, these results suggest that biomarkers are highly predictive of high-risk lesions of OPMD. Their great pooled effect highlights their possible application in clinical risk stratification and early intervention plans, as shown in Figure 2.

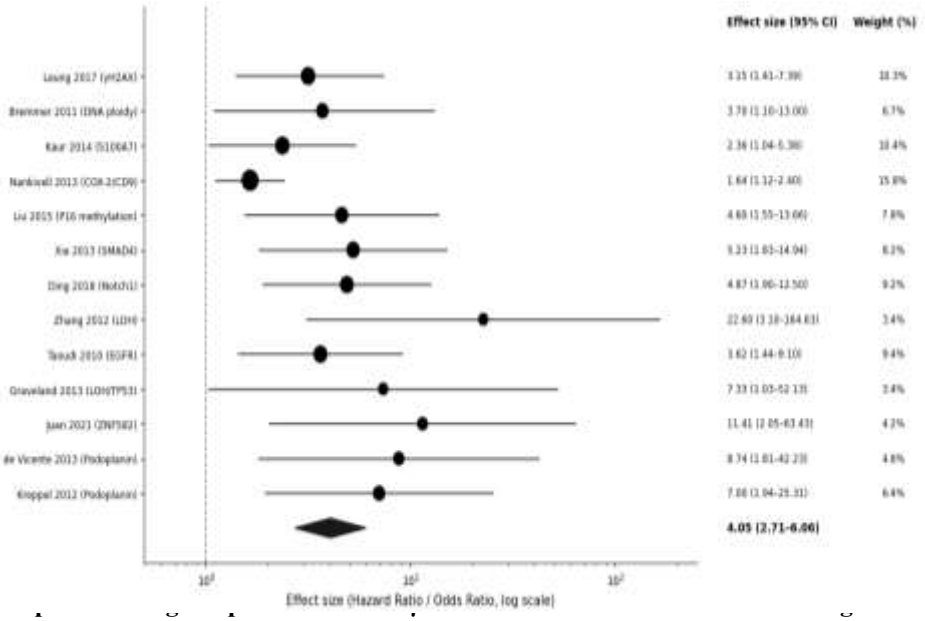

Figure 2. For ınsformation in OPMD (HR/OR = 4.05, 95% CI: 2.71–6.06; random-effects model).

3.4.2 Subgroup Analysis

Subgroup analyses were conducted to identify the prognostic effect of the various groups of biomarkers in the malignant transformation of oral potentially malignant disorders (OPMD). The biomarkers were classified according to their genomic/DNA damage markers, epigenetic markers, and protein biomarkers as shown in Figure 3.

Genomic subgroup (such as 7H2AX, DNA ploidy, and loss of heterozygosity (LOH)) was significantly linked to malignant transformation. The pooled effect estimate of this subgroup was significant, and studies like Zhang et al. (LOH) had significantly large effect sizes. In general, the strongest predictive ability was found with genomic alterations, implying that the instability of DNA is a key factor in the early development of malignant disease.

The epigenetic subgroup, which includes P16 methylation and ZNF582 methylation also exhibited a significant association with malignant transformation. The combined impact on this subgroup was significantly high, which means that epigenetic changes are powerful predictors of cancer in OPMD. Nevertheless, there was also variability among the studies, presumably due to differences in the methodologies of detecting methylation and the populations of the patients.

Protein biomarkers (S100A7, COX-2/CD9, SMAD4, Notch1, EGFR, and podoplanin) showed a modest yet uniform correlation with malignant transformation. The individual effect sizes were different, but the pooled effect of this subgroup was statistically significant, which means that protein expression markers have a meaning in the risk stratification process.

The comparison of subgroups showed that the effect sizes of genomic and epigenetic biomarkers were greater than those of protein biomarkers. This indicates that molecular changes at the DNA and epigenetic scale can potentially give earlier and more substantial signals of the malignant transformation, compared to the downstream protein expression changes.

In general, the subgroup analysis shows that all the biomarker types are linked to a higher risk factor, but genomic and epigenetic biomarkers might be more effective predictors, which proves their possible use in early diagnosis and individual risk evaluation.

