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Immuno Histomolecular Profile in Periampullary Adenocarcinoma : A Clinico-Pathological Study

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Abstract

Periampullary adenocarcinomas (PAC) are rare malignant tumors with worst survival rates due to destructive route of the disease. It comprises distal bile duct, pancreatic, ampullary and duodenal adenocarcinoma that originate within periampullary complex. The primary origin of PAC in this area is often difficult previous to the surgery into pancreaticobiliary (PB-PAC), intestinal (I-PAC) and mixed type (MT-PAC) as PB-PAC carries bad prognosis.

Objective: The main aim was to study the histomorphological features and immunohistochemical (IHC) expression of Cytokeratin (CK) 7 and 20 in PAC and assess their expression with known histopathological prognostic parameters.

Method: A total number of 31 resected pancreatoduodenectomy specimens were studied in the department of Pathology, in a tertiary care center, Karnataka, India for histomorphological features with CK7 and CK 20 expression. All the statistical methods were carried out through the SPSS for Windows (version 22.0). A p-value of ≤0.05 was taken to be statistically significant.

Results: The cases were categorized into PB-PAC (CK7+), I-PAC (CK20+) and MT-PAC (CK7+ and CK20+) based on histomorphological and histomolecular typing. There was a positive association between histomorphology and histomolecular typing. The difference in the proportion of histomolecular profiling between the expressions was statistically significant with p-value of ≤0.001.

Conclusions: The concordance in PB-PAC, I-PAC and MT-PAC by usual haematoxylin and eosin microscopy and later confirmed by the immunophenotyping advocates sub typing to be incorporated in the routine histopathology report. The importance of correctly categorizing of the PB-PAC, I-PAC and MT-PAC is mainly for the prognosis and difference in administering of the targeted chemotherapy regimens.

Key Words: Adenocarcinoma, CK7, CK20, Pancreaticobiliary, Periampullary region.

Introduction

Periampullary adenocarcinomas (PAC) are rare malignant tumors that originate within periampullary complex having a common embryologic source from the foregut. It comprises distal bile duct, pancreatic, ampullary and duodenal adenocarcinoma with worst survival rates due to destructive route of the disease. It accounts for less than 1% of all the gastrointestinal tumors. Although these tumors have different origins, the complex regional anatomy dictates a common pancreatoduodenectomy operative approach. Since it is a transition area connecting the biliary tract ductal epithelium and intestinal epithelium, the primary origin of PAC in this area is often difficult previous to the surgery into pancreaticobiliary (PB-PAC), intestinal (I-PAC) and mixed type (MT-PAC)[1,2]. The final diagnosis of histological subtype is very important along with the degree of tumor differentiation, perineural

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infiltration (PNI), lymphovascular invasion (LVI), margin status, resected lymph node status which significantly influences the outcome and different chemotherapy regimens[3,4].

The histologic subtyping of PAC into pancreaticobiliary (PB-PAC) and intestinal (I-PAC) according to Albores-Saavedra[5] is emerging as an important prognostic factor[6,7]. This classification is associated with histolpathological features which influence the overall survival, as PB-PAC type is associated with lower survival rates, when compared to good prognosis of I-PAC type[1,7-10].

Thus, the methods used to classify these tumors should be more objective and reproducible to control inter observer variability. Several studies have classified PAC subtypes using the morphologic criteria in combination of a panel of immunohistochemistry (IHC) markers[2,6,11].

CK7 and CK20 are reliable and well characterized IHC markers, and are helpful in distinguishing PB-PAC and I-PAC type respectively[2,6,10,12-14]. Data on chemotherapy options for patients with the separate periampullary subtypes are very limited but do support the conception that treatment should be tailored according to the histological subtype[15].

This study is taken up to know the histomorphological features with immuno histochemical expression of CK7 and CK20 in PAC, and its association with histopathological subtyping, grading and staging.

Materials and Methods

The material for the present study was undertaken in the department of Pathology, in a tertiary care center, Karnataka, India. Ethical clearance was obtained by the University ethical committee. A total number of 31 resected pancreatoduodenectomy specimens (Whipple's procedure) were studied for five years. All cases diagnosed histopathologically as adenocarcinomas of the periampullary region in resected specimens of pancreatoduodenectomy were included.

