ANALYSIS OF KRAS AND HER2 MUTATIONS WITH THEIR CLINICO-PATHOLOGICAL RELATION IN PERIAMPULLARY CARCINOMA UNDERGOING PANCREATODUODENECTOMY

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfilment of the requirement for the degree of

Magister Chirurgiae (MCh)
(Surgical Gastroenterology)

July 2019
AIIMS, Jodhpur

Dr Ashish Swami

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DECLARATION

I, hereby declare that the thesis entitled "Analysis of KRAS and HER2 mutations with their clinico-pathological relation in periampullary carcinoma undergoing pancreatoduodenectomy'' embodies the original work carried out by me in the Department of Surgical Gastroenterology at All India Institute of Medical Sciences, Jodhpur.

I further state that no part of the thesis has been submitted either in part or in full for my other degree of All India Institute of Medical Sciences or any other Institute/ University.

DrAshish Swami

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All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis entitled " Analysis of KRAS and HER2 mutations with their clinico-pathological relation in periampullary carcinoma undergoing pancreatoduodenectomy'' is the bonafied work of **DR. ASHISH SWAMI** carried out under our guidance and supervision in the Department of Surgical Gastroenterology, All India Institute of Medical Sciences, Jodhpur.

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It is further certified that the candidate has fulfilled the pre- requisites necessary for the submission of this thesis work.

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CONTENTS

1. Introduction

The term “periampullary cancer” (PAC) refers to neoplasms originating from four different anatomic locations within 2 cm of the ampulla of Vater (AoV): (a) adenocarcinoma of the head of the pancreas, (b) ampullary cancer, (c) duodenal cancer, and (d) distal bile duct cancer. Obstructive jaundice is the common symptom of cancers located in the vicinity of the AoV. (1)

In the ampullary/periampullary region, the pancreatobiliary epithelium of the common bile duct, pancreatic duct, and common channel merge into the intestinal epithelium of the duodenum. This is thought to be the reason why this region represents a hot spot for carcinogenesis, together with the fact that this region is also exposed to biliary juice, pancreatic juice, and duodenal juice. (2)

Among various morphological subtypes in PAC, the pancreatobiliary (PB) and intestinal subtypes are the most common. The distal CBD and pancreatic adenocarcinoma (PDAC) present with the PB subtype, whereas duodenal adenocarcinoma (DC) presents with the intestinal subtype. Ampullary carcinoma may present with either PB or intestinal subtypes as it is situated at the epithelium junction. (2) PB subtype is aggressive pathology as compared to intestinal subtype. This is supposed to be because of the difference in genomic alterations between these two subtypes. (3)

The magnitude of genetic abnormalities in periampullary carcinoma has a vast spectrum. This includes chromosomal abnormalities, point mutations, epigenetic silencing, etc. However, only a small group of mutations are predominantly required for tumor initiation and progression. Some of these mutations are more common in either the PB subtype or intestinal subtype. (4) Earlier detection of these mutations may have management and prognostic significance.

Kirsten rat sarcoma virus (KRAS) mutation and Human epidermal growth factor receptor 2 (HER2) are among the earliest mutations in the pathogenesis of PAC. Their frequencies and prognosis vary in literature among various subtypes of PAC and population-based analysis in different countries. (5) Targeted therapies are being used to manage PDAC; however, their use in pathological and morphological subtypes of PAC may need further evaluation. There is no study that evaluated the genomic alteration in PAC in the Indian population to our knowledge.

Our study evaluates the frequencies of KRAS and HER2 mutations and their relation with clinico-pathological outcomes in periampullary carcinoma in patients undergoing pancreatoduodenectomy.

2. AIM and OBJECTIVES

Aim

To study the prevalence of KRAS and HER2 mutations in periampullary carcinoma and their relation with clinico-pathological outcomes post PD.

Objectives

Primary objective

To study the prevalence of two selected gene mutations (KRAS and HER2) in periampullary carcinoma undergoing PD.

Secondary objective

To evaluate the relation of two selected gene mutations status (KRAS and HER2) in post PD specimen (as detected by FISH) with clinico-pathological parameters including age, T-stage (T1 or T2 or T3 or T4), N-stage (negative v positive nodal status), grade (well to moderate v poor), morphology (I-type v PB-type), invasion into vascular and lymphatic structures, and perineural growth.

3. Review of Literature

PACs encompass a heterogeneous group of tumors. The term “periampullary cancer” (PAC) refers to neoplasms originating from four different anatomic locations within 2 cm of the AoV. (1)

Anatomy of the ampullary region and its significance:

The AoV is a small but complex anatomical landmark. Abraham Vater first described it as ‘papilla duodeni’ (1684-1751). However, it was later named after him for his discovery. Its function remained largely unknown for two centuries until its sphincteric function was elaborated by Ruggero Oddi (1866–1913) in his landmark article ‘D’une disposition a sphincter spécial de L'Ouverture du canal choledoche (1887). (6)

The AoV is the junction of the pancreatic, biliary and digestive tracts. It contains (a) the junction of the common bile duct (CBD) and pancreatic duct (PD); (b) sphincteric of Oddi (SoO); (c) system traversing the duodenal wall, and (d) terminating at the major papilla. The papilla presents as a polypoid prominence 5 mm to 10 mm in length and 5 mm in width, hidden by transverse, circular, duodenal folds. The junction of CBD and PD has three types of presentations: A. common duct, 1- 8 mm in length (60%); (2) a “double-barreled” opening at major papilla (38%); and (3) two distinct duodenal openings for both ducts (2%). (7,8)

The AoV is the junction of the different epithelial lining of the gastrointestinal tract, the duodenal mucosa covering the papilla; the pancreatic ductal epithelium and that of the distal CBD; and the epithelium lining the common channel. In the transitional area, different cell types could be seen intimately mixed. These different mixed epithelia and bile & pancreatic juices make the AoV a potential site for carcinogenesis. The most common site of cellular atypia is found in the area of the common pancreato-biliary channel, followed by the pancreatic duct, duodenal epithelium, and Brunner's glands. (9)

Clinical features of periampullary carcinoma:

Macroscopically, the PAC may have three presentations; (1) intramural protruding (intra-ampullary), (2) extramural protruding (periampullary), and (3) ulcerating ampullary. Either of these presentations may obstruct biliary and pancreatic ducts, leading to subsequent symptoms.

