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Mucin-2 Expression in Primary and Recurrent Cases of Pleomorphic Adenoma in Tertiary Care Hospitals of Peshawar

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ABSTRACT

Background: Pleomorphic adenoma (PA) is a benign tumor, with a high recurrence rate. This study aimed to compare the Mucin-2 (MUC2) expression in primary and recurrent cases of PA in tertiary care hospitals of Peshawar.

Methods: In this study, there were 60 PA cases, 51 being primary lesions and 9 recurrent lesions. Formalin-fixed and paraffin-embedded (FFPE) blocks of the two groups were examined for the immunohistochemical expression of MUC2. The clinicopathological parameters were also examined. This study was conducted at the Pathology Department, Pakistan Institute of Medical Sciences (PIMS), Peshawar, between January 2019 to January 2021. A nonprobability sampling technique was used while collecting the samples. SPSS version 21 was used and p-value<0.05 was considered significant.

Results: The mean age was 32.3±8 years for both primary and recurrent PA cases. Out of the total, there were 30 (50%) females with 21 (35%) males, while all the recurrent cases were females 9 (15%). Most common microscopic features evaluated by H&E-staining showed Myxoid and chondroid stroma along with sheets of epithelioid and basaloid cells. Among 51 cases of primary PA, 17 (33%) showed positive MUC2 expressions and 34 (66.6%) showed negative expressions while all cases of recurrent PA showed positive MUC2 expression with a p-value of 0.05. In primary PA, only a single (1.66%) case showed a strong stain, while there were 5(55.3%) cases in the recurrent PA.

Conclusion: The expression of MUC2 is a useful marker to predict recurrence and there was a significant difference in the expression of MUC2 in primary and recurrent cases of PA.

Keywords: Mucin-2, Pleomorphic Adenoma, Salivary Gland Neoplasms.

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INTRODUCTION

Pleomorphic adenoma (PA), also known as a benign mixed tumor, is the most common salivary gland tumor. The annual global incidence of pleomorphic adenoma (PA) is approximately 2-3.5 cases per 100,000 population[1]. The PA recurrence risk ranges from 0.4% to 45%. Furthermore, recurrent PA has been linked with an increased risk of malignant transformation to carcinoma ex-adenoma pleomorphic. Among benign salivary gland tumors, the incidence of PA in Pakistan is 90%[2].

Recurrence in PA usually presents as swelling not far from the resection site. Improper surgical resection plays a vital role in the recurrence of PA[3]. The presence of the capsule around the PA is one of the important factors for recurrence. Thinning of the capsule and its infiltration by the tumor is one of the main causes of recurrence[4]. There is very limited study on immunohistochemical markers to detect the recurrence of PA after surgical resection of primary lesions[5,6]. Although some recent immunohistochemical studies have discovered the intrinsic biological characteristics of this tumor, such as over-expression of the progesterone receptor, the estrogen receptor, p53, and cell proliferation activity, there are very few markers that may help in the prediction of recurrence of PA[6].

Mucins are high molecular weight glycoproteins secreted by salivary glands and epithelial cells lining the digestive, respiratory, and reproductive tracts[7]. They are glycosylated molecules, and they show an aberrant expression in many malignancies. Mucins have two distinct classes based on structure and function: secreted glycoprotein and membrane-associated[11]. The growing body of data suggests that mucins play a significant role in shaping tumor biology, and some mucins have been identified as clinically useful tumor markers[8]. Various studies of pure mucinous adenocarcinoma of the breast have revealed the expression of proteins like MUC1, MUC2, MUC3, MUC5A, and MUC6, which have been suggested and hypothesized as prognostic variables[9].

MUC2 is an intestinal type of mucin, and it is expressed in normal goblet cells of the small intestine, colon, and airway epithelium. In mucinous carcinomas its expression is more evident, which has the same histological features as pancreas, bladder, stomach, lung, cervix, and Salivary Gland however the site of origin is different[9]. The current study aimed to compare the MUC2 expression in primary and recurrent cases of PA thus helping in the management of patients.

METHODS

This study was conducted at the Department of Pathology, Peshawar Medical College, Pakistan Institute of Medical Sciences (PIMS), Peshawar Dental College and Hospital, Peshawar. This study was carried out from January 2019 to January 2021. A non-probability sampling technique was used while collecting the samples. Approval of the study was given by the Institutional Review Board (IRB) of Prime Foundation Pakistan (IRB Approval No, PRIME/IRB/2021-295).

