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Original Research Article

Immunohistochemical Analysis of Ki-67 and CD34 in Benign and Malignant Salivary Gland Tumors

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Abstract:

Background: Salivary gland tumors constitute a heterogeneous group of neoplasms with diverse histopathological features, making accurate diagnosis and prognostic assessment challenging. Although histopathology remains the diagnostic gold standard, immunohistochemical markers may improve diagnostic precision and provide insight into tumor biology. Ki-67 is a well-established marker of cellular proliferation, while CD34 is a marker of tumor angiogenesis. This study evaluated the expression of Ki-67 and CD34 in benign and malignant salivary gland tumors and assessed their diagnostic utility.

Methods: A prospective observational study was conducted on 77 histologically confirmed salivary gland tumors received in the Department of Pathology, M.K.C.G. Medical College, Berhampur, over a two-year period (October 2015–September 2017). Routine histopathological examination was performed using hematoxylin and eosin staining, followed by immunohistochemical analysis with Ki-67 and CD34 antibodies. Ki-67 labelling index and CD34-assessed MVD (Micro-Vessel Density) were evaluated and correlated with clinicopathological parameters and tumor type.

Results: Among the 77 tumors, 45 (58.44%) were benign and 32 (41.56%) were malignant. Pleomorphic adenoma was the commonest benign tumor (46.75%), while mucoepidermoid carcinoma was the most frequent malignant tumor (25.97%). Ki-67 positivity was observed in 90.62% of malignant and 31.11% of benign tumors, with significantly higher mean labeling indices in malignant tumors (26.56±16.08 vs. 3.57±3.75). CD34-assessed MVD was also significantly higher in malignant tumors (27.59±12.00) than benign tumors (7.40±6.49). Ki-67 expression correlated with patient age but not with gender, tumor size, or site. CD34 MVD showed a significant association with age but no correlation with Ki-67 labelling index.

Conclusion: Ki-67 and CD34 are valuable adjunctive immunohistochemical markers in salivary gland tumors. Increased Ki-67 expression reflects enhanced proliferative activity, while higher CD34 microvessel density indicates increased angiogenesis in malignant lesions. Their combined assessment can aid in differentiating benign from malignant salivary gland tumors and provide useful information regarding tumor behaviour and prognosis.

Keywords: Salivary Gland Tumors, Ki-67, CD34, Immunohistochemistry, Cell Proliferation, Microvessel Density, Angiogenesis.

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Introduction

Salivary glands play a vital role in maintaining oral and systemic health through the secretion of saliva, which facilitates digestion, lubrication, speech, mastication, and immune defence. Saliva contains digestive enzymes and antibodies that help prevent microbial colonization and protect the oral cavity from infections. However, salivary glands are susceptible to a variety of pathological conditions, including metabolic disorders, infections, cysts, trauma, and neoplasms, all of which may impair their normal function.[1]

Salivary gland diseases are often asymptomatic during the early stages, except in acute infections, resulting in delayed diagnosis and presentation with advanced disease, which complicates management and adversely affects prognosis.[2] Salivary gland neoplasms constitute approximately 2–7% of head and neck tumors and less than 1% of all human malignancies. Their low incidence, coupled with considerable histological diversity and overlapping morphological features, makes diagnosis challenging even for experienced pathologists.[3–5]

Histopathological examination using H&E (Hematoxylin and Eosin) staining remains the gold standard for diagnosing salivary gland tumors. Nevertheless, IHC (immunohistochemistry) has emerged as a valuable adjunct by improving diagnostic accuracy and providing information on cellular differentiation, proliferation, and protein expression that cannot always be assessed by routine histology.[6]

Tumor cell proliferation is a key indicator of biological behaviour and prognosis. Among various proliferation markers, Ki-67 is widely accepted because of its strong association with the active phases of the cell cycle and absence in quiescent cells. Increased Ki-67 expression has been correlated with tumor aggressiveness, recurrence, and poor prognosis and is considered superior to mitotic index in evaluating salivary gland tumors.[7]

