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Immunohistochemical Assessment of Proliferation, Angiogenesis, and Viral Markers in Recurrent Laryngeal Papillomatosis

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Abstract

Recurrent laryngeal papillomatosis (RLP) is a benign but clinically aggressive condition, characterized by frequent recurrences and significant morbidity. Although infection with the human papillomavirus (HPV) represents the main etiological factor, the biological mechanisms underlying the persistence and recurrence of lesions remain incompletely understood. This study aimed to perform a comprehensive histopathological and immunohistochemical evaluation of recurrent laryngeal papillomatosis, focusing on epithelial proliferation, angiogenesis, the inflammatory microenvironment, and HPV expression.

A retrospective tissue-based study was conducted on a cohort of 32 surgically excised laryngeal papilloma specimens obtained from adult patients. Conventional histopathological examination was performed using hematoxylin–eosin staining. Immunohistochemical analysis included HPV markers, Ki-67, VEGF-A, CD31, and CD68. Proliferative activity, angiogenesis, and inflammatory infiltrates were assessed using semi-quantitative and quantitative methods. Statistical analysis was performed using standard comparative tests, with a p-value < 0.05 considered statistically significant.

Histopathological evaluation revealed a typical papillary architecture, with fibrovascular stromal cores, epithelial acanthosis, koilocytic changes, and chronic inflammatory infiltrates. HPV immunopositivity was detected in 21.9% of cases, predominantly in the suprabasal epithelial layers, displaying a mosaic pattern. All lesions showed positivity for Ki-67, with heterogeneous proliferative indices. CD68-positive macrophages were variably distributed within the subepithelial stroma. CD31 immunostaining demonstrated a dense and mature microvascular network in all cases. VEGF-A expression was present in the majority of lesions and showed a moderate positive correlation with microvascular density, indicating active angiogenesis.

Recurrent laryngeal papillomatosis is characterized by a complex interaction between epithelial proliferation, angiogenesis, chronic inflammation, and HPV-associated changes. VEGF-A-mediated angiogenesis appears to play a significant role in sustaining lesion growth. Comprehensive immunohistochemical profiling provides valuable insights into the biological behavior of RLP and may contribute to improved risk stratification and the development of targeted therapeutic strategies.

Keywords: Recurrent laryngeal papillomatosis; human papillomavirus; Ki-67; angiogenesis; VEGF-A; CD31; CD68; immunohistochemistry.

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INTRODUCTION

Recurrent laryngeal papillomatosis (RLP) is a rare but clinically significant benign neoplastic disease of the upper aerodigestive tract, characterized by the development of exophytic epithelial lesions predominantly affecting the larynx[1,2]. Despite its benign histological appearance, RLP is marked by an aggressive clinical course, frequent recurrences, and a substantial impact on voice quality, airway patency, and overall quality of life[3]. The disease represents one of the most challenging chronic conditions encountered in laryngology, often requiring repeated surgical interventions and long-term follow-up[4].

RLP affects both pediatric and adult populations, exhibiting a bimodal age distribution. Juvenile-onset RLP is typically associated with a more aggressive course, higher recurrence rates, and a greater need for repeated surgical procedures, whereas adult-onset disease often demonstrates a relatively indolent progression[5,6]. Nevertheless, adult RLP remains clinically unpredictable, with some patients experiencing rapid regrowth, distal spread, or, in rare cases, malignant transformation[7]. These features underscore the need for a deeper understanding of the biological mechanisms underlying disease persistence and recurrence.

Human papillomavirus (HPV) infection is widely accepted as the primary etiological factor in RLP, with low-risk HPV genotypes, particularly HPV-6 and HPV-11, being most frequently implicated[8,9]. Viral DNA integration into host epithelial cells leads to dysregulation of cell cycle control through the expression of viral oncoproteins E6 and E7, which interfere with tumor suppressor pathways involving p53 and retinoblastoma protein[10]. Although HPV infection is necessary for disease initiation, it is increasingly recognized that viral presence alone does not fully explain the heterogeneity of clinical behavior observed in RLP patients[11].

The discrepancy between viral infection and disease severity suggests the involvement of additional host- and tissue-related factors that influence lesion growth, recurrence, and persistence[12]. Among these factors, epithelial proliferation, angiogenesis, and the inflammatory microenvironment have gained considerable attention in recent years[13,14]. Immunohistochemical studies have emerged as valuable tools for elucidating these mechanisms, providing insights into cellular activity, stromal interactions, and biological aggressiveness of papillomatous lesions[15].

Cellular proliferation represents a fundamental process in the growth and recurrence of papillomatous lesions. Ki-67, a nuclear protein expressed during all active phases of the cell cycle except G0, is widely used as a reliable marker of cellular proliferation[16]. Increased Ki-67 labeling indices have been reported in RLP lesions, particularly in cases characterized by frequent recurrence or aggressive clinical behavior[17]. Evaluation of Ki-67 expression allows for objective assessment of proliferative activity within the stratified squamous epithelium and may serve as a prognostic indicator for disease progression[18].

