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Association of histopathologic parameters with angiogenesis and endoglin in laryngeal squamous cell carcinoma

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ABSTRACT

Aims: Laryngeal squamous cell carcinoma (LSCC) is one of the most prevalent head and neck cancers, characterized by declining survival rates. Understanding prognostic factors is essential for improving patient outcomes, particularly in the context of angiogenesis. This study was designed to comprehensively evaluate the expression of endoglin (CD105) as a marker of angiogenesis in LSCC and to determine its potential association with microvascular density (MVD) and other histopathological parameters.

Methods: Forty-two previously untreated patients who underwent laryngectomy and neck dissection were retrospectively included in the current analysis. Regions of highest vascularization ("hot spots") were delineated, and MVD was assessed using CD105 immunostaining.

Results: Findings from this study reinforce the hypothesis that MVD might not constitute a reliable prognostic biomarker in LSCC, highlighting the complex and multifactorial nature of tumor angiogenesis.

Conclusion: MVD has been reported as an independent prognostic indicator in various malignancies; however, evidence regarding CD105 expression in LSCC remains extremely limited. Previous studies have yielded conflicting results concerning the prognostic significance of MVD in head and neck SCCs, underscoring the complexity of tumor biology. This study adds to the existing discourse, attributing discrepancies to the absence of tumor-specific markers, intratumoral heterogeneity, and methodological variations.

Keywords: Larynx, squamous cell carcinoma, endoglin, CD 105, angiogenesis

INTRODUCTION

LSCC is the second most common malignancy of the head and neck, and its 5-year survival rate continues to decline globally.[1] Neoplastic angiogenesis, a hallmark of cancer, plays a critical role in tumor growth and progression.[2] MVD has been widely reported as an independent prognostic factor in various human malignancies. However, the prognostic significance of MVD in head and neck SCC remains controversial.

Endoglin (CD105) is a highly specific marker of angiogenesis and has been implicated in the prognosis of several solid tumors.[3] Compared to other commonly utilized endothelial markers, CD105 offers greater specificity for detecting active angiogenesis.[4] Nonetheless, only a limited number of studies have examined the association between CD105 expression and clinical outcomes in LSCC.

Given the paucity of data on CD105 expression in LSCC, this study aims to investigate its relationship with key clinicopathological parameters.

METHODS

This study was conducted in 2002 at Haseki Training and Research Hospital. This study was prepared in 2002 as a thesis titled "The relationship of histopathological parameters with angiogenesis and endoglin in laryngeal squamous cell carcinoma." All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Patients who underwent total or subtotal laryngectomy with neck dissection for previously untreated primary LSCC were included. Clinical data, including age, sex, histological and nuclear grade, primary tumor (T)

classification, and nodal (N) status, were retrospectively retrieved from institutional medical records.

Hematoxylin and eosin (H&E)-stained slides were reviewed for each case, and blocks without hemorrhage or necrosis were selected for further analysis. For immunohistochemistry, 4 µm-thick sections were prepared from the selected paraffin-embedded blocks and stained with CD105 antibodies to assess MVD.

Areas of highest vascularization ("hot spots") were identified under low-power magnification. Within these regions, three microscopic fields were examined at ×200 and ×400 magnification using an Olympus BX50 dual-head microscope. Evaluations were performed simultaneously by two experienced pathologists to minimize interobserver variability, and the highest microvessel counts were recorded.

Regions exhibiting inflammation, necrosis, or adjacent normal tissue were excluded from microvessel evaluation. A single CD105-positive endothelial cell or a cluster of endothelial cells clearly demarcated from tumor cells and surrounding stroma was considered a microvessel. Clusters of positively stained endothelial cells were counted as individual microvessels regardless of lumen formation[5] (**Figure 1, 2**).

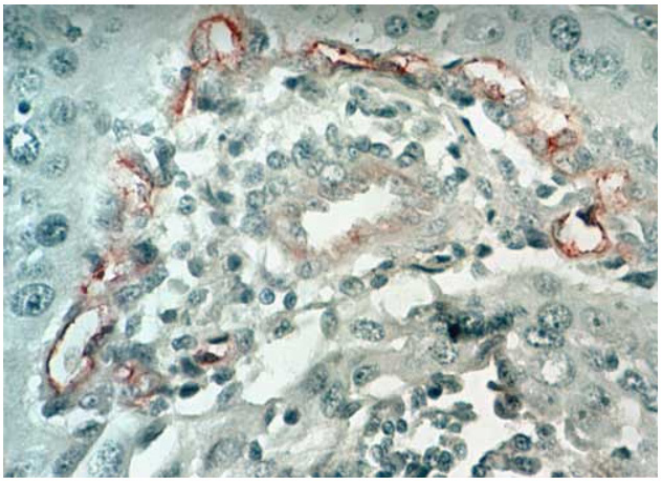

Figure 1. Proliferating microvessels between the tumor and stroma that stain positively with CD105 (CD105 x500)