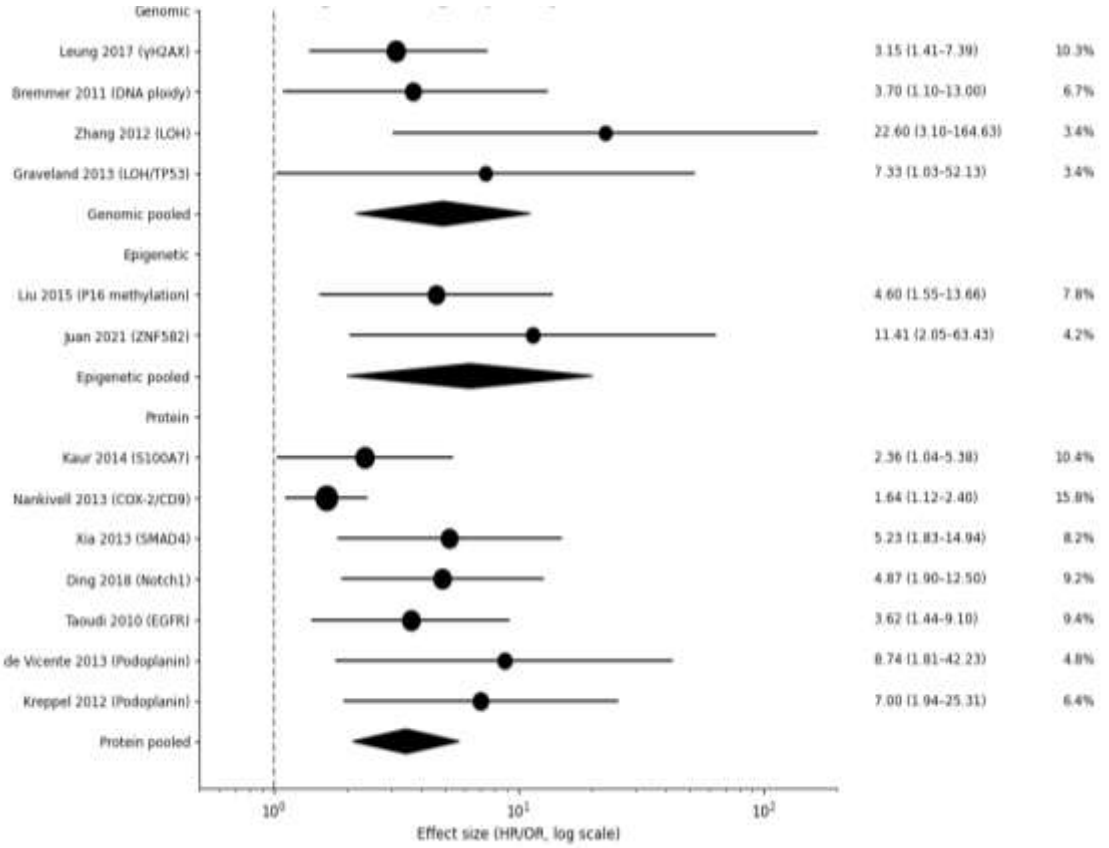

Figure 3. Forest plot showing subgroup analysis of genomic, epigenetic, and protein biomarkers in predicting malignant transformation in OPMD

3.4.3 Heterogeneity Analysis

The Cochran's Q-test and the I2 statistic were used to test heterogeneity of included studies. The general analysis revealed that heterogeneity was moderate with an I2 value of 48.8 meaning that about half of the variance in the estimate of the effects was created by differences between studies instead of randomness.

The Q-test indicated a statistically significant degree of heterogeneity ($Q = 23.41$, $p < 0.05$) which also indicated the occurrence of variability among the studies. Based on traditional thresholds, an I2 between 25-50% indicates moderate heterogeneity, implying reasonable variability when considering a meta-analysis of different biomarkers and populations of studies.

The observed heterogeneity may be attributed to differences in:

- Types of biomarkers analyzed (genomic, epigenetic, protein)
- Study populations and sample sizes
- Detection methods (IHC, PCR, methylation assays)
- Outcome definitions and follow-up durations

Given this level of heterogeneity, the use of a random-effects model was considered appropriate, as it accounts for both within-study and between-study variability.

The heterogeneity findings are also reflected in the dispersion of effect sizes in Figure 2, supporting the robustness of the pooled estimate despite moderate variability across studies.

