Original Article

Expression of MUC1, MUC2 and MUC5AC in salivary gland mucoepidermoid carcinoma: A case series with diagnostic implications

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Abstract

Context: Mucoepidermoid Carcinoma (MEC) accounts for 10–15% of all salivary gland neoplasms and its management is related to tumour grading. The expression of mucin in the tumour cells presumably affects and predicts tumour behaviour.

Aims: To analyse the expression of MUC1 (membrane bound mucin), MUC2 and MUC5AC (secreted mucins) in mucoepidermoid carcinoma and correlate with tumour grade and patient outcome.

Settings and Design: In this retrospective correlation study the expression of MUC1, MUC2 and MUC5AC were investigated using immunohistochemistry in confirmed cases of MEC.

Methods and Material: The staining patterns of MUC1, MUC2 and MUC5AC were analysed in 10 confirmed cases from the year 2013 to 2020.

Statistical Analysis Used: SPSS 23 was used for bivariate correlations.

Results: All of the tumours expressed MUC1, showing strong membranous to focal cytoplasmic localization in all cells. The goblet cell component expressed a strong apical membranous pattern. MUC2 expression was moderate, showed cytoplasmic localisation in 40% of mucinous cells and was minimal in intermediate cells and epidermoid cells. MUC 5AC expression was strong apical membranous in goblet cells and mainly negative in intermediate cells and epidermoid cells.

Conclusions: Although MUC1 is a reliable marker for all cell types of MEC but has no significant correlation with the tumour grade. MUC 2 has not been found to be a reliable diagnostic marker and has no significant correlation with the tumour grade. MUC 5AC has been found to have a significant expression in tumours with lymphoid infiltrate. There was no statistically significant correlation of MUC expression with the site, tumour grade and patient outcome.

Keywords: MUC1, MUC2, MUC5AC, mucoepidermoid carcinoma

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INTRODUCTION

Salivary gland tumours constitute approximately 11% of head and neck malignancies.[1] Mucoepidermoid carcinoma (MEC) accounts for 10–15% of all salivary gland neoplasms and 30% of all salivary gland malignancies. It is the most common malignant salivary gland tumour.[2] Histologically this tumour is characterised by cystic, solid, or mixed (cystic and solid) growth patterns and cytologically comprises of varying proportions of three cell types: mucous, epidermoid (or squamoid), and intermediate cells. These cell types resemble those of the excretory ducts of salivary glands.[3] Columnar, clear and/or oncocytic cells may also be present.

Epithelial mucins are large highly glycosylated proteins recognised by their repeat tandem domains, which are rich in threonine and serine sites for O-glycosylation.[4]

Based on sequence homologies, two main families of MUC genes can be distinguished:

(i) The MUC genes at locus 11p15, which encode secreted gel-forming mucins (MUC2, MUC5AC, MUC5B, MUC6); and
(ii) The MUC genes at loci 7q22, 3q, and 1q21, encoding mainly membrane-bound mucins.

Grading of MEC has been a topic of debate with the availability of both qualitative and quantitative grading systems. Few studies have used MUC gene expression as an aid in predicting the biological behaviour and prognosis of tumour.[5-9]

Aims and objectives: We aimed to investigate the pattern of expression of membrane bound mucin (MUC) 1, MUC 2 and MUC5AC (secreted mucins) in MEC and their correlation with tumour grade and outcome.

SUBJECTS AND METHODS

The medical records and case notes of all patients with histologically confirmed MEC of the salivary gland were reviewed retrospectively from the year 2013 to 2020. Necessary institutional ethical approvals and clearances were taken dated 9th june 2021. Out of the 13 confirmed cases, only 10 had the complete information and the blocks. The slides were reviewed and for each case, representative paraffin wax blocks were selected for immunohistochemical (IHC) evaluation. The characteristics of MUC antibodies used in the study are shown in [Table 1]. Sections were baked for 30 min at 60°C. The sections were then deparaffinised and hydrated using graded alcohol and water. Antigen retrieval was done in citrate for 35–40 min. Peroxidase was blocked for 10 min in the blocking solution (Dako). Then the slides were incubated with primary antibodies for 25–30 min and washed in tris buffer solution. The HRP polymer kit (Dako) was then applied for 30 min. After being washed in tris buffer solution, the slides were incubated with 3,3'-Diaminobenzidine (DAB) substrate chromogen solution, washed in water, counterstained with haematoxylin, washed, dehydrated and mounted.