Angiogenesis plays a critical role in sustaining lesion growth by ensuring oxygen and nutrient supply to proliferating epithelial cells[19]. Vascular endothelial growth factor A (VEGF-A) is one of the most potent proangiogenic mediators, promoting endothelial cell proliferation, migration, and increased vascular permeability[20]. Overexpression of VEGF-A has been demonstrated in various benign and malignant laryngeal pathologies, including papillomatous lesions[21]. The presence of newly formed microvessels, commonly assessed using endothelial markers such as CD31, reflects active angiogenesis and may correlate with lesion recurrence and growth dynamics[22].

In addition to epithelial and vascular components, the inflammatory microenvironment has been increasingly recognized as an important modulator of papilloma biology[23]. Chronic inflammation may facilitate viral persistence, tissue remodeling, and angiogenic signaling[24]. CD68-positive macrophages represent a key cellular component of the inflammatory infiltrate and have been implicated in tumor-associated inflammation and angiogenesis through the secretion of cytokines, growth factors, and matrix-degrading enzymes[25]. The density and distribution of CD68-positive cells may therefore provide valuable information regarding the local immune response and its role in disease chronicity[26].

While numerous studies have examined individual markers involved in the pathogenesis of RLP, comprehensive analyses integrating viral presence, proliferative activity, angiogenesis, and inflammatory response within the same cohort remain limited[27]. Such an integrative approach is essential for understanding the complex interplay between epithelial cells, stromal components, and viral factors that contribute to disease recurrence[28].

Furthermore, immunohistochemical assessment offers practical advantages in routine diagnostic

pathology, allowing for standardized evaluation of tissue biomarkers using formalin-fixed, paraffin-embedded specimens[29]. Identification of reproducible histopathological and immunohistochemical patterns may facilitate risk stratification, guide clinical decision-making, and open new perspectives for targeted adjuvant therapies aimed at reducing recurrence rates[30].

The present study aims to provide a comprehensive immunohistochemical evaluation of recurrent laryngeal papillomatosis by assessing epithelial proliferation, angiogenesis, inflammatory infiltrate, and viral markers in surgically excised tissue specimens. By correlating these parameters with morphological and biological features, this study seeks to contribute to a better understanding of the biological behavior of RLP and to identify potential markers associated with disease aggressiveness and recurrence.

MATERIALS AND METHODS

Study design and case selection

A retrospective, single-center, tissue-based study was conducted on surgically excised laryngeal papilloma specimens. The study cohort included 32 adult patients (age range: 18–68 years) with a histopathological diagnosis of recurrent laryngeal papillomatosis. All tissue samples were obtained from patients treated at the Municipal Clinical Hospital "Sfânta Treime", Chișinău, Republic of Moldova, during the period 2018–2023.

Only cases with adequate tissue preservation and sufficient material for both histological and immunohistochemical analyses were included. In total, 289 histological and immunohistochemical slides were prepared and evaluated.

Surgical procedure and tissue handling

Tissue specimens were collected during microlaryngoscopic surgical excision of papillomatous lesions using cold instruments, performed for therapeutic purposes. Immediately after excision, tissue fragments were fixed in 10% neutral buffered formalin (pH 7.2–7.4) for 16–24 hours, ensuring optimal preservation of tissue morphology and antigenicity.

Histological processing and routine staining

Following fixation, specimens were processed using an automated tissue processor and embedded in paraffin. Serial sections of 4 μm thickness were cut and mounted on positively charged glass slides suitable for immunohistochemical procedures.

Routine histopathological examination was performed on sections stained with hematoxylin and eosin (H&E). The diagnosis of recurrent laryngeal papillomatosis was confirmed based on established morphological criteria, including papillary epithelial proliferation with fibrovascular cores covered by stratified squamous epithelium.

Immunohistochemical staining procedure

Immunohistochemical analyses were carried out using an automated staining platform and a polymer-based detection system, with 3,3′-diaminobenzidine (DAB) as chromogen. The standardized protocol included slide deparaffinization, heat-induced antigen retrieval, blocking of endogenous peroxidase activity, incubation with primary antibodies, application of post-primary and polymer reagents, chromogenic visualization, hematoxylin counterstaining, followed by dehydration and permanent mounting.

Antigen retrieval was performed using either citrate buffer (pH 6.0) or high-pH retrieval solution, according to the manufacturer's recommendations for each antibody. All incubation steps were carried out under standardized conditions to ensure reproducibility.

Immunohistochemical panel

For the purposes of this study, the immunohistochemical panel included the following markers:

- Human papillomavirus (HPV) – viral detection
- Ki-67 – assessment of epithelial proliferative activity
- VEGF-A – evaluation of angiogenic signaling
- CD31 – identification of endothelial cells and microvessels
- CD68 – detection of macrophage-mediated inflammatory infiltrate

Immunohistochemical controls

Appropriate quality control measures were applied throughout the immunohistochemical procedures. Positive control tissues with known antigen expression were used to validate staining accuracy for each antibody. Negative controls were obtained by omitting the primary antibody, ensuring reaction specificity and excluding nonspecific background staining.