Painless progressive jaundice is the most common and classical presentation in PAC as lesion obstructs the biliary tract (90-100%). It is also the same reason for the early presentation of PAC compared to Pancreatic duct adenocarcinoma (PDAC) of the pancreas's head, body, and tail. Along with jaundice, the passage of clay color stools is also a prominent symptom in PAC patients. Approximately 30-40% of patients present with cholangitis, in which patients may initially present with fever with chills and rigor.

The classical reported, "waxing and waning" of obstructive jaundice is encountered in only one-third of patients. It is mainly seen in ampullary carcinoma, which causes jaundice but subsequently tumor partially sloughs off due to necrosis which leads to the waning of jaundice. This tumor slough is associated with melena due to bleeding associated with tumor necrosis. Other symptoms like gastric outlet obstruction (GOO) are rarely seen in PAC compared to PDAC, but if present, suggest advanced disease.

Icterus is the most common sign, with scratch marks on the trunk and extremities due to the pruritus from the subcuticular deposition of bile salts, high colored urine, and pale stools are pathognomonic of these tumors. The classic physical findings of left supraclavicular adenopathy (Virchow node), periumbilical adenopathy (Sister Mary Joseph node), or a firm circumferential rim of tumor at the top of the rectum on digital rectal examination (Blumer-shelf from drop metastases) are found only with advanced, disseminated disease.

Clinico-pathological presentation of PAC:

Due to its complex anatomy and physiology, the periampullary region may give rise to various neoplasms with dramatic symptoms. These neoplasms may range from benign to malignant neoplasms. Adenocarcinomas (75%) are the most common neoplasms arising in the periampullary region, followed by benign neoplasms like adenomas (20%) and neuroendocrine tumors (~5%). (9)

As far as adenocarcinomas (ADCs) are concerned, the true incidence of origin of ADC as per their site is difficult to evaluate as a tumor arising from duodenal mucosa may infiltrate the AoV and give an impression of ampullary carcinoma, and the same may go vise-versa also.

However, histological differentiation of PAC is of prime importance as the overall survival (OS) depends upon the site of tumor origin. The DC has the best prognosis among all PACs, followed

by ampullary carcinoma and distal cholangiocarcinoma (DCCA). The pancreatic variant of PACs has the worst prognostic among all. (10)

Although some authors have questioned whether this outcome reflects different biology for periampullary carcinoma, as opposed to simple detection at an earlier stage or earlier onset of symptoms, even ampullary carcinoma patients with positive lymph nodes have better survival than any other group of pancreatic carcinoma patients. However, it is also evident that these carcinoma subtypes have a difference in their clinico-pathological presentations, which may explain the difference in their OSrates.

Lymph node involvement is a critical, independent parameter affecting OS. A recent multicenter analysis demonstrated that LN involvement is the most critical adverse factor affecting OS and disease-free survival (DFS). (11) PDAC have been associated with higher LN involvement (70-90%) as compared to AC (30-70%). (10,12) Whereas 60-70% and 40-50% cases of DC and DCCA have shown LN involvement, respectively. (13–15)

Apart from LN involvement, numbers of involved LNs have been shown to affect survival. (16) Recently, American Joint Committee on Cancer (AJCC) panel had updated its TNM staging guidelines in its 8th edition. Latest N staging changes include quantitative stratification. Metastasis to regional lymph nodes is now sub-divided into N1 and N2. N1 and N2 are classified as 1-3 and > 4 lymph nodes involved, respectively. (17) It was postulated that an increased number of positive lymph nodes had been associated with poorer OS.

However, some authors have argued that lymph node ratio (LNR) may provide a better prognostic assessment than a quantitative-based system. LNR >0.2 is associated with poor OS in resected PDAC and PACs. (18,19) Some authors have shown that LNR > 0.1 and > 0.056 are associated with high recurrence-free survival in PACs. (20,21)

Perineural invasion (PNI) is another independent prognostic marker associated with poor OS. The overall frequency of PNI in PAC has been reported in 15-35% in the literature. However, their frequency may differ in various subtypes of PACs. In AC, PNI is reported in 15-22% cases, whereas in PDAC, DC, and DCCA, it is reported in >90%, 40%, and 80-90% of cases, respectively. (22–24) Despite differences in frequencies among all anatomical subtypes, PNI is associated with poor survival. AC with PNI may have a similar prognosis as PDAC, in which the

majority of patients have PNI. (23) PNI is also highly correlated with tumor volume, location, depth of invasiveness, angiogenesis, and lymph node involvement. (24)

Several biomarkers have been studied in periampullary carcinoma. CA 19-9 and CEA are the most commonly used and validated tumor antigens. CA19-9 is a sialylated Lewis A blood group antigen tumor marker, universally used in pancreato-biliary carcinomas. Its normal value is 0-37 U/ml. It is the best validated and most clinically useful tumor marker to be used in PDAC. (25) It is used for early detection, response assessment, prognostication, and surveillance in PDAC. In symptomatic patients, it has good sensitivity and specificity of 70-80% and 80-90%, respectively. However, limited studies have reported its use in PAC in western literature. Recently, Park et al. (2021) have described that elevated CA 19-9 levels are independently associated with poor OS in PAC [56 months vs. 25 months ($p < 0.05$)]. (26) Similarly, other studies have shown that higher CA 19-9 levels correlate with higher T stage, nodal status, and metastasis. (27,28)