Tumor progression and metastasis also depend on angiogenesis, which supplies nutrients and oxygen to proliferating tumor cells. Microvessel density, assessed using the endothelial marker CD34, is a widely accepted measure of angiogenic activity and has been associated with tumor stage, metastatic potential, recurrence, and survival in several malignancies, including salivary gland tumors.[8]

Aims and Objectives: The present study aims to evaluate the immunohistochemical expression of Ki-67 and CD34 in various salivary gland tumors and to assess their potential role as diagnostic biomarkers. The objectives of the study are to analyze the expression patterns of Ki-67 and CD34 across different types of salivary gland tumors and to evaluate their diagnostic utility in distinguishing benign from malignant salivary gland neoplasms.

Materials and Methods

Study Design: The present study was a hospital-based observational cross-sectional study conducted in the Department of Pathology, M.K.C.G. Medical College, Berhampur, over a period of two years, from October 2015 to September 2017**.** A total of 77 histopathologically confirmed cases of benign and malignant salivary gland tumors received from the Departments of Surgery and ENT were included in the study. The cases were evaluated histopathologically, and immunohistochemical analysis was performed to assess the expression of Ki-67 and CD34 in different salivary gland tumors and to determine their diagnostic significance in differentiating benign from malignant lesions.

Inclusion and Exclusion Criteria: The study included all patients, irrespective of age and sex, who underwent surgical excision for salivary gland lesions and were histopathologically diagnosed with salivary gland tumors. Patients with both benign and malignant salivary gland neoplasms were eligible for inclusion. Cases of non-neoplastic salivary gland lesions were excluded from the study.

Data Collection Tools

Data were collected using a structured proforma to record patients' demographic details, clinical history, examination findings, and routine investigation reports. Surgical specimens of histologically confirmed salivary gland tumors served as the primary study material. Histopathological examination was performed on hematoxylin and eosin (H&E)-stained tissue sections, followed by immunohistochemical evaluation using Ki-67 and CD34 antibodies to assess cellular proliferative activity and microvessel density, respectively. Relevant microscopic findings and immunohistochemical staining results were documented systematically for analysis.

Data Collection Procedure: After obtaining the surgical specimens, tissues were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned, and stained with H&E for histopathological diagnosis. Subsequently, representative tissue sections were subjected to immunohistochemistry using Ki-67 and CD34 antibodies following standard laboratory protocols. Ki-67 labeling index was calculated by assessing positively stained nuclei in selected high-power fields, while CD34 immunostaining was used to determine microvessel density by counting stained microvessels in vascular "hot spots." All clinical, histopathological, and immunohistochemical findings were recorded in the study proforma and analyzed statistically.

Statistical Analysis: The data were compiled, categorized, and analyzed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA)**.** Descriptive statistics were used to summarize the data. The chi-square test was applied to compare the proportions of benign and malignant tumors. The independent samples t-test was used to compare the mean Ki-67 labelling index (LI) and microvessel density between benign and malignant lesions. One-way analysis of variance (ANOVA) was used to compare the different histopathological groups and their association with clinical parameters. A p-value < 0.05 was considered statistically significant.

Results

The present study was conducted over a period of two years (October 2015 – September 2017) in the Department of Pathology, MKCG Medical College, Berhampur. A total of 77 cases were analysed and the observations are summarised below.

