Section: Pathology

Original Research Article

A RETROSPECTIVE HISTOPATHOLOGICAL STUDY OF WHIPPLE'S RESECTION SPECIMENS

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ABSTRACT

Background: The Whipple's Procedure, also known as pancreaticoduodenectomy, is performed to treat tumors of the ampulla of Vater, cancer of head of pancreas, cancer of the distal part of the bile duct, duodenal cancer, chronic Pancreatitis, etc. Pathological assessment of surgical specimens from Whipple's surgery needs special attention in order to accurately evaluate many factors that are prognostically important such as tumor size, location, extension, histological type and grade, surgical margin status, vascular & perineural invasion and lymph node status. **Aim:** To analyse and compare the incidence and different histological parameters of various tumors encountered in Whipple's resection specimens.

Materials and Methods: This is a retrospective histopathological study of total 45 Whipple's resection specimens that were received during January 2012 to October 2018 in a tertiary care hospital in Ahmedabad, India. All the cases were retrieved from the records.

Results: Out of 45 Whipple's specimens, 41 were malignant, 3 were benign and 1 was non-neoplastic. Mean age was 51.42 years with 2:1 male predominance. Peri-ampullary region was the most common site of tumor location(62.3%). We found that for grossing of pancreaticoduodenectomy specimens, Axial slicing method is more beneficial than Bi-valving except for cystic pancreatic tumors. 35 cases(85.3%) were of Adenocarcinoma and Moderately differentiated Adenocarcinoma(58.7%) was the most common histological type. The incidence of lymphnode involvement(37.1%) and perineural invasion(48.5%) was higher in cases of adenocarcinoma, associated with poor prognosis.

Conclusion: Whipple's resection specimens require meticulous histopathological evaluation and pathologists should be aware of possibility of a benign diagnosis.

Keywords: Acinar cell carcinoma, Ampullary carcinoma, Pancreaticoduodenectomy, Paraduodenal pancreatiis, Periampullary carcinoma, Whipple's resection specimen.

INTRODUCTION

The Whipple's Procedure was first demonstrated by Allen O. Whipple in 1935.[1] It involves surgical resection of pancreatic head, duodenum, distal stomach, distal common bile duct and gall bladder and it is performed to treat tumors of this region. Over 80% of the tumors in this region are adenocarcinomas. They are mostly seen among old age group and surgery is the only means of curing them. Due to intimate location of many structures in this area, even a benign condition may cause obstructive symptoms, mimicking malignancy.[2] Whipple's surgery has been done on these benign conditions and histopathology is the gold standard when such situation arises.

In this study we present the results of 7-year review of pathological findings in 45 Whipple's surgical specimens. Prognostically important factors such as tumor size, location, extension, histological type and

grade, surgical margin status, vascular and perineural invasion and lymph node status are evaluated in this study.

MATERIALS AND METHODS

This is a retrospective study of total 45 Whipple's resection specimens that were received during Jan-2012 to Oct-2018 in a tertiary care hospital in Ahmedabad, India. All the cases along with their gross examination details were retrieved from the records.

Protocols Used in Gross Examination

- When most part of the tumor was located in the ampullary region and bulged into the duodenal mucosa, it was taken as ampullary carcinoma. Adsay V et al mentioned in their study that they designate ampullary carcinoma if more than 75% of the tumor was seen in the ampullary region.[3]
- Tumor that involved the whole circumference of the ampulla was taken as periampullary carcinoma.
- Tumor that involved the circumference of the common bile duct was taken as common bile duct tumor. Longitudinal thickening of the bile duct and granular mucosal surface were taken as clues.
- Tumor with the base or epicentre in the duodenum and not involving the ampulla was taken as duodenal carcinoma.
- Tumor configuration, consistency, color, gross invasion and measurements were noted.
- Grossing of the specimen was done by axial slicing method or in cases of cystic tumors by bi-valving method. In axial slicing method, each specimen was serially sliced perpendicular to the long axis of the duodenum over its entire craniocaudal length.[4],[5] In Bi-valving method, the head of pancreas was bisected along probes placed in the common bile duct and pancreatic duct.[6],[7] After bi-sectioning, the two halves were serially sliced axially.
- **Sections submitted included:** 6 margins (Proximal duodenal/stomach resection margin, distal duodenal/jejunal resection margin, pancreatic neck margin, common bile duct margin, uncinate margin & superior mesenteric vein [SMV] margin), 3 surfaces (anterior pancreatic surface, posterior pancreatic surface & Superior mesenteric artery[SMA] surface), minimum 4 tumor sections and random sections from duodenum, pancreas, gall bladder and common bile duct.
- Lymphnodes were dissected from the specimen by orange peeling method which consists of shaving of all the peripancreatic soft tissue after multicolour inking.
- The slides were stained mainly by Haematoxylin & Eosin stain and assessed.

