ORIGINAL ARTICLE

Epithelial and stromal patterns of pleomorphic adenoma of minor salivary glands: A histopathological and histochemical study

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ABSTRACT

Background: Pleomorphic adenoma (PA) accounts for 45–74% of all the salivary gland neoplasms, of which 40–70% are present in minor salivary glands. Studies have depicted variations in histological typing and classification of these tumors. Its pleomorphism is attributed to the cytological differentiations of the epithelial components and the diverse stromal components. Biochemical investigations of saliva have revealed "mucins" to be its main component. Mucins reflect in their composition, the functional state of the mucosa, both in health and disease. Many reviews on histochemical classification and identification have been put forward to explain the intricacies of mucins; however, no attempts have been made to classify salivary gland tumors based on their mucin profiles and assess its prognostic significance. Thus, this study was executed to analyze the clinical, histopathological and histochemical behavior of PA of minor salivary glands and decipher a correlation. **Materials and Methods:** Twenty-six diagnosed cases of PA of minor salivary glands and five controls of normal minor salivary glands of the hard palate were included in the study. Blocks were retrieved, sectioned and stained with hematoxylin and eosin (H and E) stain as well as combined Alcian blue (AB)-periodic acid-Schiff (PAS) stains. **Results:** The stained slides revealed an array of epithelial and stromal patterns and varying heterogeneity of mucin expression of normal and neoplastic minor salivary glands. **Conclusion**: The study elucidated the role of mucins in tumorigenesis and its prognostic implications.
Key words: Mucins, Pleomorphic adenoma, Alcian blue-PAS stain

INTRODUCTION

Pleomorphic adenoma (PA) is the most common tumor of the salivary gland. Although benign, the rate of recurrence of PA is relatively high (2.5–32.5%). The recurrent tumors are often multinodular and frequently lack surrounding capsule, thus making surgical management difficult.[1,2]

In the human saliva, up to 26% of the salivary proteins are mucins.[3] Mucins are high molecular weight glycoproteins with oligosaccharides attached to amino acids by O-glycosidic linkages.[2] Many reviews on their histochemical classification and identification have been put forward to explain the intricacies of mucins. Mucins, elaborated by major salivary glands, reveal that a heterogeneous population of mucosubstances exist.[4,5]

Numerous alterations of mucin-associated carbohydrates have been detected in neoplastic epithelial tissues and on circulating mucins in patients with gastrointestinal tumors.[6] During the past few years, core proteins for human mucins have been identified and used as tumor markers in different neoplasms.[2]

Thus this study was executed in an attempt to correlate the clinical, histopathological and histochemical behavior of PA, so as to infer the prognostic implication.

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MATERIALS AND METHODS

Twenty-six diagnosed cases of PA of minor salivary glands and five controls of normal minor salivary acini of the hard palate were included in the study. Paraffin-embedded blocks were retrieved from the departmental archives, fresh sections

were cut and two series of slides were prepared. One series was stained with routine hematoxylin and eosin (H and E) stain, while the other series was stained with combined Alcian blue (AB)-periodic acid-Schiff (PAS) stain (Mowry 1956); to differentiate between neutral and acidic mucins.

Data assortment

Relevant clinical findings such as age, sex, site and history of recurrence were noted.

Histopathology

H and E stained slides were categorized according to Foote and Frazell's (1954) classification. Histopathological features such as capsular architecture, epithelial cells and patterns and tumoral stroma were analyzed in each slide and tabulated.

Histochemistry

The type of histochemical stain, the uniformity and intensity of staining patterns were comparatively analyzed in the epithelial and stromal components as well as in the associated salivary acini, to determine the mucin profile of the neoplasm.

All the obtained data were tabulated, compared and correlated to derive at a hypothesis.

RESULTS

Epidemiological aspects

Demographics revealed, the most common age group to be affected was 31–40 years [Table 1]. The patients were mostly females (15 cases, representing 57.7%), with a female to male ratio of 1.36:1.

The most common site of involvement was the hard palate (24 cases); accounting for 92.3% of the recorded cases [Table 2]. No cases of recurrence were recorded.

Histopathological interpretations

Based on the proportion of parenchymal and stromal tumoral components, the cases were classified into four subtypes as proposed by Foote and Frazell [Table 3]. Twelve of the 26 cases were of the type III pattern (predominantly cellular), accounting for 46.1%.