A total of 60 Formalin-fixed and paraffin-embedded (FFPE) blocks of previously diagnosed primary and recurring cases of pleomorphic adenoma were included and their respective information was gathered from their reports and put into predesigned proformas. Incisional biopsy specimens and salivary gland lesions other than PA were excluded during sample collection. Primary lesions of 51 patients with PA (parotid gland 44 and submandibular gland 7) were designated as the primary group and 9 lesions showing subsequent recurrence all from the parotid gland were retrieved that were resected between 2019 to 2021.

All the specimens were fixed in 10% formalin, embedded in paraffin wax, and cut into ^{4} µm thick serial sections for immunohistochemistry, in addition to the usual hematoxylin and eosin (H&E) staining. Immunohistochemical staining was used to stain the PA tissues. The diagnosis of PA is made using a semi-quantitative approach. The MUC2-positive cells were first estimated in accordance.

Immunohistochemical staining was carried out using an immunoperoxidase method and the Avidin Biotin Complex as described previously. Briefly, each section was deparaffinization with xylene. Endogenous peroxidase was blocked by incubating the sections in 0.3% hydrogen peroxide in absolute methanol at room temperature for 30 minutes. After hydration in decreasing concentrations of ethanol in water, the sections were washed in 10mM phosphate-buffered saline (PBS), pH 7.4. Next, 2% horse serum in PBS was applied for 30 minutes at room temperature to prevent non-specific staining. The sections were incubated with dilutions of MUC 2 antibody in PBS with 1% bovine serum albumin for 16 hours at 4°C. The sections were washed three times with PBS, incubated with the biotinylated secondary antibody, and then washed three times with PBS. All the sections were then incubated with the ABC complex for 30 minutes. After washing with PBS three times, the sections were finally reacted with diaminobenzidine substrate for 10 minutes for visualization, rinsed with tap water, counterstained with hematoxylin, and mounted. Reaction products were not present when non-immune serum or PBS was used instead of the primary antibodies.

Individual scores of the proportion of MUC2-positive cancer cells (0–5) and the staining intensity of the nuclei (0–3) were added together to produce the final scores. This is how the proportion of cancer cells

that are MUC-positive was set: Scores of 1 correspond to less than 1% of cancer cell nuclei, 2 to 10% of cancer cell nuclei, 3 to 33% of cancer cell nuclei, 4 to 66% of cancer cell nuclei, and 5 to more than 67% of cancer cell nuclei. Intensity scoring is 0 for no stain, 1 for weak stain, 2 for intermediate stain, and 3 for strong stain. The final Allred score was calculated by adding the proportion score and intensity score which is 0-1 for negative, 2-3 for weak positive, 4-6 for moderately positive, and 7-8 for strong positive.

Both the clinicopathological factors and the ratio of the numbers of patients classified into the high and low expression groups for each mucin were compared between the recurrence group and non-recurrence group, with significant ($p < 0.05$) differences determined by the Chi-Square test or Fisher's exact test. SPSS version 21 was used.

RESULTS

The mean age was 32.3±8 years for both primary and recurrent cases. The commonest age group for primary PA is 31-40 years age group having 33 (64.70%) cases. In recurrent cases, the commonest age group was also found to be 31-40 having 5 (55.3%) cases followed by 2 (22.3%) cases in the 51-60 age group. The p-value is nonsignificant, so there is no significant association between age, gender, and primary, recurrent PA.

Table 1: Demographic distribution in primary and recurrent PA cases

Variables	Primary PAs	Recurrent PAs	p-value
Overall age range (Mean± S.D.)	32.3±8	32.3±8	
18-30 (19.2 ± 5.3)	4 (7.84%)	1 (11.1%)	
31-40 (32.3 ± 8.2)	33 (64.70%)	5 (55.3%)	0.115
41-50 (41.3 ± 14.1)	7 (13.73%)	1 (11.1%)	
51-60 (62 ± 15 years)	7 (13.73%)	2 (22.3%)	
Gender			
Males	21 (35%)	0	0.329
Females	30 (50%)	9 (15%)	

p-value<0.05 is considered significant

The microscopic features of the primary and recurrent PA were evaluated on H&E-stained slides (Table 2). Myxoid and chondroid stroma was the commonest finding in primary PA 49 (87.8%) cases and 8 (13.4%) cases in recurrent PA. The second commonest finding was sheets of epithelioid and basaloid cells which were 47 (86.5%) in primary cases and 9 (14.5%) in recurrent cases. The presence of bilayered ducts and cribriform patterns was seen in 30 (90.1%) primary cases and 9 (9.9%) recurrent cases. Ductal and spindle cell proliferation with hyalinization was visualized in 19(89.3%) primary and 8 (13.4%) recurrent cases. Squamous metaplasia was found in 14 (97.2%) primary cases and only a single case (13.8%) in the recurrent group. Plasmacytoid cells were observed in 14 (74.2%) primary cases and 5 (26.8%) recurrent cases.