Table 1: Distribution of Tumors According to Histological Type and Gender

Histological Type		No. of Cases	Male	Female
Benign	Pleomorphic Adenoma	36 (46.75%)	12 (33.33%)	24 (66.67%)
	Warthin's Tumor	06 (07.79%)	03 (50.00%)	03 (50.00%)
	Basal Cell Adenoma	03 (03.89%)	02 (66.67%)	01 (33.33%)
	Total Benign	**45 (58.44%)**	**17 (37.78%)**	**28 (62.22%)**
Malignant	Mucoepidermoid Carcinoma	20 (25.97%)	09 (45.00%)	11 (55.00%)
	Adenoid Cystic Carcinoma	05 (06.49%)	02 (40.00%)	03 (60.00%)
	Acinic Cell Carcinoma	03 (03.89%)	01 (33.33%)	02 (66.67%)
	Salivary Duct Carcinoma	01 (01.29%)	-	01 (100%)
	Adenocarcinoma NOS	01 (01.29%)	-	01 (100%)
	Oncocytic Papillary Cystadenocarcinoma	01 (01.29%)	01 (100%)	-
	Lymphoepithelial Carcinoma	01 (01.29%)	-	01 (100%)
	Total Malignant	**32 (41.56%)**	**13 (40.62%)**	**19 (59.38%)**
Overall Total		**77 (100%)**	**30 (38.97%)**	**47 (61.03%)**

Table 1 illustrates the distribution of the 77 salivary gland tumors according to histological type and gender. Of the total cases, 45 (58.44%) were benign and 32 (41.56%) were malignant. Pleomorphic adenoma was the single most common tumor overall and the most common benign lesion, accounting for 46.75% (n=36) of all cases, while mucoepidermoid carcinoma (n=20) was the most common malignant tumor, followed by adenoid cystic carcinoma. Females (61.03%) outnumbered males (38.97%) overall, and this female preponderance was observed in both benign and malignant groups; basal cell adenoma and adenoid cystic carcinoma were the only tumors with a male preponderance, and Warthin's tumor showed an equal gender distribution.

Table 2: Distribution of Cases According to Age Group

Age Group (years)	Benign	Malignant	Total
10-19	05	03	08 (10.38%)
20-29	12	05	17 (22.07%)
30-39	12	06	18 (23.37%)
40-49	09	09	18 (23.37%)
50-59	06	03	09 (11.68%)
60-69	01	04	05 (06.49%)
70-79	00	01	01 (01.29%)
80-89	00	01	01 (01.29%)
Total	**45**	**32**	**77 (100%)**

Table 2 depicts the age distribution of the study population, which ranged from 11 to 85 years, with a mean age of 38.31 years. The majority of patients (46.75%, n=36) belonged to the 30–49-year age group. Of the 77 cases, 57.14% (n=44) were below 40 years and 42.86% (n=33) were above 40 years. Notably, within the 60–89-year age group, 6 of 7 cases (85.71%) were malignant, suggesting a trend toward malignancy in older patients.

Table 3: Distribution of Cases According to Site and Size of Tumor

Site of Tumor	Benign	Malignant	Total
Parotid gland	27 (60.00%)	20 (62.50%)	47 (61.03%)
Submandibular gland	07 (15.55%)	05 (15.62%)	12 (15.59%)
Minor salivary gland	11 (24.45%)	07 (21.88%)	18 (23.38%)
Total	**45 (100%)**	**32 (100%)**	**77 (100%)**
Size of Tumor	**Benign**	**Malignant**	**Total**
≤ 3 cm	19 (42.23%)	11 (34.38%)	30 (38.96%)
> 3 cm	26 (57.77%)	21 (65.62%)	47 (61.03%)
Total	**45 (100%)**	**32 (100%)**	**77 (100%)**

Table 3 shows the anatomical distribution and size profile of the tumors. The majority of tumors (59 of 77 cases) arose in the major salivary glands, with the parotid gland being the most common site overall

(61.03%), followed by the minor and submandibular glands; this pattern held for both benign (60.00%) and malignant (62.50%) parotid lesions. With regard to size, 47 tumors (61.03%) measured more than 3 cm and 30 tumors (38.96%) measured 3 cm or less. Larger tumors (>3 cm) predominated in both the malignant (65.62%) and benign (57.77%) groups.

