Biomedical and Biopharmaceutical Research
Abbreviation: Biomed. Biopharm. Res.
Volume: 22: Issue: 02 |Year: 2025
Page Number: 589-596

ASSESSMENT OF HER-2/NEU PROTEIN EXPRESSION BY IMMUNOHISTOCHEMISTRY IN DIFFERENT TYPES OF SALIVARY GLAND TUMORS

Dr Harleen Kaur[1], Dr Navneet Kaur[2], Dr Dimple Sahni[3], Dr Monika Garg[4], Dr Ninder Kumar[5], Dr Anubha Garg[6]
[1]Junior resident, Department of Pathology, Government medical college, Patiala
[2]Professor, Department of Pathology, Government medical college, Patiala
[3] Professor, Department of Otorhinolaryngology, Government medical college, Patiala
[4] Professor and Head, Department of Transfusion medicine, Government medical college, Patiala
[5] Associate Professor, Department of Pathology, Government medical college, Patiala
[6]Associate Professor, Department of Surgical oncology, Government medical college, Patiala

Corresponding Author

Dr Navneet Kaur
Professor, Department of Pathology, Government medical college, Patiala

Received: 25-06-2025

Accepted: 24-07-2025

Published: 04-08-2025

ABSTRACT

Background: Salivary gland neoplasms, though rare, present a diverse spectrum of benign and malignant tumors with varying clinical behaviors. HER2/neu overexpression, a known prognostic marker in several cancers, holds potential therapeutic implications in salivary gland tumors due to histological similarities with breast carcinomas. This study aimed to classify salivary gland tumors per the 2022 WHO classification and evaluate HER2/neu immunohistochemical expression across tumor types.

Methods: A cross-sectional study was conducted on 55 histopathologically confirmed salivary gland tumors at Government Medical College, Patiala, over 18 months. Formalin-fixed, paraffin-embedded tissue sections underwent Hematoxylin and Eosin staining for morphological assessment and immunohistochemistry for HER2/neu expression, scored from 0 to 3+, with 2+ and 3+ considered positive. Data on demographics, clinical presentation, tumor characteristics, and fine needle aspiration cytology (FNAC) were analyzed, with statistical associations assessed using chi-square tests and Cohen's Kappa for FNAC-histopathology agreement.

Results: The cohort (mean age 47.58 years) showed a slight female predominance (54.55%). The parotid gland was the most common site (89.09%), with pleomorphic adenoma (PA) predominating (63.64%). Benign tumors comprised 83.64%, and malignant tumors 16.36%, including mucoepidermoid carcinoma (MEC), adenoid cystic carcinoma (ACC), and salivary duct carcinoma (SDC). FNAC showed substantial agreement with histopathology (Kappa=0.626, $p<0.0001$). HER2/neu expression was negative (0) in 89.09%, faint (1+) in 7.27% (MEC, carcinoma ex PA), and moderate (2+) in 3.64% (SDC, ACC), with no strong (3+) expression. HER2/neu positivity was significantly associated with histodiagnosis ($p<0.0001$) but not with tumor size ($p=0.524$), grade ($p=0.143$), or site ($p=0.189$).

Conclusion: This study highlights the predominance of benign salivary gland tumors, particularly PA, and limited HER2/neu overexpression, primarily in malignant subtypes like SDC and MEC. The significant association between HER2/neu and histodiagnosis underscores its prognostic value, though its rarity limits immediate therapeutic applicability. FNAC's diagnostic reliability supports its role in preoperative assessment. These findings advocate for integrated histopathological and molecular profiling to guide personalized treatment strategies in salivary gland neoplasms.

Keywords: Salivary gland tumors, HER2/neu, immunohistochemistry, pleomorphic adenoma, mucoepidermoid carcinoma.