The tumor periphery comprised of a capsule completely surrounding the tumor, but with variable thickness. In two cases we noticed tumoral off shoots extending into the capsule [Figure 1].

The most frequent stromal pattern [Table 4] was myxoid (73.1%). Nineteen of the 26 PA revealed a myxoid component but in variable proportion from one case to another. These stromal zones were weakly basophilic, poorly delineated and disposed between the tumoral epithelial structures. The cells within these tumoral zones were undifferentiated mesenchymal cells, stellate in shape and with delicate anastomosis [Figure 2].

Table 1: Distribution of pleomorphic adenomas by age

Age group (years)	Number
<20	2
21-30	4
31-40	11
41-50	5
>50	4

Table 2: Distribution of pleomorphic adenomas by site

Site	Number
Hard palate	24
Upper lip	1
Buccal mucosa	1

Table 3: Distribution of pleomorphic adenomas based on Foote and Frazell's histopathological subtypes

Histopathological subtype		Number
Type I	Principally myxoid	2
Type II	Myxoid and cellular	5
Type III	Predominantly cellular	12
Type IV	Extremely cellular	7

Table 4: Stromal components in pleomorphic adenoma (PA)

Predominant stromal component	Number of PAs
Myxoid	19
Chondroid	2
Adipose	4
Osteoid	2
Hyalinized	1

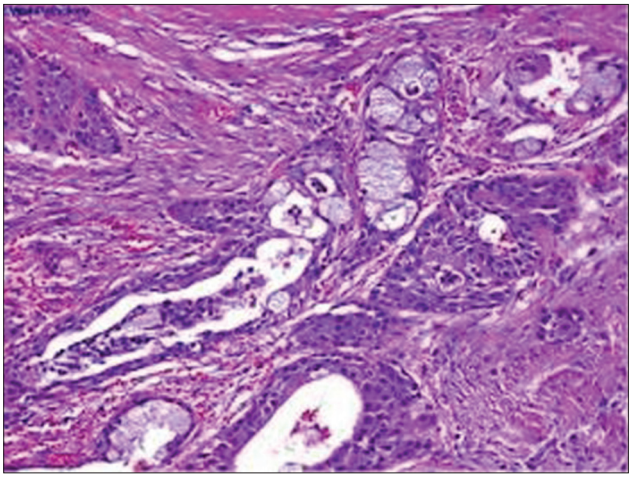

Figure 1: Photomicrograph showing tumoral off shoots within the capsule of pleomorphic adenoma (H&E stain x100)

Chondroid tumoral zones were evident in two cases. These areas resembled immature hyaline cartilage with an acidophilic ground substance and few chondroid lacunae with small oval chondroblasts. In two other cases we noticed osteoid [Figure 3] differentiation of the stromal component. The osteoid areas appeared eosinophilic with a vague lamellar disposition and rare osteoblasts. Prominent focal areas of a lipomatous stroma were evident in four of the recorded cases.

Among the morphological patterns of the epithelial component [Table 5], the ductal pattern predominated (84.6%), followed by sheets/solid patterns (61.5%) and few with trabeculae formation. These patterns were more evident in the cell rich variants [Figure 4].

Plasmacytoid cells were the most commonly found cellular type, predominantly evident in 17 cases (65.4%). Eosinophilic, plump spindle shaped cells were seen in 16 cases (61.5%), representing the second most frequent cellular type. Foci of squamous cells with keratin pearl formation were seen in 15% of the cases. Small squamous epithelium lined cyst that contain keratin were also evident. Cuboidal cells, basaloid cells and clear cells were few other cell types that were occasionally seen [Table 6].

Histochemical interpretations

The five controls of palatine minor salivary acini revealed a heterogeneous staining pattern when stained with combined AB-PAS stain. The acini took up a blend of magenta and blue color, indicating the presence of both acidic and neutral mucins. The neutral mucins predominated [Figure 5].

The histochemical staining of PA [Table 7] revealed PAS positive areas within the lumen of ducts indicative of neutral mucins [Figure 6]. The chondromyxoid areas were AB positive and indicative of acidic mucins [Figure 7]. Myoepithelial cells did not take up any stain.

