

Progressivity analysis of pleomorphic adenoma toward carcinoma ex pleomorphic adenoma

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ABSTRACT

Background: Pleomorphic adenoma (PA) is a benign salivary gland tumour with high incidence and recurrence after treatment. It may recur with the same appearance or develop toward malignancy, namely as carcinoma ex pleomorphic adenoma (CXPA). How this tumour can transform into a CXPA remains unclear. **Purpose:** The aim of this study was to analyse the possibility of pathogenesis and progressivity of PA to CXPA. **Methods:** Twenty-four samples of PA and three control samples of CXPA were stained with haematoxylin and eosin (HE), Mallory's trichrome, and Periodic acid–Schiff (PAS). All of the PA cases were identified through different kinds of stroma, tumour cells types, morphologic patterns, or else through atypical appearance of the PA similar to the CXPA. **Results:** Twenty-four samples of PA demonstrated that the most dominant stroma was myxofibrous, and the dominant tumour cell type was plasmacytoid cells with a trabecular pattern. Additionally, in the pleomorphic histological picture of adenomas we found several patterns of malignant tumour behaviour, including pseudopodia, metaplasia and hyalinisation, and cholesterol crystals that are thought to come from fat cell necrosis derived from adipose metaplasia. **Conclusions:** PA displays several atypical characteristics that have the potential to develop into malignancies such as CXPA, due to capsular infiltration, necrosis, hyalinization and high mitotic activity of cells, but all these atypical characteristics that we observed still cannot be clearly classified as CXPA because they require other specific examinations.

Keywords: pathogenesis; pleomorphic adenoma; progressivity

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INTRODUCTION

Pleomorphic adenoma (PA) is a type of salivary gland neoplasm with an incidence of over 80%. It is also called mixed benign tumour due to its heterogeneous appearance.^{1,2} Histopathologically, this tumour structure consists of duct cells, myoepithelial cells, and mesenchymal cells. It has the appearance of various extracellular matrices, called stroma, such as myxoid, fibrous, chondroid, myxofibrous and myxochondroid stroma. Pleomorphic adenoma often occurs in major salivary glands, mainly the parotid gland.^{3,4}

Carcinoma ex pleomorphic adenoma (CXPA) is often referred to as the growth of de novo malignancy. The growth of de novo malignancy in CXPA is a malignancy that arises not from the benign tumour cells that are transformed into malignant tumour cells but the malignant cells derived from normal cells around the tumour cells.

However, there are reports of CXPA which is a recurrence of PA.⁵ The pathogenesis of PA recurrence that develops towards malignancy cannot yet be explained with certainty because the recurrence rate of this tumour is low and if there is a recurrence of PA after treatment it may appear in the same pattern, or it can develop into malignancy.

Valstar et al.⁶ reported that of the 125 PA patients who had a recurrence, 20 patients (16%) had a recurrence for the second time, and 2 in 20 patients (10%) experienced a recurrence for the third time. However, only 4 patients (3.2%) of all patients who experienced recurrence showed a transformation towards malignancy. The type of malignant tumour which appears after removal is called carcinoma ex pleomorphic adenoma (CXPA).⁶

CXPA, although known as the result of pleomorphic adenoma recurrence, was also reported as de novo malignancy growth. This means that the malignancy

characteristic appeared without previous history. WHO classifies CXPA into three groups according to its carcinomatous stroma's ability to invade the capsule; widely, minimally, and non-invasive.^{6,7} The purpose of this study is to analyse the pathogenesis and progressiveness of pleomorphic adenoma towards carcinoma ex pleomorphic adenoma based on the picture of changes in the atypia of its cells in order to explain whether the cases of CXPA are de novo malignant or a continuation or transformation of the PA.