All specimens (Sep2017-Aug2019) were received in 10% of neutral buffered formalin and relevant clinical information was obtained as per the proforma. The specimen was kept for fixation for 24-48 hours. The grossing was done according to the recent 2017 CAP protocol[16] and the most representative tumor bits and other bits were taken for histopathological processing. Deparaffinised sections were stained with haematoxylin and eosin (H&E). The tumors were classified according to WHO classification[17]. Tumor (T), Node(N) and Metastasis (M) cancer staging system of the American Joint Committee of Cancer (AJCC), eighth edition[16], was followed for staging of the PAC specimens.

All the tumors were classified into PB-PAC, I-PAC and MT-PAC type according Albores-Saavedra[5] microscopic features (Table 1).

Tables with legends

Table 1: Microscopic features of pancreaticobiliary and intestinal type of periampullary adenocarcinoma.

Pancreaticobiliary	Intestinal type
The individual neoplastic glands are small ,simple branching glands, arranged in complex – papillary and micropapillary pattern.	The individual neoplastic glands are well-formed tubular glands , complex branching, arranged in cribriform and nest pattern
The lining neoplastic epithelium is cuboidal to low columnar type in a single layer	The lining neoplastic epithelium is tall columnar with cytoplasmic mucin.
Nucleus–round with marked atypia and lack pseudo stratification	Nucleus–elongated oval or cigar shaped with moderate atypia and pseudostratified
No goblet cells	Well differentiated goblet cells
Mild Necrosis	Abundant luminal necrosis
Abundant and extensive desmoplastic stroma	Mild desmoplastic stroma

All diagnostic tumor slides were re-reviewed and the most representive block was selected for cytokeratin immunochemistry CK7 (FLEX Monoclonal Mouse Anti-Human Cytokeratin 7,Clone OV-TL 12/30) and CK20 (FLEX Monoclonal Mouse Anti-Human Cytokeratin20,CloneKs20.8) to identify PB-PAC and I-PAC sub type respectively in all the 31 cases.

3-4 μm thick sections were taken on two separate Poly-L-Lysine coated slides and air dried. The slides were baked at 60° C for 1 hour in hot air oven. Slides were deparaffinized, rehydrated and heated in a pressure cooker containing antigen retrieval solution, sodium citrate buffer at pH 6. 1 liter of retrieval solution was brought to boil in the pressure cooker. Slides were placed in metal staining racks and lowered into pressure cooker ensuring that the slides were completely immersed in the retrieval solution. When the pressure cooker reached operating temperature and pressure, it was timed for 1 minute or up to 2 to 3 whistles. The pressure cooker was removed from the heat source and cooled by placing it under running cold water with the lid on. The slides were cooled, washed

with water and buffer solution. Peroxide block was applied for 10 min and washed with Trisbuffered saline (TBS) twice for five minutes. Protein block was applied for 10 min and washed with TBS twice for five minutes.The sections were incubated with primary antibody CK 7 and CK 20 for 1 hour and washed with TBS twice for five minutes. Post primary block/enhancer was applied for 30 min and washed with TBS twice for five minutes. The sections were incubated with SS label (polymer) for 30 minutes and washed with TBS twice for five minutes.

The bound antibody was visualized using a DAB-chromogen substrate which was prepared by adding 50 µl of DAB chromogen to 1 ml of DAB buffer. The sections were rinsed in running water and counter stained with hematoxylin and again rinsed in water for five minutes. External positive control tissue included sample of pancreatic tissue with a diffuse CK7 positivity for CK 7 marker and sample of normal intestinal mucosa with a diffuse CK 20 cytoplasmic immunoreactivity for CK 20 marker was taken. The negative control for CK 7 and CK 20 were taken in the selected slides which were treated with tris-buffer solution alone without the primary antibody.

The cytoplasmic membrane brown color staining was taken as positivity for CK 7 and CK 20. The staining intensity of each IHC reaction were scored semi quantitatively[12, 18].

- No staining reaction or <10% positively stained tumor cells = 0,
- 10-50%=1, 51-90%=2and 90%=3.

Scores 0 were regarded as negative and Scores 1-3 were regarded as positive. All the tumor were classified into PB-PAC where CK 7 is Positive (Fig 1a, b & c), as I-PAC when positive for CK 20(Fig 1d, e & f), and MT-PAC were both CK 7 and CK 20 are positive (Fig 1g, h & i). After compiling of H&E and IHC data then final histomolecular diagnosis was reported.