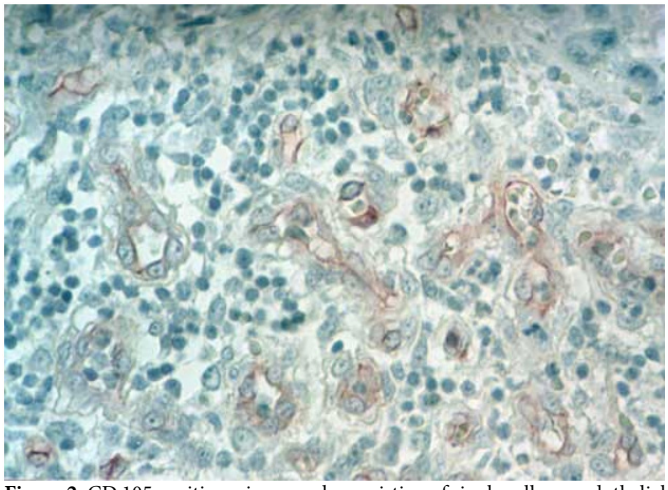

Figure 2. CD 105 positive microvessels consisting of single cells or endothelial cell clusters around the tumor. There is lymphoplasmacytic cell infiltration in between (CD105 x500)

Statistical Analysis

The data analyses were performed using SPSS software (version 22.0; IBM Corp.). Correlations between variables were assessed using Spearman's rank correlation coefficient. A p-value <0.05 was considered statistically significant.

RESULTS

Patient Demographics and Clinical Features

The study included 42 patients (41 males, 1 female) with a mean age of 58.6 years (range: 38–80). Fifteen patients (35.7%) were ≤60 years, and 27 (64.3%) were older than 60 years. Neck dissection was performed in 34 patients; 17 cases (50%) had lymph node metastasis.

Tumor Characteristics

- **Tumor grade:** Grade I in 11 patients (26.2%) (**Figure 3**), grade II in 21 (50%), grade III in 10 (23.8%) (**Figure 4**).

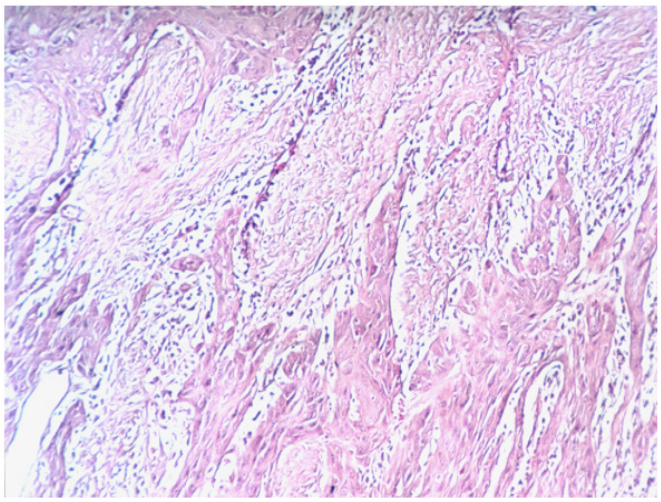

Figure 3. Grade I squamous cell carcinoma, mild lymphoplasmacytic cell infiltration (H&Ex 125)

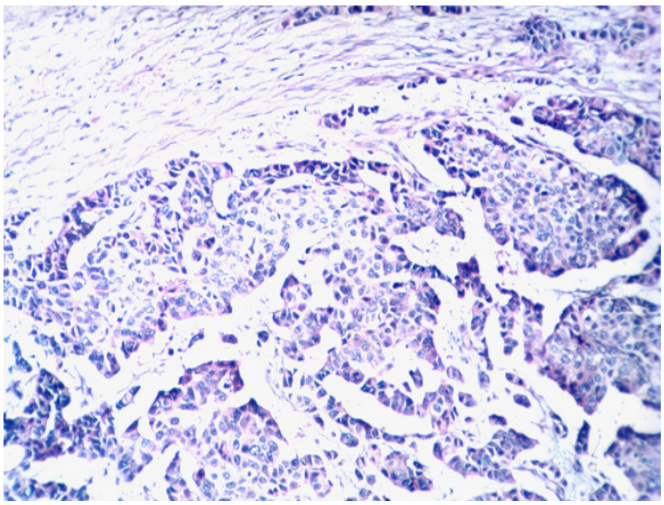

Figure 4. Grade III squamous cell carcinoma (H&E x125)