Apparently normal minor salivary acini found in association with the tumor were predominantly AB positive indicative of acidic mucins, unlike their normal counterparts [Figure 6]. However, the staining pattern varied from one case to another. In few tumors, the acini took up a uniform, homogeneous brilliant blue stain [Figure 8]; while in few others, the acini took up a heterogeneous blue and slightly magenta stain [Figure 9]. A noteworthy observation was that the acini in myxoid tumors expressed a more homogeneous stain, while those in the cellular variants expressed a more heterogeneous stain [Table 8]. These findings gave an impression that both acidic and neutral mucins are expressed by PA and as tumorigenesis progresses, the

Table 5: Epithelial components in pleomorphic adenoma (PA)

Predominant epithelial component	Number of PAs
Ducts	22
Solid/sheets	16
Trabeculae	2

Table 6: Cell types in pleomorphic adenoma (PA)

Predominant cell types	Number of PAs
Cuboidal	2
Squamous	4
Spindle	16
Plasmacytoid	17

Table 7: Histochemical profiles in pleomorphic adenoma (PA)

Site	AB	PAS
Lumen of ducts	−ve	+ve
Myxoid areas	+ve	−ve
Chondroid areas	+ve	−ve
Normal mucous acini as controls	+ve	++ve
Apparently normal mucous acini in PAs	++ve	+ve

AB: Alican blue; PAS:Periodic acid-Schiff

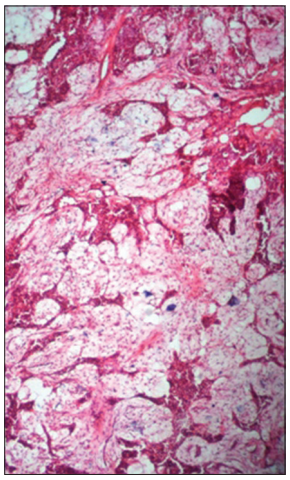

Figure 2: Photomicrograph showing myxoid stroma in pleomorphic adenoma (H&E stain, x100)

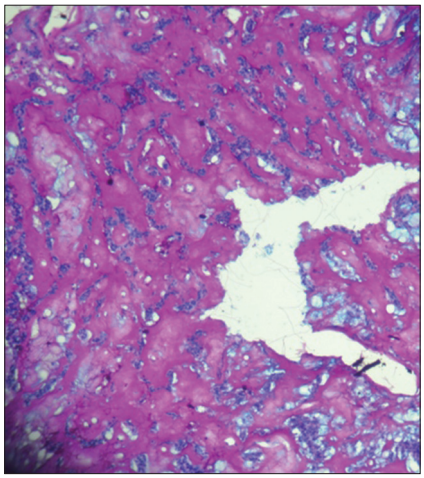

Figure 3: Photomicrograph showing osteoid-like matrix/trabecular pattern (Alcian blue-periodic acid-Schiff (AB-PAS) stain, x100)

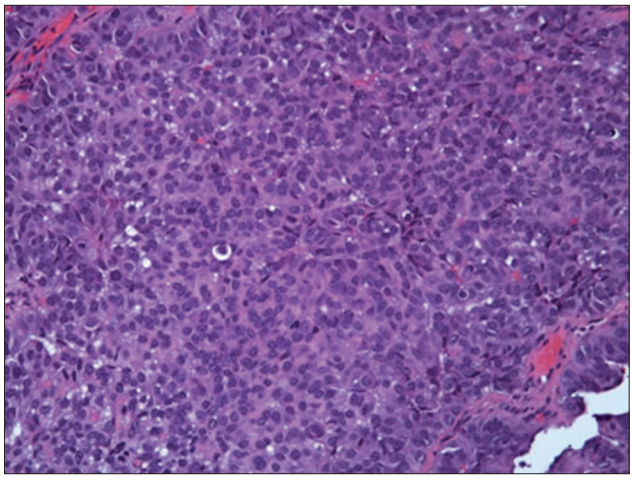

Figure 4: Photomicrograph showing cellular variant of PA (H&E stain, x400)

