

Immunohistochemical detection of α_1 -antitrypsin, α_1 -antichymotrypsin, transferrin and ferritin in ameloblastoma

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Abstract

An immunohistochemical study was performed to investigate the presence of α_1 -antitrypsin (α_1 -AT), α_1 -antichymotrypsin (α_1 -ACT), transferrin and ferritin in 32 ameloblastomas and to evaluate the co-expression of these antibodies. Histologically, we recognized the following 6 patterns in the series of 32 ameloblastomas and at least 2 patterns in variable proportions were present in each of our cases: follicular pattern (21 cases, 66%), plexiform pattern (17 cases, 53%), cystic pattern (21 cases, 66%), acanthomatous pattern (10 cases, 31%), granular cell type (2 cases, 6%), and hyalinized stromal pattern (20 cases, 63%). Neoplastic epithelia of cystic pattern were divided into superficial cell, basal cell and whole layer to compare the immunohistochemical localization. The results made on the various patterns of ameloblastomas were as follows: (1) α_1 -AT positivity in plexiform, cystic and hyalinized stromal patterns was significantly higher than that of α_1 -ACT ($P < 0.05$). (2) The incidence of transferrin in follicular and plexiform patterns was markedly higher than that of ferritin in the same patterns ($P < 0.025$ and $P < 0.01$). Transferrin strongly stained metaplastic squamous cells of acanthomatous pattern and basal cells of cystic epithelium. (3) Granular cells reacted with transferrin and ferritin. (4) In follicular and acanthomatous patterns, co-expression of α_1 -AT and α_1 -ACT, α_1 -AT and transferrin, or α_1 -ACT and transferrin was higher than that of another combination. On the other hand, co-expression of α_1 -AT and transferrin in plexiform and cystic patterns was higher than that of other antibodies. These

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results of co-expression of 4 antibodies used in the present study, suggest that the histogenesis of follicular and acanthomatous patterns is different from that of plexiform and cystic patterns.

Keywords: Ameloblastoma; Alpha-1-antitrypsin; Alpha-1-antichymotrypsin; Ferritin; Transferrin

1. Introduction

The ameloblastoma is the most common epithelial odontogenic tumour, constituting between 11% and 18% of all odontogenic tumours [3,23]. Several histologic variants of ameloblastoma have been described including follicular, plexiform, unicystic, acanthomatous, granular cell, and basal cell types [7,12,17,22,24,37]. The immunohistochemical characterization of tumours in this group is, however, still debatable, and unequivocal evidence has not yet been presented for these tissue components of ameloblastomas.

It is well known that α_1 -antitrypsin (α_1 -AT) and α_1 -antichymotrypsin (α_1 -ACT) inhibit proteases and are thereby an important factor in regulating proteases released during inflammation. Furthermore, α_1 -AT and α_1 -ACT have been demonstrated in neoplastic lesions [6,11,14,15,25,28–32]. Transferrin and ferritin are major iron-binding proteins in human biological fluids and tissues. Transferrin has a high affinity for iron for the turnover and replication of normal and neoplastic cells. Ferritin is known to be an important iron-storage protein that plays a crucial role in cell metabolism and especially cell proliferation [2,8]. By immunohistochemical study, the distribution of these proteinase inhibitors and iron-binding proteins has been investigated in normal human tissues [1,9,16,19,29,31–33] and in a wide variety of malignant states [1,14,15,26,28–33]. Recently, Takahashi et al. [30] indicated that the presence of protease inhibitors in salivary gland pleomorphic adenoma was related to the pathogenesis of this benign tumour. Furthermore, Takahashi et al. [29] described that basal cells of neoplastic oncocytes in adenolymphoma produced transferrin in order to have a greater availability of intracellular iron for their turnover. However, to our knowledge, the effect of the presence of α_1 -AT, α_1 -ACT, transferrin and ferritin on the tissue components of ameloblastomas which are categorized as the benign form of odontogenic epithelial tumour [17] has not been studied previously.

The present study was conducted to assess the incidence, distribution and co-expression of proteinase inhibitors (α_1 -AT and α_1 -ACT) and iron-binding proteins (transferrin and ferritin) in the various histologic patterns of ameloblastoma, and to investigate the participation of these 4 proteins in histogenesis of ameloblastoma.

2. Materials and methods

2.1. Patients

Thirty-two ameloblastomas were retrieved from the files of the Department of Pathology, Nagasaki University Hospital (18 cases) and Department of Oral Pathology, Nagasaki University School of Dentistry (14 cases). The tumour was

noted equally in both sexes (17 men, 15 women). Nearly two-thirds of the patients were in the second, third and fourth decades of life. The patients' ages at the time of histologic diagnosis ranged from 9 to 72 years, with the average being 33 years. About 94% of the tumours were found in the mandible; more than 70% were in the posterior body and ramus.

2.2. Conventional light microscopy

All tissues were fixed in 10% buffered formalin, and embedded in paraffin after dehydration. For each case, 3 or more sections of the tumour were stained with hematoxylin-eosin. The slides were reviewed and each tumour was histologically subclassified into the following categories: follicular, plexiform, cystic, acanthomatous, granular cell, basaloid and hyalinized stromal patterns. At least 2 of these components in variable proportions were present in each of our cases. The criteria used for the histologic classification of these tumours were those suggested by the World Health Organization (WHO) [17] and are generally used by most oral pathologists.

2.3. Immunohistochemistry

Twenty serial sections (3 μ m) of tissues fixed in 10% formalin and embedded in paraffin were used for immunohistochemical staining. The paraffin sections were studied using an avidin-biotinylated peroxidase technique. Briefly, sections were preincubated in 0.3% hydrogen peroxide in methanol for 30 min to block the intrinsic peroxidase activity and then in normal swine serum (dilution 1:20) for 30 min to decrease non-specific staining. As primary antibodies, polyclonal antibodies to α_1 -AT (dilution 1:1500), α_1 -ACT (dilution 1:100), transferrin (dilution 1:400) and ferritin (dilution 1:200) (purchased from Dakopatts, Copenhagen, Denmark) were used. Primary antibody was incubated in a moist chamber at room temperature for 60 min, and the sections were rinsed 3 times in phosphate-buffered saline (PBS), 5 min each time. The sections were next reacted with biotinylated goat anti-rabbit antibody (Vector Labs., Burlingame, CA) for 60 min, and rinsed 3 times with PBS. Sections were finally treated with avidin-biotinylated peroxidase complex for 30 min, and rinsed with PBS. For the demonstration of peroxidase activity, the sections were incubated for 10 min with 0.03% 3-3' diaminobenzidine hydrochloride (DAB) containing 0.005% hydrogen peroxide. Sections were then counterstained with Harris' hematoxylin, dehydrated, and mounted. As positive controls, normal liver and lymph node were stained with each of antibodies. Negative controls were provided by omitting the primary antibodies and replacing them by non-immune serum. The presence or absence of co-expression of α_1 -AT and α_1 -ACT, transferrin and ferritin, α_1 -AT and transferrin, or α_1 -ACT and transferrin was investigated using serial paraffin sections. The frequency of positive immunostaining was statistically examined with χ^2 analysis.