Table 4: Ki-67 Labelling Index (Quantitative and Qualitative) in Different Tumor Types

Group	Type	n	Min (%)	Max (%)	Mean Ki-67 LI (%)	Ki-67 Positive n (%)	Ki-67 Negative n (%)
Benign	PA	36	00	18	03.44±3.94	10 (27.77%)	26 (72.23%)
	WT	06	0.5	08	03.41±2.97	02 (33.33%)	04 (66.67%)
	BA	03	02	08	05.50±3.10	02 (66.67%)	01 (33.33%)
	Total	**45**	**00**	**18**	**03.57±3.75**	**14 (31.11%)**	**31 (68.89%)**
Malignant	MEC	20	03	60	25.10±14.70	18 (90.00%)	02 (10.00%)
	AdCC	05	10	36	23.20±10.82	05 (100%)	00
	ACC	03	03	20	13.66±9.29	02 (66.67%)	01 (33.33%)
	ADK NOS	01	-	-	55.00	01 (100%)	00
	OPCCa	01	-	-	38.00	01 (100%)	00
	SDC	01	-	-	30.00	01 (100%)	00
	LEC	01	-	-	68.00	01 (100%)	00
	Total	**32**	**03**	**68**	**26.56±16.08**	**29 (90.62%)**	**03 (9.38%)**
Overall Total		**77**	**00**	**68**	**13.12±15.60**	**43 (55.84%)**	**34 (44.16%)**

Table 4 presents both the quantitative and qualitative Ki-67 expression across tumor types. The mean Ki-67 labelling index (LI) was markedly higher in malignant tumors (26.56±16.08%) than in benign tumors (3.57±3.75%). Among benign lesions, basal cell adenoma showed the highest mean Ki-67 LI (5.50±3.10%), while pleomorphic adenoma and Warthin's tumor showed comparable, lower values (3.44±3.94% and 3.41±2.97% respectively); the difference among benign subtypes was not statistically significant (P=0.6663). Among malignant tumors, lymphoepithelial carcinoma showed the highest Ki-67 LI (68%), while acinic cell carcinoma showed the lowest (13.66±9.29%); mucoepidermoid carcinoma showed a wide range (3–60%, mean 25.10±14.70%). No significant difference in Ki-67 LI was found among the three most common malignant subtypes (P=0.4197). Qualitatively, Ki-67 positivity was seen in 43 of 77 cases (55.85%) overall - in 90.62% of malignant tumors versus only 31.11% of benign tumors, a highly significant difference (P=0.00001).

Table 5: Correlation of Ki-67 Labelling Index with Clinical Parameters

Clinical Parameter	Category	Ki-67 Positive n (%)	Ki-67 Negative n (%)	P value	Mean Ki-67 LI (%)	P value
Gender	Male	18 (60.00%)	12 (40.00%)	0.5573	13.58±14.27	0.8407
	Female	25 (53.19%)	22 (46.81%)	0.5573	12.84±16.54	0.8407
Age group	≤ 40 years	15 (34.10%)	29 (65.90%)	0.0009	07.57±11.99	0.0003
	> 40 years	28 (84.84%)	05 (15.16%)	0.0009	20.30±17.10	0.0003
Tumor Size	≤ 3 cm	16 (53.33%)	14 (46.67%)	0.7229	11.80±14.53	0.5553
	> 3 cm	27 (57.45%)	20 (42.55%)	0.7229	13.97±16.35	0.5553
Tumor site	Major SG	34 (57.63%)	25 (42.37%)	0.5683	13.69±15.91	0.5680
	Minor SG	09 (50.00%)	09 (50.00%)	0.5683	11.27±14.83	0.5680

Table 5 summarises the correlation between Ki-67 expression and clinical parameters. Ki-67 positivity and mean Ki-67 LI both showed a statistically significant association with patient age: 84.84% of patients above 40 years were Ki-67 positive compared with 34.10% of patients aged 40 years or below (P=0.0009), and the mean Ki-67 LI was correspondingly higher in the older age group (20.30±17.10% vs. 07.57±11.99%, P=0.0003). No significant correlation was observed between Ki-67 expression and gender, tumor size, or tumor site.