- **Tumor size:** ≤2.0 cm in 9 cases (21.4%), 2.1–4.0 cm in 23 (54.8%), ≥4.1 cm in 10 (23.8%).
- **Tumor localization:** Supraglottic (n=27, 64.3%), glottic (n=9, 21.4%), infraglottic/transglottic (n=6, 14.3%).

Histopathological Features

Lymphoplasmacytic infiltration was mild in 14 patients (33.3%) (**Figure 3**) and severe in 28 (66.7%). Desmoplastic stromal response was mild in 19 cases (45.2%) and intense in 23 (54.8%). Tumor invasion was expansile in 19 (45.2%) and infiltrative in 23 (54.8%). Increased desmoplasia correlated with infiltrative invasion (**Table 1**).

Table 1. Histopathological features and invasion patterns

Feature	Mild	Intense
Lymphoplasmacytic infiltration	14 (33.3%)	28 (66.7%)
Desmoplastic stromal response	19 (45.2%)	23 (54.8%)
Tumor invasion pattern	19 (45.2%)	23 (54.8%)

Microvessel Density (MVD)

MVD was assessed at ×200 (MVD200) and ×400 (MVD400) magnifications using CD105 immunostaining. Statistical analysis revealed:

- No significant correlation between tumor grade and MVD200 or MVD400 (p=0.350, p=0.234) (**Figure 5, 6**).

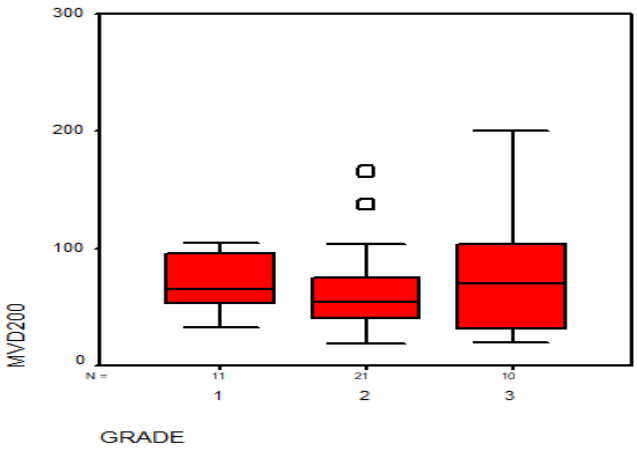

Figure 5. Distribution of MVD200 by grade

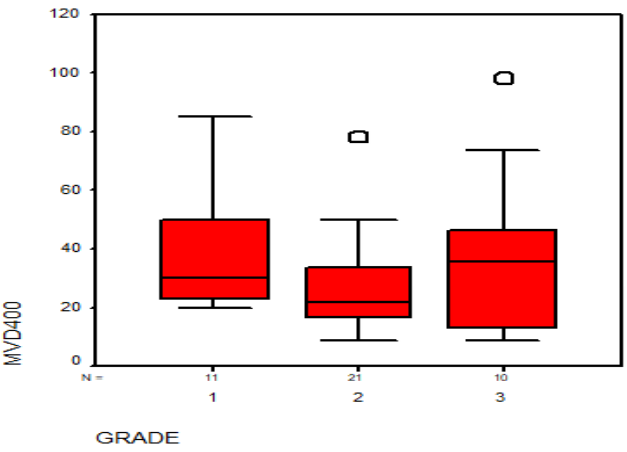

Figure 6. Distribution of MVD400 by grade

- No significant association between tumor size and MVD200 or MVD400 (p=0.498, p=0.338).
- Lymphoplasmacytic infiltration showed no correlation with MVD200 or MVD400 (p=0.367, p=0.479).
- Desmoplastic reaction: no correlation (p=0.263, p=0.259).
- Lymph node status: no correlation (p=0.970, p=0.109).
- Tumor localization: no association (p=0.374, p=0.471).
- Tumor invasion pattern: no correlation (p=0.221, p=0.490) (**Table 2**).

