

# Expression of MUC1 and MUC2 in Ampullary and Periapillary Carcinomas

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## Abstract:

**Background:** Ampullary and periampullary carcinomas may arise from two different types of mucosa, which thus reflect the broad histomorphological spectrum of these tumours. Since there are three different types of epithelia in these areas (pancreatic, biliary and intestinal), carcinomas originating from this area are classified as having intestinal or pancreaticobiliary type of differentiation. Pancreatobiliary type has consistently shown a worse prognosis. Various types of mucins are distributed in normal tissues and gastrointestinal tumours. MUC1, MUC2 and MUC5AC are known to be the most important amongst all.

**Materials and Methods:** This was a descriptive study. A total of 40 cases of ampullary and periampullary carcinoma diagnosed on histopathological examination from January 2019 to January 2020 were included. Of these 24 were periampullary and 16 were ampullary carcinomas. The histopathological slides were reviewed and the tumours were classified into intestinal type, pancreaticobiliary and other types. Representative block of tumours was subjected to immunohistochemical (IHC) study with MUC1, MUC2 antibodies. Results were subsequently analyzed.

**Results:** Pancreatobiliary type of ampullary carcinoma cases predominated over intestinal type. Carcinomas of "other types" included one case of, adenocarcinoma and five cases of mixed carcinomas. All the pancreaticobiliary type expressed MUC1 and the intestinal type carcinomas expressed MUC2. Pancreatobiliary type was associated with more number of lymph node metastasis.

**Conclusion:** Mucin markers are associated with two different subtypes of ampullary carcinomas which have different prognosis. Immunohistochemical study of mucin expression can define the different types of carcinomas of ampulla of Vater with better objective criteria. MUC1 positive staining is seen in pancreaticobiliary subtype whereas MUC2 positive staining pattern is seen in intestinal subtype. Other types of tumour subtypes such as adenocarcinoma and mixed type also showed MUC1 and MUC2 staining.

**Key Word:** MUC1, MUC2, Immunohistochemistry, Pancreatobiliary.

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## I. Introduction

The ampullary carcinomas originate from ampulla of Vater, intraduodenal portion of common bile duct and pancreatic duct. Periampullary carcinomas originate from head of pancreas, distal bile duct or duodenal tissue. The ampullary papilla is lined by intestinal mucosa while the other parts are lined by pancreaticobiliary type of simple mucinous epithelium.<sup>[1]</sup> So far, two major histological types of these tumors have been recognized: an intestinal type, arising from intestinal mucosa of the papilla, and a biliopancreatic (BP) type, deriving from the BP ductal epithelium. As patients with intestinal-type ampullary ACs have been reported to have a significantly better prognosis than patients with BP-type ACs<sup>2</sup>, the clear differentiation between the two tumor types would have clinical implications. Histological classification can be improved by using the immunohistochemical examination with antibodies directed against differentiation markers to differentiate between intestinal type and pancreaticobiliary type.

## II. Material And Methods

This was a descriptive study. A total of 40 cases of ampullary and periampullary carcinoma diagnosed on histopathological examination from January 2019 to January 2020 were included. Of these 24 were periampullary and 16 were ampullary carcinomas.

**Study Design:** Descriptive study

**Study Location:** This was a tertiary care teaching hospital based study done in Department of Pathology at Government Medical College, Kannur, Kerala.

**Study Duration:** January 2019- January 2020

**Sample size:** 40 cases.

**Sample size calculation:** Sample size is calculated based on the results of the formulas,  $n = \frac{Z_{1-\alpha/2}^2 P(1-P)}{d^2}$

where P=specificity(96%),d=5% (absolute precision).  $Z_{1-\alpha} = 95\%$  CI.

Total of 40 cases of was studied which included both ampullary and periampullary carcinomas.

