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Systematic Article

Role of biomarkers in management of laryngeal dysplasia: a systematic review

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ABSTRACT

Laryngeal dysplasia is a premalignant lesion with variable potential for progression to laryngeal squamous cell carcinoma, highlighting the need for reliable biomarkers that can improve diagnosis, risk stratification, and patient surveillance. This systematic review evaluated the current evidence regarding the role of molecular and histological biomarkers in the management of laryngeal dysplasia. A comprehensive literature search of PubMed, Scopus, Web of Science, and Google Scholar was conducted for studies published between January 2020 and September 2025 in accordance with the PRISMA 2020 guidelines. Twenty-four eligible studies were included after screening 2,847 records. Data regarding biomarker type, study design, testing methods, and diagnostic and prognostic outcomes were extracted and synthesized qualitatively. The evidence demonstrated that overexpression of p53, Ki-67, and PD-L[1] was consistently associated with an increased risk of malignant transformation. Dysregulated microRNAs, particularly miR-21, miR-146, and miR-223, together with DNA methylation markers, also showed promising diagnostic and prognostic utility. Combined biomarker panels consistently outperformed conventional histopathology alone, achieving reported sensitivity of up to 89% and specificity of up to 84% for predicting malignant transformation. Although the current evidence supports the potential clinical value of these biomarkers as adjuncts to histopathological assessment, considerable heterogeneity in study design, laboratory techniques, and reporting limits their routine clinical application. Further well-designed multicenter prospective studies with standardized biomarker assessment protocols are required before these biomarkers can be incorporated into routine clinical practice for the diagnosis, prognostication, and follow-up of patients with laryngeal dysplasia.

Keywords: Laryngeal dysplasia, Biomarkers, p53, Ki-67, MicroRNA, DNA methylation, PD-L[1], EGFR

INTRODUCTION

Laryngeal dysplasia is a premalignant condition that may progress to laryngeal squamous cell carcinoma. Dysplastic lesions that progress to malignancy can be difficult to identify accurately.[1] Traditional diagnosis relies on histopathology, which may be limited by interobserver variability and sampling bias. Therefore, scientists are studying biomarkers to accurately predict the chance of a malignant transformation.[2] Such biomarkers include p53, Ki-67, PD-L[1], microRNAs, and DNA methylation that have demonstrated the possibility of improving diagnosis and follow-up.[3,4] The use of such markers may facilitate early identification of patients in cases where one is at high risk with a view to ensuring appropriate treatment plans are guided.[5] This limitation has stimulated increasing interest in molecular and immunohistochemical biomarkers capable of capturing biological changes that precede overt malignant transformation. Earlier work has focused extensively on p53 and Ki-67 as markers of genomic instability and proliferation, whereas more recent studies have expanded toward immune-related signatures, microRNAs, chromosomal instability, oncogenic pathway alterations, and transcriptomic profiling. Contemporary

transcriptomic analysis of laryngeal dysplasia has demonstrated that progressive and non-progressive lesions may exhibit distinct immune and inflammatory gene-expression profiles, supporting concept that progression is influenced not only by epithelial abnormalities but also by surrounding immune microenvironment.

The objective of this review is to summarize the recently published knowledge in the domain of 2020 to 2025 that examined biomarkers in the management of laryngeal dysplasia.

METHODS

The PRISMA 2020 criteria guided the search of PubMed, Scopus, Web of Science, and Google Scholar for literature published between 2020 and 2025. The literature search used the following keywords: laryngeal dysplasia, biomarkers, molecular markers, p53, Ki-67, microRNA, DNA methylation, and PD-L[1].[16] Only peer-reviewed articles published in English were considered. The review included studies that analysed diagnostic, predictive, or prognostic biomarkers in laryngeal dysplasia or early laryngeal carcinoma.[7] The researcher filtered the titles, abstracts, and full texts. Disagreements were resolved through discussion. The extracted data were author, year, type of biomarker, testing method, and outcome measures.

Inclusion and exclusion criteria

Eligible studies investigated molecular, genomic, or immunohistochemical biomarkers related to laryngeal dysplasia or early malignant progression.[8] The studies had to provide quantifiable endpoints, namely sensitivity, specificity, or risk ratio. As an exclusion criterion, case reports, conference reports, review articles without new information, and animal trials were excluded, and non-peer-reviewed articles were excluded.

