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# Extracellular matrix types and distribution of pattern differentiation of ameloblastoma based on histopathological type appearance

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## ABSTRACT

**Background.** The stromal tumor as Extracellular Matrix (ECM) of Ameloblastoma is a complex structures surrounding intercellular and extracellular that supporting cells motility and regulation of cell proliferation, cells shape, and their function in connective tissue. Histological appearance of Ameloblastoma ECM particularly are fibrocollagen and fibromyxoid. The activation of stromal tumor predicted to increase tumor aggressiveness by stimulation of angiogenesis and invasiveness. Several author reported that there were several pattern of stromal tumor in the body such as focal, network and spindle pattern. **Purpose.** The purpose of this study to identify and analyze the ameloblastic stromal tumor pattern and their distribution based on their histological appearance type. **Methods.** Using fifteen paraffin embedded tissue taken from Jember district hospital that were surgically treatment from year 2010 to 2014 and diagnosed as ameloblastoma. All specimen cutted 4  $\mu\text{m}$  and then performed HE and Mallory trichrome staining. All cases observed and analyzed using light microscope with cubical micrometer scale (graticulae) 400x magnification. **Results.** ECM pattern of Ameloblastoma cases were 5 cases plexiform type showed network pattern, 1 case was focal pattern and another case was spindle. Whereas 4 cases of follicular type showed spindle pattern, 2 cases were network pattern and 2 cases were focal. **Conclusion.** The stromal tumor of ameloblastoma types particularly network pattern (54%) that distributed between tumor nest, this pattern perhaps due to local invasiveness and aggressiveness.

**Keywords :** ameloblastoma, pattern, stromal, type

## INTRODUCTION

Ameloblastoma is a benign odontogenic tumor of epithelial origin that develops most often in the jaw [1]. Ameloblastomas are slow growing, and locally invasive tumors with a high level recurrence. Clinically, Ameloblastomas are typically asymptomatic, although they

may cause jaw expansion and be able infiltrate the other area [2]. The Etiology and pathogenesis of Ameloblastomas are unknown [3].

Extracellular matrix (ECM) as stromal tumor is a complex structure surrounding tumor cells which is bordering by the body tissue. The main functions of ECM are to fill space between cells, to provide a barrier that isolates tissue from each other, to provide signals that alter cell behavior, to provide navigational cues for migratory cells, and to sequester biologically active compounds such as growth factors [4]. The activation of stromal tumor predicted to enhance tumor aggressiveness by stimulating angiogenesis, and by promoting tumor cell survival, proliferation, and invasion. The main component of ECM in this reaction is myofibroblast. Myofibroblasts are derived from granulation tissue fibroblast that synthesize ECM components such as collagen I, collagen III, fibronectin isoforms, tenascin, and versican. In addition, myofibroblasts express protease, including urokinase plasminogen activator, and matrix metalloproteinase [5].

Recently, several authors reported that the growth and development of tumor (cells proliferation, invasion, adhesion and cells migration) was mediated by ECM [6]. ECM of multicystic Ameloblastomas were fibrocollagen. Whereas, plexiform pattern was dominant fibromyxoid [7]. Invasion of fibromyxoid was higher than fibrocollagen. The type of stromal tumor predicted to influence behavior of biological cells.

In Indonesia, the optimal treatment of Ameloblastomas has been the subject of controversy to choose radical treatment or conservation treatment for many years. Because of result of histopathological anatomy observation was incomplete by oral pathologist. Whereas, the selection of treatment based on result by oral pathologist. Remember that the growth rate of Ameloblastoma was rapid with a high recurrence, which all of them influence by ECM. Therefore in this study need to observation ECM type and distribution of pattern Ameloblastoma to help identify and analyze the behavior of biological of tumor cells and a consideration of treatment in clinic.

## **MATERIALS AND METHODS.**

This research is a descriptive retrospective study. This research was in the laboratory of Pathology Anatomy, Faculty of Dentistry, Jember University on January 2015 - May 2015. The population were data of histopathological observation, paraffin embedded tissue, and slide of Ameloblastomas patients who treat in the hospital Jember distric. The sample was 15 that were selected based on criteria inclusion.