**Subjects & selection method:** Forty cases of ampullary and periampullary carcinoma were included in our study. The histopathological slides were reviewed and the tumors were classified into intestinal type, pancreatobiliary and other types based on the criteria of Albores-Saavedra T et al. Representative block of tumors was subjected to IHC. IHC study was done with antibodies which included MUC1 and MUC2. The staining intensity of each IHC reaction were scored semiquantitatively

- No staining = 0
- < 10% positively stained tumour cells = 1
- 10 to 50 % positively stained tumour cells= 2
- 50 to 80% positively stained tumour cells= 3
- > 80% stained tumour cells= 4

Scores 0 and 1 were regarded as negative and scores 2-4 were regarded as positive

**Inclusion criteria:**

- 1.All cases of ampullary and periampullary carcinomas

**Exclusion criteria:**

1. Endoscopic biopsies inadequate for reporting

**Procedure methodology**

Informed consent in both mother tongue and English will be taken from the patients before performing the study. The relevant clinical data, results of laboratory investigations already done, operative findings etc will be obtained from the request form for histopathological examination. Specimen are received at department in 10% formalin. The specimen is opened and partially sectioned to aid fixation. Colour coded inking of specimen is done following fixation. There are three approaches for specimen dissection- Bivalving technique, bread loaf slicing technique and axial slicing technique. The tumour must be examined at 3-4 mm intervals. Sections are taken to show the relationship of tumour to anatomical structures namely, duodenum, ampulla, peripancreatic tissue and common bile duct to show tumours closest distance to resection margins and surfaces. Direct extension of tumour to lymph node is classified as lymph node metastasis. Representative sections are taken, labelled and put in perforated tissue capsules for tissue processing, which involves a series of steps. Tissue sectioning and staining done and the slides are examined through the microscope. IHC is done by using both the markers, MUC1 and MUC2 and the results obtained are noted.

**Statistical analysis**

The data collected was analysed statistically by descriptive statics like frequency, percentage, mean and median. Inferential statistics like fishers exact and validity statistics are also used in this study. Fisher exact tests were performed to test for differences in proportions of categorical variables between two or more groups. The level  $P < 0.05$  was considered as the cutoff value or significance.

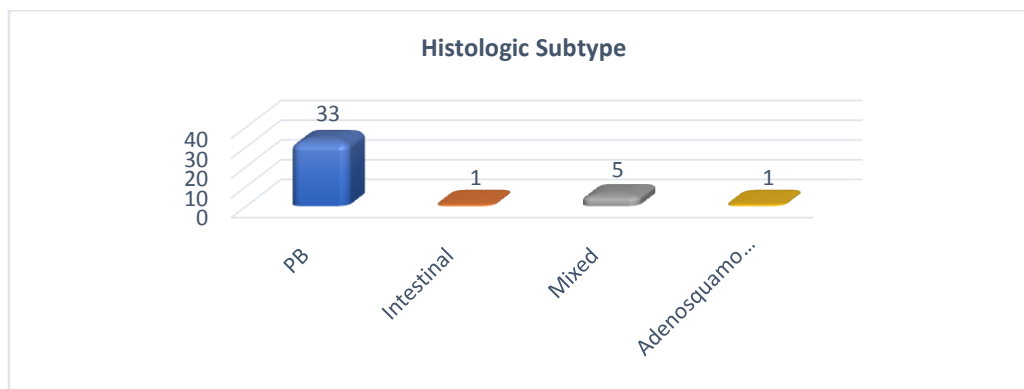
**III. Result**

A hospital based descriptive study was done to document the frequency distribution of different subtypes of ampullary and periampullary carcinoma. Immunohistochemical staining pattern was studied to confirm the histological subtypes. A total of 40 cases over a period of 1 year were studied in detail and results are given below

Histomorphologically, pancreaticobiliary type of ampullary carcinoma cases predominated (33/40), over intestinal subtype. Carcinomas of other types included adenosquamous carcinoma. Five cases with mixed pattern had features of both intestinal and pancreaticobiliary carcinoma.