3. Results

3.1. Histologic typing of ameloblastoma

Thirty-two ameloblastomas were classified according to WHO classification [17].

Almost all cases contained areas with 2 or more histologic patterns in each tumour and were counted in each category. The present study contained 21 cases (66%) of follicular pattern, 17 cases (53%) of plexiform pattern, 21 cases (66%) of cystic pattern, 10 cases (31%) of acanthomatous pattern, and 2 cases (6%) of granular cell type. In addition, we found 20 cases (63%) of hyalinized stromal pattern in direct contact with neoplastic epithelial elements.

3.2. Immunohistochemistry

Immunohistochemical results are shown in Tables 1–4. Since the co-expression of α_1 -AT and ferritin or α_1 -ACT and ferritin was hardly observed, we investigated synchronous localization of α_1 -AT and α_1 -ACT, transferrin and ferritin, α_1 -AT and transferrin, or α_1 -ACT and transferrin in ameloblastomas as shown in Tables 3 and 4.

Follicular pattern (21 cases) (Tables 1 and 3). Typical follicular ameloblastoma was composed of the centrally located cells with stellate reticulum-like morphology and the peripherally located columnar cells. The immunoreactive rate of central cells and peripheral cells for the same antibody was quite similar. The positive frequency of α_1 -AT in both cell types (Fig. 1, upper) was not significantly higher than that of α_1 -ACT. Transferrin was identified in both of central and peripheral cells in 15 (71%) of 21 cases (Fig. 1, lower), and recognition of this antibody was significantly higher than ferritin staining ($P < 0.025$) (Table 1).

In neoplastic cells of follicular pattern, the combination of α_1 -AT and α_1 -ACT, α_1 -AT and transferrin (Fig. 1) or α_1 -ACT and transferrin was more frequently observed than that of transferrin and ferritin (Table 3).

Plexiform pattern (17 cases) (Tables 1 and 3). The neoplastic cells proliferated, forming an anastomosing network. This pattern was observed in 17 ameloblastomas. For the neoplastic cells of plexiform pattern, α_1 -AT immunoreactivity was found in 13 (76%) of 17 cases (Fig. 2, upper), whereas α_1 -ACT immunoreactivity was recognized in 6 (35%) of 17 cases (Fig. 2, middle). The positive frequency between the 2 antibodies indicated significant difference ($P < 0.05$). The presence of transferrin was strongly positive in almost all the neoplastic cells in 15 (88%) of 17 cases (Fig. 2, lower). The positive frequency of transferrin was significantly higher than that of ferritin in plexiform pattern ($P < 0.01$) (Table 1).

Neoplastic cells in plexiform pattern showed frequent co-expression of α_1 -AT and transferrin (Table 3). Frequency of co-expression of α_1 -AT and transferrin was significantly higher than that of the combination of another 3 types ($P < 0.05$) (Fig. 2).

Cystic pattern (21 cases) (Tables 1 to 4). Twenty-one cases had a definite large cystic configuration and belonged to cystic pattern. Microcysts in the central parts of many epithelial islands were not classified as cystic pattern. In the cystic ameloblastoma, cystic epithelium was more frequently stained with α_1 -AT and transferrin than with α_1 -ACT and ferritin ($P < 0.05$) (Table 1). On the basis of localization of these 4 antibodies in the cystic epithelium, they were divided into 3 groups — positive cases in only the superficial cell layer, positive cases in whole layer, and positive cases in only basal cell layer of cystic epithelium. α_1 -AT was

Table 1
Immunohistochemistry in various histologic patterns of ameloblastoma

Histologic patterns	No. of cases	Positive cases of immunostain					
		α_1 -Antitrypsin		α_1 -Antichymotrypsin		Transferrin	
		<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)
Follicular	21	17	(81)	12	(57)	15	(71)
Plexiform	17	13	(76)	6	(35)	15	(88)
Cystic	21	19	(90)	13	(62)	20	(95)
Acanthomatous	10	10	(100)	9	(90)	9	(90)
Hyalinized stromal	20	16	(80)	2	(10)	10	(50)

Table 2
Immunohistochemistry in cystic pattern of ameloblastoma (21 cases)

Cell layer	Positive cases of immunostain					
	α_1 -Antitrypsin		α_1 -Antichymotrypsin		Transferrin	
	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)
Superficial cell layer	7	(33)	10	(48)	6	(29)
Whole layer	12	(57)	3	(14)	9	(43)
Basal cell layer	0	(0)	0	(0)	5	(24)
Total	19	(90)	13	(62)	20	(95)

Table 3
Immunohistochemical co-expression in various histologic patterns of ameloblastoma

Histologic patterns	No. of cases	Positive cases of co-expression			
		α_1 -Antitrypsin and α_1 -Antichymotrypsin	Transferrin and Ferritin	α_1 -Antitrypsin and Transferrin	α_1 -Antichymotrypsin and Transferrin
Follicular	21	12 (57%)	2 (10%)	15 (71%)	12 (57%)
Plexiform	17	4 (24%)	1 (6%)	11 (65%)	5 (29%)
Cystic	21	5 (24%)	0 (0%)	10 (48%)	4 (19%)
Acanthomatous	10	9 (90%)	3 (30%)	9 (90%)	8 (80%)
Hyalinized stromal	20	2 (10%)	1 (5%)	8 (40%)	2 (10%)

Table 4
Immunohistochemical co-expression in cystic pattern of ameloblastoma (21 cases)

Cell layer	Positive cases of co-expression			
	α_1 -Antitrypsin and α_1 -Antichymotrypsin	Transferrin and Ferritin	α_1 -Antitrypsin and Transferrin	α_1 -Antichymotrypsin and Transferrin
Superficial cell layer	3 (14%)	0 (0%)	4 (19%)	3 (14%)
Whole layer	2 (10%)	0 (0%)	6 (29%)	1 (5%)
Basal cell layer	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	5 (24%)	0 (0%)	10 (48%)	4 (19%)