The staining patterns of MUC 1, MUC2 and MUC5AC were analysed for the percentage of cells stained, localization [Table 2] and intensity. All MECs were classified in accordance with the Armed Forces Institute of Pathology (AFIP) grading system [Figures 1-3].[10] These were correlated with the site, tumour grade using the AFIP grading system [Table 3] and patient outcome.

RESULTS

In the present study, four patients were males while six were females. The patients' age ranged from 16 to 70 years (median 43 years). The most frequent site was the parotid (60%) [Tables 2 and 4]. The average size of the tumour was 3 cm (1.5 cm–5.5 cm). Four cases (40%) showed significant lymphocytic host response at the tumour periphery or within the tumour while stromal desmoplasia was observed in three cases (30%). None of the tumours showed keratinisation within the lesion. Perineural infiltration was seen in (20%) cases only. None of the cases showed vascular and bony invasion. Tumour necrosis was observed in only one case (10%). Two (20%) of them showed soft tissue invasion. Lymph node involvement was seen in two cases (20%) of MEC.

In normal salivary glands, MUC1 was localised to the apical membrane of columnar cells lining excretory ducts of salivary glands adjacent to the tumours. MUC2 and MUC5AC were focally expressed in the cytoplasm of a small group of luminal cells lining excretory ducts.

Expression of MUC was divided into strong, moderate

Table 1: Characteristics of MUC antibodies used in study

Antibody	Supplier	Dilution	Clone	Antibody incubation time (min)	Positive control	Staining pattern
MUC 1	Thermoscientific	1:50-1:100	MH1 (CT2)	20	Breast ca	Cytoplasmic and cell membrane
MUC 2	Thermoscientific	1:50-1:100	M53	20	Colon ca, small intestine	Cytoplasmic and cell surface.
MUC5AC	Dako Denmark A/S	Pre diluted	CLH2	20	Cervix and gastric	Cytoplasmic or perinuclear

Table 2: MUC expression in MEC according to tumour localisation

Tumor localization	MUC1	MUC2	MUC5AC
Parotid gland	7	6	6
Positive	0	1	1
Negative	100%	85.7%	85.7%
Submandibular gland	2	1	1
Positive	0	1	1
Negative	100%	50%	50%
Sublingual gland	1		
Positive	0	0	0
Negative	100%	1	1

Table 3: MUC 1, MUC 2 and MUC 5AC expression according to AFIP grading system

Tumor Grade (AFIP)	Negative	Weak	Moderate	Strong
MUC 1				
H			1	1
I			2	
L			3	3
Total			6 (60%)	4 (40%)
MUC2				
H	1	1		
I			2	
L	2	2	2	
Total	3 (30%)	3 (30%)	4 (40%)	
MUC5 AC				
H	1	1		
I				2
L	2	1	2	1
Total	3 (30%)	2 (20%)	2 (20%)	3 (30%)

Table 4: Clinical parameters with AFIP grading

Age	Gender	Site	Size (cm)	AFIP grading
36	M	Left Parotid	4×1.5	Low
70	M	R Parotid	3×3	High
44	M	Floor of mouth	3×3	High
47	M	R Parotid	5×3	Low
40	F	R Parotid	4×5	Low
16	F	L Submandibular	1×1	Low
65	F	R Parotid	5×4	Intermediate
45	F	R Submandibular	3×3	Low
20	F	R Parotid	3×2.5	Low
40	F	L Parotid	4×4	Intermediate

and weak according to the intensity and of expression; when greater than 75% of the tumor cells showed strong expression, it was considered strongly positive, 30–75% positivity indicated moderate expression, 2–30% positivity indicated weak expression and less than 2% positivity was considered focal. Some degree of MUC1 expression was seen to be accentuated in goblet cells of all MECs, 50% showed strong expression and the other 50% showed moderate expression. The predominant pattern of staining was membranous (complete, beaded and apical) [Figure 4a] followed by cytoplasmic staining [Table 5].

