Study of Pathological Findings of Whipple Pancreatectoduodenectomy Specimens in a Tertiary Care Centre

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ABSTRACT

BACKGROUND
Pancreatectoduodenectomy otherwise called Whipple surgery was first demonstrated by Allen O. Whipple in 1935. This procedure is done for periampullary carcinoma, ampullary carcinoma, pancreatic tumours, tumours of the pancreatic duct, tumours of the common bile duct, duodenal carcinoma and sometimes for non-malignant conditions.

METHODS
The present study is a cross sectional study done over a period of 18 months from October 2016 to April 2018, carried out at the upgraded department of pathology, Osmania General Hospital, Medical College/Hyderabad, a tertiary referral centre for the state of Telangana. In this study, a total of 40 cases of resected Whipple specimen were included.

RESULTS
The present study was conducted on 40 cases. Out of which, 14 cases were moderately differentiated adeno carcinoma of ampullary and periampullary region, 11 cases were of well differentiated adeno carcinoma, 5 cases turned out to be non-neoplastic lesions. 4 cases were diagnosed as pseudopapillary epithelial neoplasia, 3 cases were diagnosed as pancreatic ductal adeno carcinoma and 1 case each of mucinous cystic neoplasm of pancreas, schwannoma and granulomatous lymphadenitis.

CONCLUSIONS
Most of the resected Whipple specimens presented with adenocarcinoma of the periampullary region. Correlation with IHC markers ck7, ck20, muc1 was helpful in differentiating intestinal from pancreatobiliary type of adenocarcinoma. Though there was a strong clinical suspicion of malignancy, a significant number of cases on histopathology turned out to be non-neoplastic.

KEY WORDS
Whipple Pancreatectoduodenectomy, Periampullary Carcinoma, ck20, muc1
**BACKGROUND**

Pancreatoduodenectomy otherwise called Whipple surgery was first demonstrated by Allen O. Whipple in 1935. This procedure is done for tumours of the periampullary region which include periampullary carcinoma, ampullary carcinoma, pancreatic tumours, tumours of the pancreatic duct, tumours of the common bile duct, duodenal carcinoma and sometimes for non-malignant conditions. Advances in surgical technique, perioperative care and concentration of surgery in large volume centres have significantly improved mortality morbidity associated with the Whipple procedure. Today Whipple Pancreatoduodenectomy has increasingly been used as a safe and an appropriate resectional option for tumours of the periampullary region. This procedure involves removal of the head of the pancreas, part of the duodenum, part of the bile duct, the surrounding Lymph Nodes, the gall bladder and sometimes part of the stomach. Because of the intimate location of many structures in this area even a benign lesion can cause obstructive symptoms. Whipple surgery had been done on those benign conditions as they mimic malignancy.

Pancreatoduodenectomies (PDs) are challenging specimens, mostly because of the anatomic complexity of the region, where various structures come together, combined with lack of familiarity, owing to the rarity of the operation. Tumours in the periampullary region arise in the papilla of Vater and the two centimeters surrounding it. Histologically, they could originate in the duodenal wall, pancreatic tissue, the wall of the distal bile duct or the structures of the ampullary complex. The papilla of Vater is formed by the confluence of the pancreatic duct and the bile duct and by the sphincter of Oddi that surrounds it. The sphincter of Oddi also has components for the bile duct and pancreatic duct which are outside the papilla. The primary ampullary tumours originate in the epithelium of the bile duct, the pancreatic duct or the duodenal mucosa.

80% of the tumours in this region are adenocarcinoma and other malignancies form the rest. Ampullary carcinoma has the histological features of duodenal mucosa and the ducts. Tumours in this region are mostly seen among elderly age group around seventh decade and surgery is the only means of curing them. Proper orientation, dissection, and sampling of PD specimens is required for a more practical and accurate evaluation of pancreatic, distal common bile duct (CBD), and ampullary tumours. The distribution of resected pancreatic, ampullary, biliary and duodenal carcinomas is variable in different studies which affects patient survival. A recent large study done by Chen et al. on 501 periampullary cancers found ampullary carcinomas represented the majority of cancers subjected to pancreatoduodenectomy while other literature shows pancreatic cancer to be more frequent. Pathologic assessment of surgical specimens from PD needs special attention in order to accurately evaluate many factors that are prognostically important. These factors include tumour location, extension, size, surgical margin status, vascular or perineural invasion and lymph node status.

This procedure is performed for tumours (Mostly malignant) located in the head of the pancreas, part of the bile duct which goes through pancreas or the wall of the bowel adherent to the pancreas. Although occasionally benign ones need to be removed as well.