The procedures of research, using fifteen paraffin embedded tissue taken from Jember district hospital that were surgically treatment from year 2010 to 2014 and diagnosed as ameloblastoma. All specimen cutted 4  $\mu\text{m}$  and then performed HE and Mallory trichrome staining. All cases observed and analyzed using light microscope with cubical micrometer scale (graticulae) 400x magnification. HE staining is observate ECM type, and Mallory trichrome is observate distribution pattern of ECM

ECM type particularly such as fibromyxoid and fibrocollagen [2]. Whereas, considering the distribution pattern of myofibroblasts, the arrangement was classified into 3 groups :

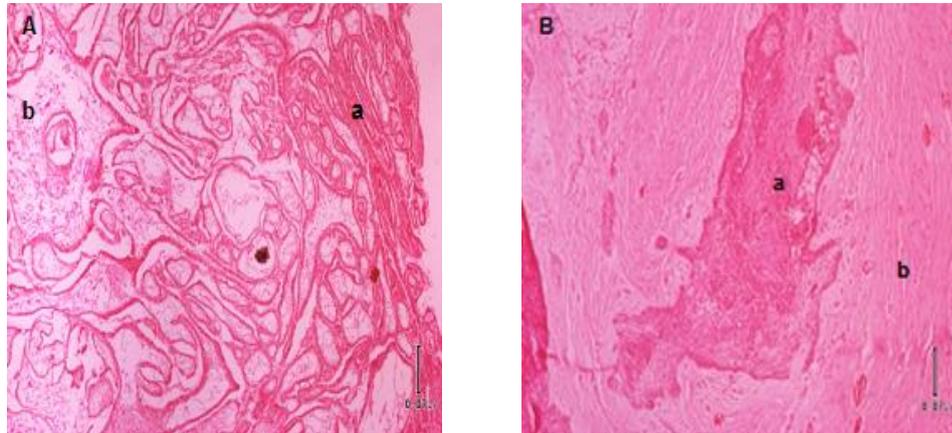
1. **Focal**, If myofibroblasts had a focal arrangement or had no special arrangement in different areas of connective tissue and stroma.
2. **Network**, Myofibroblasts with vesicular nucleus and abundant cytoplasm arranged in multiple rows with interwoven network of cytoplasmic extensions forming a network in the stroma of the connective tissue.
3. **Spindle**, Myofibroblasts arrange in one to three rows in a regular order in the periphery of the neoplasm islands or in the connective tissues with distinctive cell margins around myofibroblasts and tumor tissue [6].