Statistical Analyses

All the statistical methods were carried out through the SPSS for Windows (version 22.0). A p-value of ≤0.05 was taken to be statistically significant. Descriptive statistics, Chi-Square Test, Independent-Samples T Test, Contingency coefficient analysis (Crosstabs), and One-Way ANOVA test were used to know the association.

Results

A total of 31 cases were included in this study with clinico-pathological features (Table 2). The mean age of 59.32(±9.93) years, female predominance and jaundice as the most common presenting complaint were noted. The maximum size of the tumor documented was 7cm and minimum was 1cm, with a mean tumor size of 2.74 cm and largest size was seen in PB-PAC.

Table 2: Clinico-Pathological features in 31 cases of periampullary adenocarcinoma.

1	Age	38-75 years, Mean age: 59.32 (±9.93) years			
2	Male: Female	1:1.066			
3	Presenting complaints	Jaundice (83.87%)			
4	Mean total bilirubin	8 mg/dl			
5	Mean tumor size	2.74 cm			
6	Histopatho logical type	Total	PB	I	MT
		31	19 (61.3%)	07 (22.6%)	05 (16.1%)
7	Histopatho logical grade	Total	PB	I	MT
	Grade 1	1 (3.2%)	00	01	00
	Grade 2	27(87.1%)	17	05	05
	Grade 3	3 (9.7%)	02	01	00
8	Lymphovascu-lar invasion	Total	PB	I	MT
	Present	12(38.7%)	07	03	02
	Absent	19(61.3%)	12	04	03
9	Perineural invasion	Total	PB	I	MT
	Present	04(12.9%)	01	02	01
	Absent	27(87.1%)	16	07	04
10	pT Stage	Total	PB	I	MT
	T1B	2(6.5%)	01	01	00
	T2	21(67.7%)	15	02	04
	T3A	1 (3.2%)	01	00	00
	T3B	7 (22.6%)	02	04	01
11	pN Stage	Total	PB	I	MT
	Nx	2 (6.5%)	02	00	00
	N0	17(54.8%)	10	05	02
	N1	8 (25.8%)	05	01	02
	N2	4 (12.9%)	02	01	01

On H&E staining, PB-PAC type 19 (61.3%) was the most common tumor encountered followed by I-PAC type 07 (22.6%) and MT-PAC type 05 (16.1%). Maximum cases were Grade 2 (moderately differentiated).LVI was seen in 12 cases (38.7%) and PNI was seen in 4 cases (12.9%). There were 67.7% of T2 category, and 54.8% of N0 category (Table 2).

The IHC staining revealed CK7 was positive in 22 cases (71%) and negative in 09 cases (29%)

whereas CK 20 was positive in 14 cases (45.2%) and negative in 17 cases (54.8%). After the IHC markers CK 7 and CK 20, all 31 cases were classified as follows: 17 cases (54.8%) of PB-PAC, 9 cases (29%) of I-PAC and 5 cases (16.1%) of MT-PAC (Table 3). According to the staining intensity of each IHC reaction(Fig 2a), the CK 7 and 20 showed maximum in with grade 2 intensity.

PB- Pancreaticobiliary, I-Intestinal, MT-Mixed

The Correlation of various prognostic parameters with histomolecular diagnosis was as follows: PB-PAC showed maximum cases with grade 2(Fig 2b), 07 cases with LVI and 01 case of PNI (Fig 2c), higher pT staging with T2 and pN staging with N1 (Fig. 2d). In the final pTNM staging of PAC, there were fewer cases in higher staging indicating an early presentation. There was a positive association between histomorphology and histomolecular typing (Table 3). The difference in the proportion of histomolecular profiling between the expression was statistically significant with p-value of 0.0001(Table 4).

Table 3: Concordance relation between Histopathology and IHC diagnosis of periampullary adenocarcinoma. PB-Pancreaticobiliary, I-Intestinal, MT-Mixed.

Histopatho logial Type		Final Histomolecular diagnosis			Total
		PB (CK7+)	MT (CK7+ & CK20+)	I (CK20+)	
PB	Count	16	1	2	19
	%	94.1%	20.0%	22.2%	61.3%
MT	Count	1	4	0	5
	%	5.9%	80.0%	0.0%	16.1%
I	Count	0	0	7	7
	%	0.0%	0.0%	77.8%	22.6%
Total	Count	17	5	9	31
	%	100.0%	100.0%	100.0%	100.0%

Table 4: One way ANOVA table to compare the means of CK7 and CK20 between the groups.