INTRODUCTION

Salivary gland neoplasms are relatively rare, accounting for less than 5% of head and neck tumors, with an annual incidence of 0.5 to 2 per 100,000 individuals. The majority are benign, with only about 20% being malignant. These tumors commonly affect individuals in the sixth decade of life, with a higher prevalence in females—except in Warthin tumor and high-grade carcinomas. Among the salivary glands, tumors of the minor and sublingual glands show a higher likelihood of malignancy compared to the parotid gland. The most frequent benign tumor is pleomorphic adenoma, while

the most common malignant types include mucoepidermoid carcinoma, adenoid cystic carcinoma, and acinic cell carcinoma.[1,2] The etiology remains unclear, though factors such as viral infections, irradiation, obesity, and environmental exposures have been implicated.[3] Diagnosis typically involves imaging techniques like ultrasound, CT, MRI, and PET, but histopathological analysis remains essential for definitive diagnosis and treatment planning.[4,5,6]
HER2/neu, a proto-oncogene encoding a transmembrane receptor tyrosine kinase of the EGFR family, is overexpressed in several malignancies, including breast and salivary gland carcinomas. Its overexpression is associated with aggressive tumor behavior, poor prognosis, and resistance to conventional therapies.[7,8,9] Targeted therapies against HER2, such as trastuzumab, have shown promising results in HER2-positive cancers, including salivary gland tumors. Given the histological similarities between breast and salivary gland cancers, understanding HER2 expression in salivary neoplasms could offer prognostic value and therapeutic implications.[10,11]

AIMS AND OBJECTIVES

1. To classify the salivary gland tumors according to 2022 WHO classification.
2. To study immunohistochemical expression of HER2/neu in different types of salivary gland tumors.

MATERIALS AND METHODS

Study design- It was a cross sectional study conducted in the Department of Pathology, Department of Otorhinolaryngology and Department of Surgical Oncology, Government Medical College, Patiala over a period of one and a half year.

Inclusion Criteria-

Histopathologically diagnosed cases of Benign and Malignant Salivary Gland Tumors.

Exclusion criteria-

Cases with incomplete records.

Formalin-fixed salivary gland specimens received in the Department of Pathology were processed using standard histopathological techniques. Tissue samples were embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin (H&E) for morphological evaluation.

Immunohistochemistry (IHC) was performed on paraffin-embedded tissue sections to assess HER2/neu expression. Antigen retrieval was carried out using heat-induced methods, followed by incubation with primary antibody (c-erbB2/HER2/NEU). Detection was achieved using a chromogenic detection system, and counterstaining was performed with hematoxylin. All stained slides were examined under a light microscope, and relevant data were recorded using a predesigned proforma.

A score of 2+ to 3+ were considered as positive.

RESULTS

In this study of 55 patients, the age distribution showed the 50–59 age group as the largest, comprising 23.64% (n=13), followed closely by the 30–39 and 40–49 age groups, each at 20.00% (n=11), with the youngest patient aged 21 years and the eldest 81 years. Females outnumbered males, accounting for 54.55% (n=30) compared to 45.45% (n=25), yielding a male-to-female ratio of 1:1.08. The predominant chief complaint was swelling, observed in 78.18% (n=43) of cases, followed by swelling and pain in 21.82% (n=12). The parotid gland was the most affected site, representing 89.09% (n=49), followed by the submandibular gland at 9.09% (n=5) and the accessory parotid gland at 1.82% (n=1). Left-sided involvement was more common, seen in 56.36% (n=31), compared to 43.64% (n=24) on the right, with no bilateral cases. Symptom duration was most frequently 7 to 12 months for 40% (n=22) of patients, followed by 0 to 6 months for 27.27% (n=15). Gradual progression was observed in 96.36% (n=53) of cases, with rapid progression in only 3.64% (n=2). Tumor size predominantly ranged from 2 to 4 cm in 78.18% (n=43), with a mean of 2.37 ± 0.58 cm. On FNAC, pleomorphic adenoma (PA) was the most common diagnosis, seen in 78.18% (n=43), with benign lesions comprising 90.91% (n=50) and malignant ones 9.09% (n=5). FNAC diagnosis showed a significant association with age ($p < .0001$), with PA prevalent in younger groups, and with gender ($p = 0.036$), more common in females (90%, n=27) than males (64%, n=16), but no significant association with site ($p = 0.79$). Histopathologically, benign tumors predominated at 83.64% (n=46), with PA in 63.64% (n=35), while malignant tumors accounted for 16.36% (n=9). Histodiagnosis was significantly associated with age ($p < .0001$), with PA common in younger groups, and showed borderline significance with gender ($p = 0.051$), but no association with site ($p = 0.314$). Inter-rater agreement between FNAC and histopathology yielded a Kappa of 0.626, indicating substantial agreement, with a significant p-value (10% in 10.91% (n=6). (Table 1) Her-2/neu scores were 0 in 89.09% (n=49), 1+ in 7.27% (n=4), and 2+ in 3.64% (n=2), with no 3+ scores.(Table 3) A significant association was found between Her-2/neu score and histodiagnosis ($p < .0001$), with malignant tumors like MEC, ACC, carcinoma ex PA, and SDC showing positivity, while benign tumors lacked expression.(Table 4) No significant associations were observed between Her-2/neu score and tumor size ($p = 0.524$), grade ($p = 0.143$), or site ($p = 0.189$).