Histopathological categorisation, grading, staging, nodal status, perineural and lymphovascular invasion and marginal status were assessed. The grading of adenocarcinoma was done based on the percentage of glands seen in the tumor tissue i.e., >95% glands was taken as well differentiated, 50-95% glands as moderately differentiated, 5-49% as poorly differentiated and <5% as undifferentiated adenocarcinoma. The staging of different carcinomas was done according to their respective AJCC-TNM classification (8th edition).

RESULTS

Out of 45 Whipple's specimens, 30 cases were male and 15 were female with male: female sex ratio of 2:1. The youngest case was 21 years old male and the oldest was 70 years old male with mean age of occurrence being 51.5 years [Table/Fig-1]. Most of the patients presented clinically with the history of abdominal pain and obstructive jaundice.

Out of 45 specimens, 28 tumours(62.3%) were located in periampullary region. Other common sites were head of pancreas(13.3%) and Ampullary region(13.3%), followed by 2nd part of duodenum(11.1%). 26 tumours(57.8%) were measuring up to 2 cm. Out of which, 17 were located in Periampullary region [Table/Fig-2].

Out of 45 total cases, 42(93.4%) were found to be malignant, 2(4.4%) were benign and 1(2.2%) case was non-neoplastic. The benign conditions that we encountered were Acinar cell cystadenoma [Table/Fig-3-5] and Microcystic serous cystadenoma [Table/Fig-6]. The non-neoplastic condition was paraduodenal pancreatitis[Table/Fig-7,8]. Out of 42 malignant tumours, 24(57.1%) were moderately differentiated adenocarcinoma followed by Poorly differentiated adenocarcinoma(16.7%), Well differentiated adenocarcinoma(9.5%) [Table/Fig-9-11], Pancreatic neuroendocrine tumor (PanNET:9.5%) [Table/Fig-12,13], Non-invasive Pancreatic Intraductal Neoplasm(2.4%), Pancreatic Intraepithelial neoplasia grade II(2.4%) and Acinar cell carcinoma(2.4%) [Table/Fig.-14,15].

In our study, 14 cases(33.3%) showed presence of lympho-vascular invasion. 19 cases(45.2%) showed presence of Perineural invasion. 17(40.5 %) showed evidence of lymphnode metastasis. Lymphnodes were involved in all 4 cases of PanNET. 3 cases out of 42 malignant cases(7.1 %) showed positive surgical margin status. Out of which, 2 cases were of Moderately differentiated adenocarcinoma and 1 was of PanNET [Table/Fig.16]. In Moderately Differentiated Adenocarcinoma, one case showed SMV and Retroperitoneal margins involved while the other case showed only SMV margin involved. In Neuroendocrine tumour, only retroperitoneal margin was involved.

Tumor size (defined as the largest dimension of the tumor as assessed at microscopy) is a well-established predictor of survival in adenocarcinoma and determines T category for tumors limited to pancreas. According to AJCC-TNM 8th edition, peripancreatic soft tissue involvement is no longer a

factor in determination of T category. T1-T3 is depended only on tumor size, while T4 requires involvement of the celiac axis, superior mesenteric artery and/or common hepatic artery [Table/Fig-17]. Out of 35 cases of adenocarcinoma, 5(14%) showed evidence of chronic pancreatitis. Pancreatic fistula is the most common and serious complication of Whipple's surgery. The safety of the pancreatic anastomosis is closely related to the quality of the pancreatic remnant. In Whipple's procedure if the remnant pancreas after surgery shows histology of chronic pancreatitis (hard pancreas), the chances of anastomotic leak are around 8%.

Table 1: Distribution of cases according to age & sex

Age (in years)	Male	Female
21-30	3	1
31-40	5	2
41-50	6	5
51-60	8	4
61-70	8	3
Total	**30**	**15**

Table 2: Distribution of tumours according to their location & size

Location	Size (cm)				Total
	<2	2.1-3	3.1-4	>4	
Ampullary	6	0	0	0	6
Peri-ampullary	17	7	1	3	28
Head of pancreas	1	4	0	1	6
2nd part of duodenum	2	0	1	2	5
Total	26	11	2	6	45