		Sum of Square	Degrees of Freedom(df)	Mean Square	F	Sig.
CK_7_ score	Between Groups	32.019	2	16.009	59.535	.0001
	Within Groups	7.529	28	.269		
	Total	39.548	30			
CK20_ score	Between Groups	35.115	2	17.558	103.377	.0001
	Within Groups	4.756	28	.170		
	Total	39.871	30			

This study shows the concordance between routine H&E microscopy and immuno phenotyping of CK 7 and CK20.The concordance in PB-PAC type was 94%, I-PAC type was 100% and MT-PAC type was 80% which means these respective percentages of the cases were identified correctly by routine H&E microscopy and later confirmed by the immunophenotyping. There were cases which were reclassified by immunopheno typing but they represented only a small part of the sample. These reclassified cases belonged to the category of PB-PAC and MT-PAC (Table 3). The cases were followed up for 3 months to 3 years in the present study. The expired patients were encountered maximum in PB-PAC type suggesting the least survival over 3 months to 3 years.

Discussion

PAC was anticipated epidemiologically as the 7th cause of mortality by cancer worldwide in 2014. Many studies explain that it could turn out to be the 2nd leading cause of cancer deaths by 2020 and that its prevalence is rising in the developing countries since the relocate of risk factors like smoking , obesity, poor eating habits, and sedentary lifestyle[18].

The tumors associated with periampullary region presents early in due course of disease due to obstruction to the flow of bile, but there is an overall wide range of outcomes associated with these PAC. The prognosis mainly depends on the histomolecular subtypes and whether presence or absences of known prognostic parameters like lymph node status, LVI, PNI, surgical margins, advanced tumor stages (pT3-4) and distant metastasis[2,3,15]. In our study, we found peak involvement of fifth decade which was seen even in all the previous studies[6,10,13,18-30]. In majority of the studies, the male preponderance is known. In our study, we found female preponderance and this correlated with studies by Ferchichi et al[18] Yun S et al[19], Moriya et al[20] and Aloysius et al[21], which ranged from 1:1.056 to 1:1.29. This can be due early awareness among the female or due to the small sample size. The jaundice was the main presenting complaint in our study and 32.3% cases presents with more than 10 mg/dl of total bilirubin as in the many of the studies done[22.23]. This is mainly due to the destructive course of the tumor and causes obstruction to the bile duct leading to jaundice. In our study, the mean tumor size was 2.74 cm which involved the periampullary region as a whole. Thus, the anatomical classification like Intra-ampullarypapillary-tubular neoplasm with invasion (Intra-AMP), Ampullary-ductal, Periampullary-duodenal and Ampullary-not otherwise specified (NOS) was not possible as like in the other studies[18,19,24-27].

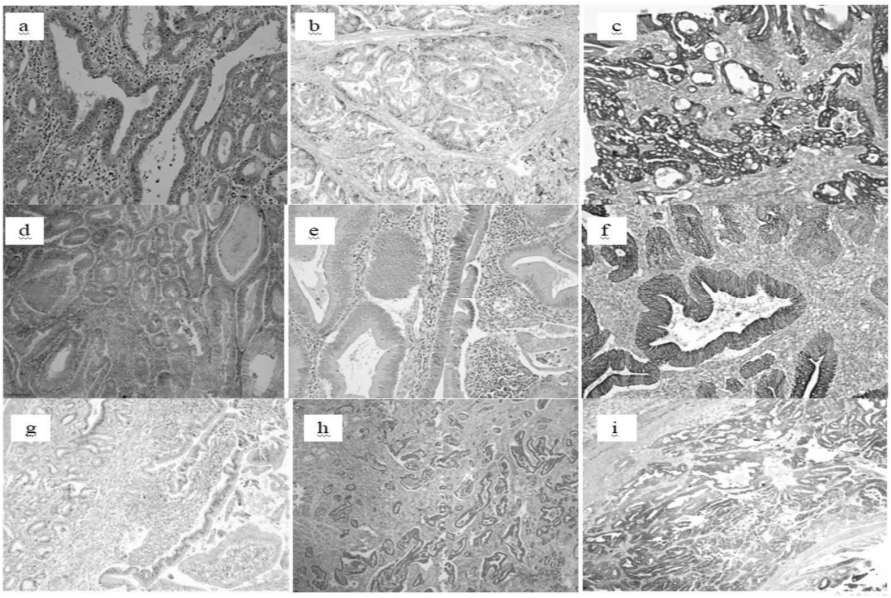

Fig. 1: Microscopic Features of Periampullary Adenocarcinomas.

