HYALINE CELLS IN SALIVARY GLAND TUMOURS

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SUMMARY

The morphology, incidence and distribution of hyaline cells in ten cases each of pleomorphic adenoma, adenoid cystic carcinoma, adenocarcinoma and mucoepidermoid tumour were studied by conventional light microscopy. Results showed that the hyaline cells were identified in 60% of pleomorphic adenoma and in 20% of mucoepidermoid tumours, but were absent in adenoid cystic carcinoma and adenocarcinoma. Relative area estimation of hyaline cells in pleomorphic adenomas showed that this may range from 2.2% to 30.4% of the total tumour area. The usefulness of the hyaline cell as a diagnostic criteria in distinguishing between some of the salivary gland tumours was also discussed.

INTRODUCTION

The salivary gland tumours form a diverse group of lesions showing pronounced variation in their histological patterns. These tumours have been classified in different ways and given a confusing variety of names. The "Histological Typing of Salivary Gland Tumours" by Thackray and Sobin [1] provides a common basis for comparison of national incidences and responses to treatment between various series. However although the histological criteria for the diagnosis of the different types of salivary gland tumours are well-established, considerable difficulties are sometimes encountered in categorising some of the salivary gland tumours.[2,3]

A pleomorphic adenoma which exhibits the typical features of myxochondroid stroma and tumour epithelium which are intermingled together, seldom presents difficulty in diagnosis. Sometimes cylindromatous, mucoepidermoid and oncocytic changes are present. If extensive areas are involved and a small specimen is examined, an error in diagnosis may occur. Similarly, a predominantly epithelial component with almost complete absence of myxochondroid tissue may sometimes be mistakenly considered as a monomorphic adenoma.

In view of this problem a diagnostic criteria was advanced by Lomax-Smith and Azzopardi in 1978.[4] They claimed that the hyaline or plasmacytoid cell is characteristic and specific to the pleomorphic adenoma. They felt that it may be justifiable to regard this cell type with the same

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diagnostic significance as myxoid or chondroid tissue which are usually regarded as the hallmark of pleomorphic adenoma.

The present study was designed to test the statement that the hyaline cell is unique to the pleomorphic adenoma. The presence and amount of this component in a series of salivary gland tumours of varying types will be determined qualitatively and quantitatively. It was also proposed to attempt to refine the criteria for the recognition of the hyaline cells.

MATERIALS AND METHOD

The materials for this study consisted of ten cases each of pleomorphic adenoma, adenoid cystic carcinoma, adenocarcinoma and mucoepidermoid tumour. These specimens were chosen from the files of the Department of Pathology, Institute of Dental Surgery, Eastman Dental Hospital, London. The specimens consisted of 32 minor and 8 major salivary gland tumours from patients aged between 14 and 89 years (Tables I–IV).

The following diagnostic criteria were applied to the salivary gland tumours for inclusion in the present study.

TABLE I

CASES OF PLEOMORPHIC ADENOMA

Age (yrs)	Sex	Site
89	F	Right cheek
17	F	Junction hard and soft palate
14	F	Right parotid gland
43	F	Left parotid gland
37	M	Junction hard and soft palate
35	F	Palate
26	M	Junction hard and soft palate
42	M	Palate
52	F	Left parotid gland
35	M	Hard palate

Pleomorphic adenoma

The tumours are generally well-circumscribed and a capsule of varying thickness and completeness is usually present. All tumours in this series consist of both epithelial tissue and stromal elements of hyaline, myxoid or chondroid appearance. However, where the latter components are relatively scanty but the epithelial component is very similar to that found in the more typical "mixed" tumour, it is included in the study. The epithelial tissue may be in the form of ducts, sheets of epithelial cells or squamous islands.

Adenoid cystic carcinoma

The cribriform pattern is the predominant pattern although a cellular pattern of solid sheets, cords and trabeculae of tumour cells may also occur. Two basic tumour cells are recognised: myoepithelial and duct-lining cells. These may form prominent duct-like structures in some cases. The stroma is fibrous, may be hyalinised and sometimes acquire a myxoid appearance but with no cells found within this area. The tumours are not encapsulated and show a highly invasive pattern with a special tendency for perineural spread.

Adenocarcinoma

The tumour is non-encapsulated, frankly malignant, exhibiting invasion of normal tissue and showing recognisable neoplastic ducts or tubules. The cells lining the ducts exhibit pleomorphism. Two tumours showing adenocarcinomatous elements but also exhibiting residues of recognisable "mixed" tumour were accepted for inclusion in the present study.