We wanted to study the histopathological findings in resected specimens of Whipple pancreatoduodenecetomy, correlate histopathological findings with IHC, aid in diagnostic dilemmas and compare the present study with literature.

**METHODS**

The present study is across sectional study done over a period of 18 months from October 2016 to April 2018 carried at the upgraded department of pathology, Osmania general hospital, medical college/Hyderabad, a tertiary referral centre for the state of Telangana and various districts of neighbouring states. In this study a total of 40 cases of resected Whipple specimen were included. Surgically resected specimens of Whipple PD from patients were collected. Periampullary lesions obtained by procedures other than Whipple surgery were excluded from the study.

Specimens were fixed in 10% buffered formalin overnight and, grossly examined with proper orientation of specimens. Representative sections were taken, processed and paraffin embedded. Sections were cut and stained with H & E and were diagnosed by histopathological examination. Lesions diagnosed as periampullary adeno carcinoma on histopathological examination were subjected to IHC with ck7 (TL12/30, dil; 1:100, dako cytometry), ck20 (k,20,8; dil; 1:50 dako cytometry), muc1 (EP85, dil; 1:100, dako,) was performed on five-micron sections utilizing standard protocols and correlation was done.

**Protocol Used in Gross Examination**

(Shifa et al) When most part of the tumour (More than 75%) is located in the ampullary region and bulges into the duodenal mucosa stretching it, it is taken as ampullary carcinoma. A tumour that involved the circumference of the ampulla was taken as periampullary carcinoma. A tumour that involved the circumference of the common bile duct (CBD) was taken as common bile duct tumours. Longitudinal thickening of the bile duct and granular mucosal surface were taken as clues. Common bile duct tumour constitutes 5% among the tumours of pancreatoduodenal origin. A tumour with the base or the epi center in the duodenum and not involving the ampulla was taken as duodenal carcinoma. Non ampullary duodenal carcinoma is different from its duodenal counterpart and the plaque like growth of the non-ampullary carcinoma is associated with microsatellite instability Tumour size, colour, consistency, gross invasion and measurements were noted. Most of the benign lesions occur around the pancreatic head and the periampullary region. They cause obstructive symptoms mimicking carcinoma leading on to Whipple surgery. The slides were viewed, histopathological categorization, grading, tumour building, staging, nodal status, perineural invasion, angioinvasion and marginal status were assessed. The grading of adenocarcinoma was done based on percentage of glands seen in the tumour tissue. If there were >95% glands it was taken as well differentiated, 50-95% glands as moderately differentiated grade, 5-49% as poorly differentiated grade and 5% as undifferentiated
adenocarcinoma.\textsuperscript{2} The staging of the Ampullary carcinoma was based on AJCC TNM classification.\textsuperscript{2} T1 - If the tumour is limited to the ampulla or sphincter of Oddi. T2 - If the tumour invades the duodenal wall. T3 - If the tumour invades the pancreas and T4 - If the tumour invades the peripancreatic soft tissue or adjacent structures. N1 - If there is regional nodal metastasis. In the case of Endocrine neoplasm, the following staging was followed. T1 - If the tumour is limited to the pancreas and it is less than 2 cm in diameter. T2 - If the tumour is restricted to the pancreas and size is between 2-4 cm. T3 - If the tumour is more than 4 cm diameter if it is limited to the pancreas or if the tumour invades the duodenum or the bile duct. T4 - If the tumour invades the adjacent organs. N1 - If the regional nodes are involved by the tumour. In case of the solid pseudo papillary tumour T1 - When the tumour is limited to the pancreas and was less than 2 cm in diameter. T2 - When the tumour is limited to the pancreas and more than 2 cm in diameter. T3 - When the tumour invades duodenal, peripancreatic tissue and the bile duct. T4 - When the tumour IHC was performed on five-micron sections utilizing standard protocols and correlation was done.

Statistical Methods
The statistical package for social science (SPSS) version 20 will be used for data analysis. Mean, median, and SD are used to describe quantitative data. Qualitative data are summarized using frequency and percentage.