CEA is another tumor marker whose levels are proposed to correlate with prognosis. It is most widely studied in colo-rectal carcinomas, and its use in pancreato-biliary malignancies is limited. Its normal range is 0-5 ng/ml. However, higher normal values may be seen in males, smokers, and elderly patients. It is primarily absorbed at the biliary ductal epithelium of bile ducts so that false elevated values may be seen in biliary obstruction. Limited studies have reported its use in PACs. Park et al. (2021) reported that elevated CEA levels did not correlate with poor OS in PAC. (26) However, some authors argue that CEA levels have significance in DAC rather than other variants of PAC. Schiergens et (al 2017) reported the potential prognostic role of CEA levels in DACs. (24, 25)

Morphological analysis of Periampullary carcinoma:

Recent advances in knowledge of the genetic mutations and morphology showed a new era for the assessment and management of PAC. Kimura et al. (1994) first proposed two morphological sub-types of PACs- intestinal subtype and pancreatobiliary (PB) subtype. (2) First, an intestinal subtype showed close similarity to tubular adenocarcinoma of the stomach or colon, and second, the PB subtype was characterized by papillary projections with scant fibrous cores. (3,30) They also noted the difference in survival between patients with these two morphological subtypes as the I subtype showed better OS than the PB subtype. (3,31,32)

This morphological sub-classification is now proposed as a better predictor of OS than anatomical location. Also, this can overcome difficulties in distinguishing these carcinomas based on histology alone, as even poorly differentiated carcinomas sustain the histological marker profile of their mucosa of origin. (33) PB subtype occurs in PDAC and DCCA, whereas intestinal subtype is more common in DAC. AC may have any of subtypes depending upon underlying genetic alteration. (26, 31)

Overall the intestinal subtype is the most common morphological subtype in PAC. (10) It arises from the intestinal mucosa, which covers the papilla, originates the intestinal subtype with well-formed tubular glands, complex cribriform areas, and solid nests, resembling colic carcinoma. (2,10,34) These tumors are composed of cribriform glands with cells having pseudostratified, elongated nuclei, showing luminal necrosis. (10) This epithelium might be arising through an adenoma-dysplasia-adenocarcinoma sequence related to colon cancer. (35)

Contrary, the PB subtype arises from the simple mucinous epithelium of the distal common bile duct, the distal pancreatic duct, or the common ampullary duct with simple or branching glands and small solid cell-nests enclosed by desmoplastic stroma. (32,34,35)

The incidence of PB and intestinal subtypes are quite variable in the literature. Kimura et al. (1994) showed that the intestinal and PB subtypes were found in 25% and 72% of cases, respectively, (2) Whereas studies from the Memorial Sloan-Kettering Cancer Center (2014) and Carter et al. (2008) found that the Intestinal and PB subtypes accounted for 49% and 22%, and 46% and 45%, respectively. (30,36)

In his meta-analysis, Kim et al. (2012) evaluated the correlation between clinico-pathological parameters and morphological types in resectable AC. Of the 104 patients, 42 (40.4%) and 62 (59.6%) patients had intestinal and PB subtypes of AC. Patients with the PB subtype showed significantly poorer disease-free survival than patients with the intestinal type (3- and 5-year DFS rates were 50.6% versus 80.0% and 47.8% versus 73.1%, respectively; $P < 0.003$). Also, the PB subtype was associated with advanced T stage, higher positive LNs, and higher lymphovascular invasion. (37)

Westgaard et al. (2008) did a unique analysis among various PAC subtypes. The authors compared clinico-pathological parameters between intestinal subtype AC and DAC & PB

subtype AC and PDAC and DCCA. Both PB and intestinal subtypes had similar adjusted OS compared to DAC and PDAC, respectively. However, PDAC tumors had larger tumor size, LN metastasis, and PNI than PB type AC and DCCA ($p < 0.01$). On the contrary, the DAC tumor had a similar histopathological outcome to intestinal subtype AC ($P > 0.1$). (3)

Recently, Zimmermann et al. (2019) published the most extensive series, which evaluated the prognostic significance of the morphological types in PACs. Among 119 patients, PB and intestinal subtype AC were seen in 69 (58%) and 41 (34.5%) patients. Mixed type AC was seen in ~ 8% of patients. PB subtype AC had significantly worse five years OS than intestinal subtype (27.5% versus 61%, $p < 0.001$). The mean OS of patients with PB, intestinal, and mixed subtype was 52.5, 115, and 94.7 months, respectively ($p < 0.001$). PB type was also associated with advanced T stage, higher positive LNs, and higher PNI, but CA 19-9 and CEA levels were comparable among three morphological subtypes. (32)

On the other hand, few studies did not find any survival difference between PB and intestinal subtype AC.

One of the recent analyses from Tata Memorial Hospital (TMH) showed no OS difference between intestinal and PB subtype of AC when the patient was treated with resection followed by Gemcitabine chemotherapy. PB subtype (n= 105) had significantly higher stage II/III, PNI, and positive LN involvement as compared to intestinal subtype AC (n= 109), but when both groups received adjuvant chemotherapy, OS difference was not significant ($p = 0.355$). (38)

Similarly, Lothe IMB et al. (2019) reported OS, disease-free survival (DFS) among PB, intestinal subtype AC and DAC patients. PB subtype AC had significantly shorter OS and DFS than intestinal subtype AC (43 vs 75 months, $p < 0.036$ & 29.4 vs 30.7 months, $p < 0.02$). But on stratification per tumor stage, PB and intestinal subtypes did not have a difference in OS.(39)

Role of immuno-histochemistry (IHC) in morphological analysis:

Modern-day pathology was revolutionized nearly a century ago after discovering the IHC technique. In this technique, antibodies detect specific proteins (antigens) on tissue blocks. This antigen-antibody reaction can be visualized under a light microscope as the antibody carries immunofluorescence.

Cytokeratins (CK) and mucoproteins (MUC) are structural proteins expressed by epithelial cells. Their structural and bio-chemical variations are tissue-specific which may help to differentiate among various morphologically undifferentiating neoplasia of different epithelial origins. Along with this, now it is also used in detecting site of origin, prognostication, and therapeutic indications in some diseases.