Mucoepidermoid tumour

For inclusion in the present study, the tumour must be shown to contain mucous-secreting cells and squamous cells with demonstrable intercellular bridges. It must not be encapsulated, and cyst formation is a commonly-seen feature. Intermediate cells as described in the literature cannot be identified with certainty and their presence is not considered essential for inclusion. The stroma is fibrous and there is no evidence of myxoid or myxochondroid material.

TABLE II

CASES OF ADENOID CYSTIC CARCINOMA

Age (yrs)	Sex	Site
52	F	Right floor of mouth
51	M	Right antrum
48	M	Right cheek
38	M	Palate
45	F	Submandibular salivary gland
61	M	Submandibular salivary gland
41	F	Palate
32	F	Right cheek
63	M	Palate
52	F	Left parotid gland

TABLE III

CASES OF ADENOCARCINOMA

Age (yrs)	Sex	Site
44	F	Left cheek
23	F	Lower right third molar region
62	M	Palate – right side
31	F	Soft palate – right side
73	F	Left submandibular gland
61	F	Hard palate
74	F	Left maxilla
56	F	Right cheek
37	F	Parotid
50	M	Soft palate

In all the 40 cases, paraffin sections of approximately 5 micron thickness were prepared. These were stained with Ehrlich's haematoxylin and eosin, tannic acid-phosphomolybdic acid and Mallory Phosphotungstic acid haematoxylin.

TABLE IV

CASES OF MUCOEPIDERMOID TUMOUR

Age (yrs)	Sex	Site
67	M	Floor of mouth – right side
54	F	Left tuberosity
30	F	Junction hard and soft palate
80	M	Lower right third molar region
40	F	Left retromolar region
45	F	Lateral border of tongue – right side
48	M	Left palate and antrum
26	M	Palate
54	F	Palate – left side
23	F	Left faucial region

Qualitative histology

The sections were studied by conventional light microscopy. In every case, any foci of tumour cells that contain not less than 20 hyaline cells with the morphology conforming to the description given by Lomax-Smith and Azzopardi[4] was regarded as positive. The morphology, arrangement and relation to epithelial tumour areas and areas of myxoid, chondroid and myxochondroid material were also studied.

Quantitative histology

For the quantitative analysis of the hyaline cells in the various salivary gland tumours, a method of relative area estimation was employed.[5] In each case, two H+E stained sections were systematically sampled. A 7x calibrated eyepiece with a square-ruled network consisting of 60 squares, and a 5x objective were used. The grid was superimposed and the entire section was sampled. The two main components calibrated were the hyaline cells and tumour area. When the hyaline cells or tumour area occupy half or more of the squares, it was recorded as positive

for that component. When it involved less than half a square, it was not considered.

The areas were estimated twice and the results averaged.

RESULTS

Qualitative histology

Incidence. Hyaline cells were found in eight of the 40 salivary gland tumours studied. They were present in six cases of pleomorphic adenomas. Of these, five were tumours of minor salivary glands and all were from the palate. The remaining case of pleomorphic adenoma was from the right parotid gland. In addition, two cases of mucoepidermoid tumour, one from the left tuberosity region and the other from the left retromolar region exhibited the presence of hyaline cells. No hyaline cells were present in both adenoid cystic carcinoma and adenocarcinoma.

As the hyaline cells were found only in the pleomorphic adenoma and mucoepidermoid tumour, the following description applies only to these cases.

Cell morphology. The hyaline cells were ovoid or polyhedral with prominent eccentric nuclei. The cytoplasm showed an eosinophilic, homogenous ground-glass appearance with no evidence of any granularity or striations. Examinations of PTAH-stained sections confirmed the absence of granular or fibrillar cytoplasm.

The eccentric nucleus was usually hyperchromatic with inconspicuous or indiscernible nucleoli and tended to be round or ovoid. However in some cases, the nuclei were vesicular and here there were often prominent, small nucleoli and also a tendency to peripheral margination of the chromatin. This combined with the eccentric location of the nucleus gave the cell a superficial resemblance to plasma cells (Fig. 1). Though the large majority of nuclei were usually ovoid, occasional indented or notched forms were seen and very rarely they appeared almost bilobed. Mononucleated cells were the predominant type but very occasionally binucleated forms were seen. The hyaline cells showed very few mitotic figures and no abnormal mitosis were seen.

Arrangement. The hyaline cells were arranged in small or large foci, or in sheets (Fig. 1). In most areas they were closely aggregated but showed a tendency of lack of cohesion or even actual separation (Fig. 1). The two cases of mucoepidermoid tumour showed hyaline cells in small foci only, and in one were found mainly in the wall of cystic cavities. Occasional foci of hyaline cells showed the presence of intercellular bridges.