a) Pancreaticobiliary type with small, well-formed glands with minimal luminal necrosis and no pseudo stratification (H&E, x100). b) Pancreaticobiliary type with desmoplasia - (H&E, x100), c) IHC of pancreaticobiliary type CK 7 positive (IHC, x40). d) Intestinal type with abundant luminal necrosis with minimal desmoplasia (H&E, x100). e) Intestinal type with tall columnar cells and pseudo stratification (H&E, x200). f) IHC of intestinal type CK 20 positive(IHC,x200). g) Mixed type having features of pancreaticobiliary and intestinal type (H&E, x40). h) IHC of mixed type CK 20 positive (IHC, x20). i) IHC of mixed type CK 7 positive (IHC, x40).

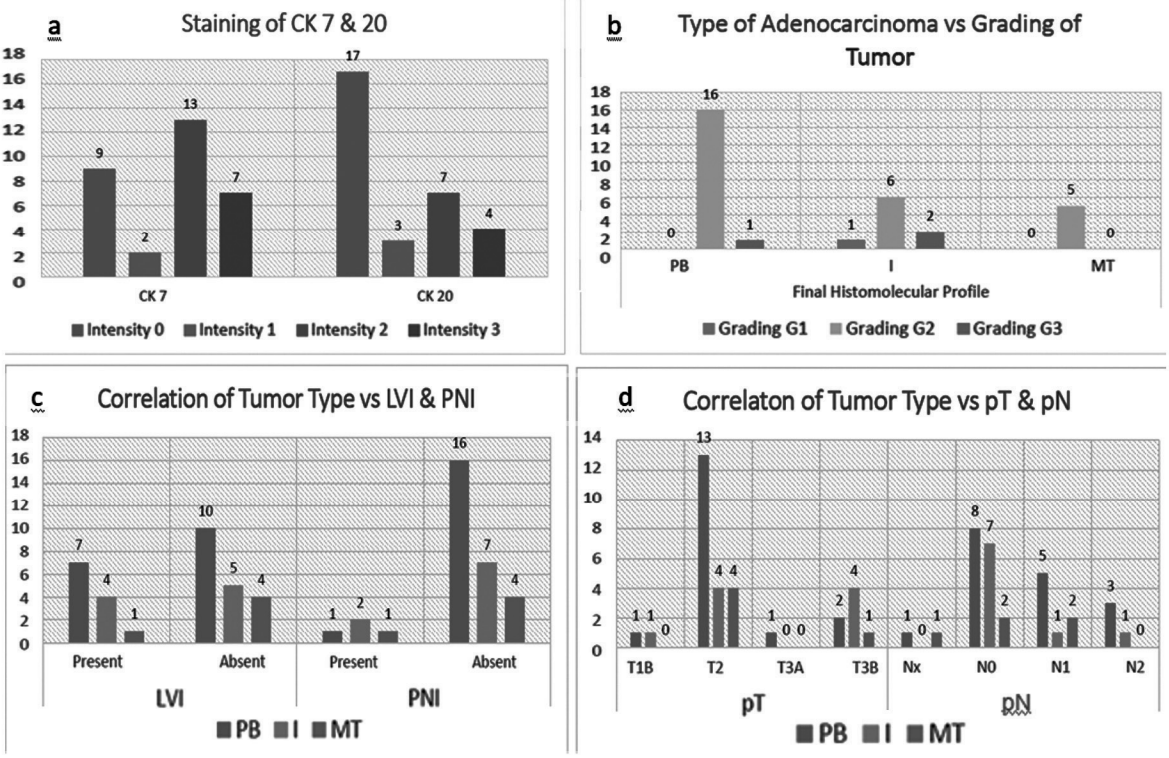

Fig. 2: Correlation of final histomolecular diagnosis of periampullary adenocarcinomas with various known prognostic factorsrevealing that pancreaticobiliary type associated with overall poor prognosis.

a) IHC intensity of CK7 & CK20. b) Grading of tumors. c) LVI and PNI correlation. d) Correlation with pTNM staging-pT & pN. PB-Pancreaticobiliary, I-Intestinal, MT-Mixed, LVI-Lymphovascular invasion, PNI-Perineural invasion.