Table 1:- Inter-rater kappa agreement between FNAC and histo diagnosis.

FNAC	HISTO DIAGNOSIS												P value	Kappa
	PA (n=35)	Warthin's tumor(n=7)	MEC (n=3)	Acinic cell carcinoma (n=1)	ACC (n=1)	Basal cell adenoma (n=1)	Carcinoma ex PA (n=1)	Malignant and mammary analogue of secretory carcinoma (n=1)	Oncocytoma(n=1)	PA with clear cell change (n=1)	PA with cystic change (n=1)	SDC (n=1)	SCC (n=1)		
Aciniccell carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (1.82%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	<0.0001	0.626
ACC	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.82%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		
Metastasis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.82%)		
Mucoepidermoidcarcinoma (MEC)	0 (0%)	0 (0%)	1 (1.82%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		
Oncocytoma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.82%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		
Papillary type of borderline tumor	0 (0%)	0 (0%)	1 (1.82%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		
PA (PA)	35 (63.64%)	1 (1.82%)	1 (1.82%)	0 (0%)	0 (0%)	1 (1.82%)	1 (1.82%)	1 (1.82%)	0 (0%)	1 (1.82%)	1 (1.82%)	1 (1.82%)	0 (0%)		
Warthin'stumor	0 (0%)	6 (10.91%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		
Total	35 (63.64%)	7 (12.73%)	3 (5.45%)	1 (1.82%)	1 (1.82%)	1 (1.82%)	1 (1.82%)	1 (1.82%)	1 (1.82%)	1 (1.					

Table 2:- Her-2 intensity distribution.

Her-2 intensity	Frequency	Percentage
No staining	49	89.09%
Faint	4	7.27%
Moderate	2	3.64%

Total	55	100.00%

Table 3:- Her-2 Staining distribution.

Staining	Frequency	Percentage
No staining/ membranous staining	49	89.09%
Incomplete membrane	4	7.27%
Complete membrane	2	3.64%
Total	55	100.00%