Relation to cellular areas. The occurrence of hyaline cells in relation to cellular areas was found to be rather variable. They occurred as foci amongst the cells or may be absent in the predominantly cellular portions or the tumours.

Relation to myxoid, chondroid and myxochondroid areas. Some of the hyaline cells were seen as small islands separated by myxoid, chondroid or myxochondroid stroma or present at the periphery of such areas (Fig. 2).

Observations with the tannic acid-phosphomolybdic acid milling dye technique

All four groups of tumours showed areas of positive staining in the epithelial areas. In pleomorphic adenomas, ductal systems were strongly outlined. Myxochondroid areas with individual tumour cells showed strong staining within the cytoplasm of these cells. Areas of hyaline cells however showed a variable response to the stain. In some specimens, areas of hyaline cells stained strongly while in other parts they stained weakly, if not at all. Most tumour cells showed some degree of staining.

Quantitative histology

Table V shows the results of the quantitative assessment of hyaline cells in relation to total tumour area in pleomorphic adenomas. This ranged from 2.2% to 30.4%. In the two cases of mucoepidermoid tumours, too few hyaline cells were present and their areas were not estimated.

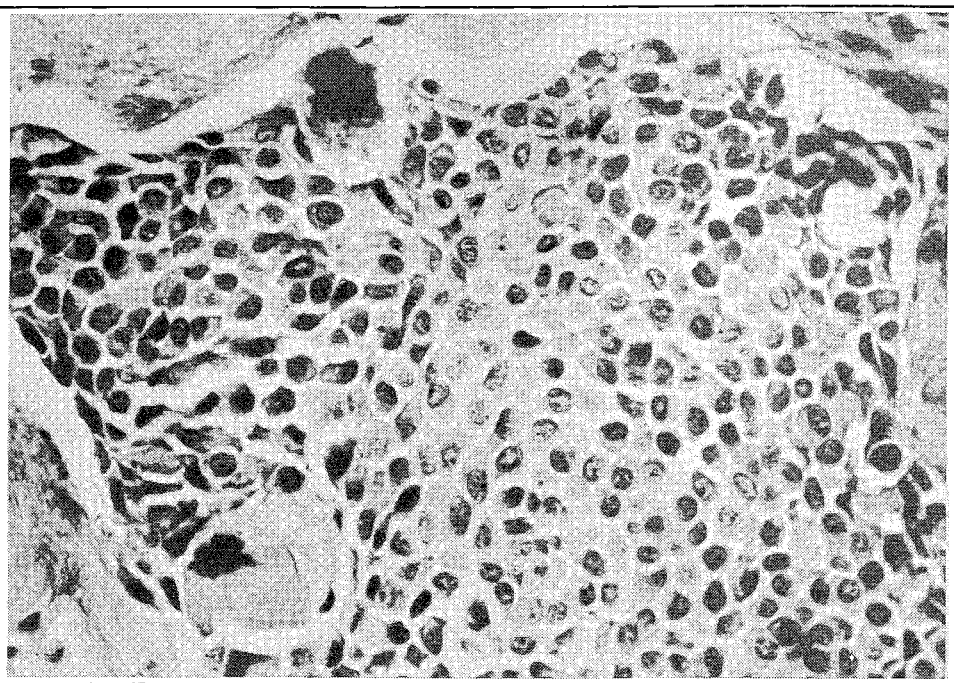

Fig. 1 Pleomorphic adenoma – sheets of hyaline cells showing the plasmacytoid appearance and lack of cohesion. (Haematoxylin and eosin. Original magnification x 300.)