The majority of the studies[6,9,13,18,19,23,26-29] showed the predominance of the PB-PAC. This correlated with the findings of this study and thus, states that the PB-PAC predominates over the other type of adenocarcinomas of the periampullary region as described under WHO Classification[17].

Histopathological grading correlated with Aloysius M et al[21] and Perysinakis et al[23]and showed the predominance of moderately differentiated type of PAC. There were few cases with LVI and majority of cases showed no PNI seen. All these findings suggest that the patients presented early in the due course of the disease as seen in other studies also.

Lymph node metastasis is considered as one of the most independent prognostic markers in the PAC. In our study, we found majority of the cases showed no metastasis to the lymph node leading to better prognosis and thus improvement in morbidity and mortality of the patients. This is mainly due to the early presentation of the cases, majority with moderately differentiated tumor, less LVI and PNI incidence. There was predominance of T2 tumors in the present study and only few cases with the lymph node metastasis which correlated with other studies[10,26,30,31].

As in many studies[10,13,15,19,23,28] there was a positive association between histomorphology and histomolecular typing. The difference in the proportion of histomolecular profiling between the expressions was statistically significant (p-value -.0001) in our study. This shows the concordance between routine H&E microscopy and immunophenotyping of CK 7 and CK 20 (Table 3). There were cases which were reclassified by immunophenotyping.

These reclassified cases belonged to the category of PB-PAC and MT-PAC type which was also seen in the previous studies [6,10,30,31]. So, if strict histopathological criteria are followed (Table 1) according to Albores-Saavedra[5] the subtyping can be done on H&E alone also and this will also reduce the interobserver variability[15,32-35]. Thus, proving the importance of histopathological features as a helpful diagnostic tool in correctly subtyping of PAC even if IHC is not used especially in poor socio economical status.

The PB-PAC showed the maximum cases with moderately and poorly differentiated tumors i.e. 94.1% and 5.9% respectively. PB-PAC also showed maximum cases with high T staging with T2 and T3 Staging i.e. 76.5% and 17.7% of cases respectively with higher number of cases with node positivity. All these are well documented prognostic markers which affect the patients directly and thus deducing that PB-PAC have worst prognosis (Fig 2).

Thus, the importance of correctly categorizing of the PAC into PB-PAC, I-PAC and MT-PAC is mainly due to the prognosis and the difference in administering of the chemotherapy regimens. There is a difference in chemotherapy response in various type of PAC. The PB-PAC may benefit from gemcitabine therapy and those with I-PAC type tumors benefit from 5-fluorouracil (5-FU) – based regimen[15,32]. Overall, the PB-PAC type has the poor prognosis and presents early with the lymph node metastasis which is confirmed in this study. In this perspective, histopathology reports should be consistently mentions all these factors in the pancreatoduodenectomy specimen.

Attempts to classify PAC face relatively a lot of challenges due to genetic characteristics of different subtypes remain imprecise and indistinct. Despite numerous studies, PAC stay behind with a clear lack of an evidence-based histopathologic subtype and standard care treatment of adjuvant therapy. The enhanced characterization of the different immuno histomolecular features of PAC will provide not only a better understanding of this tumor, but also the opportunity for discovering new targeted therapeutic agents[2,15,18,35].

Additional research is mandatory to explicate whether statistically and clinically significant differences be present that may demand a change in the existing adjuvant management strategies

The limitation of the study are small sample size and, the overall survival rate, progression, recurrence rate in this study was not assessed, as some of the cases were lost due to receiving of the treatment like chemotherapy at higher centers and even communication barriers.

Conclusions

Histomolecular profiling of PAC is considered superior to anatomic location of the tumor in prognosticating survival. The PB-PAC has the poor survival when compared to I-PAC type. Lymph node involvement is one of the most important independent prognostic factors in PAC. Thus it's important to differentiate these two subtypes by the help of histomorphology, IHC markers and

incorporating in the routine histopathology report.

There are various markers which can be used for the subtyping but in view of the resources, CK7 and CK20 are more economical. The categorizing of the PAC into PB-PAC, I-PAC and MT-PAC sub type minimizes interobserver variability, helps to know the prognosis and independently potentiates in administering of the targeted chemotherapy regimens especially in our country.