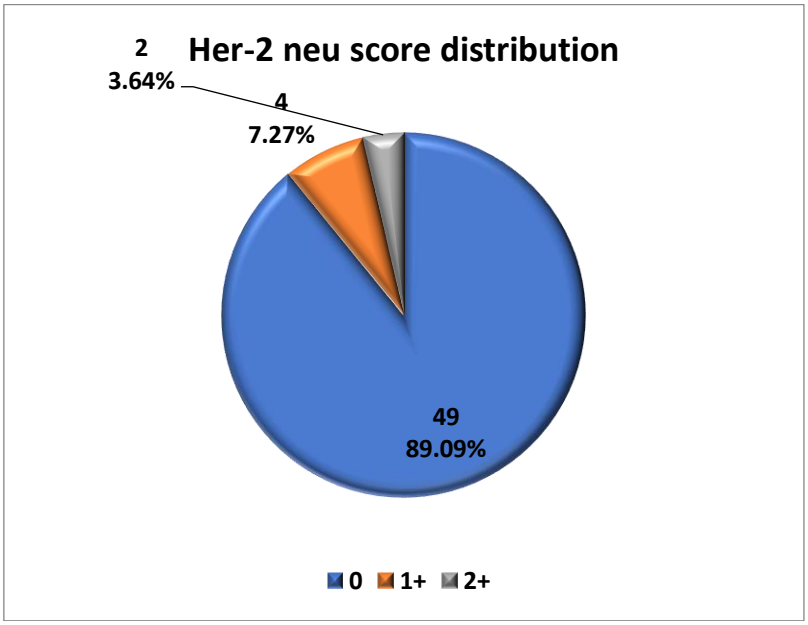


Image 1: HER2 neu score distribution

Table 4:-Association of Her-2 neu score with histodiagnosis.

Her-2 neu score	PA(n=35)	Warthin's tumor (n=7)	MEC (n=3)	Oncocytoma (n=1)	PA with clear cell change (n=1)	PA with cystic change (n=1)	Acinic cell carcinoma (n=1)	ACC (n=1)	Basal cell adenoma (n=1)	Carcinoma ex PA (n=1)	Malignant mammary analogue of secretory carcinoma (n=1)	SDC (n=1)	SCC (n=1)	Total	P value
0	35 (100%)	7 (100%)	0 (0%)	1 (100%)	1 (100%	1 (100%	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%	49 (89.09%	<.0001

					)	)							)	)	
1+	0 (0%)	0 (0%)	3 (100 %)	0 (0%)	0 (0 %)	0 (0 %)	0 (0%)	0 (0 %)	0 (0%)	1 (100 %)	0 (0%)	0 (0 %)	0 (0 %)	4 (7.2 7%)	
2+	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0 %)	0 (0 %)	0 (0%)	1 (10 0%)	0 (0%)	0 (0%)	0 (0%)	1 (10 0%)	0 (0 %)	2 (3.6 4%)	
To tal	35 (100 %)	7 (100 %)	3 (100 %)	1 (100%)	1 (10 0%)	1 (10 0%)	1 (100 %)	1 (10 0%)	1 (100 %)	1 (100 %)	1 (100 %)	1 (10 0%)	1 (10 0%)	55 (100 %)	

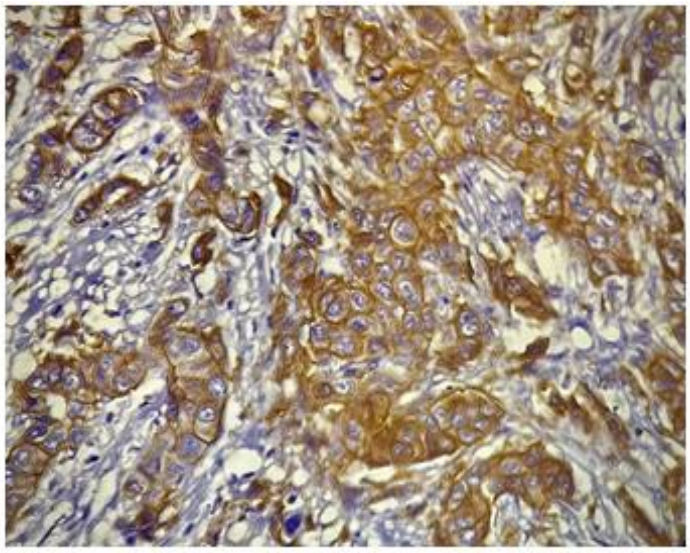


IMAGE 2: HER 2 POSITIVE CONTROL (3+)