## RESULT

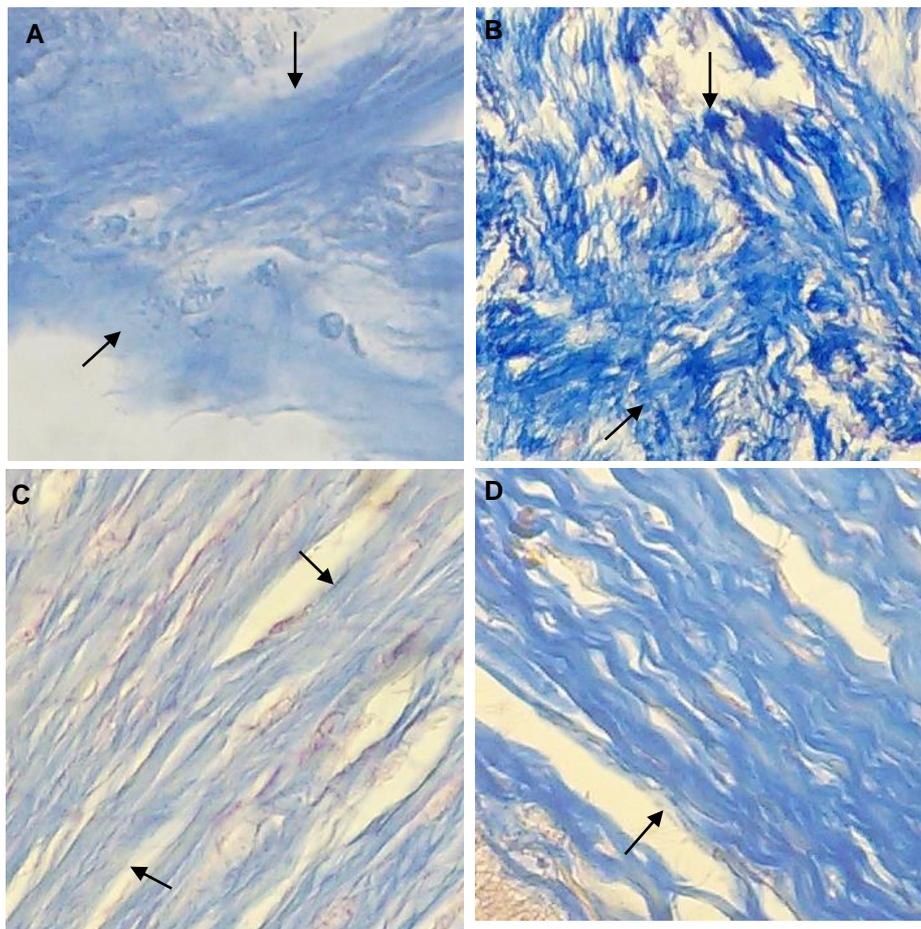
The result of Ameloblastoma's ECM type by Haematoxylin Eosin stain were 7 of 15 cases showed fibrocollagen (47%) and 8 cases showed fibromyxoid (53%). And their ECM distribution pattern by Mallory trichrome were 5 cases plexiform type showed network pattern, 1 case was focal pattern and another case was spindle. Whereas 4 cases of follicular type showed spindle pattern, 2 cases were network pattern and 2 cases were focal. The result are summarized in Table 1.

**Table 1 Result of extracellular matrix type and distribution of pattern differentiation of Ameloblastomas tumor**

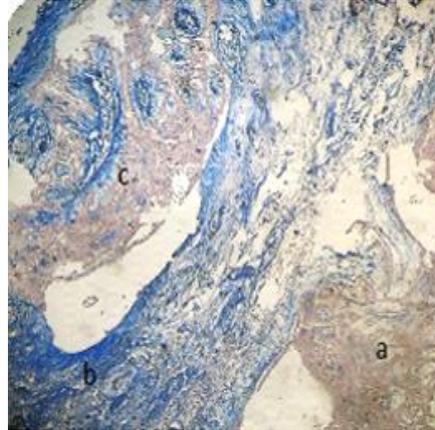
No	No. Slide	Histological Appearance	ECM type	Distribution Pattern of Myofibroblast
1	PA 09-107	Plexiform	Fibromyxoid	Spindle
2	PA 10-468	Follicular	Fibrocollagen	Network
3	PA 11-439	Follicular	Fibrocollagen	Network
4	PA 11-577	Plexiform	Fibromyxoid	Network
5	PA 12-76	Follicular	Fibrocollagen	Spindle
6	PA 12-194	Follicular	Fibrocollagen	Spindle
7	PA 12-688	Follicular	Fibrocollagen	Spindle
8	PA 13-615	Plexiform	Fibromyxoid	Network
9	PA 13-1038	Plexiform	Fibromyxoid	Focal
10	PA 13-1062	Follicular	Fibromyxoid	Focal
11	PA 14-307	Follicular	Fibrocollagen	Spindle
12	PA 14-559	Plexiform	Fibromyxoid	Network
13	PA 14-1038	Plexiform	Fibrocollagen	Network
14	PA 14-1148	Follicular	Fibromyxoid	Focal



**Figure 1 ECM type of Ameloblastoma**, odontogenic cells consist of large anastomosing cords, sheet (a) and the stroma tend to be loosely arranged and vascular (b) (A) Ameloblastoma contains an island epithelial with keratin formation in the central portions (a) and cords of odontogenic epithelium in a densely collagenized stroma (b)(B) ( HE : 100x)