Quantitative and semi-quantitative evaluation

Hot-spot selection method

Quantitative evaluation was performed using the hot-spot method, selecting areas with the highest density of immunopositive structures at ×200 magnification, corresponding to an area of approximately 0.74 mm^2. For each case, three representative hot-spot fields were analyzed, and the arithmetic mean was calculated.

Ki-67 evaluation

Ki-67 expression was assessed exclusively in epithelial

cells showing nuclear immunoreactivity. Proliferative activity was graded semi-quantitatively according to the percentage of positive epithelial nuclei:

- Score 1: <10% positive nuclei
- Score 2: 11–30% positive nuclei
- Score 3: >30% positive nuclei

VEGF-A scoring

VEGF-A expression was evaluated by integrating staining intensity and the proportion of positive epithelial and/or stromal cells, using a four-tier scoring system:

- Score 0: no detectable staining (<1% positive cells)
- Score 1: weak staining in 1–25% of cells
- Score 2: moderate to strong staining in 26–50% of cells
- Score 3: moderate to strong staining in >50% of cells

Assessment of angiogenesis and inflammatory infiltrate

Microvascular structures identified by CD31 and macrophages highlighted by CD68 were quantified in hotspot areas using the same evaluation protocol. Results were expressed as mean counts per field.

Microscopy and image acquisition

Microscopic examination and digital image acquisition were performed using an Olympus BX53 microscope equipped with an Olympus DP28 digital camera. Image adjustments were limited to brightness and contrast correction using Olympus cellSens Entry software, without altering the original histological content.

Statistical analysis

Statistical analyses were conducted using SPSS software (version 13.0) and Microsoft Excel. Group comparisons were performed using the chi-square test and Student's t-test, as appropriate. A p-value < 0.05 was considered statistically significant.

Ethical considerations

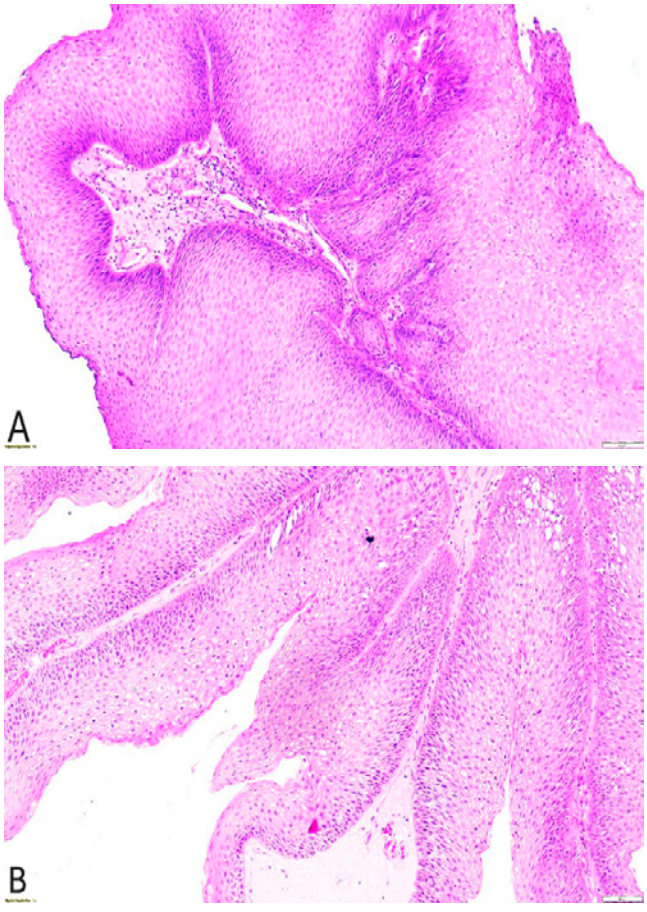
Ethical approval for the study was obtained from the Research Ethics Committee of the State University of Medicine and Pharmacy "Nicolae Testemitanu" (USMF), Chisinău, Republic of Moldova, in accordance with institutional and national regulations governing biomedical research. The study was approved by Decision No. 9, dated September 20, 2019.

All tissue samples were analyzed retrospectively, and patient data were anonymized prior to evaluation. The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki.

RESULTS

Histopathological features of recurrent laryngeal papillomatosis

Histopathological examination revealed that all lesions exhibited the characteristic architecture of exophytic papillary formations composed of finger-like projections lined by non-keratinized stratified squamous epithelium and supported by a well-defined fibrovascular core (Figure 1A). The stromal axis contained numerous congested capillaries and loosely arranged connective tissue, conferring a fragile appearance to the papillary structures (Figure 1B). The lamina propria frequently showed mild chronic inflammatory infiltrate, predominantly lymphoplasmacytic and perivascular, without evidence of necrosis or microabscess formation (Figure 1C). Interstitial stromal edema was commonly observed, resulting in a myxoid stromal appearance (Figure 1D). The covering epithelium demonstrated variable degrees of acanthosis, occasionally associated with focal hyperkeratosis.