Table 2. Correlation of clinicopathological parameters with MVD at ×200 and ×400

Clinicopathological parameter	MVD200 (p-value)	MVD400 (p-value)
Tumor grade	0.350	0.234
Tumor size	0.498	0.338
Lymphoplasmacytic infiltration	0.367	0.479
Desmoplastic stromal response	0.263	0.259
Lymph node status	0.970	0.109
Tumor localization	0.374	0.471
Tumor invasion pattern	0.221	0.490

All p-values were calculated using Spearman's rank correlation coefficient. A p-value <0.05 was considered statistically significant

DISCUSSION

Despite significant advancements in diagnostic and therapeutic approaches, the survival rate of patients with LSCC has shown little improvement over recent decades. Traditional prognostic indicators such as TNM stage and histologic grade alone are insufficient for accurately predicting clinical outcomes, necessitating the identification of additional biomarkers to better stratify patients based on their risk of recurrence.[6] This study investigated the association between CD105 expression, MVD and key clinicopathological parameters in LSCC. Angiogenesis plays a critical role in tumor growth, invasion, and metastatic dissemination. CD105 is a well-recognized marker of neovascular endothelial cells and is markedly overexpressed in newly formed blood vessels within and around tumor tissue.

The prognostic significance of MVD remains controversial, with conflicting reports in the literature. In head and neck SCCs, a strong correlation has been demonstrated between MVD measured at ×200 and ×400 magnifications (p<0.001). However, in our study, no statistically significant relationship was found between MVD and tumor grade at either magnification (p=0.350 and p=0.234, respectively).

Our findings contrast with those of Kupisz et al.[7] and Beatrice et al.,[8] who reported a positive association between histologic grade and angiogenesis. Conversely, our results align with the observations of Burian et al.[9] and Hjalmar et al.,[10] who similarly noted no significant correlation between grade and angiogenesis. One plausible explanation for this discrepancy is the predominance of grade II tumors in our cohort, which may reflect the subjectivity of histologic grading. Furthermore, the relatively small sample size could have limited the statistical power to detect subtle associations.

Consistent with previous studies by Szafarowski et al.,[5] Zvrko et al.,[4] and Hjalmar et al.,[10] we also found no correlation between MVD/CD105 expression and patient demographics (age, sex) or other histopathologic characteristics. Comparable

findings have been reported in studies of breast carcinoma, renal cell carcinoma, and esophageal squamous cell carcinoma, suggesting that angiogenesis may not uniformly correlate with histologic grade across different tumor types.[11,12]

Similarly, no significant association was observed between tumor diameter and MVD (p=0.498 and p=0.338). This is consistent with the findings of Dray et al.[13] in head and neck cancer, as well as reports from studies involving breast, esophageal, and renal carcinomas.[11,12]

The relationship between peritumoral inflammatory infiltration and angiogenesis remains controversial. While our results showed no correlation (p=0.367 and p=0.479), Hjalmar et al.[10] suggested that increased lymphoplasmacytic infiltration might enhance angiogenesis via the release of pro-angiogenic mediators. Such differences may be attributable to variability in tumor location, sample size, and inflammatory response patterns.

Similarly, no significant correlations were found between desmoplastic stromal response and MVD (p=0.263 and p=0.259), or between tumor localization and MVD (p=0.374 and p=0.471). These observations are in line with findings by Hagedorn et al.[10] and Murray et al.,[14] indicating that angiogenesis in LSCC may occur independently of anatomic subsite or stromal reaction.

With respect to lymph node status, no significant association with MVD was noted (p=0.970 and p=0.109). However, prior studies, including those by Murray et al.[14] and Marioni et al.,[15] have demonstrated a positive correlation between angiogenesis and nodal metastasis, and Shpitzer et al.[16] reported that neovascularization served as an independent predictor of nodal involvement in early-stage tongue carcinoma. These discrepancies highlight the complexity of tumor biology and the need for larger, more homogeneous study cohorts.

Finally, no significant correlation was observed between invasion pattern (expansile vs. infiltrative) and MVD (p=0.221 and p=0.490). Although infiltrative tumors tended to exhibit higher MVD values, this trend did not reach statistical significance, likely due to limited case numbers. Similarly, age was not associated with angiogenesis, corroborating prior reports in LSCC and oral cavity SCC.[8,13,17]

CONCLUSION

This study found no significant correlation between CD105-assessed MVD and various histopathologic features in LSCC. These results indicate that CD105 expression may not serve as a reliable indicator of tumor angiogenesis in LSCC. Further prospective studies with larger sample sizes and standardized protocols are required to validate these findings.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was prepared in 2002 as a thesis titled "The relationship of histopathological parameters with angiogenesis and endoglin in laryngeal squamous cell carcinoma."

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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