Figure 1: Acinar cell cystadenoma: gross appearance

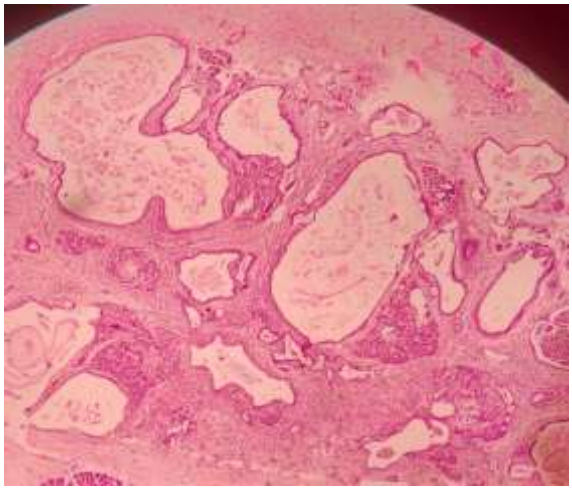

Figure 3: Acinar cell cystadenoma (H&E stain, 4X): cysts lined by acinar cells

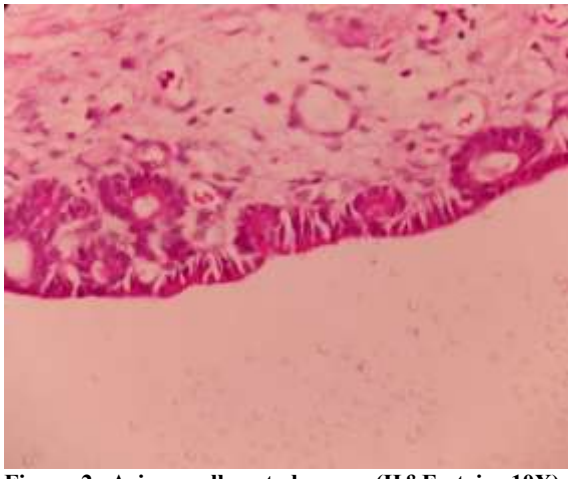

Figure 2: Acinar cell cystadenoma (H&E stain, 10X): layer of acinar cells with granular eosinophilic cytoplasm

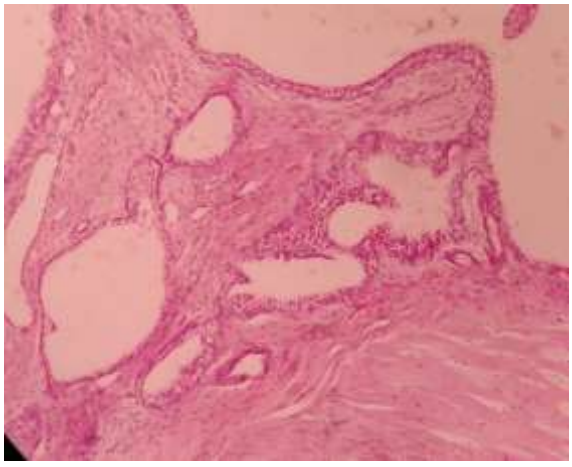

Figure 4: Serous cystadenoma (H&E stain, 10X): cysts lined by single layer of flat to cuboidal cells with clear cytoplasm

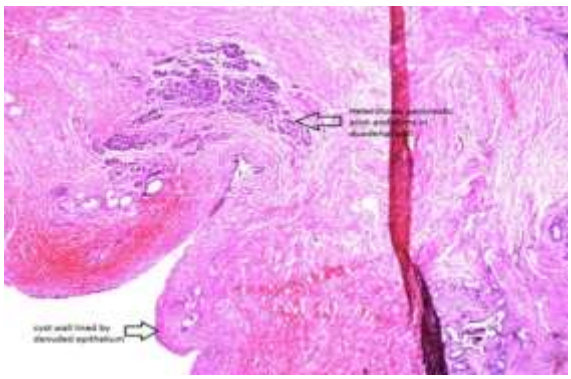

Figure 5: Paraduodenal Pancreatitis (H&E stain, 4x): Cyst wall lined by denuded epithelium with heterotopic pancreas in duodenal wall

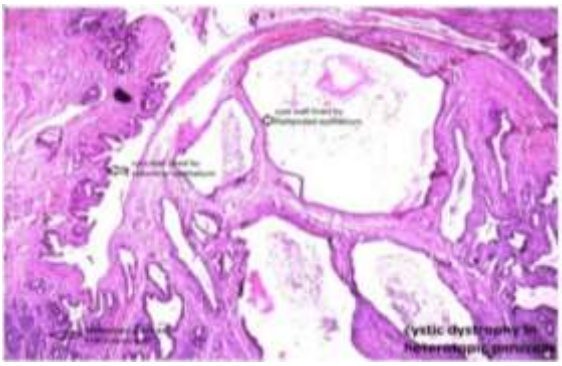

Figure 6: Cystic dystrophy in heterotopic pancreas

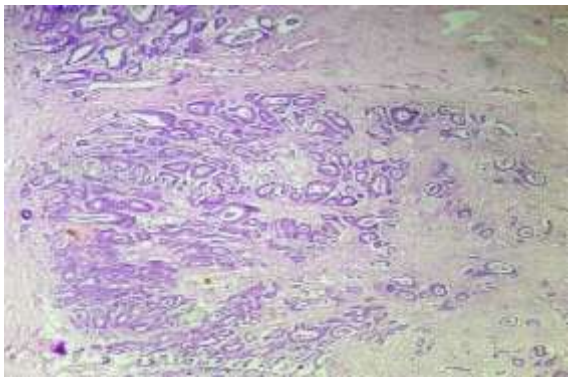

Figure 7: Ampullary Adenocarcinoma (H&E stain, 4x): tumor cells forming groups and glands