**Table no 1:** Histological classification.

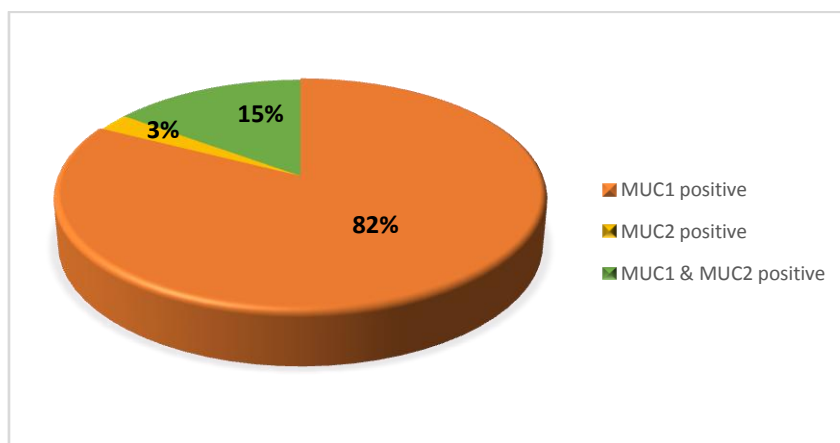
Histologic subtype	Number of cases
Pancreaticobiliary	33
Intestinal	1
Mixed	5
Adenosquamous	1



**Table no2:** Out of 40 cases, 33 showed MUC1 positivity, only 1 showed MUC2 positivity and 5 showed both MUC1 and MUC2 positivity.

**Table no2:**Subtying of ampullary and periampullary carcinoma with reference to immunohistochemistry

MUC1	MUC2	SUBTYPE	Total number
Positive	Negative	Pancreaticobiliary	33
Negative	Positive	Intestinal	1



**Table no3:**The association of pancreaticobiliary subtype with staining for MUC1 was found to be statistically significant( p value < 0.5)

**Table no3 :**Histologic subtype and MUC1 staining

			MUC1 STAINING		Total
			1	2	
HISTOLOGIC SUBTYPE	1	Count	0	33	33
		% within HISTOLOGICSUBTYPE	0.0%	100.0%	100.0%
		% within MUC1INTENSITY	0.0%	100.0%	97.1%
	% of Total		0.0%	97.1%	97.1%
	2	Count	1	0	1
		% within HISTOLOGICSUBTYPE	100.0%	0.0%	100.0%
% within MUC1INTENSITY		100.0%	0.0%	2.9%	
% of Total		2.9%	0.0%	2.9%	
Total	Count	1	33	34	
	% within HISTOLOGIC SUBTYPE	2.9%	97.1%	100.0%	
	% within MUC1 INTENSITY	100.0%	100.0%	100.0%	
% of Total		2.9%	97.1%	100.0%	

Fischer exact test  
'p value= 0.029

Table no 4: Adenosquamous carcinoma showed strong positivity for MUC1. Mixed type showed staining for both MUC1 and MUC2

**Table no 4:** MUC1 and MUC2 in other types of ampullary and periampullary carcinomas

TYPE	MUC1	MUC2
Adenosquamous type(n=1)	Strong positivity	Negative
Mixed type(n=5)	Positive	Positive

Table no5: Mixed type showed 2+ and 3+ intensity for MUC1 expression and adenosquamous type showed 4+ intensity.

**Table no 5:** Intensity of expression of MUC1 in other types of ampullary and periampullary carcinomas

Histologic Subtype	MUC 1 expression				
	0+	1+	2+	3+	4+
Mixed (5)	0	0	3	2	0
Adenosquamous (1)	0	0	0	0	1

Table no 6 shows that Mixed type has 2+ and 3+ intensity for MUC2 expression and adenosquamous has negative staining for MUC2.