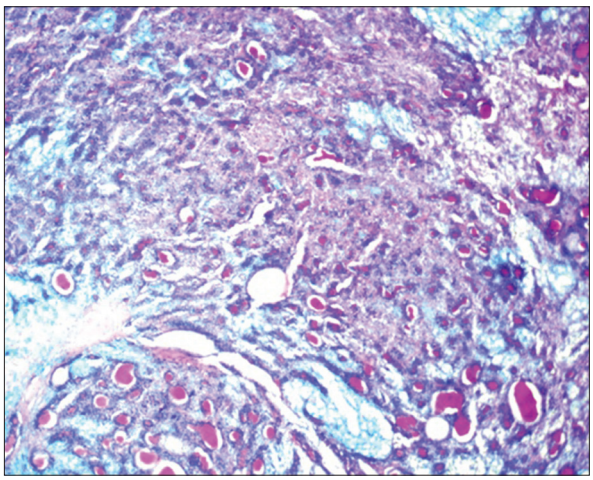

Figure 6: Photomicrograph showing cellular variant of PA (AB-PAS stain, x400)

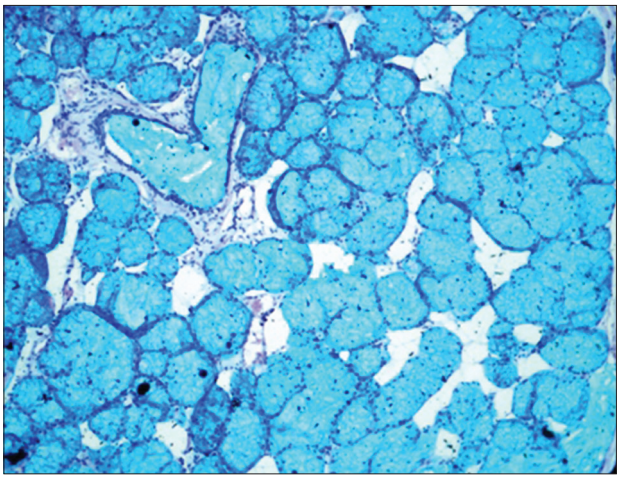

Figure 8: Photomicrograph showing mucous acini in myxoid tumors (AB-PAS stain, x400)

mucin profile of mucous salivary acini changes from neutral to acidic.

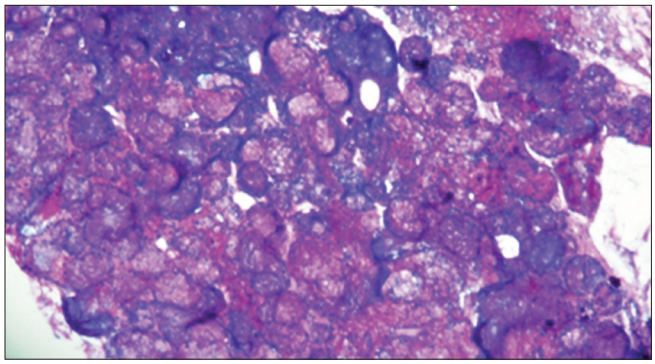

Figure 5: Photomicrograph showing cellular variant of PA (AB-PAS stain, x400)

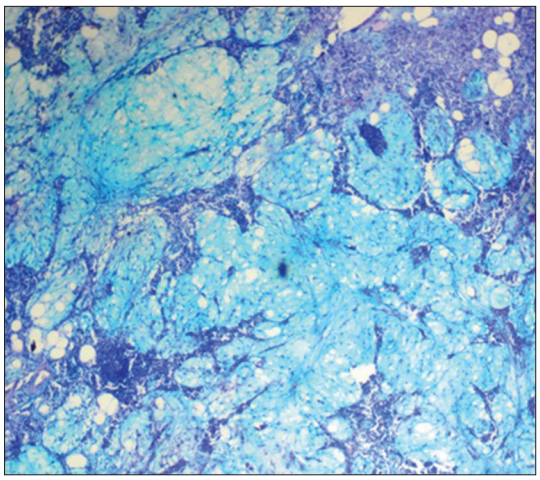

Figure 7: Photomicrograph showing AB positive chondromyxoid areas (AB-PAS stain, x100)

