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Expression of Glial Fibrillary Acidic Protein (GFAP) in Salivary Gland Tumors to differentiate Pleomorphic Adenoma from histologic mimics

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ABSTRACT

Purpose: To differentiate pleomorphic adenoma from histologic mimics by assessing the expression of GFAP in salivary gland tumors.

Study design: A Cross-sectional study

Place and duration of study: This study was conducted in the Department of Histopathology/ Oral Pathology at The Armed Forces Institute of Pathology Rawalpindi from 15th July 2024 to 31st March 2025.

Material and Methods: Forty-six biopsy specimens; 23 of pleomorphic adenoma, 3 of epithelial myoepithelial carcinoma, 7 of adenoid cystic carcinoma, 4 of acinic cell carcinoma, 6 of mucoepidermoid carcinoma, and 3 specimens of salivary duct carcinoma were stained with Glial fibrillary acidic protein. For preliminary diagnosis Hematoxylin and Eosin stains were used and expression of the immunohistochemical protein were recorded by the observer for interpretation.

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Results: Glial fibrillary acidic protein showed statistically significant positivity in pleomorphic adenomas by strong diffuse positive expression in the myoepithelial cells as well as in the stroma in some cases. However, the expression of this marker in other malignant salivary gland tumors was absent.

Conclusion: The use of Glial fibrillary acidic protein can be helpful in distinguishing pleomorphic adenoma from other malignant salivary gland tumors that can be confused with pleomorphic adenoma on the basis of histopathology. It can specially be useful in routine limited biopsy materials such as core needle biopsies where diagnosis becomes a dilemma.

Key Words: Pleomorphic Adenoma, Glial Fibrillary Acid Proteins, Histological Mimics, Salivary Gland Tumors

Running Title: Expression of GFAP

INTRODUCTION:

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Pleomorphic adenoma (PA) is the most common tumor of the salivary glands, making up over half of all neoplasms in the head and neck region. Willis proposed the term "pleomorphic adenoma," which accurately describes the lesion's unusual histologic patterns. Its name is derived from the architectural diversity observed under light microscopy.[7] Pleomorphic adenoma is a benign triphasic salivary gland tumor consisting of epithelial (ductal) cells and myoepithelial cells scattered in a myxoid stroma.[6] It is commonly recognized that pleomorphic adenomas demonstrate an almost infinite range of morphological and architectural patterns.

A broad range of salivary gland tumors, both benign and malignant, exhibit a biphasic phenotype. Tumors with a significant amount of basal and myoepithelial cells may also closely resemble Pleomorphic adenoma in terms of their cellular composition.[3] It is usually confused with its histological mimics that may include basal cell adenoma, mucoepidermoid carcinoma, adenoid cystic carcinoma, salivary duct carcinoma, and epithelial myoepithelial carcinoma. The histological diagnosis of all these tumors are dependent on the examination of the tumor slides by an expert histopathologist. However, there may be a chance of error.[4]

Glial fibrillary acidic protein, or GFAP, is an intermediate filament protein that plays a variety of roles in the cytoplasmic cytoskeleton, including structural support, organelle and enzyme scaffolding, and extracellular environment mechanosensing. It is placed in the type III intermediate filament category, which also includes vimentin (expressed in a variety of cell types), desmin (found in skeletal and cardiac muscle), and peripherin (found in neurons). Following its rapid adoption as a marker of astrocytes in the central nervous system (CNS), the

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knowledge about the role of GFAP in health and disease has steadily increased.[5]

According to previous research, Pleomorphic adenoma predominantly expresses glial fibrillary acidic protein (GFAP), which may help distinguish it from other salivary gland tumors.[3] It is used to determine glial differentiation; therefore, its expression is high in more differentiated, less malignant tumors.[10] It is usually expressed in the tumors containing myoepithelial cells, which exhibit the characteristics of both mesenchymal and epithelial tissues. The differential expression of GFAP in different tumors can help to quickly identify otherwise similar neoplasms.[9] This unique characteristic of each salivary gland tumor is comparable with other tumors. The histological features of the tumor in the background of clinical presentation are crucial for diagnostic accuracy.[8] Greater expression of GFAP in Pleomorphic adenoma as compared to the other tumors is also a unique feature aiding in the diagnosis and differentiation of the tumor.[2]

The aim of this study is to differentiate pleomorphic adenoma from histologic mimics by assessing the expression of GFAP in salivary gland tumors.