MATERIALS AND METHODS

This study is a retrospective study that was approved by the medical research ethics committee at the Faculty of Dentistry, University of Jember No.791/UN25.8/KEPK/DL/2019. In this study, cases of PA and CXPA were used which had been diagnosed in the anatomical pathology laboratory of Dr Soebandi Hospital, Jember, Indonesia, between 2017 and 2019. The research samples were obtained through purposive sampling. Samples were selected from all PA and CXPA cases based on the completeness of patient data in the histopathology report such as patient age, gender and tumour location and paraffin-embedded tissue blocks that were still in good condition. Incomplete

histopathology report samples (absent HPA report data or defective paraffin blocks) were excluded from the study. From the sample requirements above, twenty-four PA cases and four CXPA case controls were selected and used in this study.

All tissue on the paraffin-embedding block was cut using a sliding microtome (Tissue-Tek, IVS-410, Sakura Finetek, Tokyo, Japan) for a thickness of 4 µm on as many as three slides; each slide was then stained with haematoxylin and eosin staining (Merck KGaA, Darmstadt, Germany), Mallory's trichrome (BIOGNOST D.O.O, Croatia, Europe) and Periodic acid–Schiff (ScyTek Laboratories, Logan, United States). The histopathological appearance was observed by two examiners under a light microscope (Olympus Cx 43, Tokyo, Japan) with 40x, 100x and 400x magnification.

The heterogeneity of PA was examined through extracellular matrices, cell type, and morphology type. The progressivity from PA to CXPA was observed through the premalignancy atypical appearance (increase of nucleus and cytoplasm ratio, high level of mitotic activity, pleomorphism nucleus and cell, giant nucleoli, loss of cell attachment, capsule infiltration, hyalinisation, and necrosis/anaplastic) in the samples.⁸ We used Optilab Advance (Miconos, Yogyakarta, Indonesia) to perform the observation. The data has been presented in tables and images.

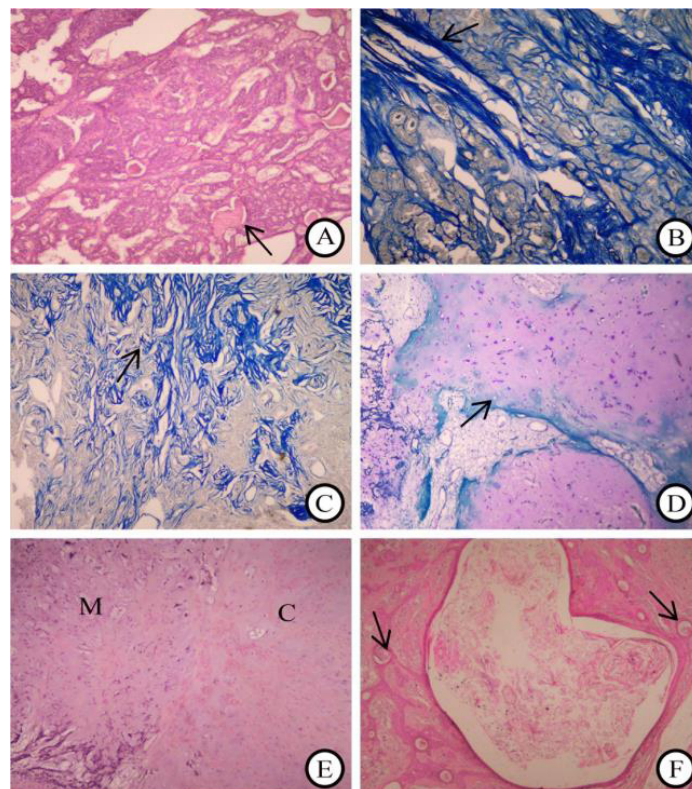


Figure 1. Variation of stroma in PA cases. A: Myxoid stroma with cribriform type showing duct-like cell appearance (black arrow), (HE, 40x). B: Fibrous stroma showing collagenfibres (black arrow), (Mallory Trichrome, 40x). C: Myxofibrous stroma, a blend of myxoid stroma and fibrous stroma (black arrow) that was stained with Mallory Trichrome (Mallory Trichrome, 40x). D: Chondroid stroma (black arrow), (PAS, 40x). E: Myxochondroid stroma, a blend of myxoid stroma (M) and chondroid stroma (C), (HE, 40x). F: Cystic type showing pseudocyst and some microcysts (black arrow), (HE, 40x).