Role of mucins in PAC: Mucins are glycoproteins expressed at cell membranes. Their structural difference is in oligosaccharide side chains comprising N-acetyl-galactosamine, which binds via an O-glycosidic linkage to specific amino acids, occurring in tandem repeats. Mucins are classified according to their structure and function as either "membrane-bound" or "secreted." The membrane-bound MUC class contains mucins MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, MUC17, and MUC21, whereas the secreted MUC class includes MUC class mucins MUC2, MUC5AC, MUC5B, MUC6, and MUC7. Both of them are used for tissue-specific analysis via IHC.

Carcinogenesis may develop by accumulating several genetic and epigenetic lesions, some of which may affect encoding for mucins. Specific mucin patterns have been studied in different neoplasms of ductal origin.

MUC1: MUC1 expression was first reported in 1991 in PDAC. In PAC, it has shown a strong correlation with the PB subtype. It is shown to be 100% specificity for PB type. So, It is now considered a biomarker for PB subtype and PDAC. It is also associated with advanced stage of the tumor, LN metastasis, PNI, vascular infiltration, and worse OS. (40) One recent study by Kulkarni et al. (2017) reported that quantitative IHC positivity correlate with tumor grade as immunoreactivity was 40%, 61%, 64% in well-differentiated, moderately differentiated, and poorly differentiated pancreato-biliary carcinomas, respectively. (41) Zhou et al. (2004) proposed that MUC1 expression may affect patient prognosis by inhibiting the formation of the E cadherin and beta-catenin complex, which would then decrease intercellular adhesion and promote the invasion and metastasis of tumors. (42)

MUC2: MUC2 was first reported in 2001 and reported to be highly specific for intestinal morphological subtypes. (43) Ang et al. (2014) reported the use of IHC in subtyping PACs. In this study, intestinal morphology was characterized by MUC2 positivity. (36) Similarly, Kulkarni et al. (2017) reported 100% specificity of MUC2 for the intestinal subtype. (41) Few

studies have studied the MUC2 role for survival assessment. Kitamura H et al. (1996) reported a positive correlation of MUC2 expression and OS and a negative correlation for MUC1. (44)

Role of cytokeratins in PAC:

Cytokeratins (CKs) are also cytomembrane proteins sub-classified based upon their structural difference. Though different types of CKs have been studied in pancreato-biliary malignancies, few studies have recently evaluated their role in PAC morphological subclassification and prognostication. Among the wide range of CKs, CK7, CK19, CK20, and CDX2 have been studied in PACs:

CK7: CK7 positivity was found in PB subtype PAC tumors and a relatively common finding between intestinal subtype PAC tumors. (36) Morini et al. (2013) reported that CK7 was found positive in > 50% of intestinal subtype PACs despite being a PB-specific cytokeratin marker. (45) On the contrary, Perysinakis et al. (2016) showed that CK7 had lower sensitivity and specificity than MUC1 for differentiation of PB subtype in PAC. Despite all these mixed results, CK7 remains an important CK marker for differentiating PB subtypes in PACs, mainly if used with MUC1. (46)

CK20: Kawabata et al. (2010) evaluated the role of CK20 and MUC1 in the differentiation of PAC tumors. They found CK20+/MUC1− expression in 100% intestinal subtype and CK20−/MUC1+ expression in 94% PB subtype. Perysinakis et al. (2016) (2017) studied the role of different MUCs and CKs in the morphological differentiation of PACs. The study reported higher sensitivity and specificity of CK20 than MUC2 for differentiation of intestinal subtypes. Univariate analysis showed that expression of CK20 ($p = 0.065$) and CDX2 ($p = 0.008$) predicted a more favorable prognosis, although the association between CK20 and survival was only slightly significant. Multivariate analysis of the same study group in 2017 indicated CK20 and MUC1 as independent predictors of morphological differentiation. (46) (47)

CDX2: In a recent study by Niraj Kumari et al. (2013), CDX2 was associated with high specificity to intestinal subtype. The author studied CK7, CK20, MUC1, and CDX2 IHC expression in 91 patients of PACs. Among them, CDX2 was highly specific for intestinal subtype with a sensitivity of 89.5% and specificity of 100%, and it was also the only factor affecting OS in multivariate analysis (CDX2+ median survival 44 months vs. CDX2– median

survival 22 months, p =0.03). (48) Similarly, Westgaard et al. (2008) found CDX2 to be positive in 54.3% intestinal-type and 14.9% PB subtype, with a sensitivity of 54% and specificity of 85%. Sessa et al. (2007) reported 100% sensitivity and 70% specificity of CDX2 IHC for the intestinal subtype. (3,49) de Paiva Haddad et al. (2010) studied a wide range of CKs and MUCs for morphological differentiation of PACs. The authors reported that CDX2 expression was seen in 86% intestinal-type and 21.3% pancreatobiliary type, with 86% sensitivity and 78.7% specificity. (50)

CK19: Limited studies were used to evaluate the morphological differentiation of PACs. CK19 shows higher specificity to the PB subtype than the intestinal subtype. Zapata et al. (2007) reported that out of 25 cases of PDAC, CK19 showed diffuse cytoplasmic positivity in 23 cases. Thus it can be a specific marker for PB subtypes. (51)

KRAS and HER2 mutation in periampullary carcinoma

The magnitude of genetic abnormalities in periampullary carcinoma has a vast spectrum. This includes chromosomal abnormalities, point mutations, epigenetic silencing, etc. (4,52) However, among these, only a small group of mutations are predominantly required for tumor initiation as well as progression. Some of these mutations are more common in either PB or intestinal subtype. (4) Earlier detection of these mutations may have management and prognostic significance.

Morphological differentiation of carcinoma may be directly used for appropriate adjuvant therapy. The intestinal type may get expected results with FOLFOX therapy, whereas the PB type may benefit from Gemcitabine-based therapy. (53) However, the study of genetic alterations may provide add-on information for prognosis and the need for targeted therapy.