MATERIAL AND METHODS:

A cross-sectional study was conducted in the Department of Histopathology/ Oral Pathology at The Armed Forces Institute of Pathology (AFIP) Rawalpindi from 15th July 2024 to 31st March 2025. All newly diagnosed cases of salivary gland tumors on excision specimens and patients of all age groups and both genders were included in the study. The exclusion criteria consisted of improperly fixed specimens, core needle biopsies of salivary gland tumors and patients not consenting to the study.

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During the above-mentioned period, all the newly diagnosed cases of salivary gland tumors on excision biopsies as described in the inclusion criteria, submitted to AFIP were selected through the consecutive sampling method. Patient´s biodata was recorded on data collection proforma and an informed written consent was obtained from all patients ensuring the confidentiality. The application of H&E stain and GFAP immunohistochemical stain simultaneously on fresh sections was done and the slides were viewed, first by the principal investigator of the study and then reviewed and confirmed by a histopathologist (supervisor) to eliminate bias. The GFAP marker by Leica Microsystem (Germany) was used to analyze the selected specimens. The tissue sections were first scanned at low power. Positive GFAP expression was predominantly cytoplasmic with strong brown staining. However, some cases showed little to no staining, that were marked as negative.

Final interpretation of the expression, pattern, intensity and distribution of GFAP was recorded in the data collection proforma and results subjected to statistical analysis through SPSS 25 software.

RESULTS:

Forty-six patients fulfilling the inclusion criteria were include in this study. Among the 46 patients, 23 were of pleomorphic adenoma (50%), 7 of adenoid cystic carcinoma (15.2%), 6 of mucoepidermoid carcinoma (13%), 4 of acinic cell carcinoma (8.7%), 3 of epithelial myoepithelial carcinoma (6.5%), and 3 cases of salivary duct carcinoma (6.5%) fig 1. These cases were evaluated according to preset criteria and included in the study by an oral pathology trainee.

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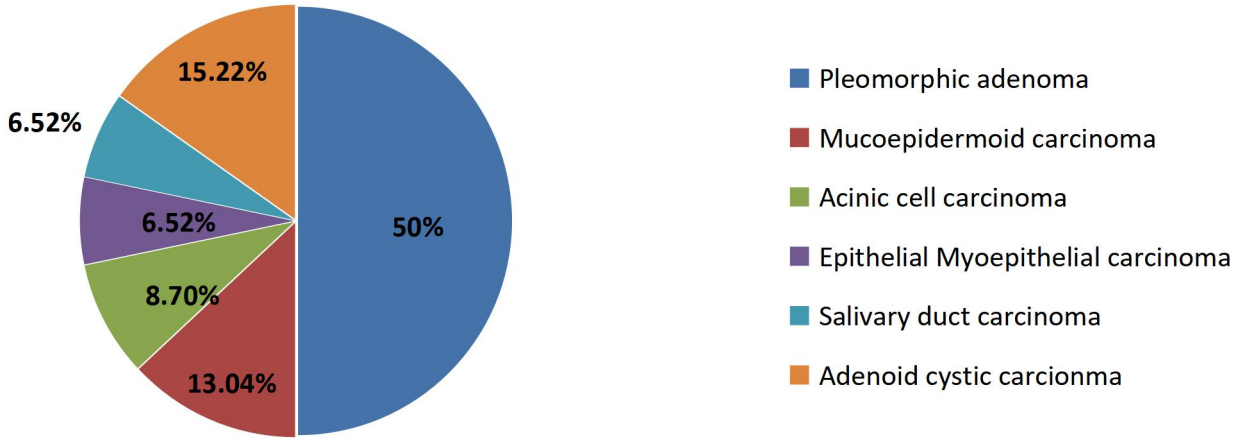

Fig-1: Frequency of Selected Cases

The mean age of patients was 47.5870 ± 16.50801 years as shown in table 1. Age varied from 16 to 78 years in both males and females. In men, the average age was 49.6563 years whereas it was 42.8571 in females.