### RESULTS

<table>
<thead>
<tr>
<th>Lesions</th>
<th>10-20</th>
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<th>40-50</th>
<th>50-60</th>
<th>60-70</th>
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<td>3</td>
<td>3</td>
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<tr>
<td>ampullary adeno carcinoma (MD)</td>
<td>5</td>
<td>6</td>
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<td>1</td>
<td>3</td>
<td>1</td>
<td></td>
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<tr>
<td>SPEN</td>
<td></td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatic ductal adeno carcinoma</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bile duct adeno carcinoma</td>
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<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwannoma</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous cystic neoplasm of pancreas</td>
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</tr>
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**Age Distribution of Cases**

<table>
<thead>
<tr>
<th>Tumours</th>
<th>1-2 cm</th>
<th>2.1-3 cm</th>
<th>3.1-4 cm</th>
<th>&gt;4 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adeno carcinoma of peripancreatic region</td>
<td>4</td>
<td>4</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>SPEN</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Pancreatic ductal adeno carcinoma</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Bile duct adeno carcinoma</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mucinous cystic neoplasm of pancreas</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Schwannoma</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

**Tumour Size Distribution in the Whipple Specimens**

**Age and Sex Distribution**
The present study was conducted on 40 cases between the age range of 10 to 70 years. Ampullary adeno carcinoma was more common in the age group of 40-50 years. Pancreatic ductal adeno carcinoma was more common in the age group of 30-60 yrs. The youngest age at which Whipple resection was performed was at the age of 12 years who presented with a clinical history of obstruction and jaundice which on histopathological examination turned out to be pancreatitis. Out of 4 cases of SPEN, 3 cases were in the age group of 40 to 50 years and 1 case was 35-year-old. Out of 40 resected specimens, 28 cases (70%) were male and 12 cases (30%) were female with the sex ratio of 2.33:1. Among the ampullary adeno carcinomas 20 were male (80%) and 5 (20%) were female. All cases of SPEN were female (100%).
ampullary region, often on the basis of a pre-existing villous adenoma or villoglandular polyp. It should therefore be distinguished from carcinomas of pancreas, terminal third of common bile duct (‘Cholangiocarcinoma’), and other portions of duodenal mucosa with secondary involvement of the ampulla. Klimstra et al had proposed the diagnostic criteria for ampullary carcinoma. According to them, the tumour should be called as ampullary when the epicentre is in the ampulla and there should be a pre-invasive lesion in the ampulla. The tumour that grows circumferentially around the ampulla is called periampullary carcinoma. The ampulla is formed by the union of 2 distinct types of mucosa. The intestinal type of mucosa, and, Pancreaticobiliary type ductal mucosa. Therefore, adenocarcinomas may arise from the intestinal-type mucosa as well as from the pancreaticobiliary type mucosa this may explain the broad histomorphological spectrum of these tumour.

When the tumour is small and confined to the ampulla, both the endoscopic and radiographic appearances may be normal. CT and ultrasound usually show dilation of the common bile duct or pancreatic duct, but the mass itself may be in apparent. Endoscopic ultrasound is said to be the best technique for staging purposes. Microscopically, nearly all ampullary malignant tumours are adenocarcinomas, often poorly differentiated. Mutations of TP53 have been detected in the majority of ampullary carcinomas, with a corresponding accumulation of the abnormal product as detected immunohistochemically. Ampullary carcinomas are less likely to show loss of DPC4 expression and of KRAS gene mutations than pancreatic ductal carcinomas. Ampullary carcinoma may invade the adjacent duodenal mucosa, duodenal wall, pancreas, and common bile duct by direct extension. Perineural invasion may also be present. Regional lymph node metastases are found in 35–50% of the cases; in general, they are restricted to one adjacent periampullary group. The diagnosis of ampullary carcinoma may be made by cytologic examination, endoscopic biopsy, or transduodenal biopsy. The biopsy preferably should be in the form of step-sectioned multiple fragments, a procedure with a diagnostic reliability greater than 90%. The treatment of choice for ampullary carcinoma is the Whipple procedure. Benign tumours and tumour like conditions of the ampulla include villous adenomas, villoglandular polyps, adenomyoma, adenomymomatous hyperplasia, and inflammatory polyp, Gangliocytic paraganglioma. Carcinoid tumours, these lesions can cause partial biliary obstruction and are best treated by local resection. Whipple resection is also performed in cases of chronic pancreatitis.

Shiha et al (2016) in their study have reviewed 30 resected specimens of Whipple pancreaticoduodenectomy, out of which twenty-one had ampullary and periampullary carcinoma, the mean age incidence of ampullary carcinoma calculated was 44 years. The sex ratio of ampullary carcinoma was 1:1. Three had pancreatic endocrine tumours, two had pancreatic endocrine tumours, both of them were female. One had a Solid pseudopapillary pancreatic tumour. Literatures were reviewed and the predominance of ampullary carcinoma was noted in their study in contrast to other studies.2

### Pathological Profile

Out of 40 resected specimens, 14 cases were moderately differentiated adenocarcinoma of ampullary and periampullary region, 11 cases were well differentiated adenocarcinoma. 5 cases turned out to be non-neoplastic lesions. 4 cases were diagnosed as pseudopapillary epithelial neoplasia, 3 cases were diagnosed as pancreatic ductal adenocarcinoma and 1 case each of mucinous cystic neoplasm of pancreas, schwannoma and granulomatous lymphadenitis.