RESULTS

All PA samples consisted of twelve men and twelve women, each with an average age of 44.88 years. PA most often occurs in the parotid gland (54%), followed by the submandibular gland (25%). It is not usual for PA to occur in the minor salivary glands; the latest study shows that 12.5% of PA cases occur in the palatal salivary glands.

The stroma of PA was categorized into five types: myxoid, fibrous, chondroid, myxofibrous, and myxochondroid (Table 1 and Figure 1), and 11 of 24 samples (45.8%) were myxofibrous stroma. During observation of tumour cells, the plasmacytoid cell type was frequently observed; other common cell types were spindle, epithelioid, and clear

cells. Morphologic patterns of PA included trabecular, cribriform, cystic and solid cell types. We observed metaplasia in squamous, chondrocyte and adipocyte cells with atypical characteristics such as capsule infiltration or pseudopodia, necrosis hyalinisation marked by cholesterol crystals, and high level of mitotic activity (Table 2 and Figure 2, Figure 3). Some of the same characteristics were also found in CXPA such as having a myxofibrous stroma and also containing high mitotic tumour cells, hyalinization and specifically found carcinomatous foci among PA tumour cells which infiltrated forming tumour nest into the capsule like pseudopodia in case of PA (Figure 4). The carcinomatous foci of CXPA displayed high mitotic levels, pleomorphism and stromal hyalinisation.

Table 1. Prevalence of pleomorphic adenoma stroma

| Stroma | Myxoid | Myxofibrous | Fibrous | Myxochondroid | Chondroid |
|--------|-----------|-------------|----------|---------------|-----------|
| Total | 7 (29.2%) | 11 (45.8%) | 2 (8.3%) | 3 (12.5%) | 1 (4.2%) |

Table 2. Prevalence of cell type, morphology type, and other variants of histopathology in PA

| Stroma | Ps | Ch | H | Mi | Metaplasia | | | Cell Type | | | Morphology Type | | | | |
|---------------|----|----|---|----|------------|---|---|-----------|----|----|-----------------|----|----|----|----|
| | | | | | Sq | C | A | Pl | Sp | Ep | Cc | Tr | Cr | Cs | So |
| Myxoid | 0 | 0 | 2 | 0 | 1 | 2 | 0 | 5 | 5 | 4 | 2 | 4 | 4 | 2 | 3 |
| Myxofibrous | 2 | 1 | 0 | 0 | 4 | 1 | 0 | 8 | 8 | 6 | 0 | 5 | 7 | 3 | 2 |
| Fibrous | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 0 |
| Myxochondroid | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 1 | 1 | 0 | 2 | 0 | 1 | 0 |
| Chondroid | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Total | 2 | 1 | 2 | 1 | 5 | 4 | 1 | 19 | 16 | 12 | 3 | 13 | 12 | 8 | 5 |

Notes: Ps (Pseudopodia); Ch (Cholesterol Crystal); H (hyalinization); Mi (Mitosis activity); Metaplasia: Sq (Squamosal), C (Cartilagenous), A (Adipose); Cell Type: Pl (Plasmacytoid), Sp (Spindle), Ep (Epithelioid), Cc (Clear cell); Morphology Type: Tr (Trabecular), Cr (Cribriform), Cs (Cyst), So (Solid)