**Figure 2 ECM distribution pattern of Ameloblastoma**, focal pattern, myofibroblasts had no special arrangement in different areas (A), network pattern, myofibroblasts had irregular arrangement and forming a network in the stroma (B), and spindle pattern, myofibroblasts arrange in several rows in regular in the connective tissue (C dan D) (Mallory trichrome : 400x)



**Figure 3.** It appears the proliferation of new tumor nest (c) in the capsule tumor (b) and plexiform pattern with fibromyxoid and network pattern (a) (Mallory trichrome : 40x)

## DISCUSSION

Ameloblastoma is a benign odontogenic tumor that the most occur in the jaw. Characteristic of Ameloblastomas are slow growing but locally invasive with a high recurrence rate [1]. Early detection of Ameloblastoma is difficult by clinical because the tumor is often asymptomatic and smaller lesions are detected only during a radiographic examination [3]. Growth and development of Ameloblastoma in the jaw involve by ECM. Because of them, in this study we need to identify and analyze the role ECM influence behavior tumor by examination of ECM type and distribution pattern. Although, until now the mechanism of ECM against locally invasive and aggressiveness Ameloblastoma is still unknown [6]. Based on the statement, we began investigated the role of ECM as driver in the growth and development of Ameloblastoma.

The result of observation by histo-pathological anatomic about ECM type of Ameloblastoma were 6 of 7 cases plexiform pattern showed fibromyxoid type and another case showed fibrocollagen. Whereas 6 of 8 cases follicular pattern showed fibrocollagen and two cases other showed fibromyxoid. This result shown differences in the behavior of invasive Ameloblastoma. Invasion of Fibromyxoid stromal is higher than fibrocollagen [7]. Because of fibromyxoid stromal has molecule which is supporting invasiveness tumor cells to penetrate the other tissue and mediating communicated between cells by homeostatic. So, the growth and development of tumor with fibromyxoid stromal still be able while tumor microenvironment is poor vascularity (hypoxia) [8].

Hypoxia can stimulate tumor cells to proliferation when condition marked by low oxygen intake [8]. Molecule of ECM can promoted angiogenesis in hypoxia with proangiogenic growth factors that is produced by fibroblast and take on cells to generated

cytokines [9]. The important molecule in the tumor environment is integrin. Integrin is a receptor for interstitial collagens involved in cell migration and collagen reorganization on mesenchymal non muscle cells that consists of two sub unit  $\alpha$  and  $\beta$ . The function of integrin is to mediate interaction between cells and between cells with ECM, to regulate transduction signal for proliferation, survival, adhesion, and invasion [10].

Compartment of tumor stromal known to play an important role in the growth characteristics and biological behavior of tumor. Tumor stromal is not only as an advocate for epithelial neoplasm, but also active and dynamic role in tumorigenesis. The aggressiveness and invasiveness of the tumor is affected by the tumor microenvironment. The tumor microenvironment is stimulating tumor cells to secrete a variety of substances which facilitate angiogenesis and invasiveness to infiltration adjacent tissue [11]. Its consists of a mixture of ECM molecule, endothelial cells, fibroblasts, hematopoietic cells and immune cells. ECM have a structure that to supports the adhesion of tumor cells to surrounding tissues and to mediated communication cells by homeostatic [12]. The growth, invasion and aggressiveness of tumor occur due to change by imbalance of ECM molecules with the other cells especially myofibroblasts and cytokine. Lynch reported that myofibroblasts act as biological factors to stimulate a variety of mediators that support, enhance and promote of tumor invasion, include enzyme urokinase, fibroblast growth factors, e-cadherin, and matrix metalloproteinases (MMPs) [13]. Matrix metalloproteinases is secreted by myofibroblasts as ECM components, play a role in the invasion of tumor through the destruction of the extracellular matrix, tumor formation, migration, invasion, metastasis, angiogenesis and induction of apoptotic clones [14].