Table 2: Histopathologic features in primary and recurrent PA cases

Microscopic Features	Primary PAs	Recurrent PAs
Bilayered Duct and Cribriform Pattern	30(90.1%)	9(9.9%)
Ductal and spindle cell proliferation with hyalinization	19(89.3%)	8(11.7%)
Squamous Metaplasia	14(97.2%)	1(3.8%)
Myxoid and chondroid stroma	49(87.6%)	8(13.4%)
Sheets of Epithelioid and Basaloid Cells	47(86.5%)	9(14.5%)
Plasmacytoid cells	14(74.2%)	5(26.8%)

The staining intensity of both primary and recurrent PA was evaluated. In the case of primary PA, the staining intensity for the majority of 19 (31.3%) cases showed weak stains, while 11 (18.33%) cases showed intermediate stains and only a single (1.66%) case showed strong stains. Meanwhile in the recurrent PA, 5(55.3%) cases showed strong stains, 3 (33%) cases had intermediate stains and only a single (11.66%) case showed weak stains but there was no significant association between the intensity of cells stained in primary and recurrent PA. (Table 3)

Table 3: Intensity and proportion of cells stained with MUC2 in primary and recurrent PA cases

Proportion score	Cells stained with MUC2 (%)	Primary PAs	Recurrent PA	Total
1	<1%	0	0	0
2	1-10%	16 (26.66%)	0	16(26.6%)
3	11-33%	27 (45%)	2(14.33%)	29(48.3%)
4	34-66%	6 (10%)	3 (25%)	9(15%)
5	67-100%	2 (3.33%)	4 (70.66%)	6(10%)
Staining intensity scoring	**Intensity of stain**			
0	No stain	0	0	0
1	Weak stain	19 (31.66%)	1 (11.66%)	20(33.3%)
2	Intermediate stain	11 (18.33%)	3 (33%)	14(23.3%)
3	Strong stain	1(1.66%)	5 (55.33%)	6(10%)

The final Allred score ranged from 2 to 7 which was grouped as follows 2-3 negative, 4-5 weak positive, and 6-7 strong positive. (Table 4)

Table 4: Final Allred score of primary and recurrent PA cases

Final Allred Score (2-7)	Primary PAs	Recurrent PAs
2-3 (negative)	34 (53.33%)	0
4-5 (weak positive)	12 (18.33%)	3(44.66%)
6-7 (strong positive)	6(10%)	6 (66.99%)

Among 51 cases of primary PA, 17 (33%) showed positive MUC2 expression and 34 (66.6%) exhibited negative expressions of MUC2. However, all cases of recurrent PA showed positive MUC2 expression. Fischer exact test was applied, and there was a significant association between primary and recurrent pleomorphic adenoma (0.05). (Table 5)

Table 5: Comparison of MUC2 expression in primary pleomorphic adenoma and recurrent pleomorphic adenoma.

Total Cases n=60	MUC2 expression		p-value
	Negative n=34	Positive n=26	
Primary pleomorphic adenoma n=51	34 (66.6%)	17 (33%)	0.05
Recurrent pleomorphic adenoma n=9	0 (0%)0	9 (100%)	

p-value<0.05 is considered significant

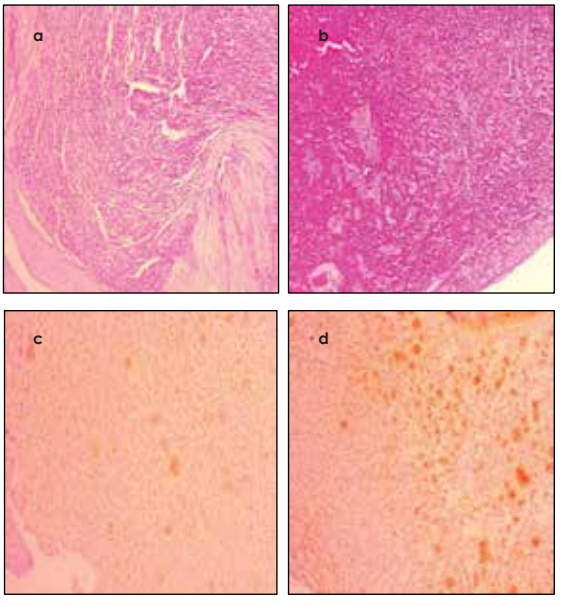

Figure 1: H&E staining of a) primary PA case, b) recurrent PA and IHC staining of c) Primary PA, d) recurrent PA (10X magnification).