Table 1

	n	dard deviation
ber	8	0

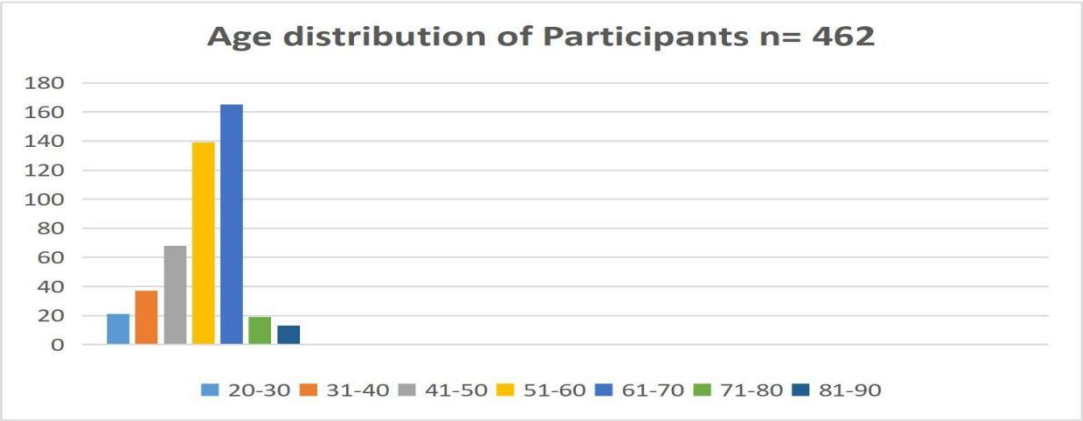

Fig-2: Age distribution of patients

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Study revealed more male predominance 32 (69.6%) as compared to females 14 (30.4%), table 2.

Table 2

Gender	Frequency	Percentage (%)
Female	14	30.4
Male	32	69.6
Total	46	100

Majority of cases were from Parotid region as shown in table 3.

Table 3

Site	Number of cases	Percent (%)
Parotid gland	36	78.2
Submandibular gland	5	10.9
Minor salivary glands	5	10.9
Total	46	100

Cases were evaluated according to the aforementioned criteria and the expression of GFAP was recorded. The p-value was found to be significant i.e., less than 0.05, table 4.

Table 4

	Diagnosis	GFAP Expression		Total	p-value	
		Positive	Negative			
1	Pleomorphic adenoma	22	1	23	0.00	
2	Mucoepidermoid carcinoma	0	6	6		
3	Acinic cell carcinoma	0	4	4		

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4	**Epithelial myoepithelial carcinoma**	**1**	**2**	**3**		
5	**Salivary duct carcinoma**	**0**	**3**	**3**		
6	**Adenoid cystic carcinoma**	**0**	**7**	**7**		
Total		**23**	**23**	**46**		

Among the twenty-three positive cases of GFAP, twenty-two were of pleomorphic adenoma and a single case of epithelial myoepithelial carcinoma. The remaining twenty-three salivary gland tumor cases were recorded as negative.

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DISCUSSION:

Pleomorphic adenoma is a mixed tumor comprising of epithelial and myoepithelial cells in the background of the chondroid or myxoid stroma. Many other salivary gland tumors may comprise of a similar histopathological pattern that can lead to a misdiagnosis. An accurate diagnosis may prove to be useful in the prediction of the prognosis which is beneficial owing to the increased recurrence and malignant potential of the tumor to carcinoma ex pleomorphic adenoma.[4] Our study implored that using GFAP along with routine microscopy can help in differentiating pleomorphic adenoma from malignant salivary gland tumors, that may mimic PA on histopathological examination.