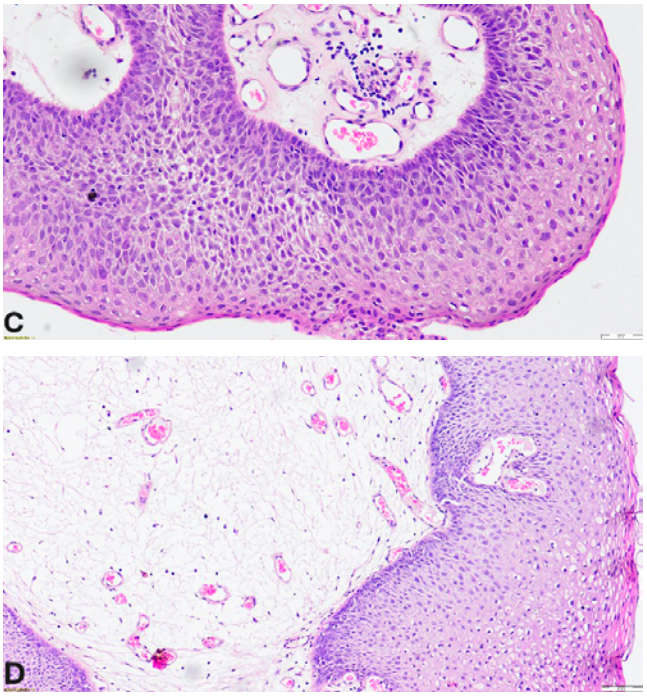


Figure1. A. Exophytic papillary projections lined by non-keratinized stratified squamous epithelium and supported by a fibrovascular core (×10). **B.** Thin papillary structures with loosely arranged stromal connective tissue and congested capillaries (×10) **C.** Mild chronic lymphoplasmacytic inflammatory infiltrate in the lamina propria, predominantly perivascular (×40) **D.** Interstitial stromal edema with myxoid appearance and epithelial acanthosis (×20).

Koilocytotic changes, characterized by perinuclear halos, were frequently identified within the intermediate and superficial epithelial layers. Mitotic figures were rare and confined to the basal and parabasal layers, without cytological atypia. In a subset of recurrent lesions, areas of subepithelial fibrosis or focal stromal hyalinization were observed, reflecting chronic tissue remodeling.

Based on histopathological criteria, lesions were classified as typical squamous papilloma (20 cases), hyperplastic papilloma (9 cases), and dysplastic papilloma (3 cases).

Immunohistochemical detection of HPV

Immunohistochemical analysis demonstrated HPV positivity in 7 of the 32 cases (21.9%). HPV immunoreactivity was restricted to the epithelial compartment and exhibited a predominantly suprabasal distribution, involving mature squamous cells of the intermediate and superficial layers (Figure 2A). The staining pattern was focal and mosaic, with scattered HPV-positive nuclei interspersed among immunonegative cells (Figure 2B). HPV-positive nuclei were primarily observed in koilocytes, appearing as granular or diffuse brown nuclear signals (Figure 2C). Basal and parabasal epithelial layers consistently lacked HPV immunoreactivity. No staining was detected in the underlying stroma (Figure 2D).