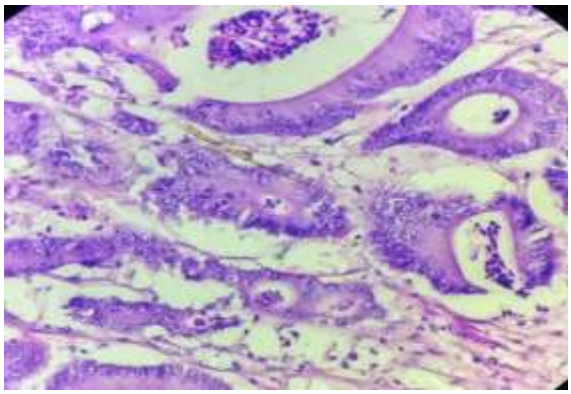

Figure 8: Ampullary Adenocarcinoma (H&E stain, 40x): Tumor cell nuclei are pleomorphic, hyperchromatic and vesicular. Prominent nucleoli and chronic inflammatory cells are seen

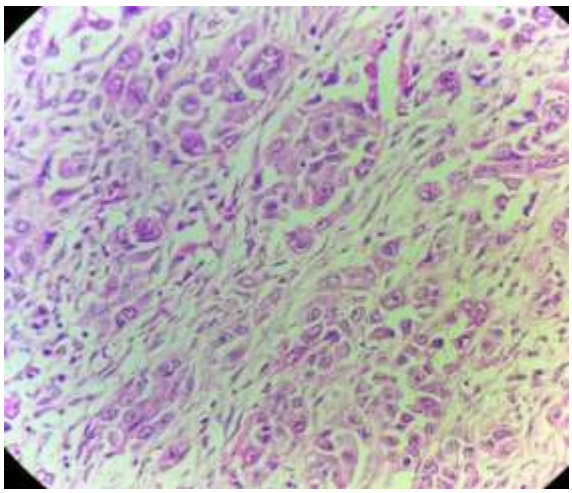

Figure 9: Poorly Differentiated Adenocarcinoma (H&E stain, 10X): Poorly formed ductal and glandular structure with nuclear pleomorphism and small nucleoli

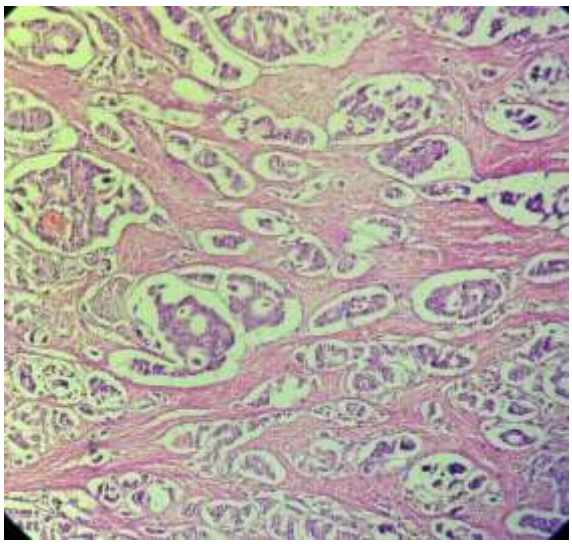

Figure 10: PanNET (H&E stain, 4x): Nesting pattern of cell arrangement

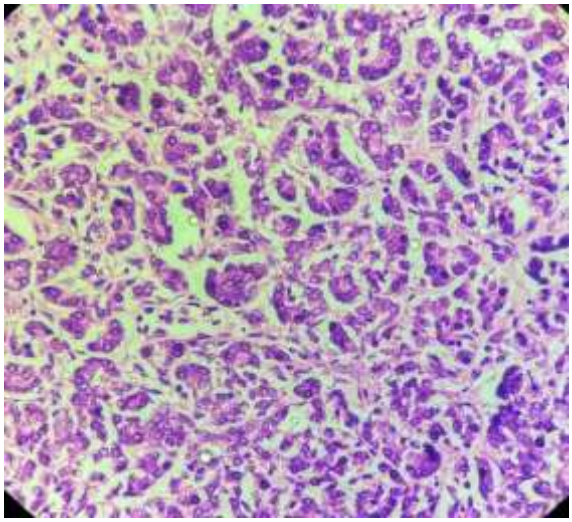

Figure 11: PanNET (H&E stain, 40x): small round cells with centrally located nuclei with salt and pepper chromatin