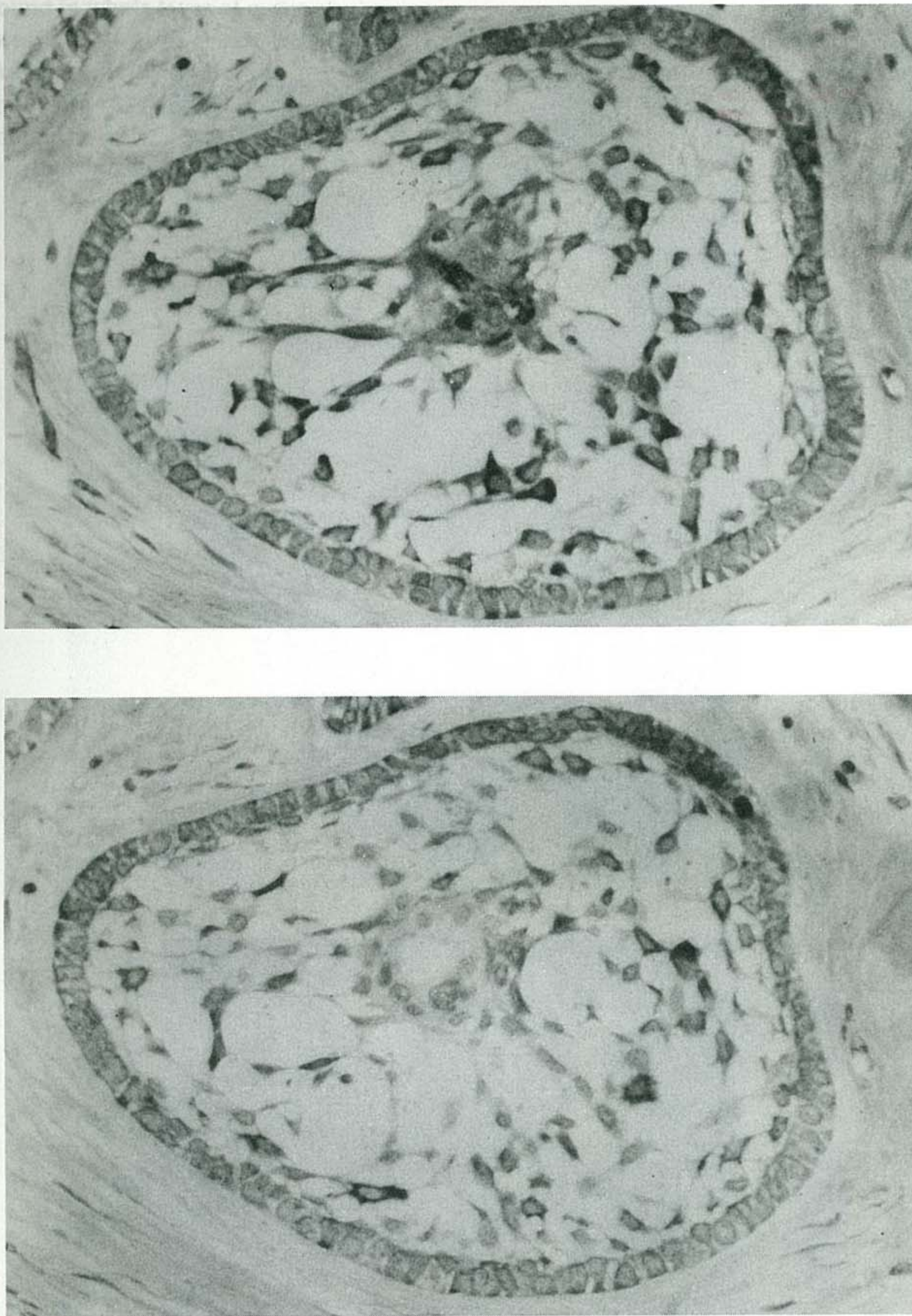


Fig. 1. Immunohistochemistry of follicular ameloblastoma: serial paraffin sections stained with α_1 -antitrypsin and transferrin. Many of the central stellate cells and peripheral columnar cells are reactive with the antibody α_1 -antitrypsin (upper). Some of both cell types are reactive with the antibody transferrin (lower). (Hematoxylin counterstain, $\times 120$).

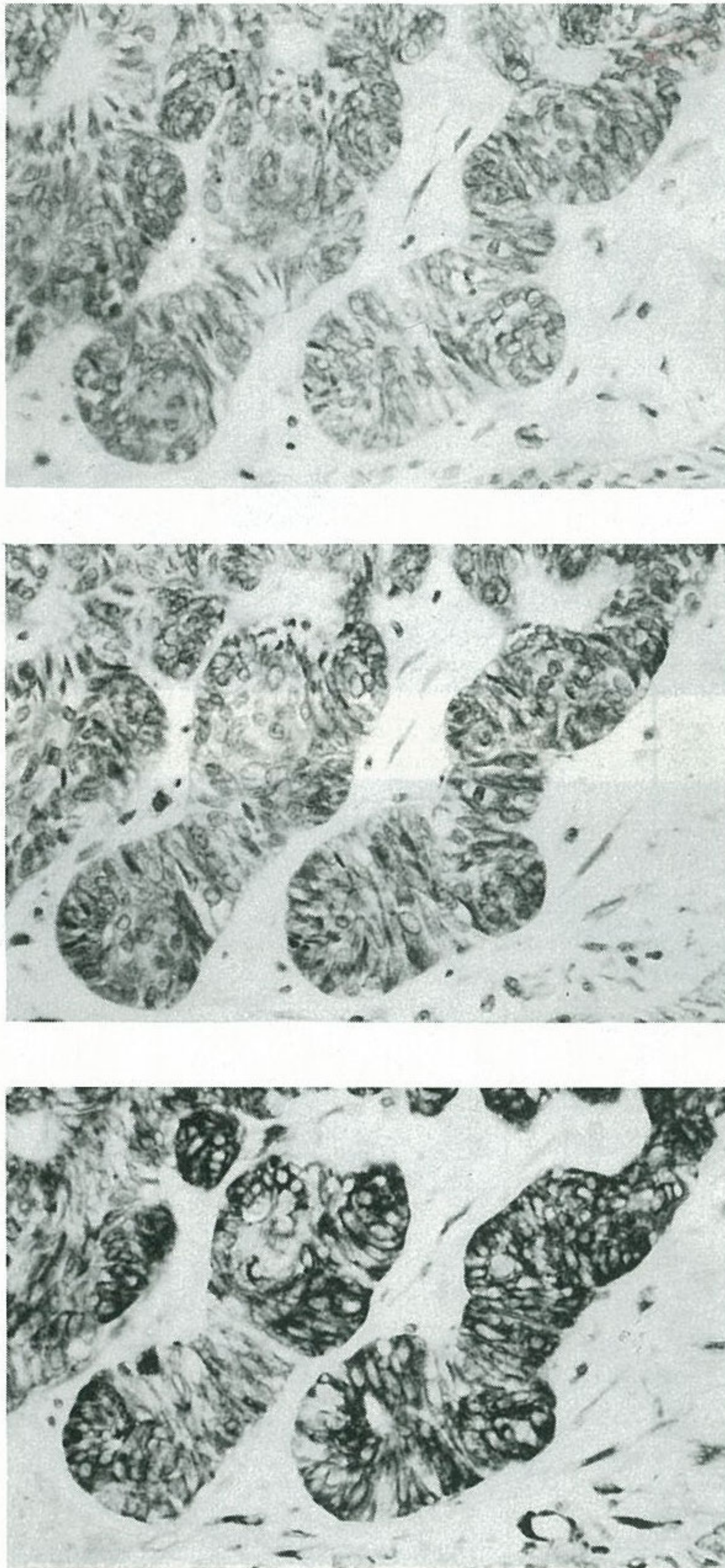


Fig. 2. Immunohistochemistry of plexiform ameloblastoma: serial paraffin sections stained with α_1 -antitrypsin, α_1 -antichymotrypsin and transferrin. All of the central stellate cells and peripheral columnar cells are weakly stained with α_1 -antitrypsin (upper). By contrast, numerous cells show strong staining with transferrin (lower). Some of the peripheral cells reveal strong positive reaction with α_1 -antichymotrypsin (middle), and staining intensity of α_1 -antichymotrypsin in the majority of neoplastic cells shows moderate intensity between the α_1 -antitrypsin and transferrin antibodies. (Hematoxylin counterstain, $\times 100$)