In this study, we identify distribution pattern of ECM based on composition myofibroblasts. The examination result showed that 3 of 15 cases were focal pattern, 5 cases were spindle pattern and 7 other cases were network pattern. This pattern predicted to correlate by their type of Ameloblastomas. Plexiform pattern tends network pattern, while follicular pattern is spindle pattern. This is due to differences by proportion of myofibroblasts in stromal tumor. Arrangement myofibroblasts of plexiform pattern have a high proportion, loosely and large anastomosing cords, and it difference with follicular type. The proportion of myofibroblasts are loosely and large anastomosing cords that support to easy penetration and migration cells of tumor. If the proportion of myofibroblasts are slightly, the collagen fibers in the tumor stromal become dense and thick tends to show a spindle pattern [6].

Increasing the proportion of myofibroblasts by Mallory trichrome stained showed blue, implies the stromal tumor typical a loss of smooth muscle cells is accompanied by a decrease in collagen in the tumor stromal. In this condition, myofibroblasts predict to undergo remodeling and activation as a signal response of aggressiveness and growth tumor. it marked by a presence of myofibroblasts layer thickened in some area tissues [5]. Furthermore, increasing of myofibroblasts make tumor stromal became network. When the distribution pattern of tumor stromal is network, it shows more aggressive than spindle and focal pattern.

In other research, Ayala reported that the distribution pattern result were analyzed to correlate with recurrence-free survival data. Interpretable data were obtained from 545 patients. Analyze of distribution pattern of tumor stromal, the network pattern has a high recurrence rate than the other pattern. It due to a high percentage of the reactive stromal in the

network pattern that impact on progression and invasion of tumor. furthermore, it can transform became malignant neoplasm [15].

The type and distribution pattern of Ameloblastoma stromal hold an important role in determining the biological behavior of tumor include aggressiveness, locally invasion and recurrence of tumor. It can be taken into consideration of Ameloblastomas treatment in the clinic. It is supported by the result of research that found a plexiform pattern with fibromyxoid and network pattern which newly formed tumor nest penetrate into the tumor capsule. Based on these result, it can be assumed that fibromyxoid and network pattern have a rapid aggressiveness, and invasiveness with a high recurrence rate.

It is important to understand the growth characteristics and to remove the full extend of the tumor, including the surrounding tissues when planning the treatment of Ameloblastoma. Otherwise, the remaining tumor cells may lead to multiple morbidities of recurrence. Resent advancements in the understanding of biological behavior of Ameloblastoma have revealed that lesions with fibromyxoid and network pattern of tumor stromal are characteristic by a rapid aggressive infiltration to adjacent tissue with a high recurrence rate [1][6]. Theoretically, the treatment of Ameloblastoma with fibromyxoid and network pattern of tumor stromal was radical treatment. To prevent the recurrence of the tumor, patients were scheduled for clinical examination twice a year for the first 3 years and after that only once a year. If it found any recurrence, can be done re-surgically [16]. Because our analyze result of the growth characteristics of Ameloblastoma showed a rapid aggressiveness, high recurrence rate and locally invasion with infiltration to adjacent tissue.

The proportion can be different from the reality incident because objects in this research are patients who check up and treat into the hospital of Jember district Besides, the presence of latent tumor without syptoms to progress to malignancy suffers even though death can occur.

In summary, the present result justify investigations on a huge scale to assess whether the frequency of tumor cells undergoing such modifications may be correlated with variations in the biological behavior of Ameloblastoma and clinical out comes. Realizing that myofibroblasts are part of the tumor that contribute to its aggressiveness and invasiveness. Myofibroblasts as the tumor cells are in a dynamic state of changing phenotypes of ameloblast toward a neoplasm differentiation. This result will relate with treatment of tumor on future especially Ameloblastoma Implying that future tumor therapies would have to target stromal constituents and should not focus solely on conventional tumor cells. [6].

## **CONCLUSION.**

1. ECM type of plexiform pattern tends fibromyxoid, while follicular pattern is fibrocollagen.
2. Distribution pattern of plexiform stromal is network pattern and follicular stromal tends spindle pattern.
3. Fibromyxoid and network pattern have a high aggressiveness, invasiveness, and recurrence rate of tumor.

4. Role of the type and distribution pattern of tumor stromal in this study proved to be able determine the aggressiveness, invasion, and recurrence of Ameloblastoma. It can be innovated in the clinical applications and the data fields tumor especially Ameloblastoma on future for therapeutic management.

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