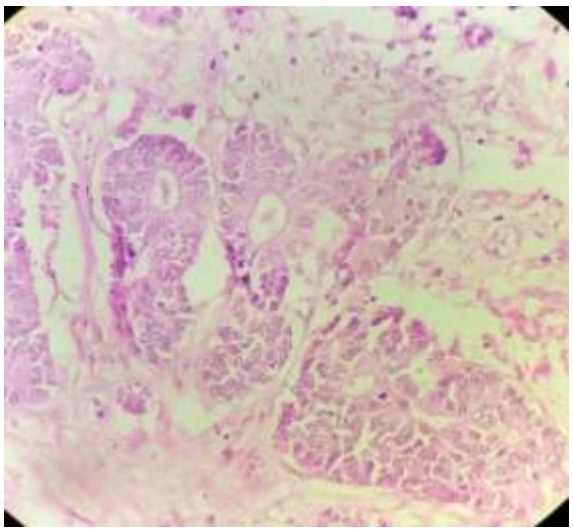

Figure 12: Acinar cell carcinoma (H&E stain, 10x): Neoplastic glands arranged in acinar pattern with central lumen

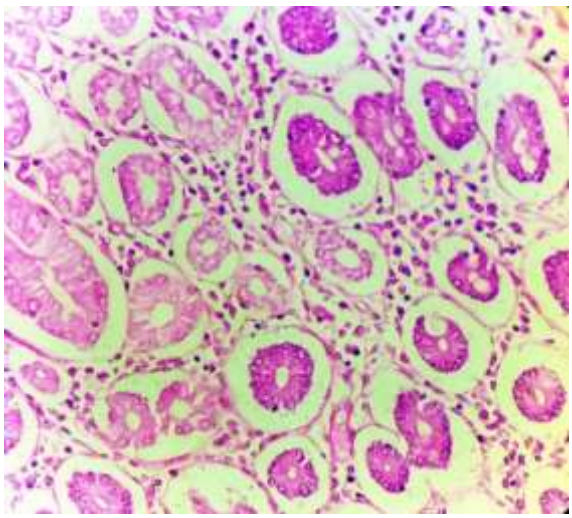

Figure 13: Acinar cell carcinoma (H&E stain, 40x): cells with pleomorphic, hyperchromatic, vesicular nuclei with eosinophilic cytoplasm

Table 3: Distribution of histological tumour types according to lympho-vascular invasion, Perineural invasion, lymphnode status and surgical margin status

Histological type	Lymphovascular invasion		Perineural invasion		Lymphnode status		Surgical margin status	
	Present	Absent	Present	Absent	Uninvolved	Involved	Involved	Free
Well differentiated adenocarcinoma	0	4	2	2	3	1	0	4
Moderately differentiated adenocarcinoma	9	15	12	12	15	9	**2**	22
Poorly differentiated adenocarcinoma	4	3	3	4	4	3	0	7
Non-invasive intraductal papillary neoplasm	0	1	0	1	1	0	0	1
Pancreatic neuroendocrine tumor	0	4	1	3	0	4	**1**	3
Acinar cell carcinoma	1	0	1	0	1	0	0	1
Pancreatic Intraepithelial Neoplasm	0	1	0	1	1	0	0	1
Total	**14**	28	**19**	23	25	**17**	**3**	39