Table 6: CD34 Microvascular Density (MVD) in Different Tumor Types

Group	Type	n	Min MVD	Max MVD	Mean CD34 MVD (/mm²) - P value
Benign	PA	36	1.25	28.93	06.18±6.15
	WT	06	7.54	22.64	13.83±6.11
	BA	03	05.03	12.58	09.22±3.84
	Total	**45**	**1.25**	**28.93**	**07.40±6.49 (P=0.0210)**
Malignant	MEC	20	12.58	40.25	25.84±8.82
	AdCC	05	16.35	33.96	23.89±7.27
	ACC	03	15.09	20.12	18.02±2.61
	ADK NOS	01	-	-	38.99
	OPCCa	01	-	-	55.35
	SDC	01	-	-	32.70
	LEC	01	-	-	65.41
	Total	**32**	**12.58**	**65.41**	**27.59±12.00 (P=0.3191)**
Overall Total		**77**	**1.25**	**65.41**	**15.79±13.54**

Table 6 outlines CD34 MVD across tumor types. The mean MVD was substantially higher in malignant tumors (27.59±12.00/mm²) than in benign tumors (7.40±6.49/mm²). Among benign lesions, Warthin's tumor showed the highest MVD (13.83/mm²) and this difference among benign subtypes was statistically significant (P=0.0210). Among malignant lesions, lymphoepithelial carcinoma showed the highest MVD (65.41/mm²), while no significant difference in MVD was found among the three most common malignant subtypes (P=0.3191).

Table 7: Correlation of CD34 Microvascular Density with Clinical Parameters

Clinical Parameter	Category (n)	Mean CD34 MVD (/mm²)	P value
Gender	Male (30)	15.03±13.53	0.5433
	Female (47)	16.97±13.70	0.5433
Age Group	≤ 40 years (44)	10.14±11.68	0.0001
	> 40 years (33)	23.32±12.24	0.0001
Tumor Size	≤ 3 cm (30)	13.24±11.83	0.1883
	> 3 cm (47)	17.42±14.41	0.1883
Tumor Site	Major SG (59)	16.71±14.03	0.2841
	Minor SG (18)	12.78±11.66	0.2841

Table 7 reveals the relationship between CD34 MVD and clinical parameters. A statistically significant correlation was observed between patient age and MVD, with patients above 40 years showing a markedly higher mean MVD (23.32±12.24/mm²) than those aged 40 years or below (10.14±11.68/mm², P=0.0001). MVD did not correlate significantly with gender, tumor size, or tumor site.

Table 8: Correlation Between Ki-67 Labelling Index and CD34 Microvascular Density

Tumor Category	No. of Cases	Mean Ki-67 LI (%)	Mean CD34 MVD (/mm²)
Benign	45	03.57±3.75	07.40±6.49
Malignant	32	26.56±16.08	27.59±12.00
P value	**-**	**0.0001**	**0.0001**
Marker	**Mean Value**	**No. of Cases**	**P value**
Ki-67 LI (%)	13.12±15.60	77	0.2585
CD34 MVD (/mm²)	15.79±13.54	77	

Table 8 compares Ki-67 LI and CD34 MVD between benign and malignant groups and examines their correlation across all cases. Both markers were significantly higher in malignant tumors than in benign tumors (Ki-67 LI: 26.56±16.08% vs. 3.57±3.75%; CD34 MVD: 27.59±12.00/mm² vs. 7.40±6.49/mm²; P=0.0001 for both), confirming that both proliferative activity and microvascular density increase with malignant transformation. However, when Ki-67 LI and CD34 MVD were correlated with each other across all 77 tumors, no statistically significant correlation was found between the two markers (P=0.2585).