Being an early-stage mutation, KRAS may be shared between these subtypes. It is the most common oncogenic mutation in PDAC and other biliary cancers. It occurs in 90-95% of these carcinomas; however, the frequency in AC and DA is slightly low (30-35%). (54) It codes for GTPase, which is involved in MAPK and PI3K/Akt signaling pathways, leading to abnormal growth factors production. Most KRAS mutation was located at codon 12, and the most common mutation types were G12D and G12V. (55,56) Among all the histological and morphological subtypes, KRAS mutation has a highly variable frequency in literature. (55–58) Schultz et al. (2012) showed that frequencies of KRAS were 80% and 67% in PDAC and A-AC, respectively.

(55) Chung et al. (1996) showed that 40% of patients with A-AC had KRAS mutation, whereas Jarnagin et al. (2017) showed that KRAS mutation is present in 90-90% of PDAC patients. (54,57)

In early literature, the mutation was associated with poor prognosis in PAC but not in patients with PDAC. (55–58) However, a recent meta-analysis demonstrated that KRAS mutation was a potential poor prognostic marker for PDAC.(58)(59). Lundgren et al. (2019), retrospective cohort of 175 patients with resected PAC, showed the incidence of KRAS mutation 45% with its significant association with reduced OS in intestinal subtype tumors (P = .018), but not in PB subtype tumors. (59)

The association between KRAS mutation and histological subtype was analyzed in a few studies. Mikhitarian et al. (2014) analyzed the frequency of KRAS mutation in PAC and reported that intestinal and PB subtype cancers had 52% and 42% incidence, respectively. (60) Contrary, Hechtman et al. (2015) showed an increased frequency of KRAS mutation in the PB subtype cancers (61% vs. 29% intestinal). (61) Targeted therapy is available for wild-type KRAS mutation patients. Hence, low incidence of this mutation may provide good results using EGFR targeted therapy in duodenal and ampullary carcinoma as seen in wild type of metastatic colon carcinoma. (62)

HER2 or ERBB2 is human epidermal growth factor 2 and is involved in MAPK, PI3K, STAT, PLC, and PKC pathways via tyrosine kinase signaling. (5) Overexpression of this gene is associated with the unregulated proliferation of cells. There is limited literature on HER2 overexpression in PDAC. Also, the incidence of HER2 overexpression has varied widely (7–82%), likely because of differences in methodology and patient selection as these studies have primarily included patients with early-stage, resected disease, focusing on the role of HER2 in precursor lesions. (63,64) Sarfran et al. (2001) showed that HER2 overexpression was more common in metastatic lesions; however, there was no association between the grade of carcinoma. (65,66) However, no study in our knowledge evaluated the association of morphological changes in PACs with HER2 mutation. One study had evaluated the role of Trastuzumab with Gemcitabine for HER2 positive PDAC with limited success. (66) However, evaluation of this mutation in PAC may facilitate the use of targeted chemotherapy (Trastuzumab) in this set of patients.

4. Methods and materials

Study design

- It is a Single-center, In-hospital, prospective, uncontrolled, observational study.
- All the patients undergoing pancreatoduodenectomy (PD) for periampullary carcinoma and meeting inclusion criteria during the study period will be recruited from the Department of Surgical Gastroenterology at AIIMS, Jodhpur.

Place of study

The study was carried out in the Department of Surgical Gastroenterology, Pathology, and Anatomy at the All India Institute of Medical Sciences (AIIMS), Jodhpur.

Time of study

January 2020 - September 2021

Study Population

A total of 30 patients were recruited in the study.

Ethics Approval

Ethical approval for this study was obtained from the AIIMS Ethics committee with reference no AIIMS/IEC/2019-20/952.

Inclusion criteria

a) Patients who undergo pancreatoduodenectomy for periampullary carcinoma and confirmation made by histopathology.
b) Patient age > 18 years

Exclusion criteria

a) Patients who undergo palliative bypass
b) With PNET tumors, benign CBD stricture, chronic pancreatitis induced pancreatic head mass.
c) Patients with carcinoma of the head of the pancreas
d) ASA grade > 3

Methodology

The study was carried out in the Department of Surgical Gastroenterology, Pathology, and Anatomy, All India Institute of Medical Sciences, Jodhpur. A total of 30 patients who visited the Surgical Gastroenterology OPD were recruited under the study protocol. After assessing the inclusion and exclusion criteria and taking consent, patients were taken up for surgery.

Counseling

All patients were provided with a patient information leaflet and were counseled regarding:

- Surgical management of periampullary carcinoma
- Benefits and procedure-related details of pancreatoduodenectomy
- The concept of genetic/mutational analysis
- Benefits of genetic/mutational analysis of KRAS and HER2

Pre-operative workup

Informed consent

The patients were explained about the purpose of the study. A detailed written informed consent was taken.

History

The patients with obstructive jaundice/cholangitis were evaluated. History for the mode of onset, progression, and duration of jaundice with any associated symptoms like pain, fever, nausea, vomiting, etc., were evaluated. The patient's demography and comorbidities like hypertension, diabetes mellitus, and others were noted. All the parameters were noted in the patient's proforma.

Physical examination:

- General physical examination
- Abdominal examination

Investigations:

A. Laboratory investigations
   - Complete blood count
   - Liver function tests
   - Renal function tests
   - Serum electrolyte
   - Prothrombin time/INR
   - Tumor markers (CA19-9 and CEA)

B. All patients underwent a comprehensive multi-disciplinary evaluation, which included-
   - Radiologist evaluation – A pancreatic protocol CT examination.
   - Pre-operative anesthesia evaluation– To rule out associated risk factors and immediate /delayed morbidity.
   - Medical gastroenterology evaluation: For side-viewing endoscopy ± biopsy of tumor and ERCP and CBD stent placement for jaundice (optional).
   - Pathology – Pre-operative assessment of biopsy (if done).

Imaging

- Patients were subjected to a Chest radiograph as a part of a pre-anesthesia check-up.
- Patients were subjected to pancreatic protocol CT for assessment of :
   - Tumor morphology
   - Local extent
   - Vascular anatomy and involvement
   - Lymph node involvement
   - Rule out distant metastasis etc.