80% of the pleomorphic adenomas show GFAP positivity, whereas the histological mimics are either negative or weakly positive for the protein. Glial fibrillary acidic protein is not typically found in the salivary gland tissue, but its presence in the salivary gland tumor indicates different types of cells at its origin. The pattern and distribution of GFAP in the myoepithelial cells of the tumor can distinguish it from the histological ambiguities. It is now commonly used with other immunohistochemical markers such as S-100 protein, cytokeratins, and p63. Molecular and genetic testing advances have transformed the evaluation of tumors, especially those with histological similarities.

Several studies have utilized glial fibrillary acidic protein (GFAP) as supplementary diagnostic tool to differentiate PA from other salivary gland tumors.[3] One study used other immunohistochemical markers like SMA, CD 117 and CD 43 along with GFAP to assess their expression in tumors of minor salivary glands.[11] Another study proposed the use of DOG-1, GFAP, and B-catenin to distinguish benign salivary gland tumors that displayed

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histopathological overlap.[4] A recent study assesssed the utility of p63 immunomarker to highlight myoepithelial cells for the diagnosis of PA.[1]

In this study, pleomorphic adenoma, a benign tumour, was analyzed for the expression of GFAP along with other salivary gland tumours. A total of 23 cases of pleomorphic adenoma were selected. The immunomarker GFAP showed strong diffuse positivity in the myoepithelial cells of pleomorphic adenoma whereas the expression of this immunomarker was negative in malignant salivary glands including adenoid cystic carcinoma, mucoepidermoid carcinoma, salivary duct carcinoma, epithelial myoepithelial carcinoma, and acinic cell carcinoma. There was a strong positive expression in a single case of EMC. Also, the distribution, pattern, and intensity of expression of GFAP was noted in the cases that showed positive expression of this protein. Sixteen of GFAP positive cases of pleomorphic adenoma showed expression in myoepithelial cells, 2 in the stroma while the remaining 5 positive cases showed expression in multiple components. Out of 23 positive cases, 19 displayed diffuse staining while 4 cases showed focal expression. In the light of intensity, 12 cases were strong, 10 were moderate and only one case showed weak positivity of GFAP. It was also noted that myoepithelial cells of other biphasic malignant tumors did not show any positivity of GFAP.

Studies have also been conducted on the profiling of the tumors of the salivary glands, where the maturation of the salivary glands should also be studied to know the step-by-step process of the gland's development. This will help us understand the relationship of every biochemical marker with the tissue undergoing development. Immunochemical markers like GFAP present various opportunities and challenges in the diagnostic fields of salivary gland tumors. The understanding of the tumors can be improved by further research on tumorigenesis, its origins,

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and molecular basis. Molecular chemistry, genetics, and histology can contribute to the further development of diagnostic accuracies.

H&E staining is still the gold standard in routine microscopy. Immunohistochemical markers like GFAP can be useful adjunct in detecting difficult cases of salivary gland tumors. The right diagnosis is critical for a successful treatment plan, predicting prognosis, therapeutic interventions and determining patient survival rates.

CONCLUSION:

The use of Glial fibrillary acidic protein (GFAP) immunohistochemical marker has been shown to differentiate pleomorphic adenoma from its histologic mimics. This diagnostic tool offers pathologists crucial information, allowing for more accurate and consistent diagnosis of salivary gland tumors especially in small biopsies like incisional biopsies or core needle biopsies where due to limited material extensive immunohistochemical workup cannot be done as timely the diagnosis is mandatory. It helps in making proper clinical decisions, optimizing patient management, and improving overall patient outcomes by improving diagnostic precision.

RECOMMENDATIONS

Studies focusing on salivary gland tumors, with a bigger sample size, should be done in the future, for the universality of the results. Also, the effectiveness of GFAP immunohistochemistry should be evaluated in core biopsy cases. A better staining process and careful handling of the specimen can help in achieving even more consistent results.

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CONFLICT OF INTEREST

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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