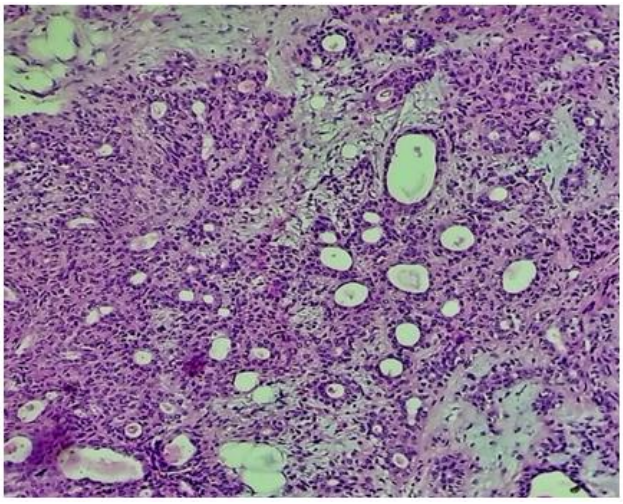


IMAGE 3: HISTOLOGICAL IMAGE OF PLEOMORPHIC ADENOMA SHOWING EPITHELIAL AND MYOEPITHELIAL COMPONENTS ALONGWITH CHONDROMYXOID STROMA (H&E, 100x)

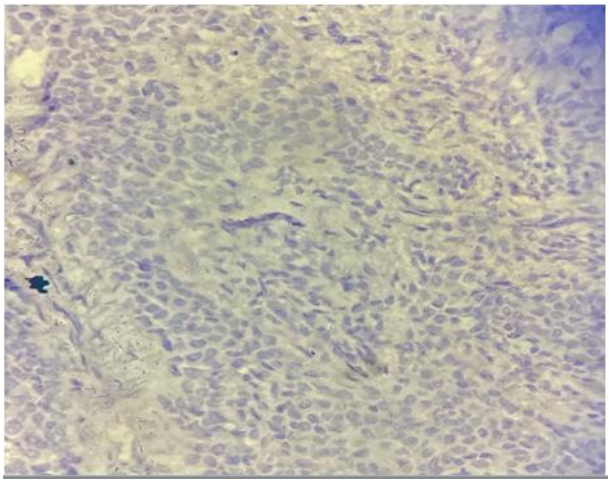
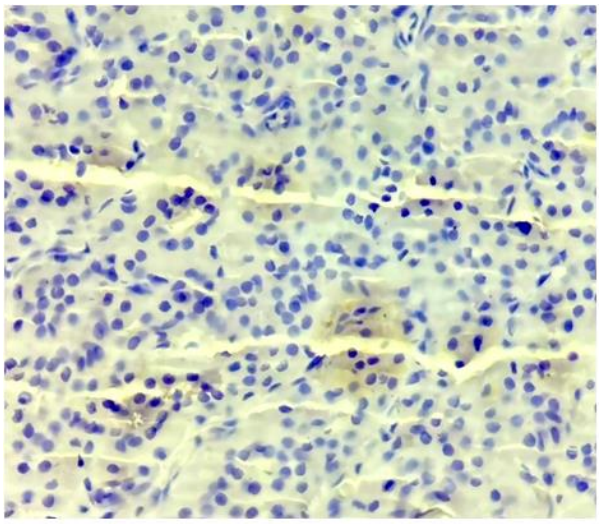


IMAGE 4: PLEOMORPHIC ADENOMA SHOWING HER2 EXPRESSION OF 0

IMAGE 5: ONCOCYTOMA SHOWING HER2 EXPRESSION OF 0



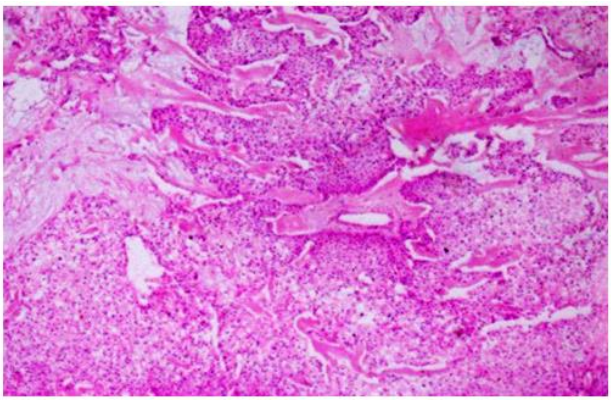


IMAGE 6: HISTOLOGICAL IMAGE OF LOW GRADE MUCOEPIDERMOID CARCINOMA SHOWING VARYING PROPORTIONS OF EPIDERMOID CELLS, INTERMEDIATE CELLS AND MUCOCYTES. (H&E, 100x)