Study selection and data extraction

A total of 2,847 records were identified. After 542 duplicates were removed, records underwent title and abstract screening.[2] Sixty-seven articles were assessed in full text, and 24 met the inclusion criteria. Extracted data included sample size, biomarker, testing method, and diagnostic or prognostic outcomes. Figure 1 presents the PRISMA flow diagram for study selection.

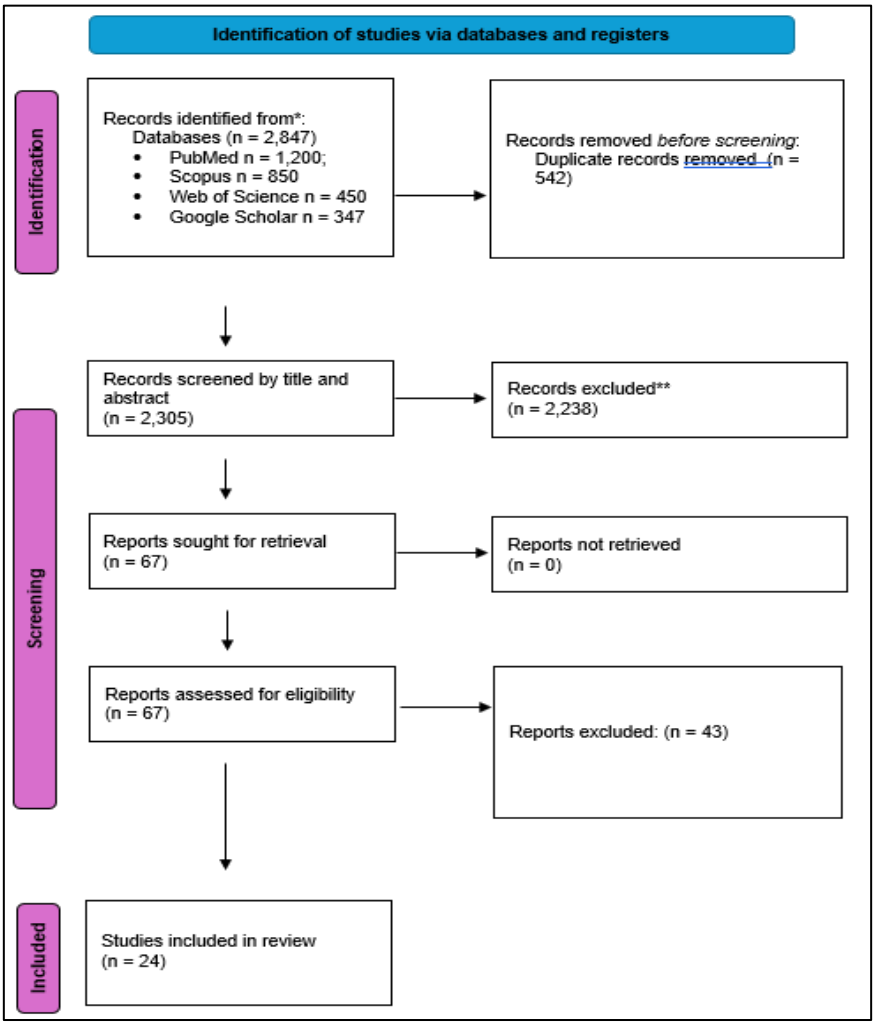

Figure 1: PRISMA flow diagram.

Statistical analysis

Descriptive statistics were performed to summarize results in the selected studies. Sensitivity, specificity, odds ratios, and hazard ratios were considered for comparison. Similar biomarkers were amalgamated in the studies.[11] Since there was a significant difference in study design and biomarker testing procedures, meta-analysis was not carried out. Relative predictive values of major using biomarkers were, however, correlated to detect common trends.[12]

RESULTS

The final synthesis included studies published between 2020 and 2025. Most were molecular or clinical studies of laryngeal dysplasia or early squamous cell carcinoma.[13,24] The most common biomarkers explored were p53, Ki-67, PD-L[1], microRNAs, and patterns of DNA methylation.[10,14] Biomarkers were analyzed using immunohistochemistry, PCR-based assays, or RNA sequencing techniques.[15] Across several studies, p53 overexpression was strongly associated with malignant progression. Abnormal p53 expression was associated with an approximately threefold increased risk of recurrence.[16] High Ki-67 and p53 expression have also been linked with increased malignant potential in vocal fold leukoplakia.[2]