found in whole layer of cystic epithelium in 12 (57%) of 21 cases (Fig. 3, upper). The presence of α_1 -ACT was strongly positive in only superficial cells in 10 cases (48%). Anti-transferrin antiserum strongly stained throughout the entire cystic epithelium in 9 cases (43%). Staining reaction with transferrin in only the basal cell layer was identified in 5 cases (24%) (Fig. 3, lower) (Table 2).

Co-expression of α_1 -AT and transferrin in the neoplastic cells of cystic ameloblastoma (Fig. 3, lower) was observed in 6 cases (29%).

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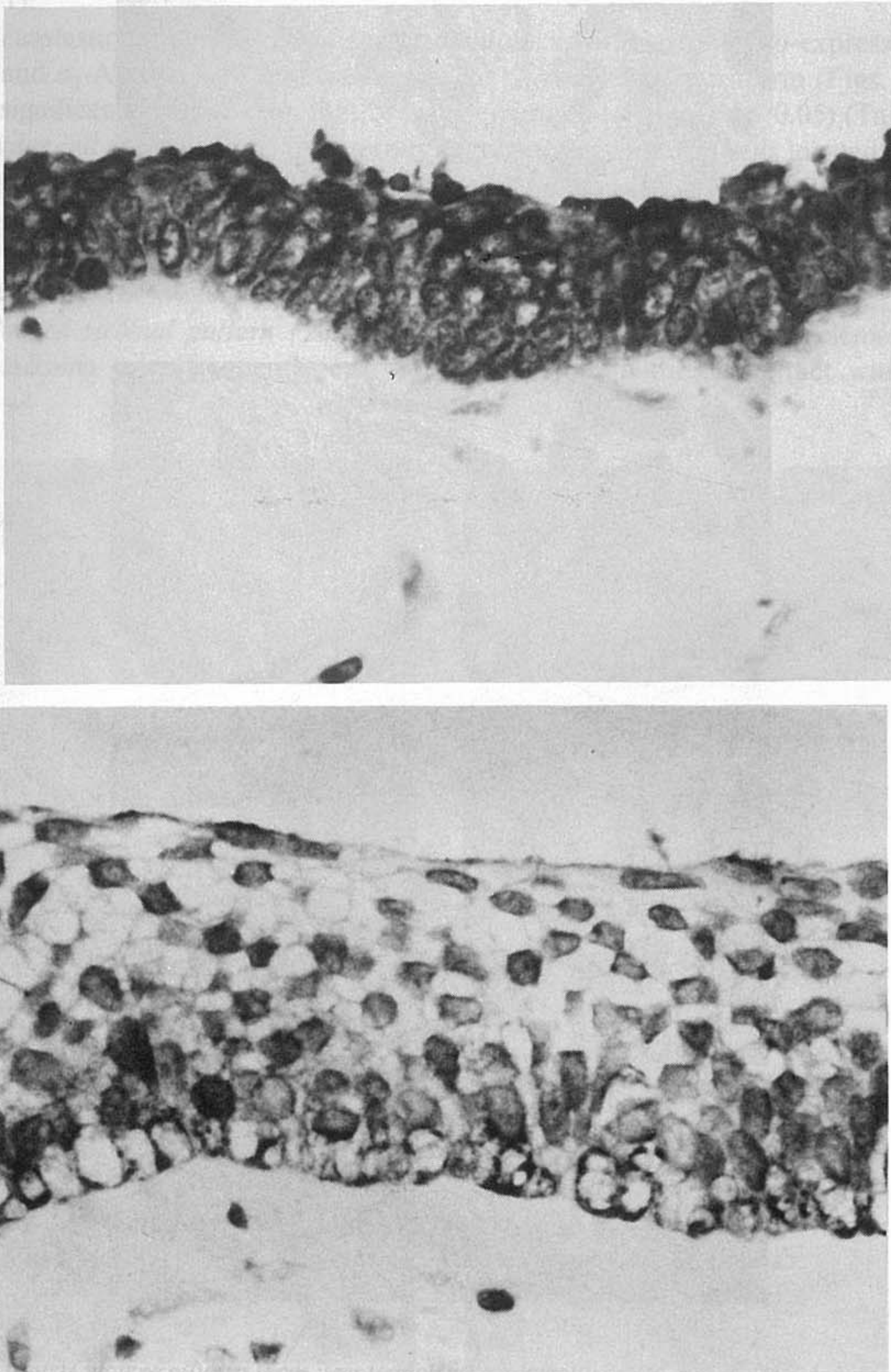


Fig. 3. Immunohistochemistry of cystic ameloblastoma. All the layers of the cystic epithelium show strong staining for α_1 -antitrypsin (upper). Transferrin staining is positive on the cell membrane of basal cell layer (lower). (Hematoxylin counterstain, $\times 240$).