Operative workup

Pre-operative preparation

- Perioperative antibiotic prophylaxis was given in the form of Inj. Cefoperazone - sulbactam 1.5gm half an hour before surgery and then continued for three days till patient allowed oral soft diet.

Decision for a surgical procedure-

- Decisions for surgical procedures were made based on the patient's general condition, clinical examinations, radiological findings of CT.
- All patients underwent pancreatoduodenectomy as per standardized protocol in the Department of Surgical Gastroenterology, AIIMS Jodhpur.

Different methods of Pancreato-duodenectomy were employed in the study population:-

A) Open
B) Laparoscopic assisted
C) Robotic-assisted
D) Total robotic

Post-operative management-

In the immediate post-operative period, patients were kept in the recovery room. After proper evaluation and clearance from anesthetics, the patients were shifted to the ward. Patients were started on oral liquids after 2-3 days of the post-operative period in the ward. Then, patients were subsequently introduced to semi-solid and solid foods. Patients were managed actively in the ward for any surgical complication in the Department of Surgical Gastroenterology as per standardized protocols. On discharge, all patients were prescribed oral medications after full recovery.

Histopathology- Post-operative histopathological examination and morphological sub-classification of PAC tumors were done in the Department of Pathology, AIIMS, Jodhpur as per standard protocols. (Figure 1-5)

- All the clinic-pathological parameters of the study population were filled in the patient proformas.

Immunohistochemistry of mucin and cytokeratin markers: (Figure 6-11)

Sample processing, staining, and immunohistochemistry

1) Steps of block preparation and section cutting

After the representative sections were taken from the specimen or the cores placed in cassettes, tissue was processed through various stages.

1. Dehydration was carried out by passing the biopsy/sections through a series of ascending grades of ethyl alcohol 50%, 70%, 95%, and absolute alcohol.

2. The clearing was done by passing the tissue through xylene, two changes.
3. Impregnation was done in molten paraffin wax, which had a melting point of 54 – 62˚C.
4. Embedding: An embedding station (Leica EG 1150 H) was used through which a small amount of liquid paraffin was layered into aluminium molds. Properly oriented tissues were placed inside the molds, then filled with liquid paraffin (60 – 62˚C and allowed to cool and harden. The lower portion of the cassette with the identification number was used as the final block.
5. Microtomy: Microtome (Leica-RM 2255) was used, and thin ribbons (4 μm) were cut and floated in warm water (~56˚C) for expansion of the curled sections. These sections were then collected on frosted glass slides and kept for drying.

2) Staining of sections: (For H and E Stain)

1. Deparaffinization – The glass slides containing the tissue sections were kept over a hot plate at 60 0C for 10 minutes, followed by two changes in xylene (Xylene I & Xylene II), 10 minutes each.
2. Hydration – Through graded alcohol (100%, 95%, 70%, 50%) to water, 10 minutes each.
3. Hematoxylin – The sections were kept in Harris's hematoxylin for 5 minutes.
4. Washing – The sections were washed well in water for 2 minutes.
5. Differentiation – Done in 1% acid alcohol (1% HCl in 70% alcohol) for 10 seconds.
6. Washing – Done under running tap water (usually 15 – 20 minutes) until the sections 'blue'.
7. Eosin – Stained in 1% eosin Y for 10 seconds.
8. Washing – Done in running tap water for 2 minutes.
9. Dehydration – Through graded alcohol (50%, 70%, 95%, 100%), 10 minutes each.
10. Clearing –Through xylene (Xylene II & Xylene I), 2 minutes each.
11. Mounting – The sections were mounted in DPX with a cover slip.

Results:

- Nuclei – blue/black
- Cytoplasm – varying shades of pink
- Muscle fibers – deep pink/red
- Red blood cells – orange/red
- Fibrin – deep pink

3) Immunohistochemistry

Steps of IHC staining:

A. Preparation of Buffer–Two types of buffers were used

1. Wash Buffer: 6 gm powdered TRIS buffer salt was dissolved into 1 liter of distilled water, and pH was set at 7.4.

2. Antigen Retrieval Buffer (ARB): 6.05 gm TRIS salt and 0.744 gm EDTA salt were dissolved in 1 liter of distilled water, pH was set at 9.0.

NOTE:

- To increase the pH, NaOH solution was added drop by drop, and pH was titrated.
- To decrease the pH, HCl was added drop by drop, and pH was titrated.

B. Preparation of Poly-L-Lysine Solution (PLL Solution):

1 ml of PLL was diluted with 9 ml of distilled water (1 in 10 dilutions).

C. Slide Coating Procedure:

Step 1: Diluted PLL solution was taken in a clean container/Coplin jar

Step 2: Both sides of the glass slides were cleaned with tissue paper

Step 3: The clean slides were immersed in PLL solution for 5 minutes

Step 4: After 5 minutes, the coated slides were removed and kept overnight for air dry. The coated slides were kept at room temperature. Tissue sections of 4 µ thickness were obtained on the PLL coated slides.

Baking: The slides were kept at 60˚C for 1 hour and then cooled to room temperature.

IHC staining procedure

Step 1: Deparaffinization –

- The slides were kept in Xylene I (10 minutes), followed by Xylene II (10 minutes).

Step 2: Rehydration –

- The slides were kept in 100% alcohol for 5 minutes, followed by 70% alcohol for 5 minutes and 50% alcohol for 5 minutes.

Step 3: Running tap water for 5 minutes

Step 4: Antigen retrieval – by pressure cooker method

200 ml of clean tap water was taken in the empty pressure cooker and heated up to the steam formation. The slides were placed in a rack. 300 ml of ARB was put in the container, and the rack with slides was placed inside the container. Then the container containing the rack with slides was placed inside the pressure cooker, and the lid was closed. After two whistles, the pressure was released by lifting the air vent and cooling until it reached room temperature.

Step 5: Wash – Slides were washed in Wash Buffer (pH7.4) thrice at a 1-minute interval.

Step 6: Peroxide blocking – Blocking reagent was added to the sections and incubated for 10 minutes in the Humidity chamber at room temperature. This step prevents unwanted, non-specific background staining.