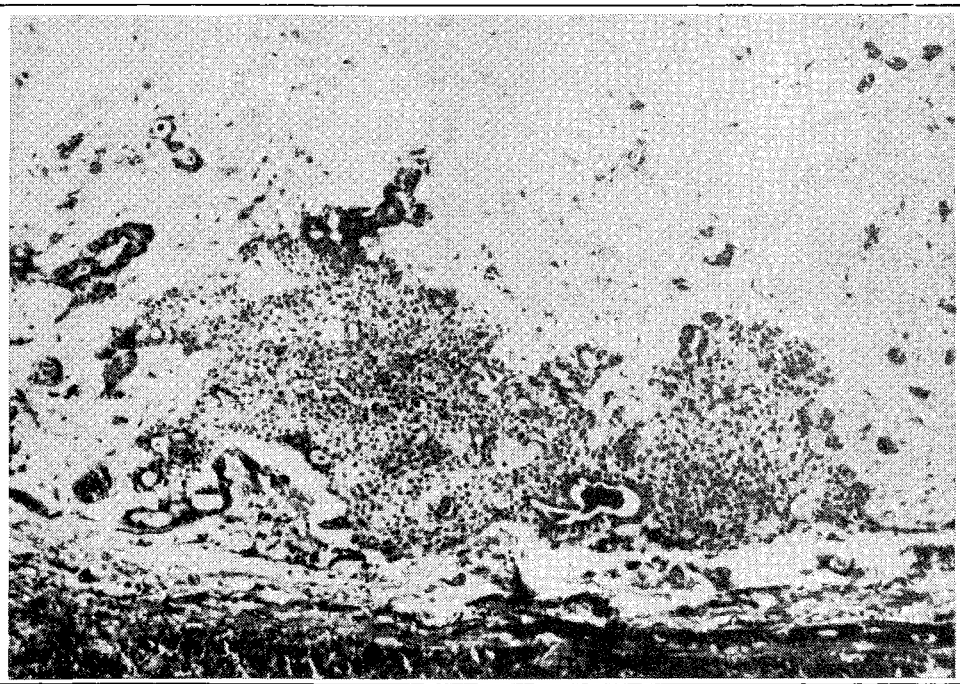

Fig. 2 Pleomorphic adenoma – an island of hyaline cells at the periphery of a myxochondroid area. (Haematoxylin and eosin. Original magnification x 75.)

TABLE V

SCORE FOR HYALINE CELLS IN PLEOMORPHIC ADENOMAS

Age (yrs)	Sex	Hyaline cells (average no. of squares)	Tumour area (average no. of squares)	(%)
35	M	45.5	149.5	30.4
17	F	321.5	1,128.5	28.5
14	F	76.5	432.0	17.7
37	M	57.5	532.5	10.8
26	M	29.0	1,083.5	2.7
42	M	40.0	1,817.5	2.2

DISCUSSION

Although the incidence rate of hyaline cells in pleomorphic adenomas varies in different studies, it is generally noted that this tends to be lower in lesions of the major than in the minor salivary glands.[4,6,7] In the present study the incidence rate for the minor salivary gland lesions was comparable with the findings of Lomax-Smith and Azzopardi.[4] However as only one of the three major salivary gland lesions in the present study contained hyaline cells, it is felt that the number of cases studied is too small to have any significance for comparison with other studies. The higher incidence of hyaline cells in minor salivary gland lesions has been attributed to the fact that these lesions usually contain less myxochondroid material and that the hyaline cells are frequently more abundant in the cellular parts of these tumours.[8] A similar trend was also observed in the present study.

The identification of hyaline cells in two cases of mucoepidermoid tumours in the present study refutes Lomax-Smith and Azzopardi's[4] claim that the hyaline cells are unique to the pleomorphic adenoma. Similarly Warner and Seo[6] found that 7% of their adenoid cystic carcinoma contained hyaline cells. This infers that though hyaline cells are more commonly found in pleomorphic adenomas they are not pathognomonic of these lesions.

The tannic acid-phosphomolybdic acid-milling dye staining technique was first developed by Putchtler *et. al.*,[9] for the demonstration of myofibrils in endothelial cells for examination by direct, polarisation and fluorescence microscopy. Macartney *et. al.*,[10] also observed that this technique shows selective staining of the myoepithelium in normal salivary glands tissue and around areas of intraductal carcinoma of the breast. In the present study, many types of cells and structures in the pleomorphic adenoma showed staining by this technique and it is therefore unlikely that the use of this staining method alone would be useful in the identification of hyaline cells in salivary gland tumours.

Previous investigations[4,7] have mainly carried out subjective assessment of the presence of hyaline cells. Lomax-Smith and Azzopardi[4] did not provide any information of their criteria of subjective grading or their method of quantitation though they provided some brief information of the percentage of hyaline cells in relation to the total cell population. They found that in major salivary gland tumours the hyaline cells do not comprise more than 2% of the total cell population in a given section. In contrast the hyaline cells in palatal tumours may form up tc 30% of the total cell populations in some sections. All their cases of pleomorphic adenoma with hyaline cells occurring in major salivary glands were given a subjective grading of 1+.

In Buchner *et. al.*,'s study,[7] 8% of their cases of pleomorphic adenoma of the major salivary glands contained several relatively large islands of hyaline cells. These tumours were given a subjective grading of 2+.

In the present study quantitative assessment showed that the proportion of hyaline cells to tumour cells in pleomorphic adenomas may range from 2.2% to 30.4%. The tumours with the highest score were composed almost entirely of hyaline cells. A case of pleomorphic adenoma of the parotid gland which would probably be consistent with the subjective grading of 2+ by Buchner *et. al.*,[7] was found on quantitation to contain hyaline cells which occupied about 17.7% of the total tumour area.

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