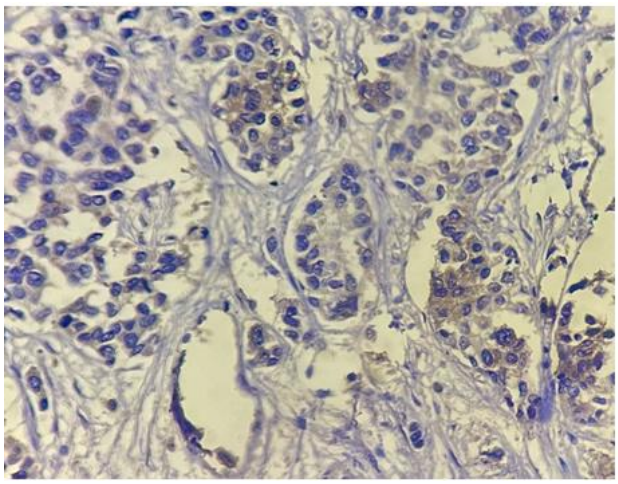


IMAGE 7: MUCOEPIDERMOID CARCINOMA SHOWING HER2 EXPRESSION OF 1+

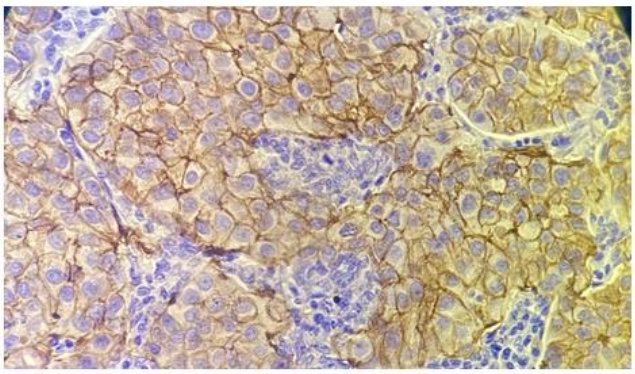


IMAGE 8: SALIVARY DUCT CARCINOMA SHOWING HER2 EXPRESSION OF 2+

DISCUSSION

In this study of 55 salivary gland tumors, the mean age of 47.58 years aligns with literature, where Bobati et al. reported 43 years however Ghartimagar et al. noted 48.45 years for malignant tumors. The 50–59 age group predominated (23.64%), consistent with middle-aged peaks in prior studies. A slight female predominance (54.55%, male-to-female ratio 1:1.08) mirrors findings by Bobati et al. (1:1.8), Ghartimagar et al. (1:1.36), and Kumari et al. (1:1.24), likely due to the high prevalence of benign tumors like PA. Swelling was the primary symptom (78.18%), typical of benign and low-grade tumors, with swelling and pain (21.82%) suggesting more aggressive lesions, aligning with Bobati et al. and Patel et al. The parotid gland was the most affected site (89.09%), consistent with Sugano et al. (59.32%), Patel et al. (75.71%), and Kumari et al. (62.5%). Gradual progression dominated (96.36%), supporting the benign-heavy cohort, as noted by Bobati et al. and Patel et al. Benign tumors comprised 83.64%, higher than the 65–80% in studies by Bobati et al., Rachakonda et al., and others, with PA (63.64%) and Warthin's tumor (12.73%) most common. Malignant tumors (16.36%) were lower than the 20–35% in literature. HER2/neu expression was absent in 89.09% of cases, aligning with high negativity rates (80–95%) in Sugano et al., S Dori et al., and Clauditz et al. Faint (1+, 7.27%) expression occurred in low-grade MEC and carcinoma ex PA, while moderate (2+, 3.64%) expression was seen in SDC and ACC, with no strong (3+) expression. This contrasts with high 3+ rates in SDC-heavy studies by Skálová et al. (100%) and Cornolti et al. (77%). Benign tumors showed universal HER2 negativity, consistent with de Souza et al. and Can et al. The significant association ($p < .0001$) between HER2/neu and histodiagnosis highlights its relevance in malignant subtypes, though the absence of 3+ expression and high benign proportion limit targeted therapy applicability in this study.