Table 4: Distribution of Adenocarcinomas according to stage

Stage	T1	T2	T3	T4
No. of cases	2	12	17	4

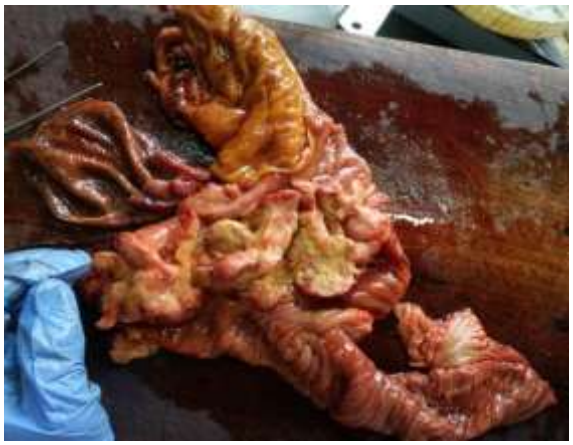

Figure 14: Whipple's specimen: Gross appearance

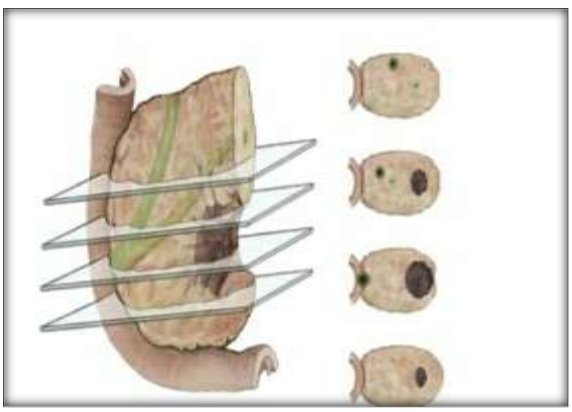

Figure 15: Axial slicing method

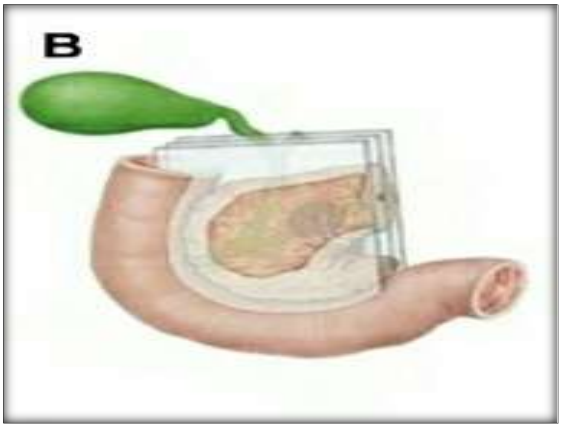

Figure 16: Bi-valving method

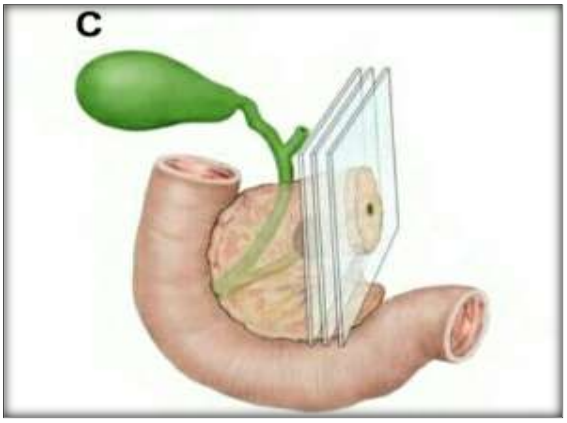

Figure 17: Bread loafing method

Table 5: Comparison of Age &Gender incidence in different studies

Study	Total cases	Male (%)	Female (%)	Age range (years)	Mean age (years)
Present study	45	66.7	33.3	21-70	51.4
Dhakhwa et al[15]	35	54.3	45.7	36-77	60.45
Foroughi F et al[16]	51	72.5	27.5	18-82	57
Fatima et al[17]	40	70	30	10-70	39
Kokandakar et al[18]	31	66	33	38-76	-
Shifa et al[19]	30	50	50	35->70	44

Table 6: Comparative analysis of our study with other studies

Studies	Adenocarcinoma-Intestinal type	Adenocarcinoma-Pancreaticobiliary type	Acinar cell carcinoma	Duodenal carcinoma	Bile duct carcinoma	PanIN	Solid pseudo papillary tumor	PanNET	Mucinous cystic neoplasm	Benign lesions & others
Our study	68.90%	2.20%	2.20%	6.70%	-	2.2	-	8.90%	-	4.40 %
Shifa et al[19]	63%	6.70%	-	-	-	-	3.3	6.70%	-	20%
Howe et al[13]	76%	27%	-	-	-	-	-	-	-	-
Yeo el al[20]	81%	-	-	7%	12%	-	-	-	-	-
Chan C et al[21]	91%	-	-	3%	5%	-	-	-	-	-
Michelassi F et al[22]	89.30%	-	-	2.50%	6.20%	-	-	-	-	-
Fatima et al[17]	62.50%	7.50%	-	-	2.50%	-	10%	-	2.50%	15%
Dhakhwa et al[15]	55.90%	4.10%	-	20%	5.70%	-	-	2.90%	2.90%	8.50 %

DISCUSSION

Pancreaticoduodenectomy is one of the most technically challenging and complex operations performed for pathology arising from pancreatoduodenal complex. Initially it was associated with very high mortality rate of approximately 40-50%, but with surgical advances it has come down to <5%.[8] Long term survival is largely dependent on the pathology within the resected specimen, which emphasizes the need of meticulous evaluation of Whipple's specimens.[9]

Standard Kausch-Whipple's pancreatoduodenectomy specimen comprises of distal stomach, duodenum, head of pancreas, CBD, gall bladder and regional lymphnodes[Table/Fig-18]. There are mainly 3 techniques for grossing of Whipple's specimen.