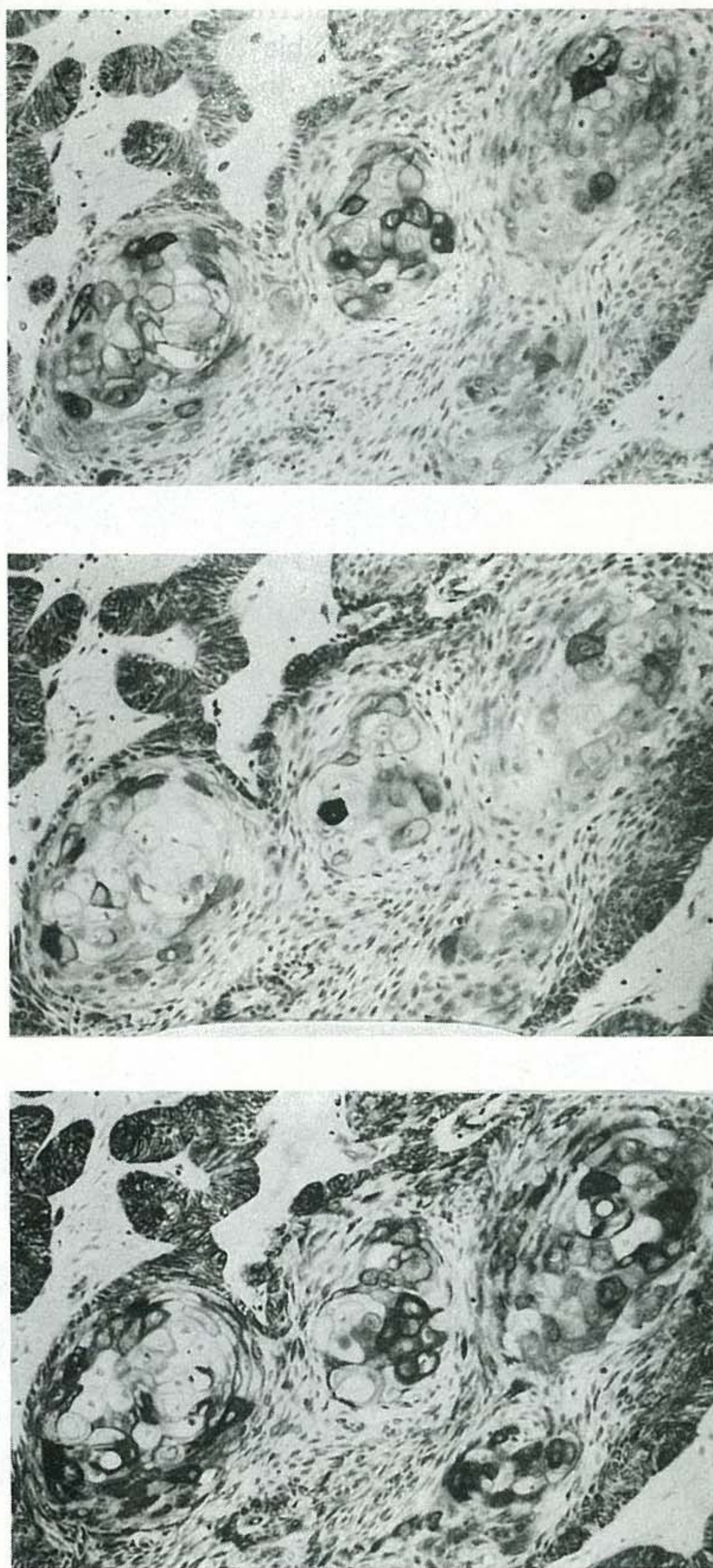


Fig. 4. Immunohistochemistry of acanthomatous ameloblastoma: serial paraffin sections stained with α_1 -antitrypsin, α_1 -antichymotrypsin and transferrin. Many of the metaplastic squamous epithelial cells in acanthomatous foci show co-expression of α_1 -antitrypsin (upper), α_1 -antichymotrypsin (middle), and transferrin (lower). (Hematoxylin counterstain, $\times 50$).

ameloblastoma was higher than that of α_1 -AT and α_1 -ACT, transferrin and ferritin, or α_1 -ACT and transferrin (Tables 3 and 4).

Acanthomatous pattern (10 cases) (Tables 1 and 3). Squamous metaplasia and conspicuous keratinization were seen in the center of follicular and plexiform epithelial nests in 10 cases of 32 ameloblastomas. α_1 -AT was found in metaplastic squamous cells in all 10 cases (100%) (Fig. 4, upper). α_1 -ACT (Fig. 4, middle) and transferrin (Fig. 4, lower) recognized acanthomatous pattern in 9 (90%) of 10 cases (Table 1).

In metaplastic squamous cell of acanthomatous ameloblastoma, co-expression of α_1 -AT and α_1 -ACT, α_1 -AT and transferrin, or α_1 -ACT and transferrin (Figs. 4 and 6) was significantly higher than that of transferrin and ferritin ($P < 0.05$) (Table 3).

Granular cell type (2 cases). Numerous aggregates of granular cells located within the follicles were demonstrated in only 2 cases. The granular cells were large, round or polyhedral in configuration, and densely packed with eosinophilic granules when viewed in sections with hematoxylin and eosin. Granular cells produced strongly positive results for transferrin (Fig. 5, left) and ferritin (Fig. 5, right).

Hyalinized stromal pattern (20 cases) (Tables 1 and 3). Epithelial elements of ameloblastoma were frequently surrounded by, and in direct contact with, the