References

1. Bronsert P, Kohler I, Werner M, Makowiec F, Kuesters S, Hoeppner J, et al. Intestinal-type of differentiation predicts favorable overall survival: confirmatory clinicopathological analysis of 198 periampullary adenocarcinomas of pancreatic, biliary, ampullary and duodenal origin. BMC Cancer. 2013;13(1):428.
2. Rosa VD, Peria FM, Brunaldi MO. Carcinoma of Ampulla of Vater: Carcinogenesis and Immunophenotypic Evaluation. J Clin Epigenet. 2017;3(S2):25.
3. Chen JWC, Bhandari M, Astill DS, Wilson TG, Kow L,Smith M B, et al. Predicting patient survival after pancreaticoduodenectomy for malignancy: histopathological criteria based on perineural infiltration and lymphovascular invasion. HPB.2010; 12(2):101-8.
4. Plichta JK, Godambe AS, Fridirici Z, Yong S, Sinacore JM, Abood GJ, et al. The association between survival and the pathologic features of periampullary tumors varies over time. HPB Surgery: a World Journal of Hepatic, Pancreatic and Biliary Surgery. 2014 ;2014:890530.
5. Albores-Saavedra J. Tumors of the gallbladder, extrahepatic bile ducts, and ampulla of Vater. Atlas of tumor pathology. Armed Forces Institute of Pathology. 2000; 27:259- 316.
6. Kumari N, Prabha K, Singh RK, Baitha DK, Krishnani N. Intestinal and pancreatobiliary differentiation in periampullary carcinoma: the role of immunohistochemistry. Hum Pathol.2013; 44(10):2213-2219.
7. Westgaard A, Tafjord S, Farstad IN, Cvancarova M, Eide TJ, Mathisen O, et al. Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma. BMC Cancer.2008; 8(1):170.
8. Pomianowska E, Grzyb K, Westgaard A, Clausen OP, Gladhaug IP. Reclassification of tumor origin in resected periampullary adenocarcinomas reveals underestimation of distal bile duct cancer. EJSO.2012; 38(11):1043-50.
9. Westgaard A, Pomianowska E, Clausen OP, Gladhaug IP. Intestinal-type and pancreatobiliary-type adenocarcinomas: how does ampullary carcinoma differ from other periampullary malignancies? Ann. Surg. Oncol.2013; 20(2):430-9.
10. Morini S, Perrone G, Borzomati D, Vincenzi B, Rabitti C, Righi D et al. Carcinoma of the ampulla of Vater: morphological and immunophenotypical classification predicts overall survival. Pancreas. 2013; 42(1):60-6.
11. Ang DC, Shia J, Tang LH, Katabi N, Klimstra DS. The utility of immunohistochemistry in subtyping adenocarcinoma of the ampulla of vater. Am J Surg Pathol.2014; 38(10):1371-9.
12. Duval JV, Savas L, Banner BF. Expression of cytokeratins 7 and 20 in carcinomas of the extrahepatic biliary tract, pancreas, and gallbladder. Arch Pathol Lab Med.2000; 124(8):1196-200.
13. Kawabata Y, Tanaka T, Nishisaka T, Inao T, Nishi T, Yano S. Cytokeratin 20 (CK20) and apomucin 1 (MUC1) expression in ampullary carcinoma: Correlation with tumor progression and prognosis. Diagn Pathol. 2010; 5(1):75.
14. Malla BR, Rodrigues G. Periampullary carcinoma: an audit of an institutional experience with literature review. Surg. Chron.. 2017; 22(1):4-6.
15. Zhou YM, Liao S, Wei YZ, Wang SJ. Prognostic factors and benefits of adjuvant therapy for ampullary cancer following pancreatoduodenectomy: A systematic review and meta-analysis. Asian J. Surg.2020 Apr 2. (Article in press).
16. Kakar S, Shi C, Adsay NV, Fitzgibbons P,Frankel WL, Krasinskas AM ,et al. Cancer Protocols for the examination of specimens from patients with carcinoma of ampulla of vater. College of American Pathologists, Northfield.Version: Ampulla vater June 2017.
17. Flejou JF. WHO Classification of digestive tumors: the fourth edition. In Annales de pathologie 2011 Nov (Vol. 31, No. 5 Suppl, p.S27).
18. Ferchichi M, Jouini R, Koubaa W, Khanchel F, Helal I, Hadad D, Bibani N, Chadli-Debbiche A, BenBrahim E. Ampullary and pancreatic adenocarcinoma—a comparative study. J Gastrointest Oncol 2019; 10(2):270-275.
19. Yun SP,Seo HI.Prognostic impact of immunohistochemical expressionof CK7 and CK20 in curatively resected ampulla of Vater cancer. BMC Gastroentero. 2015; 15(1):165.
20. Moriya T, Kimura W, Hirai I, Takasu N, Mizutani M. Expression of MUC1 and MUC2 in ampullary cancer. Int J Surg Patho.2011; 19(4):441-7.
21. Aloysius MM, Zaitoun AM, Awad S, Ilyas M, Rowlands BJ, Lobo DN. Mucins and CD56 as markers of tumour invasion and prognosis in periampullary cancer. Br J Surg. 2010; 97(8):1269-78.
22. Jakhmola CK, Kumar A. Whipple's pancreaticoduodenectomy: Outcomes at a tertiary