A) **Axial slicing[4,5]:** Each specimen is serially sliced perpendicular to the long axis of the duodenum over its entire craniocaudal length [Table/Fig-19]. The dissection plane is therefore the same as that of CT scanning, facilitating in identification of all anatomic structures and their relations. Another advantage is that circumferential margins are

readialy assessable.[10,11] But ampullary region sometimes falls between sections, hindering accurate assessment of tumor origin. And in case of IPMN, axial slicing does not allow to distinguish between lesions originating from the main pancreatic duct or side branches.[6,7]

B) **Bi-valving/ Multi-valving[6,7]:** It involves bisecting the head of pancreas along probes placed in the common bile duct (CBD) and pancreatic ducts [Table/Fig-20]. After bi-sectioning, the two halves can be serially sliced in three different planes: either by axial slicing, multi-valving (serial slicing along each half of pancreas), or bread loafing (parallel to neck of the pancreas). This method is technically more challenging than axial slicing, especially if one or both ducts are narrowed by tumor. And pancreatic surface is disturbed so it hinders with margin assessment.[4,5] But the important advantage compared to axial slicing is that periampullary region is always visualized and the primary origin of periampullary tumors can be more reliably appreciated. Additionally, after bi-sectioning, the main pancreatic duct can be completely evaluated and a distinction can be made between the CBD, main pancreatic duct, and side branches, facilitating diagnosis of main and/or side branch IPMN. Thus, bi-sectioning allows much more accurate documentation of cystic tumors and their relationships to the ducts.[6,7]

C) Breadloafing[10]: Specimen is sectioned perpendicular to the neck of pancreas [Table/Fig-21]. This technique becomes difficult in the region of the duodenum and may distort the relationship of the tumor to the ampulla and the insertion of the pancreatic and bile ducts.

Pancreatic tumours are predominantly seen in males in their seventh decade. We also obtained male dominance which was comparable to most of the other studies. In Henson's study mean age of incidence was 69.7 years,[12] in Howe et al's study it was 65.6 years[13], and in Yeo JC et al's study mean age was 64 years.[14] [Table/Fig-22]

Periampullary region was the most common site(62.2%) involved in present study, which is comparable to studies by Kokandakar et al,[18] and Dhakhwa et al.[15] who also found the predominant site of involvement to be Periampullary region. Whereas Howe et al.[13] and Shifa et al.[19] reported ampullary carcinoma as the predominant cancer. This discrimination could be due to our strict adherence to the diagnostic criteria proposed by Klimstra et al for the diagnosis of Ampullary carcinoma.[13]

Histopathologically, pancreatic adenocarcinoma can be intestinal, pancreatobilliary, mixed or undifferentiated type. Categorization is important because prognosis of intestinal type is better than pancreatobilliary type.[13] We found only 1 case of pancreatobiliary type adenocarcinoma.[Table/Fig-23]

Usually PanNETs are non-functional but in our study, 1 case was functional, histologically correlating with Somatostatinoma. It was a known case of Neurofibromatosis-1and the patient had multiple well defined skin nodules. In the specimen, there were multiple small nodules in small intestine which showed the histology of Gastro Intestinal Stromal Tumor.

Yeo et al performed multivariate analysis of 443 cases and reported 4 factors found to adversely affect the survival,[14]: 1) tumour diameter >3cm; 2) positive resection surgical margins; 3) lymphnode metastases; and 4) the presence of poorly differentiated tumour. They also reported ampullary carcinomas to have the smallest tumor dimension with lower incidence of positive resection margins. We also found the mean tumor diameter to be smallest in ampullary tuomrs (mean 1.6cm). Howe et al,[13] and Dhakhwa et al,[15] found mean size of ampullary tumors to be 2.7cm and 2.2cm respectively. This is in agreement with our study.

In Howe et al's study, well and moderately differentiated graded tumours predominated.[13] In Foroughi et al's study, well differentiated tumors were 59.1%.[16] In Kokandakar's study well and moderately differentiated tumors were 28% and 58% respectively,[18] which correlates with our study that showed 53.3% tumours to be moderately differentiated adenocarcinoma. The pathological stage of majority of adenocarcinomas was T3(49%), followed by T2(34%) in our study. Foroughi et al found 50% of their carcinomas having T3 stage,[16] and Kokandakar et al also found T3 stage in 52% cases.[18] This indicates late detection of malignant lesions and therefore they recommended more precise evaluation of clinical signs and symptoms for early detection so as to increase the resectability of the tumor.