**Table no 6:** Intensity of expression of MUC2 in other types of ampullary and periampullary carcinomas

Histologic Subtype	MUC 2 expression				
	0+	1+	2+	3+	4+
Mixed (5)	0	0	2	3	0
Adenosquamous (1)	1	0	0	0	0

Table no 7 shows pancreaticobiliary has higher lymph node metastasis and TNM staging.

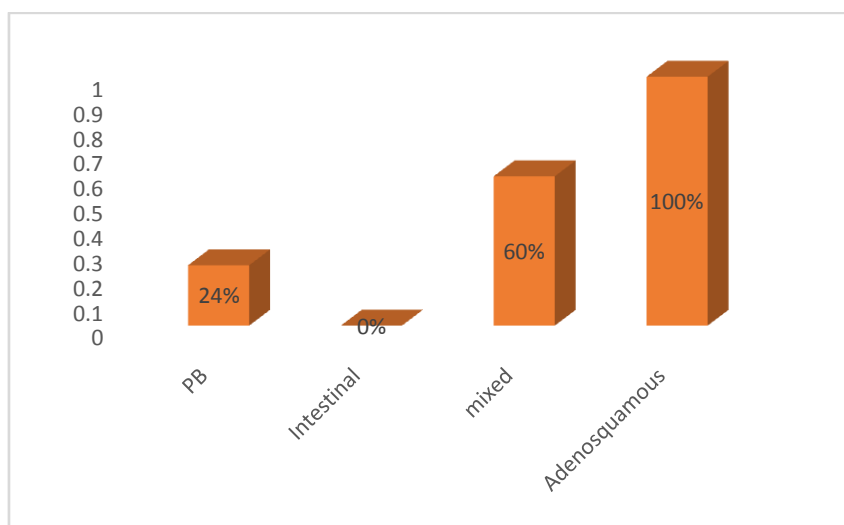
**Table no 7:** Comparison of pancreaticobiliary type and intestinal type of ampullary and periampullary carcinoma

PARAMETER	PANCREATICOBILINARY TYPE (N=33)	INTESTINAL TYPE (N=1)
Mean Age at diagnosis	62.27	58
Male:Female Ratio	7:4	1:0
<b>TNM Staging</b>		
T1	13	
T2	7	1
T3	12	
T4	1	
<b>Lymphnode metastasis</b>		
Absent	15	1
Present	18	0
<b>MUC 1</b>	33	0
<b>MUC 2</b>	0	1

Table no 8 shows that the intensity of MUC1 expression is more with higher grade of tumours. Moderately differentiated tumours has more intensity of MUC1 expression when compared to well differentiated. Perineural invasion was more in pancreaticobiliary type.

**Table no 8:**MUC1 expression with grade of tumour

Grade of tumour (N)	MUC 1 expression				
	0+	1+	2+	3+	4+
WDT(31)	0	0	14	10	7
MDT(8)	0	0	0	3	5



#### IV. Discussion

The objective of the study was to study the expression of MUC1 and MUC2 in the two main histologic subtypes of ampullary and periampullary carcinoma and also to compare the main two subtypes with prognostic factors. A total of 40 cases which satisfied the inclusion criteria were included in the study. Demographic data showed the mean age group of the study population was 61.8 with an age range from 40 to 77 years. Majority of the study population were males (67%), similar to study conducted by A Schueneman et al<sup>3</sup> and Maithili et al<sup>4</sup> where the percentages were 58 and 60% respectively. Histomorphologically, pancreaticobiliary type of ampullary carcinoma cases predominated (33/40), over intestinal subtype, similar to studies done by kimura et al<sup>5</sup>, Fischer HP et al<sup>6</sup>, and Maithili et al<sup>4</sup> where the pancreaticobiliary subtype accounted for 75%, 44%, and 50% respectively and the intestinal type accounted for 25%, 23% and 25% respectively. When compared with other studies the number of intestinal subtype was very low which accounted for only 1, out of 40 cases.