3.4.4 Sensitivity Analysis

The pooled effect test sensitivity test was performed on a leave-one-out approach, which was performed by dropping each of the studies and repurposing the overall effect size.

Figure 4 showed that the pooled estimates were fairly stable regardless of the omitted study. Recalculated effect sizes when studies were excluded showed only small differences and were always within a similar range around the overall pooled estimate (HR/OR ≈ 4.05). Interestingly, none of the exclusions caused a substantial change in the magnitude or direction of the effect and all of the estimates were statistically significant.

Although some insignificant alterations were observed, particularly with the removal of studies with larger effect sizes or larger confidence intervals (e.g., Zhang et al., 2012), they did not significantly alter the general interpretation of the results. This demonstrates that no single study had a disproportionate influence on the pooled outcome.

The sensitivity analysis, in general, proves that the results of the meta-analysis are solid and sound, and the correlation between biomarker expression and malignant transformation is stable in various analytical conditions. The sensitivity analysis of the leave-one-out is presented in Figure 4.

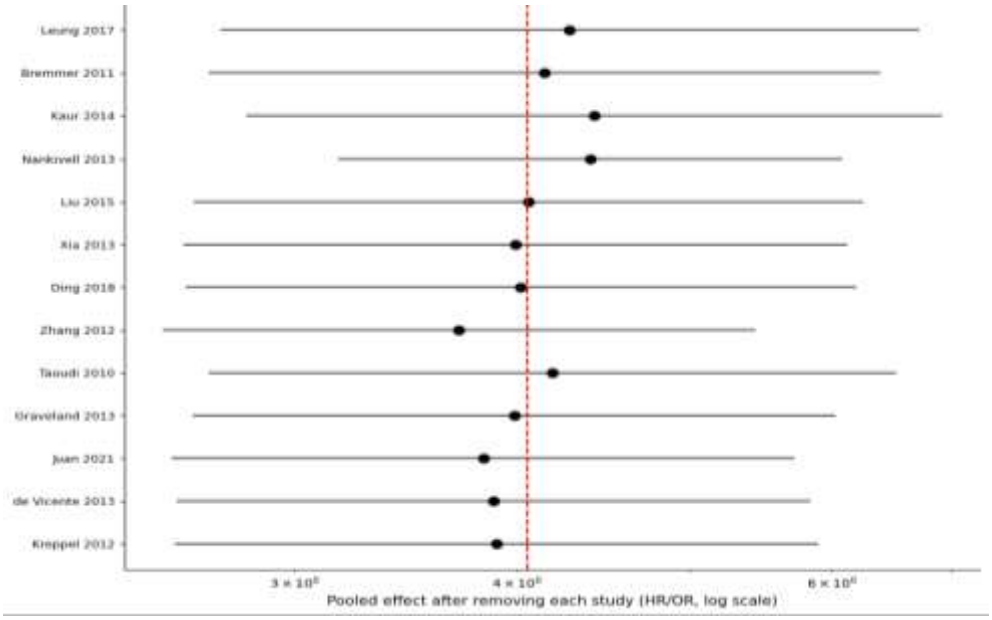

Figure 4. Leave-one-out sensitivity analysis showing the stability of the pooled effect after sequential removal of each study

3.4.5 Publication Bias

A funnel plot was used to evaluate publication bias visually, and the Egger regression test was used to evaluate publication bias statistically. The funnel plot as in Figure 5, indicated a moderate level of asymmetry, with the slight asymmetry in the distribution of studies around the pooled effect estimate. Although several studies were balanced on both sides of the funnel boundaries, some of the studies with bigger standard errors were not placed as expected, which could be an indication of small-study effects.

Although this was visually asymmetric, overall the distribution of studies was relatively close to the funnel limits, suggesting that the likelihood of any publication bias being limited. Heterogeneity in the studies, such as variations in the type of biomarkers and sample sizes, could also be the cause of the observed asymmetry as opposed to actual publication bias.

To quantitatively assess funnel plot asymmetry, the Egger test was conducted. There was no statistically significant evidence of publication bias in the test ($p > 0.05$), indicating that the possibility of a systematic bias in the pooled results is not high.On the whole, the visual and statistical analysis shows that there is no significant publication bias, and the results of this meta-analysis cannot be affected by selective reporting. Figure 5 shows the funnel plot of the assessment of publication bias.