DISCUSSION

Pleomorphic salivary adenoma is known for its tendency to reappear after excision There have been limited studies that have compared the non-recurrence group with the primary lesions of the recurrence group. In our study, primary and recurrent cases of PA were evaluated for their age gender distribution, and site involved, along with the comparison of immunohistochemical expression of MUC2 was assessed. We observed that MUC2 was strongly expressed in all the recurrent cases of PA. The present study aimed to evaluate MUC2 as a predictive marker to find out the recurrence of PA, while also assessing the value of quantitative MUC2 expression as a predictive marker.

In the present study, the majority of both primary and recurrent PA cases belonged to the age group 31-40 years. The mean age for both primary and recurrent pleomorphic adenoma was found to be 32± 8 years. A study by Albert in the USA reported that primary PAs mostly occur in young- and middle-aged adults, between 30 and 40 years similar to our study findings. Another study in Brazil by Alves et al. reported that the primary tumor occurred commonly between the 3rd and 4th decades of life. A study from the Netherlands by Valster M et al also reported a mean age of 48 years in males and 49 years in females which is in contrast to our findings[12,13].

The current study showed that females were more commonly affected with primary PA as compared to males (M: F ratio = 7:10), while recurrent PA was exclusively found in females. Our findings were in concordance with the findings by Zhan et al in Poland, who found a slightly higher female preponderance in females than in males (1:2 ratio)

[14]. A study done by Aro et al in Finland reported that the PA cases occurred more in females while Almeslet at Riyadh also reported a definite female predilection (M: F ratio = 8:13) in primary cases of PA[15,16].

In this study regarding the site, the parotid gland was found to be the most frequent site of involvement in both primary and recurrent cases of PA followed by the submandibular gland. None of our cases were found in minor salivary glands. A study conducted in Malaysia by Baharoom et al also reported the parotid gland to be the commonest site involved in primary PA[17].

In the current study, cases of both primary and recurrent PA presented with histology of varied morphological patterns, having epithelial and myoepithelial cells with interspersed areas of mesenchymal differentiation. Epithelial cells typically formed duct-like structures associated with non-ductal cells presenting morphological variants. Myxoid, cartilaginous, hyaline, or osseous differentiation was also appreciated in the stromal component. These findings corresponded with findings reported by Nagger Et al.[18].

MUC2 expression was assessed in 51 primary cases and 9 recurrent cases in the present study. In the primary group, 34(53.3%) were negative, with 12 (18.3%) being weak positive, and only 6 (10%) were strongly positive whereas, in the recurrent group, 3(44.4%) cases were weak positive while 6(66.9%) were strong positive. A study by Sebastian Mannweiler et al. reported MUC2 expression in non-neoplastic salivary gland tissues FFPE blocks and strong expression of MUC2 was noted in Warthin tumors with weak expression in PA. This was in accordance with our study which showed a weak expression of MUC2 in 53.3% of primary cases of PA[19,20].

In contrast to this, a study conducted by Hamada et al in Japan analyzed immunohistochemical expression of mucins in 9 recurrent PA cases and 40 of primary PA. MUC2 displayed no expression in primary cases but showed weakly positive expression in only two cases of recurrent PA. There were no cases belonging to the strong positive expression group for MUC2. According to them, there was no significant relation between the expression of MUC2 [21,22,23]. Thus, a comparison of MUC2 expression in primary and recurrent PA cases could be used as a predictive marker for recurrence in cases of primary PA showing characteristics of histopathologic features, particularly the presence of sheets of epithelioid cells and abundance of chondromyxoid stroma.

However, in the present study, we had certain limitations such as a small sample size, as it was study-based research so there was a lack of sufficient time available, financial constraints and a small number in recurrent PA may have added bias to the ratio of males and females.

CONCLUSION

In conclusion, we report that there is an over-expression of MUC2 in recurrent PA which could be a significant risk factor and can be an interesting area for future research. MUC2 could also be used as a predictive marker in the recurrence of PA. A detailed clinical, histopathological and histochemical analysis in salivary gland tumors will provide a better insight to the pathophysiology of the disease, tumor differentiation and prognostic implications.

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None

CONFLICT OF INTEREST

There is no conflict of interest.

ETHICAL APPROVAL

Approval of the study was taken from the Institutional Review Board (IRB) of Prime Foundation Pakistan (IRB Approval No, PRIME/IRB/2021-295).

AUTHORS CONTRIBUTION

All authors contributed equally

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