In our study Carcinomas of other types included adenosquamous carcinoma (1 out of 40) and mixed types (5 out of 40). The five mixed type had features of both intestinal and pancreaticobiliary carcinoma. In our study, 33 pancreaticobiliary subtype stained positive for MUC1 and negative for MUC2. Whereas the intestinal subtype stained positive for MUC2 and negative for MUC1. This pattern of staining for MUC1 and MUC2 markers were found to be similar to many other studies. Kawabata et al<sup>7</sup> found that MUC1 had high sensitivity for the pancreaticobiliary type and that MUC2 and CK20 had a high sensitivity for the intestinal type. In our study, we used only MUC1 and MUC2 to classify into PB-type and I-type ampullary cancer. However because there was only one intestinal subtype and 33 pancreaticobiliary subtype, the validation of these markers were difficult in this study. But the statistical significance of MUC1 with pancreaticobiliary subtype was found to be significant (p value= 0.029).

The adenosquamous type of ampullary carcinoma was found in a young age patient with history of familial adenomatous polyposis. The adenosquamous subtype showed strong diffuse positivity for MUC1 and negative staining pattern for MUC2 and T staging was found to be T4 with lymph node metastasis and no distant metastasis. This finding was similar to the study conducted by Maithili et al<sup>4</sup> which also had one case of adenosquamous type with strong diffuse positivity for MUC1 and MUC2, MUC5AC negative.

There were 5 mixed type of carcinomas, staining positive for both MUC1 and MUC2. Three of them showed 2+ staining for MUC1 and two of them showed 3+ staining for MUC1. Two of them showed 3+ staining for MUC2 and three showed 2+ staining for MUC2. The study conducted by Maithili et al<sup>4</sup> also had a case of mixed type with similar staining pattern for both MUC1 and MUC2.

In our study we found that out of the 33 cases of PB subtype, 13 cases were T1, seven cases were T2, 12 cases were T3, and only one case was T4. The intestinal subtype was T2 stage. Others studies also showed

that the PB subtype had higher T staging when compared to intestinal. According to the study done by Maithili et al<sup>4</sup> out of 10 PB cases, 7 cases were T3 and 3 cases were T2, and all intestinal cases were T2.

In another study done by Moriya et al<sup>8</sup> out of 14 PB type, 10 had higher Tstage (T3 and T4), whereas out of 18 intestinal subtype, 11 cases were T1. In this present study, we also found that, 18 of 33 PB subtype showed lymph node metastasis and the intestinal type did not show lymph node metastasis. Studies done by Moriya et al<sup>8</sup> Maithili et al<sup>4</sup> also showed that the lymph node metastasis was more in the PB type when compared to intestinal type, similar to our study.

Grade of tumour and the MUC1 intensity was also analyzed in this present study and it was found that immunoreactivity increased with increase in grade of the tumour. There were 31 cases of well differentiated tumours and 8 cases of moderately differentiated. Eight cases of moderately differentiated had 3+ and 4+ intensity. Moderately differentiated tumour had higher staining pattern when compared to well differentiated. In this present study we did not have cases of poorly differentiated carcinoma grade

Another prognostic factor which was analyzed in our study was perineural invasion. Only 8 out of the 33 cases of pancreaticobiliary showed perineural invasion accounting for only 24%. But in other studies, the presence of perineural invasion in pancreaticobiliary subtype was higher. Study by Moriya et al<sup>8</sup>, showed that 50% of cases with pancreaticobiliary subtype showed perineural invasion when compared to intestinal which was only 16%.

## V. Conclusion

Pancreatobiliary carcinoma expressed MUC1 and intestinal subtype of carcinoma expressed MUC2. Prognostic factors of pancreaticobiliary type includes, T staging, perineural invasion, grade of tumour and lymph node metastasis. Unlike other studies where, all the above factors were unfavourable, this study showed only lymph node metastasis as an unfavourable prognostic factor

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