### Discussion

Whipple pancreaticoduodenectomy is one of the most complex surgeries performed for the management of a variety of tumours involving the head of pancreas, ampulla of Vater, distal common bile duct or duodenum. Although regarded as a lifesaving procedure, long term survival is largely dependent on the pathology within the resected specimen which emphasizes the need of meticulous evaluation of PD specimens.

Ampullary adeno carcinoma constitutes about 0.2% of all the gastrointestinal tumours. It constitutes 6% among the tumours of the periampullary region. Ampullary carcinoma is the term employed for any malignant epithelial tumour centred in the ampulla of Vater. Although originally defined on topographic grounds, the term ampullary carcinoma also implies origin from the intestinal-type mucosa of the

### Table

**Pathological Profile - Categorisation Based on HIC & HPE**

<table>
<thead>
<tr>
<th>Type of Tumour</th>
<th>No. of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal Type</td>
<td>18 cases</td>
<td>72%</td>
</tr>
<tr>
<td>Mixed Type</td>
<td>5 cases</td>
<td>20%</td>
</tr>
<tr>
<td>Pancreaticobiliary Type</td>
<td>2 cases</td>
<td>8%</td>
</tr>
<tr>
<td>Total number of Cases</td>
<td>25 cases</td>
<td>10%</td>
</tr>
</tbody>
</table>

**Distribution of Various Lesions in the Per-Ampullary Region**

- Well differentiated adenocarcinoma: 5 cases (25%)
- MD adenocarcinoma: 2 cases (10%)
- Benign: 7 cases (35%)
- SPEN: 2 cases (10%)
- Pancreatic ductal Adenocarcinoma: 2 cases (10%)
- bile duct Adenocarcinoma: 1 case (5%)
- Schwannoma: 1 case (5%)
- Mucinous cystic neoplasm: 1 case (5%)

**No. of Cases of periampullary adeno carcinoma Showing Lymph Node Metastasis and Perineural Invasion**

<table>
<thead>
<tr>
<th>Invasion</th>
<th>No. of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node Metastasis</td>
<td>12 cases</td>
<td>18%</td>
</tr>
<tr>
<td>Perineural invasion</td>
<td>5 cases</td>
<td>2%</td>
</tr>
</tbody>
</table>

**Lymph node Metastasis and Perineural Invasion**

- Lymph node Metastasis: 12 cases (18%)
- Perineural invasion: 5 cases (2%)
Ramesh Dhakwa, Neeta Kafle (2016) have done a descriptive study in 35 patients who underwent Whipple PD procedure in a period of 36 months. They found that malignant tumour was present in 31 cases and 4 cases had benign lesions. Among the malignant tumours periampullary mixed carcinoma was the predominant tumour. lymphovascular and perineural invasion varied in different tumour types.3

Ductal adenocarcinoma

Ductal adenocarcinoma of the exocrine pancreas comprises about 85% of all cases of pancreatic malignancy9. Carcinomas of the pancreas share embryologic origin, differentiation pathways, population-related development, and microscopic features with tumours of gallbladder, extra hepatic bile ducts, and ampulla of Vater, suggesting a field effect in carcinogenesis.

Pancreatic carcinoma has been reported in the past in workers exposed to β-naphthylamine or benzidine. Cigarette smoking is also a risk factor. About 10% of pancreatic cancers show familial aggregation consistent with a genetic susceptibility.10,11 At least five such syndromes have been identified, to wit: (1) familial breast cancer with germ line mutation of BRCA2 (2) familial atypical multiple mole melanoma syndrome with germ line mutation in the P16 gene (3) Peutz–Jeghers syndrome with germ line mutations in the STK11/LKB1 gene (4) Hereditary nonpolyposis colorectal cancer with germ line mutations in one of the DNA mismatch repair genes; and (5) Hereditary pancreatitis with germ line mutations in the cationic trypsinogen gene. Most patients with pancreatic carcinoma are elderly, and there is a slight male preponderance. However, it can also occur in patients younger than 40 years.12 In a well differentiated pancreatic adenocarcinoma, microscopic diagnosis can be extremely difficult. Close attention must be given to cytologic details. At low-power examination, the glands are often well formed, have a large lumen, and are lined by one or a few layers of cylindrical or cuboidal epithelium.

