

Mitotic Index and Immunohistochemical Expression of Ki-67 in Pleomorphic Adenoma of Salivary Glands

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ABSTRACT

Background: Pleomorphic adenomas (PAs) are the most common type of salivary gland tumors (SGTs) which may undergo malignant transformation to Carcinoma ex PA (CaExPA). The purpose of this study was to determine mitotic index (MI) and expression of Ki-67 in Pleomorphic adenomas of salivary gland tumors.

Methodology: This study was carried out on sixty patients of pleomorphic adenoma. Slides with specimens were stained with Hematoxylin & Eosin to count Mitotic index. Labeling Index (LI) of Ki-67 was determined immunohistochemically. Mitotic figures (MF) were counted in 10 HPF selected in the proliferative area. MI was scored as **1** (0 to 4 MF per 10 HPF); **2** (5-9 MF per 10 HPF) and **3** (> 9 MF per 10 HPF). Immunoexpression of Ki-67 was scored as '0-negative' when <5% of neoplastic cells stained, '1-weak positive' when 5-19% of neoplastic cells were stained, '2-moderate positive' when 20-49% of neoplastic cells were stained, and '3-strong positive' when >50% of neoplastic cells were stained with Ki-67. Data were analyzed by SPSS 21.

Results: Mean age was 38.7 ± 12.86 years. Out of 60 cases, 26 (43.33%) were of males while 34 (56.67%) were of females. Expression of Ki-67 was negative in 50% while remaining were weak positive. Only score 01 of MI was observed.

Conclusion: Ki-67 is more sensitive marker than mitotic index in pleomorphic adenomas even in small sized tumors and it can help in detection of malignant transformation of PAs.

Keywords: Adenomas, Immunohistochemistry, Ki-67 Antigen, Parotid Neoplasms, Pleomorphic

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^{1,2}Conception; Literature research; manuscript design and drafting; ^{2,3} Critical analysis and manuscript review; ^{5,6} Data analysis; Manuscript Editing.

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Introduction

Pleomorphic adenoma is the most common salivary gland tumor, also named as mixed tumor. It is reported in both minor and major salivary glands. More commonly it is reported in 4th to 5th decades of life and involves females more than males. It mainly

involves parotid salivary (PSG) but can also be present in submandibular salivary gland (SMSGs). Clinically when it involves parotid gland, it typically presents as a slow growing swelling in front of the ear which is difficult to differentiate clinically from other SGTs. Swelling is initially mobile and not fixed to underlying tissue, without pain and palsy of 7th

cranial nerve.^{1,2,3} When it involves minor SGs, most commonly it occurs at palate followed by upper lip and buccal mucosa accounting approximately for 50%, 27% and 17% of intraoral tumors respectively. PAs which are present on palate are not mobile; however those which occur on lip or buccal mucosa are moveable.⁴ Microscopically, PA is a biphasic tumor which possesses epithelial and mesenchymal components. Typically, it is a well circumscribed, encapsulated tumor; however it maybe incompletely and partially infiltrated by tumor cells. Intraoral tumors particularly palatal PAs lack complete capsule. Glandular epithelial and myoepithelial tumor cells present against the background of mesenchymal like tissue with variable proportion. In various tumors, connective tissue stroma is far more as compared to epithelial components. Some tumors may consist almost entirely of background "stroma". Certain tumors are highly cellular in nature with little background alteration. PA which contains abundant cellularity is called cell rich type and if it contains high amount of connective tissue, it is called stroma rich type.^{5,6} Epithelial component may be arranged in ducts and cystic shape or may occur as islets or sheets of cells. It may also show squamous cells keratinization. Mucous producing cells are also observed. Other type of cells which may give diversity to this tumor are myoepithelial cells. These cells often make up a large percentage of the tumor cells in PAs. These cells have a variable morphology as angular or spindle shaped. In some tumors, myoepithelial cells appear round like plasma cells. Presence of both types of cells help in diagnosis of PAs.⁷

Histopathological diagnosis becomes difficult in certain cases of PAs. It is particularly true when biopsy is small and tissue possesses diversity because of pleomorphism and heterogeneity.² Due to this pleomorphism it may look like chondroid, osteoid and plasmacytoid like tissue. Diagnosis further becomes difficult due to variation in capsule thickness which is difficult to detect, especially in

PAs with mainly mucous parts. Its transformation into Carcinoma (CaExPA) further augments difficulty in diagnosis of PAs due to presence of focal necrosis, extensive hyalinization, vascular or capsule invasion, hypercellularity and atypical mitosis. Its conversion to CaExPA is about 5 to 10 %. Recurrent cases may create difficulty on histopathological diagnosis particularly in cases where stroma is rich and capsule is infiltrated.⁸ Lastly, it also gives challenge due to resemblance to other SGTs like low-grade adenocarcinoma (LgAdC), adenoid cystic carcinoma (AdCC), basal cell adenoma (BCA) and epithelial-myoeplithelial carcinoma (EMC).^{2,3}