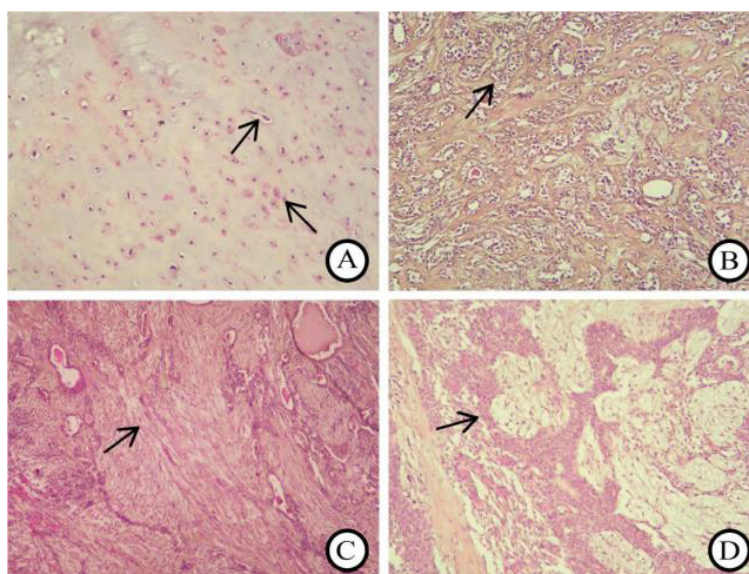


Figure 2. Cell type in PA cases. A: Plasmacytoid type with mitotic activity (black arrow) in chondroid stroma (HE, 100x). B: Clear cell type (black arrow) in fibrous stroma (HE, 100x). C: Spindle type (black arrow), (HE, 100x). D: Epithelioid type (black arrow) in myxoid stroma with trabecular pattern type (HE, 100x).

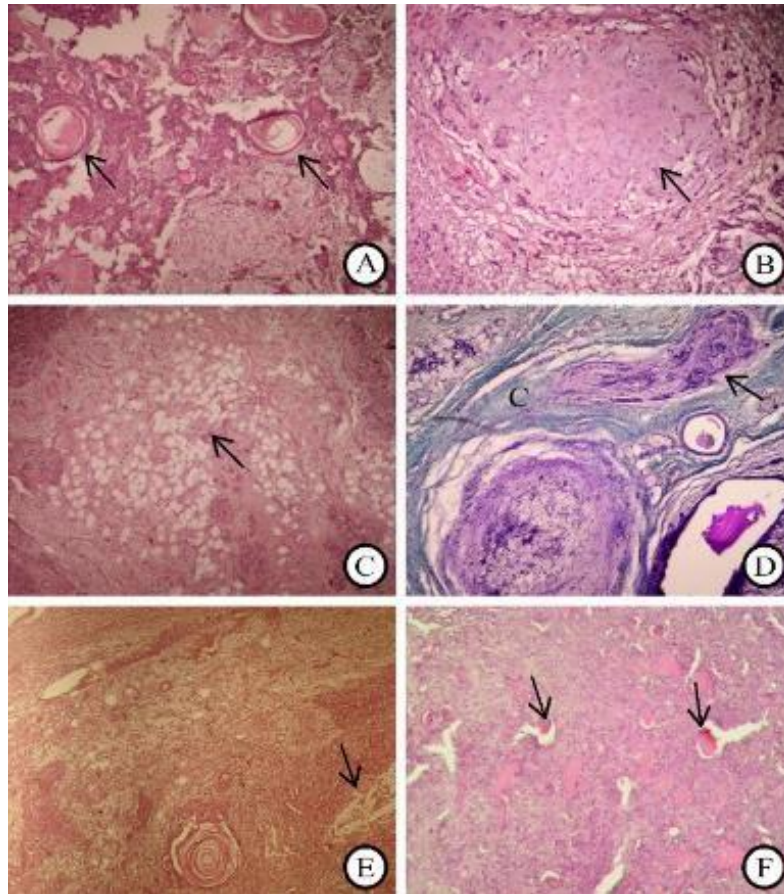


Figure 3. Atypical appearance (pre-malignancy) in PA cases. A: Squamous metaplasia produces keratin and showing keratin pearls appearance (black arrow), (HE, 40x). B: Cartilage metaplasia (black arrow), (HE, 40x). C: Adipose metaplasia (black arrow) is rarely metaplasia in pleomorphic adenoma, (HE, 40x). D: Pseudopodia (black arrow); there are two pseudopodia in one view that are surrounded by fibrous capsules (C), (HE, 40x). E: Cholesterol crystal (black arrow), result of adipose necrosis (HE, 40x). F: Hyalinisation appearance (black arrow) in solid pattern (HE, 40x).