Another vital marker was Ki-67. Combined p53 and Ki-67 expression achieved 87% accuracy in one predictive model.[17] Ki-67 positivity of 30% or greater was associated with a3-fold increase in malignant risk.[2,18] There was an inverse correlation between PD-L[1] expression and tumoral aggressiveness and immune escape. The optimal findings were reported by PD-L[1] overexpression and high Ki-67 index along with poor prognosis.[19] Laryngeal dysplasia and cancer were consistently dysregulated by MicroRNAs, which included miR-21, miR-223, and miR-146.[20] Circulating miRNAs showed diagnostic sensitivity of 89% across study groups.[21] It was also confirmed that miR-[21] was persistently upregulated, whereas miR-375 was downregulated in malignant lesions.[4]

Another promising direction was provided by the DNA methylation markers. Promoter hypermethylation of MGMT and RASSF1A correlated with tumor stage and nodal metastasis.[22] Methylation profiling of oral brushing samples showed 88% sensitivity and 83% specificity for detecting recurrence.[23] Generally, the multimarker panel, compared to individual markers, was better. Multimodal integrations involving the use of immunohistochemical and molecular biomarkers had a maximum sensitivity (89%) and a specificity (84%) of malignant transformation.[24]

Table 1: Summary of key biomarker findings in laryngeal dysplasia (2020-2025).

Studies (years)	Biomarker(s)	Methods	Major finding	Diagnostic/ prognostic metric
Wan et al[2] (2020)	p53, Ki-67	IHC	High p53 and Ki-67 linked with higher malignant potential in vocal fold leukoplakia	OR=2.1; sensitivity= 78%; specificity=81%
Eckel et al[24] (2020)	Histopathology + biomarker adjuncts	Clinical guideline	Integrating molecular markers improves diagnostic reproducibility	-
Ghafouri-Fard et al[16] (2020)	microRNAs (miR-21, miR-31)	RT-qPCR	Dysregulated miRNAs predict poor differentiation and early recurrence	AUC=0.84
Tuncturk et al[23] (2021)	miR-21, miR-34a, miR-146b	RT-qPCR	Stepwise increase from benign to premalignant to malignant lesions	P<0.01
Falco et al[1] (2022)	PD-L1, p53, Ki-67	IHC	PD-L1 correlates with proliferation index and tumour grade	OR=1.9
Al Amen et al[20] (2022)	PD-L1, p53, Ki-67	IHC	Combined PD-L1 and Ki-67 overexpression predict poor outcome	Sensitivity=82%; specificity=76%
Broseghini et al[15] (2023)	microRNAs (miR-375, miR-21)	Systematic review	miR-21 consistently upregulated; miR-375 downregulated	Diagnostic accuracy=85%
Genc et al[22] (2023)	Exosomal miR-21, miR-223, miR-146	Plasma assay	Exosomal miRNAs associated with nodal involvement	AUC=0.87
Jeong et al[9] (2023)	p53, Ki-67	IHC and predictive modelling	Combined p53 and Ki-67 predictive model achieved 87% accuracy	Accuracy=87%
Tan et al[5] (2023)	p53, PTEN	IHC	Combined loss predicts shorter disease-free survival	HR=2.6 (95% CI 1.2-4.8)
Verro et al[3] (2023)	Multiple biomarkers	Literature review	Identified several validated biomarkers for LSCC	-
Inchanalkar et al[12] (2023)	DNA methylation	Whole-genome bisulfite sequencing	Hypermethylation in multiple gene promoters linked with progression	OR=2.8
Maffini et al[2] (2024)	Transcriptome-wide	RNA-seq	Upregulation of TP53, EGFR, and immune checkpoints in high-grade lesions	-
Ko et al[13] (2024)	p53 mutation	IHC + sequencing	Abnormal p53 expression predicts higher recurrence	HR=3.2
Liu et al[6] (2024)	p16, Ki-67	IHC	Dual positivity associated with improved survival	HR=0.62

Continued.