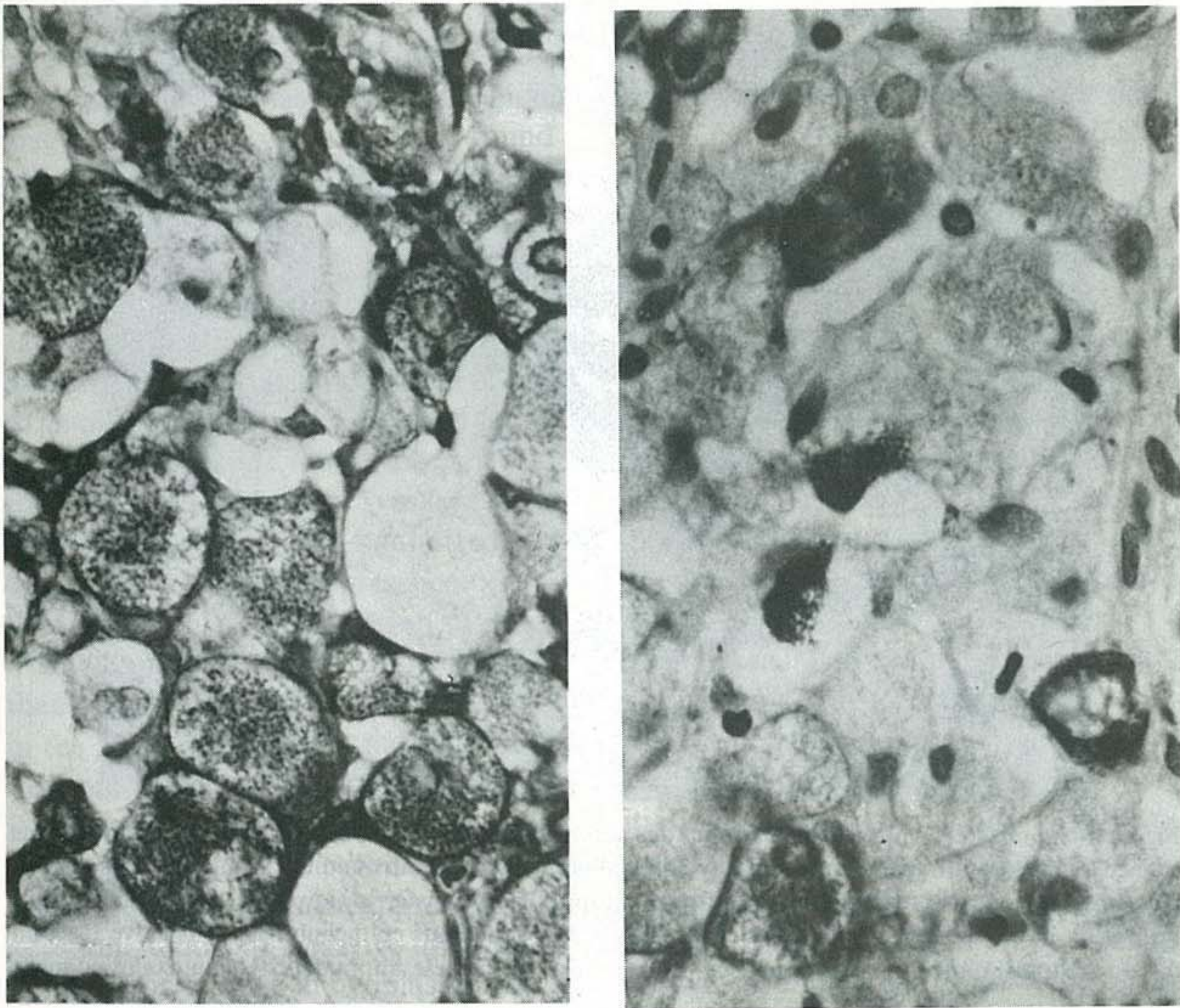


Fig. 5. Immunohistochemistry of granular cell ameloblastoma. Many of the granular cells are strongly reactive with anti-transferrin antibody (left). Some of the granular cells are well stained with anti-ferritin antibody (right). (Hematoxylin counterstain, $\times 200$).

hyalinized connective tissue stroma. Twenty of the 32 cases showed narrow zones of hyalinized stroma adjacent to the epithelial islands. In 16 (80%) of 20 cases α_1 -AT stained hyalinized stroma (Fig. 6, upper), and transferrin stained this stromal component in 10 cases (50%) (Fig. 6, lower) (Table 1).

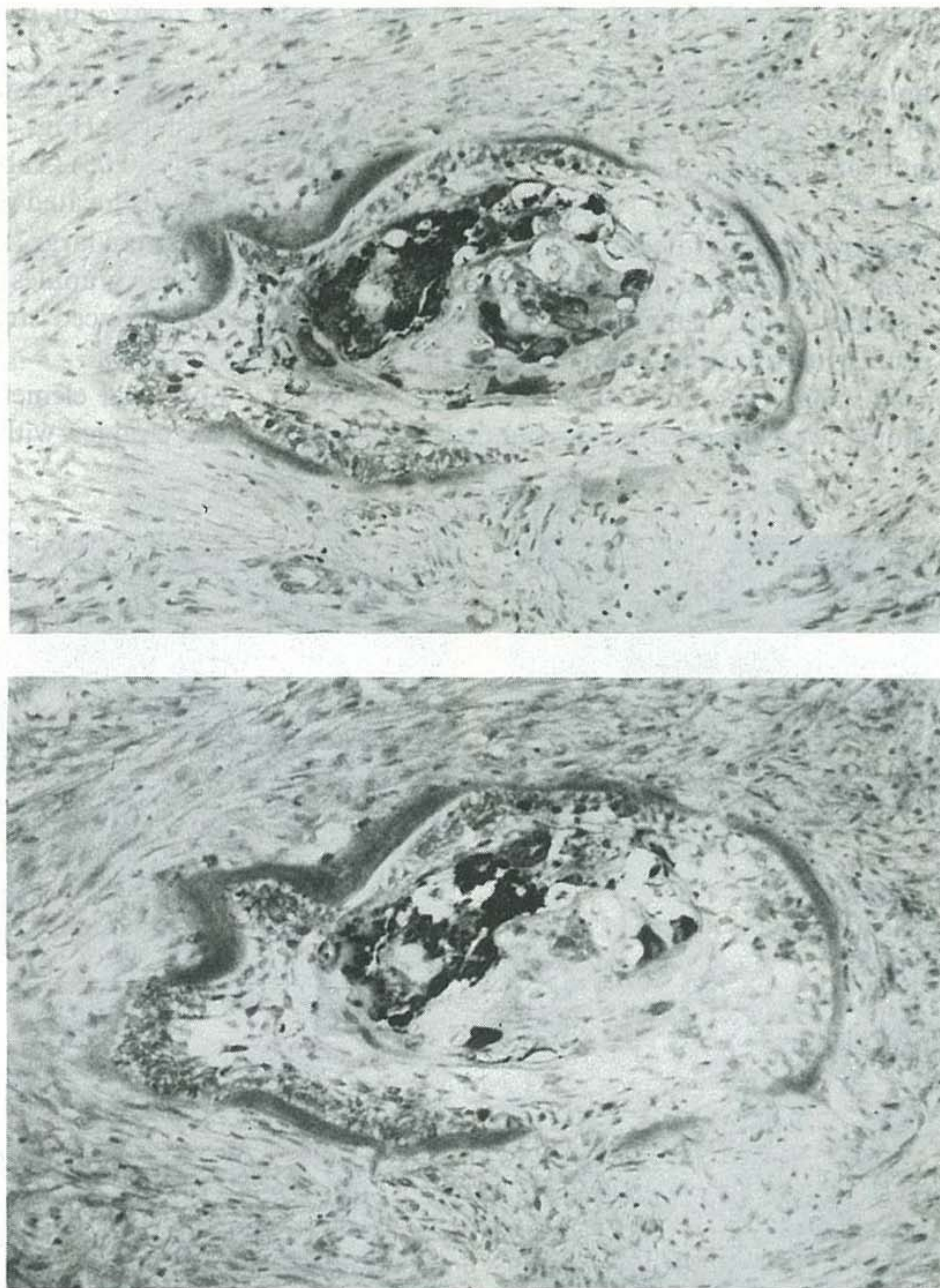


Fig. 6. Immunohistochemistry of acanthomatous and hyalinized stromal patterns: serial paraffin sections stained with α_1 -antitrypsin and transferrin. Hyalinized stroma surrounding acanthomatous ameloblastoma shows strong reaction for α_1 -antitrypsin (upper) and transferrin (lower). This lesion shows co-expression of α_1 -antitrypsin and transferrin. Metaplastic squamous epithelium shows positive reaction for α_1 -antitrypsin and transferrin, and co-expression of both these antibodies. (Hematoxylin counterstain, $\times 50$).

Co-expression of α_1 -AT and transferrin was observed in 8 (40%) of 20 cases with hyalinized stromal lesion. This combination in the hyalinized stroma (Fig. 6) was significantly higher than that of α_1 -AT and α_1 -ACT, transferrin and ferritin, or α_1 -ACT and transferrin ($P < 0.05$) (Table 3).