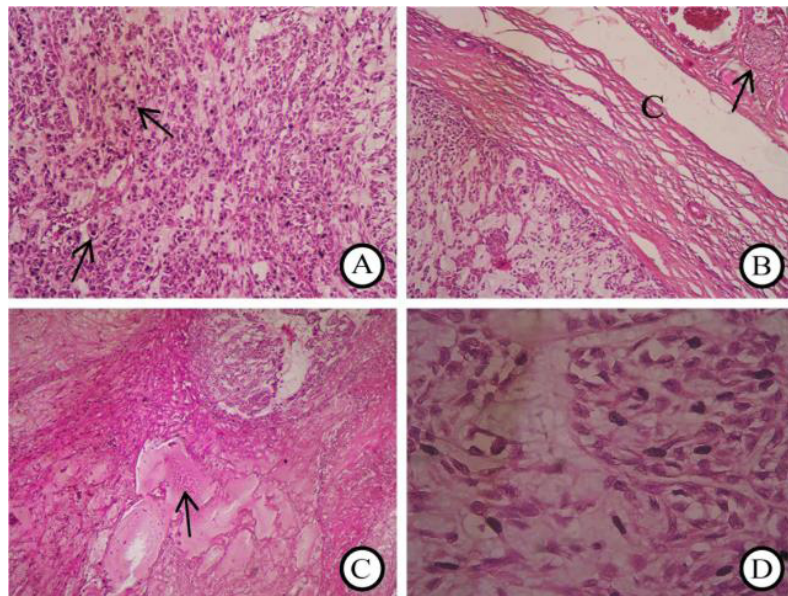


Figure 4. Atypical appearance (malignancy) in CXPA cases. A: Hypercellularity with high mitotic activity (black arrow), (HE, 100x). B: Capsule of tumour (C) with tumour nest infiltration (black arrow), (HE, 100x). C: Hyalinization with necrosis appearance (black arrow), (HE, 100x). D: Loss of cell attachment (HE, 400x).

DISCUSSION

Histopathologically, pleomorphic adenoma demonstrates pleomorphism of its stroma and various cells. The stroma types include myxoid, fibrous, chondroid, myxofibrous and myxochondroid stroma. The formation of PA is mainly attributed to the PLAG1 (pleomorphic adenoma gene 1) and p63/p40 genes. p63 is a known tumour suppressor gene located inside the myoepithelial cell.⁹ p63 and p40 are related to the p53 gene, which is the most commonly mutated oncogene in malignant head and neck tumours. p53 is the last defence when over-proliferation occurs; if this gene undergoes mutation, the apoptosis cycle will be disrupted. Mutations in the p53 gene result in errors in protein formation that will trigger a failure to stimulate and attach other proteins in DNA Binding Domains (DBD). Their mutations, such as translocation, deletion, amplification or point mutation in several codons, can occur in exons 5 to 10. Previous studies have shown that p53 activation was observed in more than 50% of neoplasm cases, especially in malignant cases.¹⁰

In a normal environment, myoepithelial cells could differentiate into ductal cells as cell regeneration occurs. It is one of the reasons why this cell is presumed to play an important role in PA formation when the p63 and p40 genes are mutated and PLAG1 is activated. In PA, this cell can differentiate into other cell types, such as plasmacytoid, spindle, epithelioid, and clear cells.¹¹ Plasmacytoid and spindle cells are the most common types of cell in PA composition. However, Koutlas et al.¹² showed that plasmacytoid cells are not the differentiation form of myoepithelial cells. The negative result of a myoepithelial cell marker test was the reason they concluded that plasmacytoid cells are the result of epithelial-mesenchymal transformation.¹²