Ki-67 is a protein (Antigen) which is observed in all proliferating cells in active phases of cell cycle except G0. This antibody reacts selectively with the nuclei of the proliferating cells. It is used as a marker of nuclear proliferation. Literature revealed role of Ki-67 in Pleomorphic adenoma and its variable expression.^{9,10,11,12} The biological behavior of PA is variable in few cases due to its transformation into CaExPA. Its diagnosis is usually made by conventional histopathological examination and potential malignant transformation is determined by counting mitotic activity however role of immunohistochemistry i.e. Ki-67 need to be investigated. Earlier changes of its transformation into malignancy can be detected with the help of Ki-67 expression, so the aim of this study was to determine mitotic count and expression of Ki-67 in Pleomorphic adenoma, to find percentage of mitotic figures in conventional histopathological examination on H&E staining and percentage of cells showing positive expression of Ki-67 on immunohistochemistry.

Methodology

This descriptive study was conducted in three hospital settings in Lahore from January 2019 to December 2020 after taking ethical permission from Institutional Review Board (IRB) de'Montmorency College of Dentistry, Lahore. A total of 60 cases from

the Department of Surgery, Lahore General Hospital/Post Graduate Medical Institute, Department of Oral and Maxillofacial Surgery, Mayo Hospital, Lahore were included after taking consent from the patients. Sample size was based on a previous study conducted by Muhammad et al.¹¹ Non probability purposive sampling was used for data collection. Recurrent cases of PA (carcinoma ex pleomorphic adenoma), patients on radiotherapy and chemotherapy were excluded from this study. Demographic characteristics such as age, gender, and occupation, along with clinical information such as size of tumor, site (intraoral and extraoral), and type of biopsy of each individual were recorded in a proforma. Gross examination of specimens of PAs were performed in Histopathology Department of LGH/PGMI and information such as size, color and consistency were recorded as per protocol. Routine H&E and Immunohistochemistry were performed. Two histopathologists examined PAs for their histological characteristics using Olympus BH-2 microscope and (×40) objective using this equipment, one high Power field visualizes an area of 0.14 mm.²

Mitotic figures were counted in 10 High Power Field (HPF) selected in the active proliferative area. Scoring was done as **score 1** (0 to 4 mitotic figures per 10 HPF); **score 2** (5 to 9 mitotic figures per 10 High Power Field) and **score 3** (> 9 mitotic figures per 10 HPF). Scoring of Ki-67 was done as negative or positive. Its expression was taken as 0-negative when neoplastic cells were less than 5 (<5%). Positive expression was taken as '1-Weak Positive' when neoplastic cells ranged from 5 to 19% (5-19% of neoplastic cells), '2-Moderate Positive' when neoplastic cells ranged from 20 to 49% (20-49% of neoplastic cells), and '3-Strong Positive' when neoplastic cells were more than 50% (>50% of neoplastic cells). Data was entered in SPSS 21. Numerical variables like Mitotic Index (MI) and cells stained with Ki-67 Labelling Index (LI) were described in terms of mean and standard deviation. Categorical variables like intensity of Mitotic Index

and expression of Ki-67 were presented as frequencies and percentages.

Results

The clinicopathological features of pleomorphic adenoma and Immunohistochemical expression of Ki-67 and Mitotic Index in Pleomorphic Adenoma are given in Table 1 & 2 respectively.

Variables	Sub variables	N (%)
Age in years	20- 40	44 (73.34)
	41-60	4 (6.66)
	61-80	12 (20)
Gender	Male	26 (43.33)
	Female	34 (56.67)
Occupation	Industry	6 (10)
	Farmer	6 (10)
	Labor	34 (56.67)
	Office job	0 (0)
	Household	14 (23.33)
Site	Parotid Gland (PG)	38 (63.33)
	Submandibular SG (SMSG)	16 (26.67)
	Sublingual SG (SLSG)	0 (0)
	Palate	6 (10)
	Tongue	0 (0)
	Labial mucosa	0 (0)
	Buccal mucosa	0 (0)
Laterality	Right	28 (46.67)
	Left	32 (53.33)
Size	<1cm	0 (0)
	1-2cm	0 (0)
	2-5cm	60 (100)
	> 5 cm	0 (0)

*Mean age (38.7 ± 12.86 years)

Table II: Immunohistochemical expression of Ki-67 and Mitotic Index in Pleomorphic Adenoma (N=60)		
Sr. No	Score	Cases N (percentage)
Ki-67 Expression	0-Negative	30 (50)
	1-Weak positive	30 (50)
	2- Moderate positive	0 (0)
	3-Strong positive	0 (0)
Mitotic Index	Score 1 (0-4 MF/ 10 HPF)*	60 (100)
	Score 2 (5 to 9 MF/10 HPF)	0 (0)
	Score 3 (> 9 MF/ 10 HPF)	0 (0)