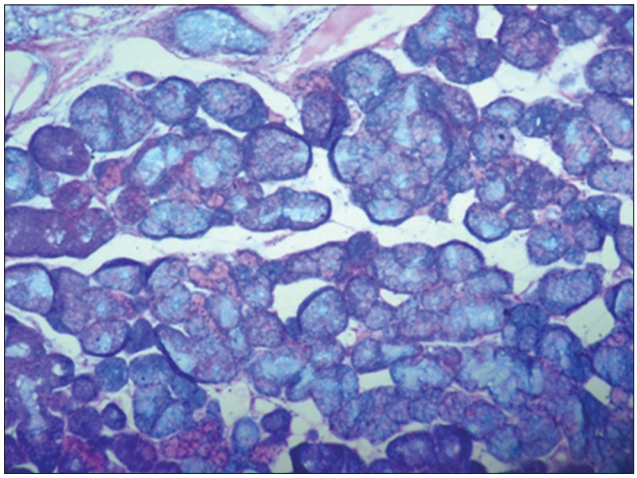

Figure 9: Photomicrograph showing mucous acini in cellular tumors (AB-PAS stain, x400)

DISCUSSION

In this study, 92.3% of PA developed in the minor salivary glands of the palate. A marked female predominance of 57.7% was recorded. In various studies conducted, the universal agreement is that, the most common sites of involvement of

Table 8: Histochemical profile of mucous acini in histological subtypes of pleomorphic adenoma (PA)

Subtypes of PA	AB	PAS	Staining pattern
Type I (myxoid)	++	−	Homogeneous
Type II (myxoid and cellular)	+	−	Homogeneous
Type III (predominantly cellular)	+	+	Heterogeneous
Type IV (extremely cellular)	+	+	Heterogeneous

AB: Alican blue; PAS:Periodic acid-Schiff

minor salivary glands are hard palate, upper lip and buccal mucosa. These minor salivary gland tumors present as slow growing, painless submucosal masses. The more common palatal mixed tumors are located laterally and rarely cross the midline. This presentation is due to the distribution and arrangement of minor salivary tissues of the palate.[7,8] Similar clinical manifestations were recorded in this study.

All the excised specimens were well circumscribed, but with ill-defined capsules. The tumors were in close proximity to the overlying mucosa. In two cases, both with a myxoid rich stroma, tumoral off shoots into the capsule were noted. However, no signs of atypia were detected in these extensions. In PA of the major glands and in predominantly mucoid variants, the capsule is generally incomplete with tumoral extensions into the surrounding tissues. The extensions beyond the capsule without nucleocytoplasmic atypia are not interpreted as a sign of malignancy.[9,10]

According to the relative proportion between stromal and epithelial component of these tumors, 46.2% were of type III, 27% were of type IV, 19.2% were of type II and 7.7% were of type I.

Foote and Frazell first submitted such histopathological classification of PA in 1954. Seifert *et al*., proposed similar findings in 1976. According to these authors, type I comprised of principally myxoid variant, type II comprised of myxoid and cellular variant, type III comprised of predominantly cellular variant and type IV comprised of an extremely cellular variant.[11,12] PA of the major salivary glands generally belong to the type I or II categories while the minor salivary gland tumors are more cellular in nature.[13]

This distinction has no therapeutic significance but helps emphasize the broad morphological spectrum possible within this neoplasm. Observations made in this study, revealed the great diversity of morphological aspects in minor salivary gland tumors.

The most common stromal type was the myxoid type, encountered in 19 cases and accounting for 73.1%. Histochemically, the prevalence of neutral mucins within the ductal lumens and acid mucopolysaccharides in the myxoid areas were appreciated. Review data referring to type of mucins produced in PA, have specified the existence of two types of mucins. One of epithelial origin, with a high content of neutral glycoproteins and one of mesenchymal origin, rich in sulfated and non-sulfated glycosaminoglycans.[14,15]

Zhao *et al*., in a study concerning glycosaminoglycans localization in PA reveals that both epithelial and mesenchymal-like tissues contain glycosaminoglycans.

It is suggested that the glycosaminoglycans in PA are mainly produced by non-luminal cells and influence the proliferation, secretory activity and shape of tumor cells, thus contributing to the morphological diversity of this tumor.[16]

In two cases, a chondroid differentiation surrounded by a myxoid stroma was noted, suggesting the possibility of chondroid metaplasia of the myxoid stroma. These areas reflected the presence of acid mucopolysaccharides, histochemically. According to Aigner and colleagues, the chondroblastic cells, derived from stellate cells of the myxoid areas, through mucopolysaccharides synthesis developed cartilaginous areas in the PAs.[17]

Only two cases with osteoid stromal differentiation were recorded. The review data have quoted few cases of PAs with osteoid stromal differentiations.[13,18]