Discussion

Salivary gland neoplasms form a rare but markedly heterogeneous group, whose diagnosis is often complicated by overlapping morphological features between subtypes. This diversity has repeatedly prompted revisions of the histological grading

system and description of new tumour subtypes,[4] although only adenoid cystic carcinoma, mucoepidermoid carcinoma and carcinoma ex pleomorphic adenoma display a histologic spectrum broad enough to permit meaningful grading.[9] The variety of subtypes is largely attributed to the histogenetic origin of the tumours.[10] Batsakis et al., (1977) proposed that stem cell progenitors in the proximal and distal salivary duct system determine tumour origin, with mucoepidermoid, adenoid cystic and acinic cell carcinomas arising from excretory duct, intercalated duct and acinar cells, respectively.[11] Since salivary carcinomas can metastasize to distant sites and must be considered in the differential of tumours of unknown primary,[12] immunohistochemistry has become central to their diagnosis and classification, beginning with early studies of keratin, vimentin and desmin.[13] Subsequently, antibodies to cell-cycle antigens such as PCNA and Ki-67 were introduced to assess proliferative fraction and histogenesis, proving useful both as prognostic indicators and as diagnostic adjuncts for distinguishing tumour types.[14]

Benign tumours characteristically show low S-phase and G2/M-phase fractions compared with malignant tumours, a difference chiefly explained by variation in the duration of the G1 phase; Horri et al., (1998) suggested that a prolonged G1 phase accounts for the comparatively high Ki-67 fraction in benign lesions.[15] Angiogenesis, the second proliferative parameter studied, is a multistep process of basement-membrane degradation, endothelial migration, proliferation and lumen formation,[16] essential for nutrition of both benign and malignant tumour tissue. Tumour cells release angiogenic and antiangiogenic factors governing neovascularization,[7] and immunohistochemical counting of microvessels has become the standard method of assessing angiogenic activity, most often using the pan-endothelial marker CD34, which stains both newly formed and pre-existing vessels. Since metabolic demand is the principal driver of vessel density,[17] high vascular density is associated with increased metastatic potential and reduced survival.

Age in this series ranged from 11 to 85 years, with peak incidence between 20 and 49 years, consistent with Kaza et al.,[7] however, reported a predominance beyond 40 years. Benign tumours outnumbered malignant tumours in every age group, though the proportion of malignancy rose with age. A female preponderance (M:F 1:1.56) was noted, as also reported by Luukkaa et al., (2006)[18] found a male predominance - a discrepancy possibly reflecting population differences and women's greater tendency to seek early medical attention. The parotid gland was the commonest site, followed by minor salivary and submandibular glands, similar to Luukkaa et al., (2006)[18] found the submandibular gland most common. Benign tumours predominated overall, consistent with Kaza et al.[7] Pleomorphic adenoma was the commonest benign and mucoepidermoid carcinoma the commonest malignant tumour, matching Kaza et al.[7]

Ki-67 was expressed in 31.11% of benign and 90.62% of malignant tumours. Among pleomorphic adenomas, positivity was weak, more frequent in males and in parotid tumours, and higher in the epithelial than stromal component when epithelium predominated, which linked stromal proliferation to recurrence; no recurrences occurred in this cohort. Warthin's tumour showed positivity in only 2 of 6 cases, Kaza et al., (2016)[7] found no epithelial positivity. Basal cell adenoma had the highest Ki-67 index among benign tumours, consistent with Batsakis et al., who linked this to its higher recurrence rate.[11] Among mucoepidermoid carcinomas, 18/20 were Ki-67 positive, with strong positivity concentrated in high-grade, epidermoid-rich tumours, as also reported.