Step 7: The peroxide was decanted and not washed with buffer

Step 8: Wash – After that, slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval.

Step 9: Amplifier – Amplifier was added over the sections and incubated for 30 minutes in the Humidity chamber at room temperature.

Step 10: Wash – The slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval.

Step 11: HRP label – The HRP was added and incubated for 30 minutes in the Humidity chamber at room temperature.

Step 12: Wash – The slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval.

Step 13: DAB – The DAB chromogen was applied to the sections and incubated in the Humidity chamber for 10 minutes, avoiding light exposure as much as possible.

Step 14: Wash – The sections were washed in distilled water twice at a 1-minute interval.

Step 15: Counterstain – Slides were counterstained using Harris hematoxylin for 2-3 minutes.

Step 16: Wash – The slides were washed in running tap water for 5 minutes.

Step 17: Dehydration – was done in graded alcohol (50%, 70%, 95%, 100%), 1 minute each.

Step 18: Mounting – Slides are air-dried, mounted with DPX, and examined under the microscope.

IHC was performed using commercially available ready-to-use antibodies. With each batch, appropriate controls were also run.

Antibodies used in IHC:

Marker	Antibody	Clone
MUC1	Thermo-scientific	E29
MUC2	Dako	CCP58
CK7	Thermo-scientific	OV-TL 12/30
CK19	Thermo-scientific	A53-B/A2.26
CK20	Thermo-scientific	KS 20.8
CDX2	Dako	DAK-CDX2

FISH protocol for KRAS and HER2 mutational analysis:

1) Steps of block preparation and section cutting

After the representative sections were taken from the specimen or the cores, tissue was processed as follows:

1. Dehydration was carried out by passing the sections through a series of ascending grades of ethyl alcohol, from 50%, 70%, 95% to absolute alcohol.
2. Clearing was done by passing the tissue through two changes of xylene.
3. Impregnation was done in molten paraffin wax, which had a melting point of 54 – 62˚C.
4. Embedding: Embedding station (Leica EG 1150 H) was used through which a small amount of liquid paraffin was layered into aluminium moulds. Properly oriented tissues were placed inside the moulds, which were then filled with liquid paraffin 60 – 62˚C and allowed to cool and harden. The lower portion of the cassette with the identification number was used as the final block.
5. Microtomy: Microtome was used, and thin ribbons (3 μm) were cut and floated in warm water (~56˚C) for expansion of the curled sections. These sections were then collected on frosted glass slides and kept for drying.

2) Staining of sections: (For H and E Stain)

1. Deparaffinization – The glass slides containing the tissue sections were kept over a hot plate at 60 ˚C for 10 minutes, followed by two changes in xylene (Xylene I & Xylene II), 10 minutes each.
2. Hydration – Through graded alcohol (100%, 95%, 70%, 50%) to water, 10 minutes each.
3. Haematoxylin – The sections were kept in Harris's Haematoxylin for 5 minutes.

4. Washing – The sections were washed well in water for 2 minutes.

5. Differentiation – Done in 1% acid alcohol (1% HCl in 70% alcohol) for 10 seconds.

6. Washing – Done under running tap water (usually for 15 – 20 minutes) until the sections 'blue'.

7. Eosin – Stained in 1% Eosin Y for 10 seconds.

8. Washing – Done in running tap water for 2 minutes.

9. Dehydration – Through graded alcohol (50%, 70%, 95%, 100%), 10 minutes each.

10. Clearing –Through xylene (Xylene II & Xylene I), 2 minutes each.

11. Mounting – The sections were mounted in DPX with a cover slip.

Steps for Fluorescent in situ Hybridization (FISH) (Figures 13-16)

1. Cut 5 µm of tissue sections onto salinized slides, incubate at 56°C overnight.

2. Treat with xylene 2 × 10 min to remove wax. Xylene solutions should be changed regularly to avoid the build-up of wax residues.

3. Rehydrate slides through 100%, 85%, and 70% alcohols, 1 min each wash. Wash in running tap water, rinse in distilled water.

4. Pretreat with 0.2 N HCl for 20 min, then wash in distilled water for 3 min

5. Place slides in 8% sodium thiocyanate in distilled water at 80°C for 30 min.

6. Wash in 2X saline citrate buffer (dissolve 175.3 g NaCl and 88.2 g sodium citrate in 800 mL distilled water, pH to 7.0 with 10 M NaOH, make up to 1 L with distilled water, autoclave; dilute 1:10 with distilled water for 2X SSC) for 3 min.

7. Digest in 0.5% pepsin in 0.2 N HCl at 37°C for 26–32 min. The digestion time will depend on the tissue used. We used a digestion time of 30 min.

8. Immerse slides in distilled water for 1 min and saline citrate buffer for 5 min.

9. Dehydrate slides through 70%, 85%, and 100% alcohol, 1 min in each alcohol. Air-dry.

10. Apply Vectashield with 0.5 µg/mL 4,6-diamidino-2-phenylindole-2 hydrochloride (DAPI) added, and then apply coverslips.

11. View with a fluorescence microscope that incorporates a filter block specific for the excitation and emission wavelengths of DAPI.

12. Place slides in 2X SSC pH7.0 buffer until the coverslips fall off, then dry in an oven at 45°C before proceeding with in situ hybridization

For K-RAS

KRAS FISH analysis was performed using spectrum green-labeled chromosome enumeration probe 12(CEP12), and a spectrum orange labeled KRAS locus-specific (RP11-29515) probe. Amplification was defined as a KRAS/CEP12 ratio of >2.

Chromosome 12 hyperploidy was defined as >40% cells with >2CEP12 signals.

Chromosome 12 monosomy was defined as >40% cells with one CEP signal.

For HER 2

The scoring and evaluation for in situ hybridization was performed by counting HER2 and CEP17 signals from 100 nuclei per case.

Non-tumor tissue (normal colon mucosa) was used as a negative internal control. Samples with a HER2/CEP17 ratio > 2.0 were considered amplified (positive).