Out of 10 MECs, nine had intermediate cell component and some degree of MUC1 expression was seen in all these nine cases of MEC, out of which two cases (22.2%)

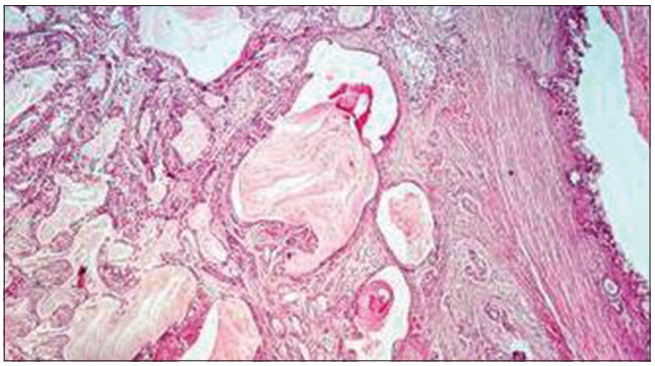

Figure 1: Low grade MEC (×100, H&E)

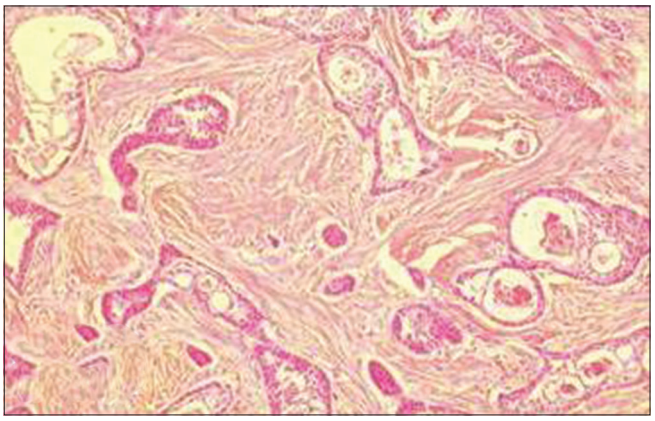

Figure 2: Intermediate grade MEC (×100, H&E)

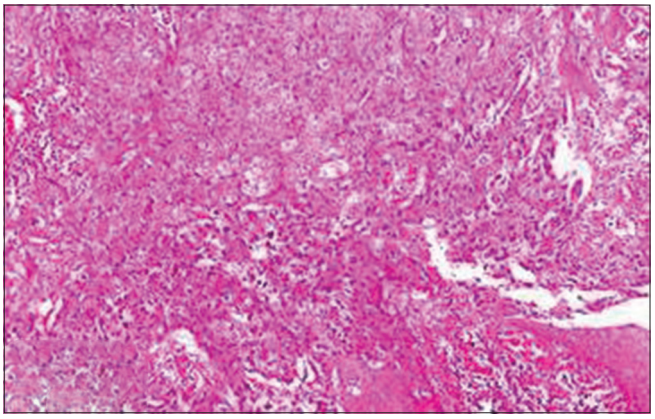

Figure 3: High grade MEC (×100, H&E)

showed strong expression, four (44.4%) showed moderate expression [Figure 4b], two (22.2%) showed weak expression and one (11.1%) had focal positivity. All of them showed membranous (beaded) as well as the cytoplasmic pattern of staining. Three MECs showed the presence of epidermoid cells of which MUC1 was expressed in two of them, out of which, one showed strong expression and the other showed moderate expression. The pattern of staining was membranous [Figure 4c].

MUC2 was expressed in varying degrees in the mucinous cell [Figure 5a] of all cases of MEC, with 50% tumours showing

moderate expression, 40% showing weak expression of MUC2 and other 10% tumours showing focal expression. The pattern of staining was membranous and cytoplasmic. Nine out of 10 MECs had intermediate cell components, of which only two cases showed some degree of cytoplasmic expression of MUC2. One of the tumour (50%) showed weak expression and the other (50%) had focal positivity. The rest of the MECs were negative for MUC2 [Figure 5b]. Epidermoid cells were present in three MECs, of which MUC2 was expressed by one (33.3%) tumour, which showed weak expression [Figure 5c] and the other two (66.6%) showed negative expression. The pattern of staining was membranous [Table 5].