Their overall low-power appearance may not be particularly suggestive of carcinoma, except for the irregularities in the shape and distribution of the glands and the prominent concentric desmoplastic stroma that surrounds them. However, high-power examination of the lining epithelium will show one or more features that, indicative of malignancy: marked nuclear pleomorphism, loss of polarity, prominent nucleoli, and mitotic activity. This disparity between the high degree of cytologic atypia and the low level of architectural atypia is typical of tumours of the pancreaticobiliary region.12 Pancreatic ductal adenocarcinomas are positive for mucins, keratins, EMA, CEA, CA 19-9 and mesothelin.

The progression as per Figure 10 from histologically normal epithelium to low-grade pancreatic intraepithelial neoplasia (PanIN) (PanIN1 and PanIN2), to high-grade PanIN3, to invasive carcinoma (left to right) is associated with the accumulation of specific genetic alterations. Solid pseudopapillary epithelial neoplasia is a tumour of the young female and middle-aged women, with the median age of 20 years. Martin RC et al14 in their study had mentioned the median age as 39 years, which correlated with present study. It is a cystic and solid neoplasm involving the head and tail of the pancreas.15 Histopathologically, this tumour has an appearance of the endocrine neoplasm composed of small round cell crowding around the blood vessels. The extensive necrosis of the cells that are away from the blood vessels gives it a pseudo papillary appearance. Individual cells are smaller with oval and folded nucleus. Mitosis is few in number. This tumour expresses beta catenin, Vimentin, CD10 and CD56.15 This is a tumour of intermediate malignant potential with frequent metastasis to the liver. For localized tumour surgery is the treatment and for metastatic tumours, aggressive management is required.

![Figure 10. Genetic Progression Model of Pancreatic Carcinogenesis](image)

**Incidence of Various Lesions among the Whipple Specimens** - Comparison with Other Studies

While histopathological typing is a useful method for classifying ACs, some cases cannot be easily classified by using histomorphology. Determination of the cytokeratin (CK) and amylin (MUC) immune phenotypes of an AC can facilitate identification of the primary tumour site. Most pancreaticobiliary adenocarcinomas express CK7 and low levels of CK20. Among ACs, the pancreaticobiliary type expresses CK7 but does not express CK20, while the intestinal type expresses CK20 but does not express CK7. The pancreaticobiliary type of ACs usually express MUC1 but do not express MUC2. Most intestinal-type ACs express MUC2. In the present study, we analysed the spectrum of CK and MUC expression in 25 patients with adenocarcinomas. We then evaluated the immunohistochemical subtypes of ACs by analysing the expressions of CK7, CK20, MUC1, in these tumours. We found that 18 cases were of intestinal type and 2 were of pancreaticobiliary type. And the remaining (5) were inconclusive to be categorised as intestinal or PB type further, we assessed the correlations between the histomorphological findings and the defined immunohistochemical subtypes. The classification of ACs on the basis of their immunohistochemical characteristics may be useful to predict the clinical outcome. Six cases were diagnosed as chronic pancreatitis on the Whipple specimen suspected as periampullary carcinoma. De la Fuente SG in their study had...
mentioned that in the Whipple specimen they have received they have encountered 7% benign cases. Endoscopic biopsies have limited diagnostic accuracy in case of ampullary carcinoma. FNAC and other investigative modalities have limited application in accurately diagnosing the tumours in this area because of the complex and intimate anatomy of the ampullary area.

**CONCLUSIONS**

Out of all the Whipple resected specimens, periampullary adenocarcinoma (intestinal type) was the predominant tumour, this is in contrast to other literature reports where pancreatic ductal carcinoma was predominant. The predominant age group in which ampullary carcinoma was detected is in the range of 40-50 years and men were the dominant population. While in other studies ampullary carcinoma was detected in elderly (50-60 years) age group. All these cases of adenocarcinoma were of well & moderately differentiated. Correlation with IHC markers (CK7, CK20, MUC1) was helpful in differentiating intestinal from pancreatobiliary type of adenocarcinoma in the present study. Though there was a strong clinical suspicion of malignancy, a significant number of cases on histopathology turned out to be non-neoplastic. As the predominant tumour encountered in the present study is periampullary adenocarcinoma which is in contrast to other literature reports where pancreatic ductal adenocarcinoma was predominant, more studies are needed to analyse the genetic makeup, dietary or environmental factors among the people who are responsible for this contrast.

**REFERENCES**