4. Discussion

Although a few studies of immunohistochemical staining for keratin and involucrin have been reported in ameloblastomas [20,35,38], immunohistochemical detection of α_1 -AT, α_1 -ACT, transferrin and ferritin has not yet been investigated in this neoplasm. We have demonstrated that the expression of α_1 -AT, α_1 -ACT, transferrin and ferritin differs in histologic patterns in ameloblastoma. The positive frequency of α_1 -AT was higher than that of α_1 -ACT in plexiform and hyalinized stromal patterns. Moreover, transferrin was detected more frequently than for ferritin in all the histological patterns of ameloblastoma. Our results suggest that the presence of α_1 -AT and transferrin in ameloblastoma may serve as tumour tissue markers of ameloblastoma.

α_1 -AT and α_1 -ACT are major proteinase inhibitors that share considerable amino acid sequence homology i.e. 42% [4]. This view is also supported by the fact that immunoreactivity for α_1 -AT and α_1 -ACT is frequently detected in the same tumours [6,11,14,25]. However, in the present study there was relatively different immunoreactivity for α_1 -AT and α_1 -ACT in ameloblastomas. The incidence of α_1 -AT within the neoplastic cells of follicular pattern was not significantly higher than that of α_1 -ACT. By contrast, we found that α_1 -AT immunoreactivity in plexiform pattern was statistically higher than that of α_1 -ACT in the same pattern. In the cystic lesion, although α_1 -AT was frequently detected in whole layer of cystic epithelium, the distribution of α_1 -ACT was frequently localized in the superficial cell layer. Hyalinization of stroma was sometimes found around the follicular pattern and beneath the cystic epithelium. In the present study, α_1 -AT was positive for basal membrane substances in 16 of 20 cases. On the other hand, hyalinized stromal parts of the tumour were positive for α_1 -ACT in only 2 of 20 cases. The application of antibody against α_1 -AT may be helpful for the identification of the hyalinized stroma. Ameloblastoma is characterized by a high frequency of α_1 -AT in the follicular, plexiform and hyalinized stromal patterns, in contrast to the lower incidence of α_1 -ACT. This presence of α_1 -AT reaction may be associated with inhibition of control of degradation of proteinase in ameloblastoma.

In the present study, the incidence of transferrin was higher in plexiform pattern than in follicular pattern. In some of the cystic pattern, transferrin was found only in the basal cell layer. It has been reported that transferrin has a high affinity for iron and that neoplastic cells require increased amounts of iron for their replication [8]. It is noteworthy that iron is crucial for initiating and maintaining DNA synthesis [23]. Therefore, in our opinion, neoplastic cells of ameloblastomas produce transferrin in order to have a greater availability of intracellular iron for their turnover. In addition, almost all negative immunohistochemical reaction for ferritin allows us to exclude iron storage in neoplastic cells. The cellular iron uptake appears to be medi-

ated by specific molecules like transferrin receptors [8]. Transferrin receptors have been demonstrated in much larger amounts on proliferating cells and on cells having undergone malignant transformation [13,27,36]. From these facts, it is considered that the plexiform pattern is in a stage of high cellular activity. Hereafter, investigation of the oncogenes and cellular proliferation by using Ki67 and proliferating cell nuclear antigen in ameloblastomas should be accumulated.

In 2 of the 32 ameloblastomas, the neoplastic cells, which usually occurred in large masses within the follicles, showed a remarkable granular change. The origin of the granular cells in such oral lesions as the granular cell ameloblastoma has long been a matter of speculation. There is now general agreement that the granular cells in ameloblastoma are of odontogenic epithelial origin and that transition between these cells and columnar or stellate cells is a notable histologic feature [12,18]. Immunohistochemical and ultrastructural studies confirmed the epithelial origin of the granular cells [5,21,34]. Electron microscopy revealed that the cytoplasmic granules were pleomorphic electron-dense bodies resembling organelles usually described as lysosomes [21,34]. Gold and Christ [10] suggested that the cytoplasmic granules represent a metabolic phenomenon rather than a degenerative process. In the present study, granular cells were positive for transferrin and ferritin. This result suggests that granular cells contain iron-binding proteins and such cells indicate cellular activity.

There are 4 categories of co-expression profile of 4 antibodies in ameloblastoma. These include tumours with predominant co-expression of α_1 -AT and α_1 -ACT, α_1 -AT and transferrin, or α_1 -ACT and transferrin, tumours with predominant co-expression of α_1 -AT and α_1 -ACT or α_1 -AT and transferrin, tumours with predominant co-expression of α_1 -AT and transferrin, and tumours that show low frequency of all co-expression patterns. Co-expression of α_1 -AT and α_1 -ACT, α_1 -AT and transferrin or α_1 -ACT and transferrin is predominantly observed in follicular pattern. Neoplastic cells of the follicular ameloblastoma showed a higher frequency of co-expression of α_1 -AT and α_1 -ACT than those of the plexiform pattern. Co-expression of α_1 -AT and α_1 -ACT, α_1 -AT and transferrin, or α_1 -ACT and transferrin is observed in the acanthomatous pattern. The present immunohistochemical study indicated that neoplastic cells of acanthomatous pattern had strong similarity to that of the follicular pattern in comparison with the plexiform pattern. Therefore, ameloblastoma of the acanthomatous pattern is thought of as a variant of the follicular pattern which undergoes squamous metaplasia in the central stellate cells. On the other hand, as the immunostaining co-expression in plexiform pattern is similar to that in cystic pattern, both these patterns may share common histogenesis.

A significant finding of the present study is that human ameloblastomas are divided into 2 groups (follicular and acanthomatous patterns and plexiform and cystic patterns) by the co-expression pattern of α_1 -AT, α_1 -ACT, transferrin and ferritin. These 2 histological groups may indicate different histogenesis.

References

- [1] Aroni K, Kittas C, Papadimitriou CS, Papacharalampous NX. An immunocytochemical study of the distribution of lysozyme, α_1 -antitrypsin, α_1 -antichymotrypsin in the normal and pathological gall bladder. *Virchows Arch A Pathol Anat* 1984;403:281–289.