All MECs showed some degree of MUC5AC expression in mucinous cells, with 42.8% tumours showing strong expression [Figure 6a], 28% showed moderate expression of MUC5AC and other 28% tumors showing focal expression. The pattern of staining was membranous (complete and beaded) and cytoplasmic in most cells and was apical membranous in goblet cells. Nine out of the 10 MECs had intermediate cell components of which only one tumour (11.1%) showed focal expression of MUC5AC with the cytoplasmic pattern of staining [Figure 6b]. Three out of 10 MECs showed the presence of epidermoid cells of which MUC5AC was negative in 66.6% of cases [Figure 6c] and showed weak expression in only 1 tumor (33.3%). The pattern of staining was cytoplasmic [Table 5].

According to AFIP histological grading system, there was six low grade (60%), two intermediate grade (20%) and two high grade (20%) tumours. MUC 1 was expressed by all the tumors whereas MUC2 and MUC5AC were expressed by four low grade, two intermediate grade and one high grade tumours [Table 3].

Out of 10 patients, one showed recurrence 3 months after resection which had low grade carcinoma with lymph node

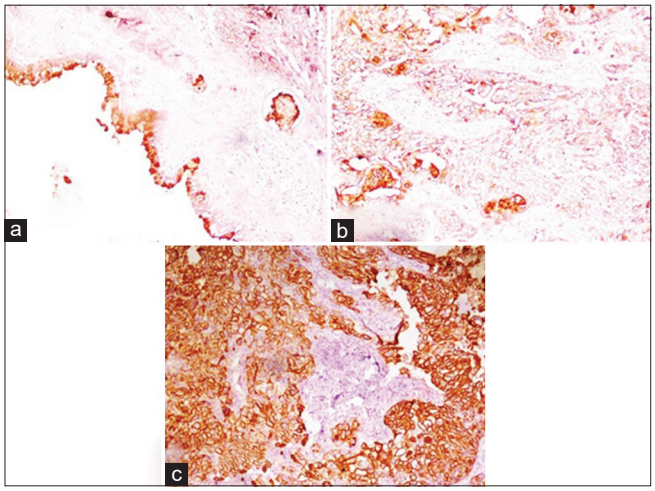

Figure 4: MUC 1 immunoexpression in (a). Strong expression with apical accentuation mucinous/goblet cells (×100) (b). Moderate expression in intermediate cells (×100) (c). Strong membranous positivity in epidermoid cells (×400)

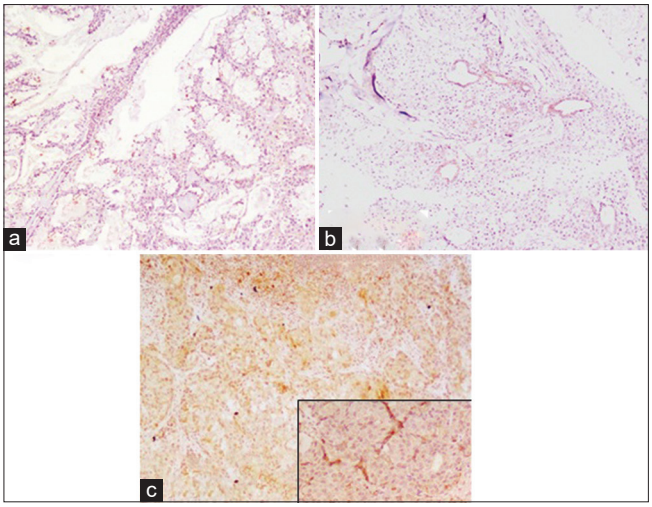

Figure 5: MUC 2 immunoexpression in (a). Negative expression in mucinous/goblet cells (×100) (b). Negative in intermediate cells (×100) (c). Weak expression in epidermoid cells (×100) Inset: weak expression in epidermoid cells (×400)