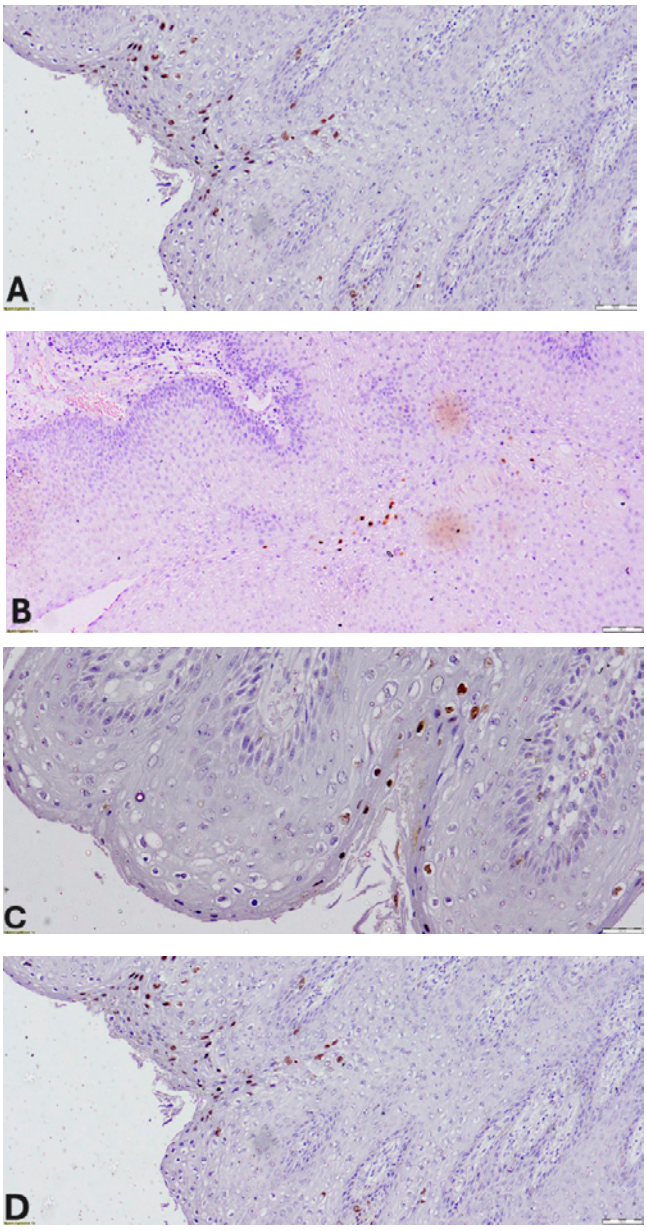
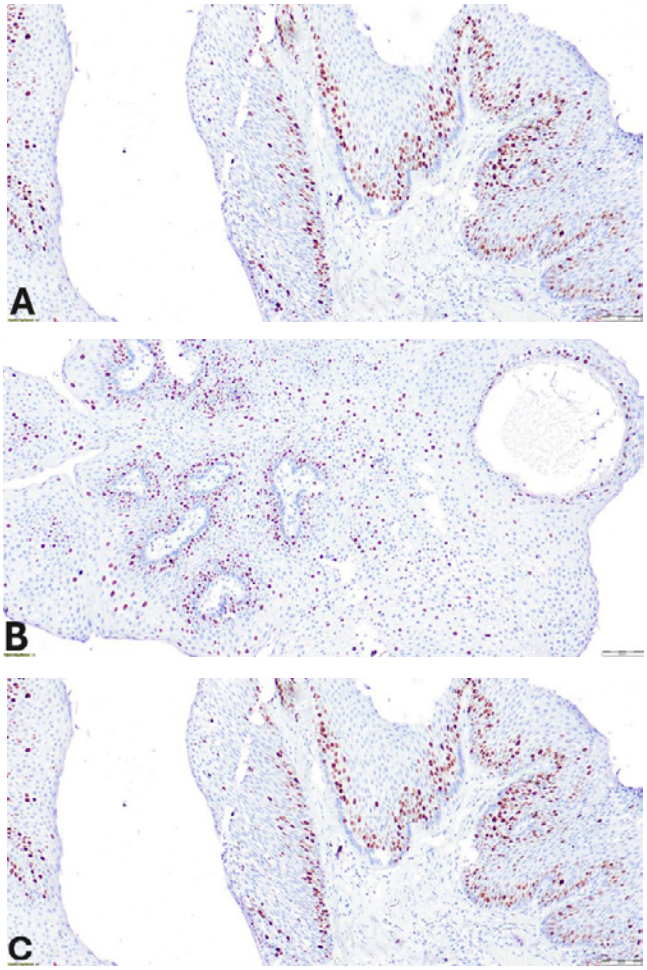


Figure 2. A. Suprabasal distribution of HPV-positive epithelial cells, limited to intermediate and superficial layers (×20). **B.** Mosaic pattern of HPV nuclear immunoreactivity within the stratified squamous epithelium (×10). **C.** Koilocytes exhibiting distinct nuclear HPV positivity with granular and diffuse staining (×40). **D.** Absence of HPV immunoreactivity in basal epithelial layers and underlying stroma (×20). (Immunohistochemistry for HPV, DAB chromogen.)

Proliferative activity assessed by Ki-67

Ki-67 immunohistochemistry revealed nuclear positivity in all cases, confirming the proliferative nature of recurrent laryngeal papillomatosis. Ki-67-positive nuclei were predominantly localized within the basal and parabasal epithelial layers (Figure 3A), with increased labeling in areas of epithelial thickening and prominent rete ridges (Figure 3B). The distribution of Ki-67 expression showed a clear gradient, with decreasing positivity toward the superficial epithelial layers (Figure 3C). Semi-quantitative analysis demonstrated heterogeneous proliferative indices, ranging from low to high scores (Figure 3D). Specifically, score 1 was observed in 8 cases, score 2 in 15 cases, and score 3 in 9 cases.