Four cases revealed considerable adipose tissue amidst the tumor mass, which could be either a result of herniation of adipose from the surrounding stroma or as a result of metaplasia.[19]

In the current study, seven of the 26 (26.9%) PAs belonged to the extremely cellular variants. Plasmacytoid cells were the most frequent cell type followed by the spindle cells. Ellis *et al*., related that these cells appear to be in transition from one form to another.[13] Additionally, in PA, plasmacytoid cells seem to originate from luminal rather than myoepithelial cells. This feature is more prominent in mixed tumors of minor salivary glands than in those arising from the major glands.[13,20,21] Squamous cells organized in abrupt islands and areas of keratin in whorls, microcysts or irregular masses were found in the solid cellular zones or in proximity to the myxoid areas.[13,18,21]

Mucins are composed of a number of chemical substances which differ chemically, depending on the cell from which they are derived. Epithelial mucins, include neutral mucins and acidic mucins (sulfo- and sialomucins); while the connective tissue mucins show acid mucosubstances like chondroitin sulfates, keratin sulfates, hyaluronic acids and dermatan sulfates. By using different combination of stains, these mucins can be categorized.[22-25]

Mucin profiles in developing fetal salivary glands revealed their role in maturation and maintenance of the ductal network. No mucins were expressed by the acinar cells.[26]

Eversole (1972) studied the mucin histochemistry in normal salivary glands. The mucous acini of the sublingual and the submandibular glands showed a varied heterogeneity with the AB-PAS stains.[27] The present study was in concordance with these features, with respect to the normal mucous acini of the hard palate. In apparently normal acini in relation to the tumor; however, the histochemical profile revealed the predominant presence of acidic mucins.

An important role of mucins is recognized in cancer development and invasion. Like normal epithelial tissue, cancer cells use mucins to control the environment, regulate differentiation and proliferation and enhance invasive and metastatic properties.[28]

Histochemistry of salivary gland tumors depicts the presence of mucosubstances, but does not comment on the refined and detailed constituents of those mucosubstances nor does it emphasize on the prognosis or longevity of the neoplastic diseases based on the mucin pattern which has been secreted. There have been no views on the different gradations of the tumor based on pure histochemical analysis.[29]

In the current study on PAs, the ductal lumens revealed strong PAS positivity, while the chondromyxoid areas showed strong AB positivity with the AB-PAS stain. This was in accordance with the study done by Azzopardi and Smith (1959) who conducted a survey on 100 cases of salivary gland tumors.[30]

The grading of the tumors based on the mucin production was also noted in the gastrointestinal tract. The inference drawn from the study on the gastrointestinal tumors, is that they generally show sulfomucin predominance and as the differentiation of the tumour increases, the sulfated mucin content decreases with significant increase in the neutral mucin content.[31,32]

The evidence that malignant transformation develops through a sequence of changes with gradual loss of cellular differentiation and the reappearance of the fetal phenotype can also be implicated in salivary gland tumors. A hypothesis derived at from this study could be that the myxoid variants express more acidic mucins due to lack of differentiation; hence, have a higher recurrence rate and poorer prognosis. Whether the change in mucin expression causes the malignant transformation or whether malignancy causes the change in mucin expression, remains speculative and debatable.

CONCLUSION

This study demonstrated the great diversity of morphological aspects of the stroma in PAs of the minor salivary gland. The role of myoepithelial cells in stromal histogenesis was confirmed. The neoplastic myoepithelial cells differentiate to stellate cells, which produce the myxoid matrix and then further differentiate to chondroblastic cells, which via mucopolysaccharide synthesis develop cartilaginous areas. We also noted the change in mucin profile from neutral mucins to acidic mucins as the neoplasm progresses.

The future scope of this study lies in studying the true nature of fetal mucins and its role in tumorigenesis. A larger sample size needs to be assessed in order to hypothesize the prognostic implications. Histochemistry of malignant tumors and its correlation to benign tumors would further highlight the role of mucins in tumorigenesis. Finally, an attempt should be made to classify these tumors based on their histochemical profile.

A detailed clinical, histopathological and histochemical analysis in salivary gland tumors will provide a better insight to the pathophysiology of the disease, tumor differentiation and prognostic implications. Thus, emphasizing on a more 'pleomorphic' approach to PAs.

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