Table 5: MUC staining in mucous, intermediate and epidermoid cells

	MUC1	MUC2	MUC5AC
Normal	Apical membrane of columnar cell lining excretory ducts	Focal expression in cytoplasm (luminal cells lining excretory ducts)	'do'
MEC			
Mucous cell 10/10 cases			
Number	10 cases	10 cases	10 cases
Pattern	memb > cytoplasmic	memb + cytoplasmic	memb + cytoplasmic
Intensity	5- strong 5-mod	6-mod, 3-weak, 1-focal	5-strong 3-mod, 2-focal
Intermediate cell 9/10 cases			
Number	9 cases	2 cases	1 case
Pattern	memb + cytoplasmic	cytoplasmic	cytoplasmic
Intensity	2-strong, 4-mod, 2-weak, 1-focal	1-weak, 1-focal	1-focal
Epidermoid cell 3/10 cases			
Number	2 cases	1 case	1 case
Pattern	membranous	membranous	Cytoplasmic
Intensity	1-strong. 1-mod	1-weak	1-weak

mod - moderate, memb - membranous

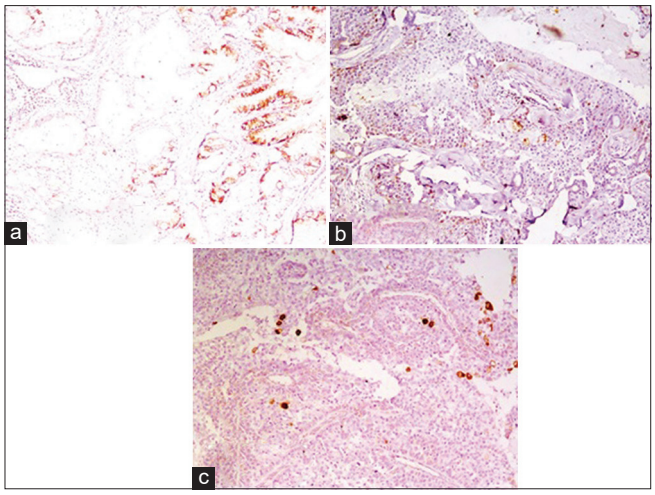

Figure 6: MUC 5AC immunoexpression in (a). Strong cytoplasmic in mucinous/goblet cells (×100) (b). Weak expression in intermediate cells (×100) (c). Negative in epidermoid cells. (×100)

metastasis. The tumour showed strong membranous MUC1 expression in mucinous cells, and MUC2 and MUC5AC were negative. One patient died within one month of resection and had high grade carcinoma with desmoplasia. It showed moderate membranous expression of MUC1 in mucinous and intermediate cells and negative MUC2 and MUC5AC. Two other cases showed desmoplasia, onewith low grade tumour showed strong membranous and cytoplasmic MUC1 expression in mucinous and intermediate cells with weak MUC2 and strong moderate cytoplasmic MUC5AC. The other was intermediate grade carcinoma showing moderate cytoplasmic and membranous staining for MUC1 and MUC2, and strong apical expression for MUC5AC in mucinous cells whereas intermediate cells showed weak expression of MUC1. Pearson bivariate correlation was done. There was no significant relationship between any of the mucin types with histological grade, tissue invasion, desmoplasia and outcome.

DISCUSSION

MEC is the most common type among malignant salivary gland tumours.[11-13] Histological grading and tumour staging are among the most important tools in the hands of clinicians in determining the appropriate management and prognostication in patients presenting with salivary gland MEC. In this study, we examined type of expression of both membrane-bound mucins (MUC1) [Figure 4] and secreted mucins (MUC2 and MUC5AC) [Figures 5 and 6] in MECs using the standard IHC protocol [Table 5].

In this study MUC1 expression was seen to be accentuated in goblet cells of all MECs, 50% showed strong expression and the other 50% showed moderate expression.