When lymphnode involvement by malignant tumour is taken into consideration, 17 cases(40.5%) showed lymphnode metastasis which is higher as compared to a study done by Ibrahim et al(10%).[23] Among cases of adenocarcinoma, the nodal involvement was 37.1% in our study. In Warren KW et al's study,[24] nodal metastasis ranged from 29-52% and in Allema JH et al's study.[25] it was 40%, which correlates with our study. Optimal histologic examination of a pancreaticoduodenectomy specimen should include analysis of a minimum of 12 lymphnodes.[26]

Margin status has been shown to relate to survival outcomes. The SMV margin is most frequently involved by the tumor cells, due to lack of peripancreatic soft tissue in this area. Yeo et al showed that 5 year survival in those with negative surgical margins was 26% (median survival 12 months) compared to those with a positive margin: 8% (median survival 10 months).[27] While margin positivity appears to be critically important, not all margins may have a similar impact on patient survival. Delpero et al. reported that positive SMA or SMV margin had a significant impact on progression-free survival, while a positive posterior margin had no impact[28]. In our study, two cases were retroperitoneal margin positive and one case was

SMV margin positive. While Kokandakar's study had 3 cases with SMA margin positivity and 1 case with positive posterior margin.[18]

In the large series of 1175 resected pancreatic cancers by Winter et al, the incidence of perineural invasion was 91% whereas vascular involvement was lower (53%).[29] In our study, patients with adenocarcinoma had 37.1% incidence of lymphovascular invasion and 48.6% incidence of perineural invasion.

Studies have shown that incidence of benign histopathology on pancreaticoduodenectomy specimens could be as high as 13%.[30] In a report from Myo clinic, Smith et al. reviewed 484 patients who underwent Whipple's procedure for suspected periampullary malignancy and found chronic inflammatory disease in 24 cases(5%).[31] VanGulik et al. reviewed 220 patients and reported 6% benign findings.[32] In many complicated cases of chronic pancreatitis, presenting as a periampullary mass, Whipple's procedure is now considered reasonable.[13]

Two(4.4 %) cases suspected of malignancy in our study turned out to be benign on histopathology. They were Microcystic serous cystadenoma and Acinar Cell Cystadenoma. As both of them may cause abdominal mass and discomfort, they mimic malignancy. However serous cystadenoma is more common in body or tail of pancreas and they usually present grossly as large multiloculated cystic mass filled with clear fluid and lined by small, flat/cuboidal cells containing abundant glycogen and small round nuclei[33,34]. Acinar cell cystadenoma is a rare entity consisting of unicystic or multi-cystic lesion lined by well-differentiated acinar cells, usually not connected with pancreatic ductal system.[35,36]

One case(2.2%) was found to be non-neoplastic - cystic dystrophy of heterotropic pancreas in duodenal wall (paraduodenal pancreatitis). This form of chronic pancreatitis typically occurs in male alcoholics, and it is clinically significant because the dense fibrosis and irregular borders mimic neoplasia. Adsay et al noted that many of the 'pseudo tumors' in their study were found to have paraduodenal pancreatitis.[37]

Other reported rare non-malignant cases in whipple's specimen include 1 case of Brunner's gland hyperplasia in Kokandakar's study,[18] cholesterol cyst in CBD in Shyr et al's study,[38] and schwannoma in Fatima et al's study.[17]

CONCLUSION

Pancreaticoduodenectomy specimens require meticulous histopathological evaluation for proper categorisation of histopathological types and other features which affect patient survival. Possibility of a benign diagnosis remains in Whipple's specimens which have been resected presuming malignancy based on clinical judgement and radiological data. We found two benign lesions: Microcystic serous cystadenoma, and Acinar cell cystadenoma& one Non-neoplastic case of paraduodenal pancreatitis. Periampullary region was the most common site for tumorlocation(62.3%). 35(85.3%) cases were adenocarcinoma and Moderately differentiated Adenocarcinoma(58.7%) was the most common histological type. One case of PanNET was functional and showed histology of Somatostatinoma. It was a known case of Neurofibromatosis-1 and multiple nodules were found in small intestine which showed histology of GIST. Youngest case was 21 years old and oldest case was 70 years. Mean age was 51.42 years with 2:1 male predominenace. We found that for grossing of Whipple's specimen, Axial slicing method is more beneficial in cases of Periampullarytumors for accurate margin assessment, whereas Bi-valving method is more benificial than axial slicing in cases of cystic pancreatic tumors to determine its primary origin. The incidence of lymphnodeinvolvement(37.1%) and perineural invasion(48.5%) was high in cases of adenocarcinoma, associated with poor prognosis. 3 cases showed margin involvement. In 2 cases retroperitoneal margin was involved and in 1 case SMV margin was involved.

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