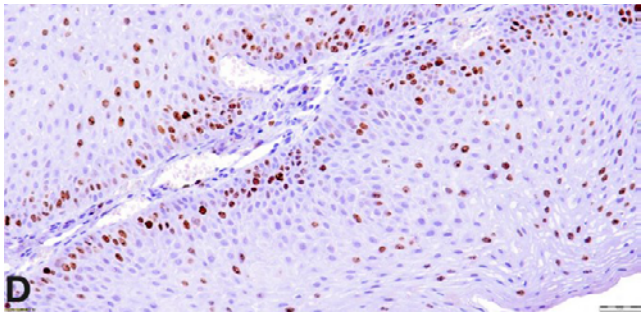
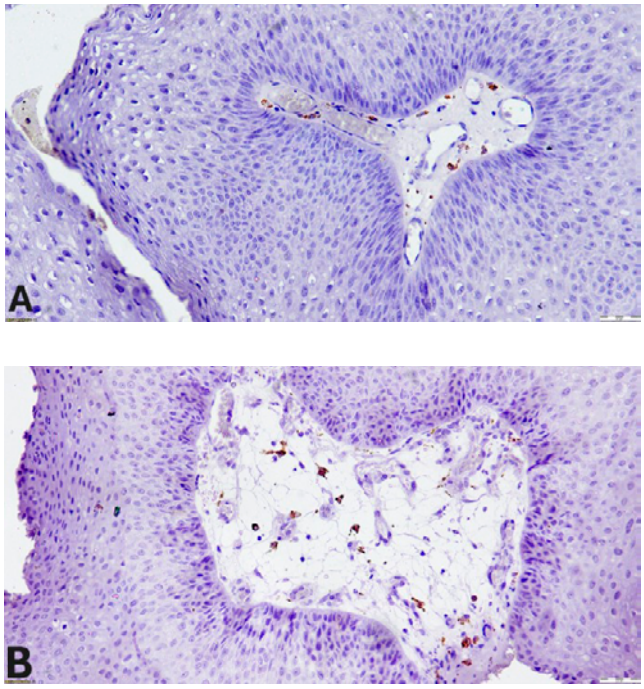


Figure 3. A. Nuclear Ki-67 positivity predominantly in basal and parabasal epithelial layers (×40). **B.** Increased Ki-67 expression in areas of epithelial thickening and prominent rete ridges (×10). **C.** Gradient distribution of Ki-67-positive nuclei, with decreasing labeling toward superficial epithelial layers (×10). **D.** Representative example of high proliferative index (Ki-67 score 3) (×40).

Chronic inflammatory infiltrate and CD68-positive macrophages

CD68 immunostaining highlighted macrophages within the stromal compartment of papillomatous lesions. CD68-positive cells were predominantly located in the subepithelial stroma, frequently arranged in a perivascular distribution (Figure 4A). Morphologically, macrophages exhibited large cell size, abundant granular cytoplasm, and eccentrically positioned nuclei (Figure 4B). Quantitative analysis revealed considerable variability in macrophage density, with counts ranging from 2 to 102 CD68-positive cells per high-power field (×20), with a mean value of 20.1 ± 4.58 cells per field (Figure 4C).



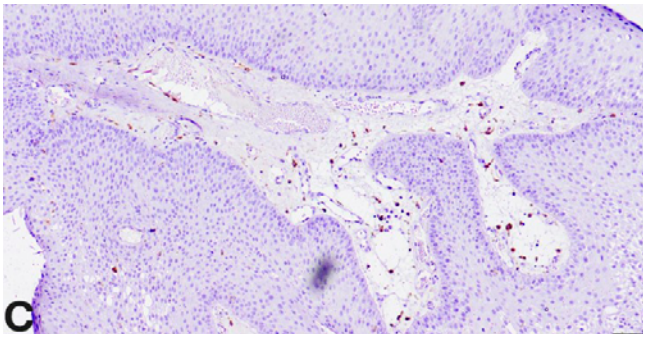


Figure 4. A. CD68-positive macrophages located predominantly in the subepithelial stroma, frequently in a perivascular distribution (×10) **B.** Activated macrophages exhibiting large size, abundant granular cytoplasm, and eccentrically positioned nuclei (×20). **C.** Variable density of CD68-positive macrophages within the stromal compartment (×20).

Microvascular density assessed by CD31

Immunohistochemical labeling for CD31 revealed a rich microvascular network in all examined specimens. Numerous CD31-positive vessels were observed within the stromal cores of papillary projections and in the subepithelial connective tissue (Figure 5A). The endothelial cells exhibited strong membranous and cytoplasmic staining, and the vascular structures displayed features of mature microvessels, including thin walls and well-defined lumina (Figure 5B). Microvessel density ranged from 8 to 39 CD31-positive vessels per field (×20), with a mean value of 17.31 ± 1.21 vessels per field.

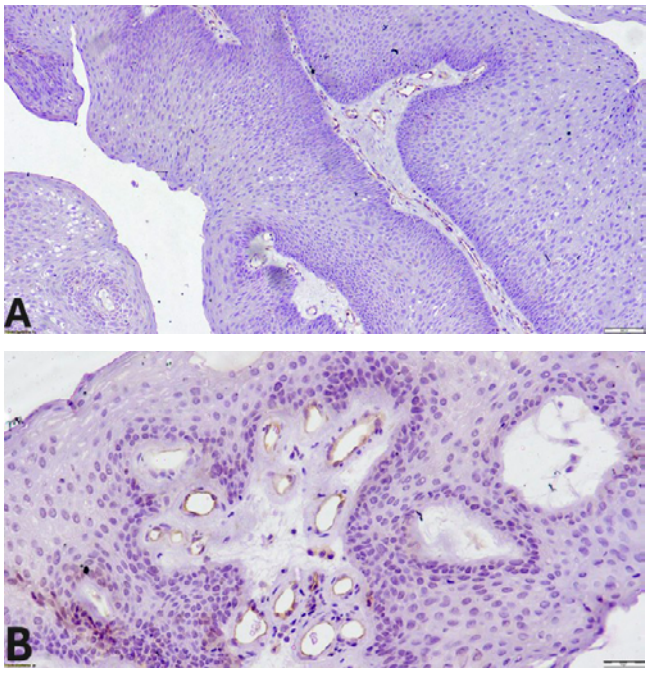


Figure 5. A. Numerous CD31-positive microvessels within the stromal cores of papillary projections and subepithelial connective tissue (×10). **B.** Mature microvessels with thin walls, well-defined lumina, and uniform endothelial lining highlighted by CD31 staining (×20).