- [2] Barresi G, Tuccari G. Iron-binding proteins in thyroid tumours: An immunocytochemical study. *Pathol Res Pract* 1987;182:344–351.
- [3] Bhaskar SN. Oral pathology in the dental office: Survey of 20 575 biopsy specimens. *J Am Dental Assoc* 1968;76:761–766.
- [4] Chandra T, Stackhouse R, Kidd VJ, Robson JH, Woo SLC. Sequence homology between human alpha-1-antichymotrypsin, alpha-1-antitrypsin and antithrombin III. *Biochemistry* 1983;22:5055–5061.
- [5] de Wilde PCM, Slootweg PJ, Müller H, Kant A, Moesker O, Vooijs P, Ramaekers FCS. Immunocytochemical demonstration of intermediate filaments in a granular cell ameloblastoma. *J Oral Pathol* 1984;13:29–39.
- [6] du Boulay CE. Demonstration of alpha-1-antitrypsin and alpha-1-antichymotrypsin in fibrous histiocytomas using the immunoperoxidase technique. *Am J Surg Pathol* 1982;6:559–564.
- [7] Gardner DG. Plexiform unicystic ameloblastoma: a diagnostic problem in dentigerous cysts. *Cancer* 1981;47:1358–1363.
- [8] Gatter KC, Brown G, Trowbridge IS, Woolston RE, Mason DY. Transferrin receptors in human tissues: their distribution and possible clinical relevance. *J Clin Pathol* 1983;36:539–545.
- [9] Geboes K, Ray MB, Rutgeerts P, Callea F, Desmet VJ, Vantrappen G. Morphological identification of alpha-1-antitrypsin in human small intestine. *Histopathology* 1982;6:55–60.
- [10] Gold L, Christ T. Granular cell odontogenic cyst. *Oral Surg* 1970;29:437–442.
- [11] Gonzales B. Benign fibrous histiocytoma of the skin. An immunohistochemical analysis of 30 cases. *Pathol Res Pract* 1985;180:486–489.
- [12] Hartman KS. Granular cell ameloblastoma. A survey of twenty cases from Armed Force Institute of Pathology. *Oral Surg* 1974;38:241–253.
- [13] Judd W, Poodry CA, Strominger JL. Novel surface antigen expressed on dividing cells but absent from nondividing cells. *J Exp Med* 1980;152:1430–1435.
- [14] Kindblom L-G, Jacobsen G, Jacobsen M. Immunohistochemical investigations of tumors of supposed fibroblastic-histiocytic origin. *Hum Pathol* 1982;13:834–840.
- [15] Kittas C, Aroni K, Kotsis L, Papadimitriou CS. Distribution of lysozyme, α_1 -antichymotrypsin and α_1 -antitrypsin in adenocarcinomas of the stomach and large intestine. *Virchows Arch A Pathol Anat* 1982;398:139–147.
- [16] Kittas C, Aroni K, Matani A, Papadimitriou CS. Immunocytochemical demonstration of α_1 -antichymotrypsin in human gastrointestinal tract. *Hepato-Gastroenterology* 1982;29:275–277.
- [17] Kramer IRH, Pindborg JJ, Shear M. Histological typing of odontogenic tumours. International histological classification of tumours, 2nd edn. Geneva: WHO, 1992.
- [18] Lucas RB. Pathology of Tumours of the Oral Tissues, 3rd edn. Edinburgh, London and New York: Churchill-Livingstone, 1976.
- [19] Mason DY, Taylor CR. Distribution of transferrin, ferritin, and lactoferrin in human tissues. *J Clin Pathol* 1978;31:316–327.
- [20] Matsuo A, Ueno S. Immunohistochemical demonstration of keratin in ameloblastoma as an indication of tumor differentiation. *J Oral Maxillofac Surg* 1991;49:282–288.
- [21] Navarrete AR, Smith M. Ultrastructure of granular cell ameloblastoma. *Cancer* 1971;27:948–955.
- [22] Regezi JA, Kerr DA, Courtney RM. Odontogenic tumours: analysis of 706 cases. *J Oral Surg* 1978;36:771–778.
- [23] Robbins E, Pederson T. Iron: its intracellular localization and possible role in cell division. *Proc Natl Acad Sci USA* 1970;66:1244–1251.
- [24] Robinson L, Martinez MG. Unicystic ameloblastoma. A prognostically distinct entity. *Cancer* 1977;40:2278–2285.
- [25] Roholl P, Kleyne J, Elbers H, van der Vegt M, Albus-Lutter CH, van Unnik J. Characterization of tumour cells in malignant fibrous histiocytomas and other soft tissue tumours in comparison with malignant histiocytes. Immunohistochemical study on paraffin sections. *J Pathol* 1985;147:87–95.
- [26] Rossiello R, Carriero MV, Giordano GG. Distribution of ferritin, transferrin and lactoferrin in breast carcinoma tissue. *J Clin Pathol* 1984;37:51–55.

- [27] Sutherland R, Delia D, Schneider C, Newman R, Kemshead J, Greaves M. Ubiquitous cell-surface glycoprotein on tumour cells is proliferation-associated receptor for transferrin. *Proc Natl Acad Sci USA* 1981;78:4515–4519.
- [28] Tahara E, Ito H, Taniyama K, Yokozaki H, Hata J. Alpha 1-antitrypsin, alpha 1-antichymotrypsin, and alpha 2-macroglobulin in human gastric carcinomas: A retrospective immunohistochemical study. *Hum Pathol* 1984;15:957–964.
- [29] Takahashi H, Tsuda N, Tezuka F, Okabe H. Difference of immunohistochemical reactions in epithelial cells of adenolymphoma. *J Oral Pathol* 1988;17:287–292.
- [30] Takahashi H, Fujita S, Tsuda N, Tezuka F, Okabe H. Immunohistochemical demonstration of α_1 -antichymotrypsin and α_1 -antitrypsin in salivary gland pleomorphic adenomas of children. *Tohoku J Exp Med* 1990;162:79–93.
- [31] Takahashi H, Tsuda N, Fujita S, Tezuka F, Okabe H. Immunohistochemical investigation of vimentin, neuron-specific enolase, α_1 -antichymotrypsin and α_1 -antitrypsin in adenoid cystic carcinoma of the salivary gland. *Acta Pathol Jpn* 1990;40:655–664.
- [32] Takahashi H, Fujita S, Okabe H, Tsuda N, Tezuka F. Immunohistochemical characterization of basal cell adenomas of the salivary gland. *Pathol Res Pract* 1991;187:145–156.
- [33] Takahashi H, Fujita S, Tsuda N, Tezuka F, Okabe H. Iron-binding proteins in adenoid cystic carcinoma of salivary glands: An immunohistochemical study. *Tohoku J Exp Med* 1991;163:1–16.
- [34] Tandler B, Rossi EP. Granular cell ameloblastoma: electron microscopic observations. *J Oral Pathol* 1977;6:401–412.
- [35] Thesleff I, Ekblom P. Distribution of keratin and laminin in ameloblastoma. Comparison with developing tooth and epidermoid carcinoma. *J Oral Pathol* 1984;13:85–96.
- [36] Trowbridge IS, Domingo DL. Anti-transferrin receptor monoclonal antibody and toxin-antibody conjugates affect growth of human tumour cells. *Nature* 1981;294:171–173.
- [37] Vickers RA, Gorlin RJ. Ameloblastoma: delineation of early histopathologic features of neoplasia. *Cancer* 1970;26:699–710.
- [38] Vigneswaran N, Whitaker SB, Budnick SD, Waldron CA. Expression patterns of epithelial differentiation antigens and lectin-binding sites in ameloblastomas: a comparison with basal cell carcinomas. *Hum Pathol* 1993;24:49–57.