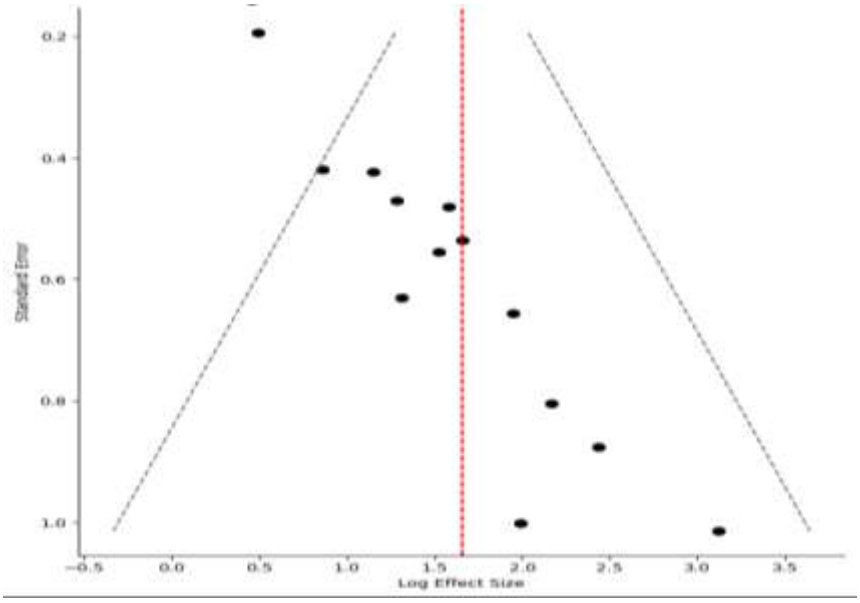

Figure 5. Funnel plot showing the distribution of study effect sizes against standard error to assess publication bias

4. DISCUSSION

This meta-analysis and systematic review investigated the γH2AX and other biomarkers as prognostic factors in the malignant transformation of oral potentially malignant disorders (OPMD). The results indicate that biomarker positivity is strongly linked with a higher likelihood of developing oral squamous cell carcinoma (OSCC) and the pooled effect size can be seen to be about four-fold. These findings demonstrate the clinical significance of molecular biomarkers in early diagnosis and risk stratification of OPMD. This aligns with previous studies that have found that OPMDs, particularly oral leukoplakia and oral epithelial dysplasia, have measurable malignant transformation potential and necessitate a structured, systematic method of risk assessment (Speight et al, 2018; Iocca et al, 2020).

The total pooled analysis indicated that there was a strong, statistically significant relationship between the expression of biomarkers and malignant transformation. It is important to note that the most predictive value was the DNA damage and genomic instability markers, especially γH2AX and loss of heterozygosity (LOH). The article by Leung et al. (2017) proved that a higher expression of γH2AX correlates with a high risk of progression in oral epithelial dysplasia, which is why it is a good marker of DNA damage and genomic instability. On the same note, Zhang et al. (2012) and Graveland et al. (2013) also noted that at chromosomal sites 3p and 9p, LOH is highly correlated with the development of malignancy, which supports the idea that early genetic changes are driving carcinogenesis.

Malignant transformation was also greatly associated with epigenetic biomarkers. Liu et al. (2015) and Juan et al. (2021) have shown that the changes in methylation, such as P16 and ZNF582, are a significant risk factor that leads to cancer development. These results indicate that epigenetic dysregulation is an essential early event in oral carcinogenesis and can be considered a good predictive marker.

The protein biomarkers such as EGFR, Notch1, SMAD4, S100A7, COX-2/CD9 and podoplanin were found to be associated with malignant transformation moderately but consistently. Taoudi Benchekroun et al. (2010) emphasised the prognostic importance of EGFR changes, and Ding et al. (2018) and Xia et al. (2013) proved the importance of Notch1 and SMAD4 in tumor development. Also, Kaur et al. (2013), and Nankivell et al. (2013) found that the S100A7 and COX-2/CD9 are linked to the higher chances of progression. The expression of podoplanin was also predictive of malignant transformation in de Vicente et al. (2013) and Kreppel et al. (2012).