No statistically significant correlation was observed between microvessel density and Ki-67 proliferative index ($r = -0.32$, $p > 0.05$).

VEGF-A expression and its association with angiogenesis

VEGF-A immunoreactivity was predominantly localized to the cytoplasm of epithelial cells, exhibiting a granular staining pattern (Figure 6A). Endothelial cells also demonstrated VEGF-A positivity in several cases. Only two cases were negative or weakly positive for VEGF-A, whereas the majority of lesions demonstrated clear expression. Moderate expression (scores 1–2) was observed in 19 cases, while strong expression (score 3) was identified in 11 cases (Figure 6B).

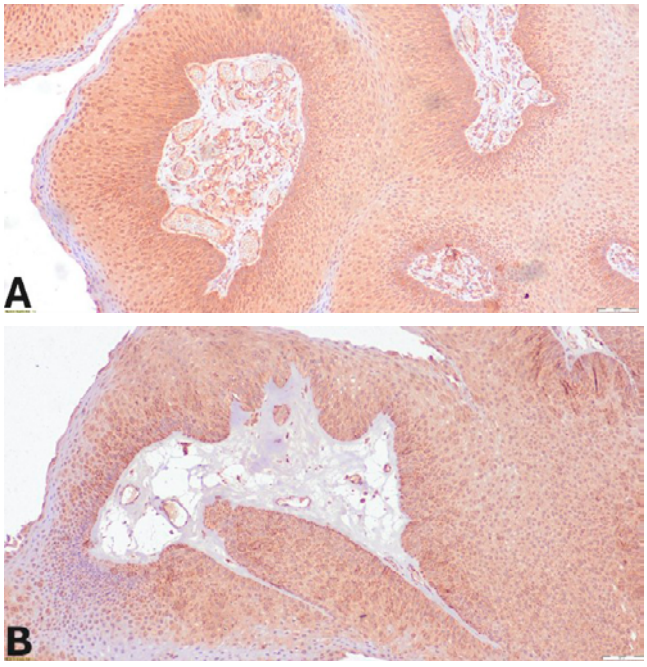


Figure 6. A. Granular cytoplasmic VEGF-A immunoreactivity in epithelial cells of papillomatous lesions (×10). **B** Comparative example of lower VEGF-A expression intensity (score 1) (×10).

A moderate positive correlation was identified between VEGF-A expression and microvessel density ($r = 0.368$), suggesting that increased VEGF-A expression is associated with enhanced angiogenesis in recurrent laryngeal papillomatosis.

DISCUSSION

Recurrent laryngeal papillomatosis (RLP) remains a challenging benign neoplastic disorder due to its unpredictable clinical course, high recurrence rate, and substantial impact on airway function and voice quality. Although infection with human papillomavirus (HPV) is widely accepted as the primary etiological factor, increasing evidence suggests that viral presence alone is insufficient to explain the biological behavior of the disease[1,8,11].

In the present study, histopathological evaluation confirmed the classical morphological features of RLP, including exophytic papillary architecture, fibrovascular stromal cores, epithelial acanthosis, and koilocytotic changes. Chronic inflammatory infiltrates, stromal edema, and variable degrees of fibrosis were frequently observed, reflecting the chronic and recurrent nature of the disease[1,4]. Importantly, all lesions preserved a non-invasive growth pattern, consistent with their benign classification, despite repeated surgical interventions.

HPV detection and epithelial distribution

HPV immunopositivity was identified in 21.9% of cases, a finding that aligns with previous reports demonstrating variable detection rates depending on the diagnostic technique employed[8,9,31]. The suprabasal and mosaic distribution of HPV-positive cells observed in our study is characteristic of productive HPV infection, in which viral replication occurs predominantly in differentiated epithelial layers (10,31). The absence of HPV immunoreactivity in basal and parabasal cells further supports the concept that viral persistence may occur at levels below immunohistochemical detection thresholds or in latent forms[11,32].

These findings reinforce the notion that HPV detection by immunohistochemistry reflects active viral protein expression rather than mere viral DNA presence, which may explain the relatively low positivity rate compared to molecular techniques such as PCR[9,31].