CONCLUSION

This comprehensive investigation of 55 salivary gland tumors has elucidated critical insights into demographic, clinical and histopathological profiles, with a particular emphasis on HER-2/neu expression as potential prognostic and therapeutic marker. The rarity of HER-2/neu overexpression in this study suggests limited current applicability of anti-HER-2 therapies, yet the presence of 2+ staining in selected malignant subtypes emphasizes the need for targeted molecular testing to identify potential therapeutic candidates. The highly significant association between HER-2/neu scores and histopathological diagnosis ($p < 0.0001$) underscores its utility as a molecular marker of malignancy,

particularly in aggressive subtypes like SDC and MEC.The lack of significant correlations between HER-2/neu expression and tumor size ($p = 0.524$), grade ($p = 0.143$), or anatomical site ($p = 0.189$) suggests that HER-2/neu status is primarily influenced by tumor histology rather than these parameters. Further, FNAC exhibited robust diagnostic reliability, evidenced by a Cohen's Kappa of 0.626 ($p < 0.0001$) for concordance with histopathological diagnosis, reinforcing its efficacy as a critical preoperative tool for salivary gland tumor assessment. These findings thus advocate for an integrated diagnostic approach, combining histopathological evaluation with molecular profiling, to optimize risk stratification and treatment planning.

Funding: None
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. Shinde A G, Patil A, More S. Salivary gland tumors-Three-year Retrospective Study. Ann. Int. Med. Den. Res. 2019; 5(3):PT33-PT37.
2. Sathish Babu N, Mahadev N H, Kumar G V. A clinical study of the incidence of salivary gland tumors in a tertiary care teaching hospital. Int Surg J 2019;6:2110-3.
3. Saldanha C,Yaranal P,Upadhyaya K.A clinicopathological study of salivary gland tumors. Trop J Path Micro 2018;4(7):532-538.
4. Lee YY, Wong KT, King AD, Ahuja AT. Imaging of salivary gland tumours. Eur J Radiol. 2008 Jun;66(3):419-36.
5. Ettl T, Schwarz-Furlan S, Gosau M, Reichert TE. Salivary gland carcinomas. Oral Maxillofac Surg. 2012 Sep;16(3):267-83.
6. Burke CJ, Thomas RH, Howlett D. Imaging the major salivary glands. Br J Oral Maxillofac Surg. 2011 Jun;49(4):261-9.
7. Sugano S, Mukai K, Tsuda H, Hirohashi S, Furuya S, Shimosato Y et al. Immunohistochemical study of c-erbB-2 oncoprotein overexpression in human major salivary gland carcinoma: an indicator of aggressiveness. Laryngoscope. 1992 Aug;102(8):923-7.
8. Klapper LN, Glathe S, Vaisman N, Hynes NE, Andrews GC, Sela M, Yarden Y. The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. Proc Natl Acad Sci U S A. 1999 Apr 27;96(9):4995-5000.
9. King CR, Kraus MH, Aaronson SA. Amplification of a novel v-erbB-related gene in a human mammary carcinoma. Science. 1985 Sep 6;229(4717):974- 6.
10. Javaheripour A, Saatloo MV, Vahed N, Gavgani LF, Kouhsoltani M. Evaluation of HER2/neu expression in different types of salivary gland tumors: a systematic review and meta-analysis. J Med Life. 2022 May;15(5):595-600.
11. Limaye SA, Posner MR, Krane JF, Fonfria M, Lorch JH, Dillon DA et al. Trastuzumab for the treatment of SDC. Oncologist. 2013;18(3):294-300.