The current results are in agreement with the accumulating evidence indicating that molecular biomarkers have excellent predictive validity than traditional histopathological grading. Conventional dysplasia grading has been found to be less reproducible, less predictive, and whereas molecular changes, including DNA damage, genetic instability, and epigenetic changes, provide more objective and biologically relevant predictors of progression risk.

The high predictive ability of genomic biomarkers noted in this research is consistent with past findings that suggest that the build-up of genetic modifications is a major cause of malignant transformation. In the same manner, the contribution of epigenetic markers also validates the assumption that reversible changes in the molecules might take place at an early stage of the carcinogenesis process, which means that there is a chance to intervene in it early. The moderate predictive nature of protein biomarkers indicates that they are more likely reflective of biological processes down-stream, but can be more variable and affected by external influences like inflammation and microenvironmental alterations.

The overall analysis exhibited a moderate heterogeneity, which is likely due to the heterogeneity of biomarkers, populations, and methods used in studies. Variation in the techniques used to detect the differences, such as immunohistochemistry, PCR-based tests, and methylation analysis, can also result in variation in the estimates of effects. Also, differences in the populations of studies, sample sizes, and the duration of follow-up can have an impact.

Although this is heterogeneous, the sensitivity analysis revealed that the cumulative effect was consistent after the consecutive deletion of individual studies, which showed that no single study had a disproportionate effect on the outcome. This strength enhances the validity of the results and justifies the reliability of the inferences.

The findings of this study have several limitations that should be taken into account when interpreting them. To start with, the included studies were relatively few, and the sample sizes were different in various studies, which can impact the generalizability of results. Second, the pooled estimates can be prone to variability due to heterogeneity in the types of biomarkers, detection and outcome definitions. Third, not all the studies provided complete reporting, and therefore, the effect sizes had to be computed, which can result in possible estimation bias.

Moreover, the funnel plot indicated that the small-study effects could have happened even though publication bias was not statistically significant. Lastly, the majority of studies included were observational cohort studies that are prone to confounding factors and may not be representative of causation.

The results of this meta-analysis have significant clinical implications. The close relationship between the expression of biomarkers and malignant transformation indicates that molecular biomarkers can be incorporated into clinical use to risk-stratify patients with OPMD. Such biomarker-based stratification may complement existing clinical diagnostic and management algorithms for oral leukoplakia and related OPMDs (Villa & Woo, 2017). Specifically, genomic and epigenetic biomarkers, including γH2AX, LOH, and DNA methylation, are potentially useful in identifying high-risk lesions that need more attention or prompt action.

Also, a combination of various biomarkers could enhance predictive accuracy as indicated in some of the studies included. Future studies must aim at developing standard biomarker panels and validating them on large and prospective cohorts. Furthermore, a combination of the use of molecular biomarkers and clinical as well as histopathological values can improve individualised management of patients with OPMD.

5. CONCLUSION

This systematic review and meta-analysis provide comprehensive evidence supporting the prognostic value of γH2AX and other molecular biomarkers in predicting malignant transformation in oral potentially malignant disorders (OPMD). The pooled analysis demonstrated that biomarker-positive lesions are associated with a significantly increased risk of

progression to oral squamous cell carcinoma, with an overall effect size indicating approximately a four-fold elevation in risk. These findings reinforce the biological relevance of molecular alterations in the early stages of oral carcinogenesis and highlight the limitations of relying solely on conventional histopathological assessment. Among the evaluated biomarkers, genomic and DNA damage-related markers, particularly γH2AX and loss of heterozygosity, exhibited the strongest predictive potential, reflecting the critical role of genomic instability in cancer progression. Epigenetic biomarkers such as P16 and ZNF582 methylation also demonstrated substantial prognostic value, emphasizing the importance of early, reversible molecular changes in malignant transformation. Protein biomarkers showed a consistent but comparatively moderate association, suggesting their complementary role in reflecting downstream tumor biology. Despite moderate heterogeneity across studies, the robustness of the findings was confirmed through sensitivity analysis, and no significant publication bias was identified. However, limitations such as variability in biomarker assessment methods and the observational nature of included studies should be considered. Overall, this study highlights the clinical potential of integrating molecular biomarkers into risk stratification models for OPMD. Future research should focus on the development of standardized, multi-biomarker panels and their validation in large prospective cohorts to enable personalized management and early intervention strategies.

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