Proliferative activity and Ki-67 expression

All examined RLP lesions demonstrated Ki-67 positivity, confirming their proliferative nature. The predominance of Ki-67-positive nuclei within basal and parabasal epithelial layers, with a decreasing gradient toward the surface, resembles the physiological renewal pattern of stratified squamous epithelium, albeit at an amplified level[16,17]. This observation supports the concept that RLP represents a hyperproliferative epithelial disorder rather than a truly dysregulated neoplastic process.

The heterogeneous distribution of Ki-67 scores among cases suggests biological variability in proliferative potential, which may partly explain differences in clinical behavior and recurrence frequency[17,18,33]. However, the lack of correlation between Ki-67 expression and microvascular density in our cohort indicates that epithelial proliferation and angiogenesis may be regulated through partially independent mechanisms.

Chronic inflammation and macrophage involvement

CD68-positive macrophages were consistently identified within the stromal compartment of RLP lesions, predominantly in a subepithelial and perivascular distribution. Tumor-associated macrophages are known to play a dual role in tissue remodeling, immune regulation, and angiogenesis through the secretion of cytokines, growth factors, and matrix metalloproteinases[25,26,34].

The wide variability in macrophage density observed in our study reflects heterogeneity in the local inflammatory response. Chronic inflammation has been implicated in promoting viral persistence, epithelial proliferation, and stromal remodeling in RLP[12,14]. Although macrophage density did not show a direct statistical correlation with proliferative indices in our cohort, their strategic localization suggests a potential modulatory role in disease chronicity and recurrence.

Angiogenesis and microvascular density

Angiogenesis represents a key biological process sustaining lesion growth in RLP. CD31 immunostaining revealed a dense and well-organized microvascular network within the stromal cores of papillomatous projections. The morphological characteristics of these vessels—thin walls, uniform endothelium, and absence of atypia—are consistent with mature, functional microvessels rather than aberrant neovascular formations[19,22].

The increased microvascular density observed in RLP compared to normal laryngeal mucosa supports previous findings highlighting the angiogenic nature of papillomatous lesions[13,21]. However, the absence of a significant correlation between microvascular density and Ki-67 expression suggests that vascular supply alone may not directly dictate epithelial proliferative activity.

VEGF-A expression and its role in angiogenesis

VEGF-A was expressed in the vast majority of lesions, predominantly within the epithelial compartment and, to a lesser extent, in endothelial cells. VEGF-A is a

central mediator of angiogenesis and has been shown to promote endothelial proliferation, migration, and increased vascular permeability[20].

The moderate positive correlation identified between VEGF-A expression and microvascular density in our study supports the role of VEGF-A as a key angiogenic driver in RLP[13,21]. Lesions exhibiting high VEGF-A expression tended to display a denser microvascular network, suggesting that VEGF-mediated signaling contributes to sustaining lesion growth and recurrence.

These findings are consistent with emerging therapeutic approaches targeting angiogenesis in severe or refractory cases of RLP, including the use of anti-VEGF agents as adjuvant therapy[30,35].

Integrated pathogenic perspective

Taken together, our results support a multifactorial pathogenic model of RLP, in which HPV infection initiates epithelial alteration, while enhanced proliferative activity, chronic inflammation, and angiogenesis collectively sustain lesion persistence and recurrence. The lack of strong correlations among individual markers underscores the complexity of biological interactions within the papilloma microenvironment.

Comprehensive immunohistochemical profiling, as performed in this study, provides valuable insights into the biological behavior of RLP and may contribute to improved risk stratification and identification of potential therapeutic targets. Further studies integrating molecular analyses and clinical outcome data are warranted to better define prognostic markers and optimize management strategies.

CONCLUSIONS

Recurrent laryngeal papillomatosis is characterized by typical papillary histopathological architecture associated with enhanced epithelial proliferative activity, chronic stromal inflammation, and active angiogenesis, reflecting its biologically persistent and recurrent nature.

HPV immunohistochemical positivity was identified in a subset of cases and exhibited a predominantly suprabasal, mosaic distribution, supporting the role of productive viral infection while indicating that HPV expression alone does not fully explain disease behavior.

Angiogenesis represents a central pathogenic mechanism in recurrent laryngeal papillomatosis, as demonstrated by increased microvascular density and widespread VEGF-A expression, with a moderate positive correlation between VEGF-A levels and vascular proliferation.

Integrated histopathological and immunohistochemical evaluation of proliferative, inflammatory, and angiogenic markers provides valuable insight into the biological behavior of recurrent laryngeal papillomatosis and may contribute to improved prognostic assessment and the development of targeted therapeutic strategies.

Ethics Statement and Conflict of Interest Disclosures
Financial support and sponsorship: All authors have declared that no financial support was received from any organization for the submitted work.
Ethics Consideration: The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national laws. Written informed consent was provided by the patient participant in this study. This study was approved by the Institutional Research Board and Ethics Committee.
Conflict of interest: No known conflict of interest correlated with this publication.
Availability of data and materials: The data used and/ or analyzed throughout this study are available from the corresponding authors upon reasonable request.
Competing interests: The authors declared that they have no competing interests.
The use of generative AI and AI-assisted technologies: The authors did not use in this article generative AI and AI-assisted technologies.

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