care hospital. Med J Armed Forces India.2014; 70(4):321-6.

23. Perysinakis I, Minaidou E, Leontara V, Mantas D, Sotiropoulos GC, Tsipras H, Zografos GN, Margaris I, Kouraklis G. Differential expression of β-catenin, EGFR, CK7, CK20, MUC1, MUC2, and CDX2 in intestinal and pancreatobiliary-type ampullary carcinomas. Int J Surg Patho. 2017 Feb; 25(1):31-40.
24. Zhao XQ, Dong JH, Zhang WZ, Liu Z. Prognosis of ampullary cancer based on immunohistochemical type and expression of osteopontin.Diagn Pathol.2011;6(1):98.
25. Schmuck RB, Brokat C, Andreou A, Felsenstein M, Klein F, Sinn B et al. Clinicopathological Stratification and Long-term Follow-up of Patients with Periampullary Carcinomas. Anticancer Res.2018; 38(9):5379-86.
26. Reid MD, Balci S, Ohike N, Xue Y, Kim GE, Tajiri T et al. Ampullary carcinoma is often of mixed or hybrid histologic type: an analysis of reproducibility and clinical relevance of classification as pancreatobiliary versus intestinal in 232 cases. Mod Pathol.2016; 29(12):1575.
27. Kimura W, Futakawa N, Yamagata S, Wada Y, Kuroda A, Muto T et al. Different clinicopathologic findings in two histologic types of carcinoma of papilla of Vater. Jpn. J. Clin. Oncol.1994; 85(2):161-6.
28. BaumhoerD, Zlobec I, Tornillo L, Dietmaier W, Wuensch PH, Hartmann A et al. Immunophenotyping and oncogene amplifications in tumors of the papilla of Vater. Virchows Archiv.2008; 453(6):579.
29. Adsay V,Ohike N,Tajiri T,Kim GE,Krasinskas A, Balci S etal. Ampullary region carcinomas: definition and site specific classification with delineation of four clinicopathologically and prognostically distinct subsets in an analysis of 249 cases. Am. J. Surg. Pathol.2012; 36(11):1592-608.
30. Bakshi N, Dhawan S, Nundy S, Rao S, Chopra P, Bhalla S. Role of Immunohistochemistry in the Subtyping of PeriampullaryAdenocarcinoma. Int J Surg Pathol.2019:1066896919837606.
31. Goldstein NS, Bassi D. Cytokeratins 7, 17, and 20 reactivity in pancreatic and ampulla of vater adenocarcinomas: percentage of positivity and distribution is affected by the cut-point threshold. Am J Clin Patho. 2001; 115(5):695-702.
32. Schiergens TS, Reu S, Neumann J, Renz BW, Niess H, Boeck S et al. Histomorphologic and molecular phenotypes predict gemcitabine response and overall survival in adenocarcinoma of the ampulla of Vater. Surgery.2015; 158(1):151-61.
33. Leo JM, Kalloger SE, Peixoto RD, Gale NS, Webber DL, Owen DA et al. Immunophenotyping of ampullary carcinomata allows for stratification of treatment specific subgroups. J Clin Pathol.2016; 69(5):431-9.
34. Balachandar V. Review on Clinical and Molecular Diagnosis of Periampullary carcinoma. Int. J. Adv. Res. Biol. Sci.2016; 3(9):210-6.
35. Pathy S, Mallick S, Sharma A, Shukla NK, Sahni P, Pal S, et al. Compliance and outcomes of concurrent Chemo- radiation in patients with peri-ampullary cancer undergoing curative resections. Indian J Cancer.2017; 54(3):519-525.