Discussion

Pleomorphic adenomas are seen more commonly in females. Findings of this study are consistent with this fact and similar results are reported in other studies.^{4,6} Size of tumor is a predictive factor for complication however, in current study it was consistently found to be 2 to 5cm.¹³ Mean age was 38.7 ± 12.86 years which shows that PAs are more frequently found in young age, this is also reported in other studies like Hussain et al., who reported mean age of Pleomorphic Adenoma to be 30.36 ± 4.838 years in their study and Khan et al (2017) who reported mean age of pleomorphic adenoma to be 34 years in population of Lahore.^{14,15}

Diaz et al., reported male predominance as compared to females. The mean age was 43.1 ± 18.0 years (ranging from 13 to 84 years). PAs mainly affected parotid gland followed by submandibular gland. Comparing the results with current study, mean age of subjects with PAs was 38.7 ± 12.86 (ranging from 20 to 80 years). Out of 60 Pleomorphic Adenomas, 38 (63.33%) were located in Parotid, 16 cases (26.67%) in Submandibular gland and 6 (10%) cases were found on the Palate. In Diaz et al study, the average tumor size was 1.6 cm, ranging from 0.8 to 2.4 cm. The mean percentage of Ki-67 immunostaining of PA was 0.06%, ranging between 0 and 0.40% while in this study all 60 cases of Pleomorphic Adenomas (100%) were 2-5 cm in diameter which is greater in diameter reported by

Diaz et al. 50% of PAs were negative while remaining had weak scores for Ki-67. All PAs had 0-4 mitotic figures/HPF. Further, it is also note-worthy that 30 (50%) cases of Pleomorphic adenomas (out of 60) also had weak positive scores for Ki-67 which is quite higher than Diaz study. This difference may be due to difference in methodology because they excluded weak nuclear staining during cell count however we included it in our study.¹⁶

Raja et al., reported that Ki-67 LI was more expressed in cell rich type as compared to stroma rich type 2.49 ± 1.5 and 1.19 ± 1.8 respectively however in this study we did not measure separately in cell rich type and stromal rich type.¹⁷ Cunha et al also reported that Ki-67 LI was less expressed in PAs compared to CaExPA.¹⁸ Ki-67 LI expressed moderate positive in PA in a study by Omitola and Iyogun however there was only one case of Pleomorphic Adenoma.

In another study, Ki-67 LI in benign SGTs ranged from 0.76% to 13 % with highest reported in recurrent Pleomorphic adenoma of the palate of cell rich type. The mean value in PAs was 4.60 with range 1.2 to 13.¹⁹ In another study, the mean \pm SD of Ki-67 LI (%) in normal salivary gland parenchyma was $0.27 \pm 0.31\%$, range (0–0.88%) while in benign salivary gland tumors (15 case out of which 11 were PAs) mean \pm SD of Ki-67 LI was $0.76 \pm 2.02\%$, and there was negligible proliferative activity in pleomorphic adenoma however in this study, 30 cases of PAs expressed weak positive expression.²⁰

Higher values of Ki67 were found among cases with larger size ($p = 0.0061$) and showing greater cellularity ($p = 0.0004$).²¹ In another study, expression of Ki-67 was not observed in benign mixed SGTs however in current study, 50% cases expressed weak positive (5 to 19 cells) and 50% expressed negative.²² Finding of another local study showed that mean age of patients of PAs was 34.13 years with male predominance contrary to our study and most common site was parotid salivary glands similar to current study.²³

Another histopathological study of PA concluded that the pathogenesis and progression of pleomorphic adenoma to CaExPA can be observed on routine H&E section by counting mitotic figures along with metaplasia, pseudopodia, cholesterol crystals, hyalinization, and lymphoid tissue. However still it should be confirmed by applying immunomarkers.²⁴ This finding is consistent with the current study that Ki-67 expression in PA determines certain areas which expressed more than others.

Markkanen's study reported Ki-67 in PAs, recurrent PAs and CaExPAs. Expression of Ki-67 was increased in recurrent PAs and CaExPAs than PAs. Mitotic activity (0-2) in PAs was low in 25 cases (out of 26) while increased mitotic activity (>3) was observed in CaExPAs. Findings regarding mitotic activity in PAs are similar to our study however Ki-67 expression is high in our study in contrast to their study. The majority of studies reported Ki-67 expression is negative to weak positive and some studies reported moderate to strong positive.^{11, 12, 16,17,18,19,20, 21, 25} The results of these studies indicate that biological behavior of PAs is different in cases which recur and transform to malignancy and also show high expression of Ki-67. During routine histopathological examination of PAs when mitotic figures are observed more, then Ki-67 expression may be suggested to observe its early transformation to malignancy (CaExPAs).

One of the limitations of the study is that small sized PAs were included in the study, whereas in literature it was observed that with large size tumors Ki-67 was expressed more, so a large size of PAs may be included in another study to find out a relationship between size of the tumor and Ki-67 expression. Another limitation of this study is that the period of existence of pleomorphic adenomas was not correlated with the expression of Ki-67.

Conclusion

Ki-67 is more sensitive marker than mitotic index in pleomorphic adenomas even in small sized tumors

and it can help in detection of malignant transformation of PAs.

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