MUC1 overexpression reduces cell–cell and cell–matrix adhesion, favouring stromal invasion of the tumour cells.[5,14] In normal epithelia, mucins are localized to the apical borders of the cell membrane. In MEC, expression of transmembrane mucins, e.g., MUC1 is not restricted to apical borders of tumour cells. Mucins are repositioned over the entire cell membrane as the cells lose polarity.[15] With several carcinomas showing high MUC1 expression compared with normal tissue it is considered a promising biomarker.[14] MUC1 overexpression in breast carcinoma, papillary thyroid carcinoma and prostate carcinoma was found to be associated with aggressive tumour behaviour and poor clinical outcome.[6,16,17] Alos *et al*.[5] found that MUC1 expression in more than 50% of tumour cells was associated with a higher histological grade, increased risk of metastasis and poorer prognosis. Siyi *et al*.[8] were in agreement with the findings of Alos *et al*.,[5] however their study suggested a MUC1 expression level of greater than 75% in tumour cells. Shemirani *et al*.[9] evaluated mucin expression using quantitative polymerase chain reaction techniques, found that in tumour cells greater expression of MUC1 correlated with a less aggressive disease process and an increased survival rate. However, studies by Handra-Luca *et al*.[7] and Llupi and Qoku[6] did not find any prognostic significance of MUC1 expression in MECs. The current study found that all of the tumours expressed MUC1 as seen in previous studies of Robinson *et al*.[18] and Alos *et al*.[5] No significant correlation was found between MUC1 expression and any prognostic indicators in our study, these findings were in support of previous studies by Handra-Luca *et al*.[7] and Llupi and Qoku.[6] The pattern of staining intensity ranging from strong membranous to focal cytoplasmic in our study was also observed by Alos *et al*.[5]

Studies found that MUC2 gene expression is consistently positive in mucinous carcinomas of the colon, stomach, pancreas, breast and ovary, linking its expression to the so-called "mucinous pathway of carcinogenesis".[19,20] Other studies highlight the tumour suppressor nature of MUC2, indicating that overexpression of MUC2 by pancreatic and biliary tumours was associated with a low degree of invasiveness and better overall prognosis.[14] Alos *et al*.[5] and Robinson *et al*.[18] assessed MUC2 expression in MECs, they found an overall lack of expression, suggesting that MECs develop along a different pathway from other mucinous tumours. In the current study, 70% of the tumours expressed MUC 2, and among them, 40% tumour showed a moderate membranous and cytoplasmic pattern of staining in mucinous cells of low and intermediate-grade tumors The other studies reported cytoplasmic expression of MUC2.[5-7,18] None of the high grade tumour expressed MUC2 in our study similar to the previous studies.[7] Our

study showed negative expression of MUC2 in intermediate cells and epidermoid cells whereas Handra-Luca *et al*.[7] showed its expression in intermediate cells.

MUC5AC is gel-forming gastric mucin, expressed in mucous cells in normal gastric mucosa.[21] However, in many precancerous and malignant lesions, MUC5AC is highly expressed. This means a significant up-regulation of MUC5AC gene transcription and therefore, expression of the secreted mucin MUC5AC in salivary tumour tissue seems to be a metaplastic feature in relation to the lack of expression in normal salivary glands.[22] MUC5AC may suppress immune cells and produce potent anti-apoptotic effects, thereby playing an important role in creating a suitable environment for cancer cell growth.[23] In our study, we observed that MUC5AC was expressed by 70% of the tumours, mainly in mucinous cells. Similar results were found in previous studies.[5-7] Handra-Luca *et al*.[7] found that the expression of MUC5AC was 53% in intermediate cells whereas in our study it was weakly expressed by 10% of the tumour. Tumours with lymphoid infiltrate showed moderate to strong expression of MUC5AC in our study as also observed by Handra-Luca *et al*.[7]

CONCLUSION

MUC1 is a reliable marker for all cell types of MEC. It has a characteristic membranous pattern in mucous cells, intermediate cells, and epidermoid cells and can be utilised in cases of diagnostic dilemma. MUC 2 has not been found to be a reliable diagnostic marker. MUC 5AC has been found to have a significant expression in tumours associated with lymphoid infiltrate. However, none of these have any significant correlation with the tumour grade, site or patient outcome. A large scale study is needed to validate the findings.

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Conflicts of interest
There are no conflicts of interest.

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