Studies (Years)	Biomarker(s)	Methods	Major finding	Diagnostic/ prognostic metric
Rivera Peña et al[19] (2024)	DNA methylation (MGMT, RASSF1A)	PCR	Promoter hypermethylation correlates with tumour stage and nodal spread	P<0.05
Rapado-González et al[10] (2024)	DNA methylation	Systematic review	Validated methylation markers in oral/laryngeal lesions	AUC=0.82
Komitova et al[21] (2024)	miRNAs	Review	miR-21 and miR-375 were among the most reproducible prognostic miRNAs	-
Falco et al[7] (2024)	Serum miRNA panel	Bioinformatic signature	Serum miRNA panel distinguished early LSCC	AUC=0.91
Horton et al[11] (2024)	Dysplastic vocal fold lesions	Retrospective analysis	Malignant transformation rate reported over long-term follow-up	-
Gissi et al[18] (2024)	DNA methylation panel	Oral brushing assay	Detects recurrence in treated patients	Sensitivity=88%; specificity=83%
Wang et al[14] (2024)	Surgical outcome biomarkers	Clinical cohort	Biomarker-based selection associated with improved surgical outcomes	P<0.05
Cívico-Ortega et al[4] (2025)	EGFR	Meta-analysis	EGFR overexpression increases malignancy risk	OR=3.5 (95% CI 2.1-5.8)
Banta et al[17] (2025)	Circulating miRNAs	Systematic review	miR-21 and miR-223 validated across multiple cohorts	Diagnostic sensitivity=89%

Table 2: Sensitivity and specificity of selected biomarker approaches.

Biomarker approaches	Studies	Sensitivity (%)	Specificity (%)
p53 + Ki-67	Wan et al (2020)	78	81
PD-L1 + p53 + Ki-67	Al Amen et al (2022)	82	76
DNA methylation panel	Gissi et al (2024)	88	83

Table 3: Distribution of biomarker categories in included studies.

Biomarker categories	Number of studies	Percentage (%)
Immunohistochemical biomarkers	7	29.2
microRNA biomarkers	7	29.2
DNA methylation biomarkers	4	16.7
Transcriptomic biomarkers	1	4.2
EGFR	1	4.2
Other/multimarker/clinical approaches	4	16.7
Total	24	100.0

DISCUSSION

This review suggests that molecular biomarkers may be valuable adjuncts in the clinical management of laryngeal dysplasia. p53 and Ki-67 are the most extensively studied indicators of malignant risk.[2,16] Their overexpression reflects dysregulated cellular proliferation and impaired genomic stability, both of which contribute to carcinogenesis.[3] PD-L[1] has received increasing attention because of its role in immune escape. When combined with proliferation markers, PD-L[1] may help identify high-risk lesions that require closer surveillance or early intervention.[19] MicroRNA and DNA methylation markers are emerging as promising minimally invasive biomarkers. They may be assessed using brushing samples or serum-based assays, offering less invasive alternatives to repeated biopsies.[21,23] The reproducibility is good between various groups of cohorts of microRNA markers, like miR-21, when it comes to clinical translation.[20,21]

RASSF1A and MGMT are other examples of DNA methylation signatures with a good predictive recurrence and progression case.[22,23] A mixture of these molecular discoveries and histological grading would definitely contribute to enhancing the patient follow-up plans and performing individual practice.[6,18] The detection method, however, differs, and the sample sizes used are very small, which complicates comparisons of studies. Hegemonic testing protocols must be developed before biomarkers can be used in the clinical field.[10,14]

Limitations

The evidence base remains limited by heterogeneity in patient selection, histological classification, biomarker

assays, positivity thresholds, and duration of follow-up. Many available studies are retrospective and involve relatively small cohorts, while some assess biomarkers in established laryngeal squamous cell carcinoma rather than strictly in premalignant laryngeal lesions. This limits direct inference regarding malignant transformation. Furthermore, several biomarkers included in present review supported partly by evidence from oral epithelial dysplasia/broader head-and-neck cancer populations, which may not be biologically interchangeable with laryngeal dysplasia. Absence of standardized testing protocols and externally validated prediction models also restricts quantitative pooling and clinical translation. Recent systematic evidence similarly identifies incomplete confounder adjustment, limited follow-up and risk of bias as major weaknesses in this field.

CONCLUSION

Current evidence supports biomarkers as promising adjuncts to histopathology rather than standalone tools for the management of laryngeal dysplasia. p53 and Ki-67 remain among the most investigated conventional markers, while emerging evidence increasingly implicates immune signatures, microRNAs, chromosomal instability, oncogenic pathway alterations, and transcriptomic profiles in malignant progression. The most promising future strategy is likely to involve validated multimarker models integrating histopathological, molecular, immune, and clinical information. Before routine clinical implementation, these approaches require standardized assays, predefined thresholds, external validation, and prospective demonstration that biomarker-guided management improves clinically meaningful outcomes.

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