Pleomorphic adenomas have several morphological patterns: trabecular, cribriform, cystic, and solid. The cribriform pattern is strongly dominated by cells such as ducts, differentiated types of myoepithelial cells or the ductal cells themselves, which are also capable of producing mucous (mucoïd) secretions. Accumulation of mucoïd material may occur between tumour cells, resulting in a myxomatous background.¹⁰ In this study, it was seen in eosinophilic images in duct-like cells, which also showed myxoid stroma images. Dead cells observed with haematoxylin and eosin staining showed cell remnants and cytoplasmic components in the form of pseudocysts/microcysts.

Myoepithelial cells' proliferation and differentiation ability to other cell types is quite recognisable. It may differentiate into epithelial cuboid cell of ductus, and become a hallmark of malignancy. The duct-like cell also could undergo non-cancerous change (metaplasia) into a squamous cell, which is known as squamous metaplasia. Squamous metaplasia also produces keratin and causes the appearance of keratin pearls. Furthermore, this cell mutation sometimes continues to occur and develops into dysplasia, marked by the increasing presence of mitotic figures and pleomorphism nuclear that signal the beginning

of a malignant tumour. However, there are researchers who report that the pleomorphic adenoma transformation into CXPA is not affected significantly by the number of keratin pearls appearing. Adipocyte and chondrocyte cell metaplasia was also observed in PA, which supports the possibility of metaplastic change in plasmacytoid.¹³

Pseudopodia in PA are tumour nodules bulging from the tumour edges and separated by fibrous tissue from the main tumour mass, but still localised within the main tumour capsule because it appears inside the capsule of PA; this is known as capsule infiltration.¹⁴ It is possible that this infiltration into the capsule occurs due to mutations in the metastatic gene allowing tumour cells to destroy capsules through their proteolytic enzymes. The components of the plasminogen activation system (PAS) and the metalloproteinase family [mainly matrix metalloproteinases (MMPs)] are overexpressed in malignant tumours.¹⁵ It has been reported that pseudopodia are found in the myxoid matrix, and this increases the risk of recurrence, mostly in cases with enucleated treatment choice.

Our study of PA also found necrotic foci inside the tumour; we observed tissue necrosis caused by insufficient blood supply. The tumour cell has no supply of oxygen or nutrition which leads to its death.¹⁶ Normal cells surrounding tumour cells can be necrotised due to tumour cells producing pro-inflammatory cytokines such as TNF- α , IFN. IL-1 β , IL-3, IL-5, IL-6, IL-8.¹⁷ In this study, we observed one case showing cholesterol crystal, the hallmark of adipocytes necrosis, Metaplastic adipocytes also experienced no oxygen and nutrition supply which led to a necrotic state. This process is important to limit the tumour cells' growth; however, it is also one of the main indicators of malignancy. The rapid growth of malignant cells is often impossible to stop through the necrosis process. The tumour only needs several months or even weeks to develop into malignancy.^{5,6}

Hyalinisation is also a signature of malignancy. PA with hyalinisation is usually the turning point condition into premalignancy.^{15,18} We also observed a high level of mitotic cells in one case of PA samples, even though it was not as high as the CXPA (Figure 4). From all the PA samples, only three cases (12.5%) of atypical appearance were observed as similar to CXPA appearance, such as mitotic figure appearance, squamous metaplasia with keratin pearls, nuclear pleomorphism and capsule infiltration (pseudopodia).

Based on this study, we can conclude that the pathogenesis and progression of pleomorphic adenoma to CXPA can be seen in atypical features that serve as markers of premalignancy, such as metaplasia (squamous, adipose, and cartilage), pseudopodia, cholesterol crystals, hyalinisation, lymphoid tissue, and mitotic features. These factors can give pleomorphic adenomas the potential to transform into malignancy, although in those cases we have not been able to classify them as CXPA because specific examination (immunostaining) is required to confirm transformation from PA to CXPA.

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