The mean Ki-67 index was significantly higher in malignant tumours overall, consistent with the findings of Horri et al.,[15] found no difference at all – the former uniquely using flow cytometry on fresh tissue rather than immunohistochemistry. The strong correlation between Ki-67 and histological grade in mucoepidermoid carcinoma reflects that both measure proliferative activity, directly and indirectly (via solid areas), respectively.[7] Expression was high in high-grade and low in low-grade mucoepidermoid carcinoma, as also found by Skalova et al.,[19] and Siddique et al.,[20] who proposed Ki-67 as a prognostic adjunct; formal grading by labelling index was not attempted here due to small, unevenly distributed grade-wise numbers. A low index was similarly seen in tubular/cribriform and a high index in solid adenoid cystic carcinoma. One basal cell adenoma simulating adenoid cystic carcinoma was correctly diagnosed based on solid, non-invasive architecture supported by a lower Ki-67 index, consistent with Kaza et al (2016).[7]

Inter-study variation in Ki-67 expression may reflect differences in tissue handling, staining protocol, antibody clone and counting method.[20] A persistent limitation is the absence of a universal cut-off: Skalova et al.,[19] Luukkaa et al.,[18] found an index >10% favourable and <5% indolent, a 5% cut-off. Ki-67 positivity here was significantly higher beyond 40 years, with no significant association with gender, site or size found. It had a significant correlation with gender and site.

Malignant tumours showed significantly higher microvessel density than benign tumours, corroborating Luukkaa et al.,[18] supporting a link between neovascularization and malignant

behaviour. Among benign tumours, Warthin's tumour showed the highest MVD, consistent.[21] Vessels in pleomorphic adenoma were confined to cellular areas, whereas in basal cell adenoma they were more dispersed with greater neovascularization. Asttributable to the myxoid/chondroid stroma of pleomorphic adenoma requiring less vascular support, versus the stroma-poor, cellular basal cell adenoma requiring new vessel formation to meet metabolic demand.[22]

Myoepithelial differentiation, absent in mucoepidermoid and salivary duct carcinoma but characteristic of adenoid cystic carcinoma and pleomorphic adenoma, confers distinct biological behaviour, as myoepithelial cells secrete high levels of proteinase and angiogenesis inhibitors and low levels of angiogenic factors, leading them as angiogenesis inhibitors. Accordingly, mucoepidermoid and salivary duct carcinoma showed higher MVD than adenoid cystic and acinic cell carcinoma. Adenoid cystic carcinoma formed large, hypovascular aggregates rimmed by larger vessels, while mucoepidermoid carcinoma showed smaller aggregates with more surrounding small vessels, attributable to the angiogenesis-inhibitor-rich, vessel-poor matrix produced by myoepithelial cells, as described by Nguyen et al.[23] linked MVD in mucoepidermoid carcinoma to stage, grade and recurrence, with higher MVD in high-grade tumours, MVD in adenoid cystic carcinoma correlated with size, stage, vascular and perineural invasion and metastasis, using different markers and longer follow-up.[24]

Despite both markers rising in malignant tumours, no significant correlation was found between Ki-67 and CD34-defined MVD, suggesting angiogenesis and proliferation are governed by largely independent mechanisms, with metabolic demands possibly met through oxygen-independent pathways such as glycolysis.

Conclusion

The present study demonstrated that malignant salivary gland tumors exhibit significantly higher proliferative activity and angiogenesis than benign salivary gland tumors, as evidenced by increased Ki-67 labelling index and CD34-assessed MVD. Ki-67 proved to be a reliable marker for assessing tumor proliferative potential and identifying tumors with a greater risk of aggressive behavior, local recurrence, and metastasis. Similarly, CD34 was found to be an effective marker for evaluating tumor angiogenesis, with higher MVD reflecting the increased metabolic demands and biological aggressiveness of malignant tumors. These findings contribute to a better understanding of the biological behaviour of salivary gland tumors and suggest that the combined assessment of Ki-67 and CD34 may serve as valuable prognostic indicators. Furthermore, the increased angiogenic activity observed in malignant tumors highlights the potential role of anti-angiogenic therapy, particularly in highly vascular neoplasms lacking myoepithelial differentiation, as a promising adjunct in their management.

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