Statistical analysis

All data were acquired in a specified format as in proforma and entered in SPSS v 26/MS-Excel software for analysis. Measured data were expressed as median with interquartile range (IQR) at the 25^{th} and 75^{th} percentiles or as percentages. Proportions were compared using Chi-square, whichever is applicable and numerical data were compared using the Mann Whitney U test. P-value ≤ 0.05 was considered significant in all statistical evaluations.

Ethical considerations

All the patients enrolled in the study received the standard care management, and participation in the study has not led to any change in their usual diagnostic workup, follow-up, or management. All personal data collected during the study were kept strictly confidential.

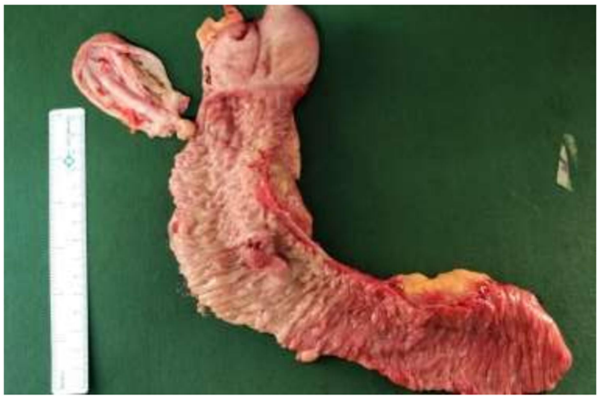


Figure 1: Post-operative specimen picture of pancreato-duodenectomy for periampullary carcinoma

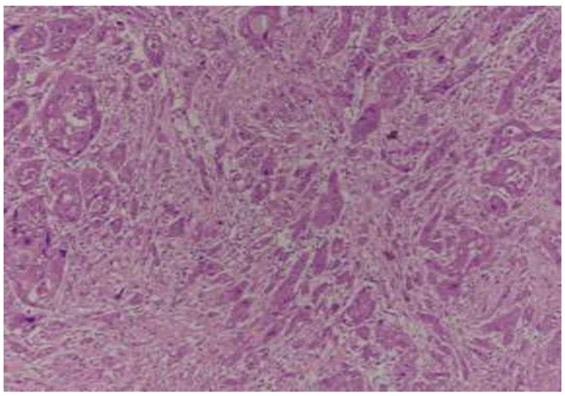


Figure 2: Invasive adenocarcinoma with desmoplastic response, 100X, H&E stain

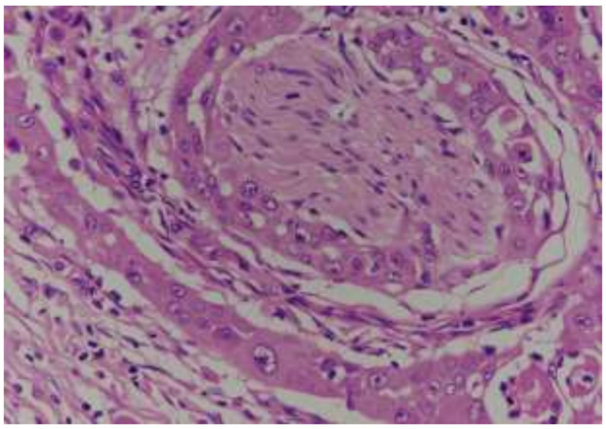


Figure 3: Adenocarcinoma with PNI (centre of image), 400X, H&E stain

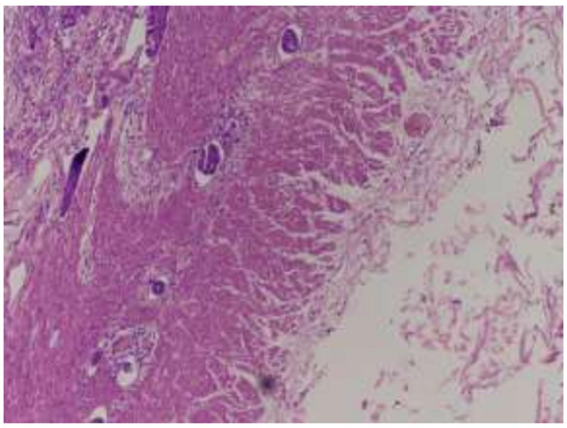


Figure 4: Several LVSI, 100X, H&E stain

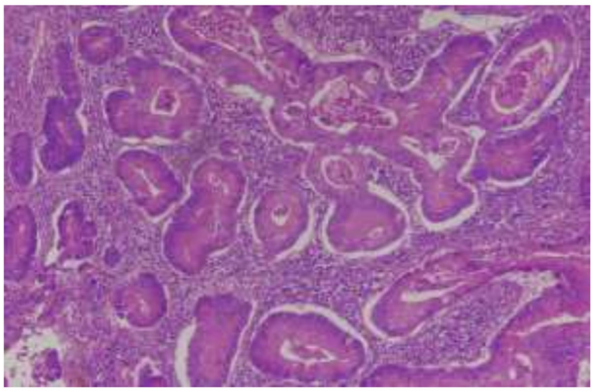


Figure 5: Adenocarcinoma of intestinal type with large infiltrating glands lined by stratified nuclei and containing luminal debris, 100X, H& E

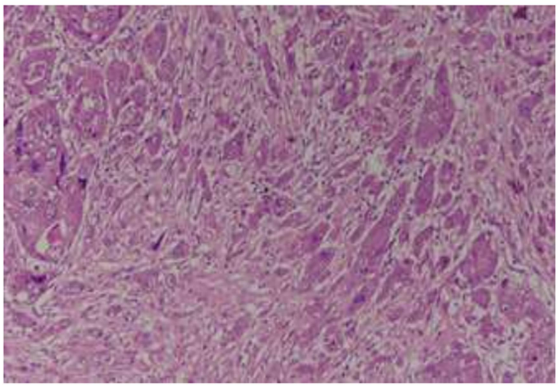


Figure 6: Adenocarcinoma of pancreatobiliary subtype

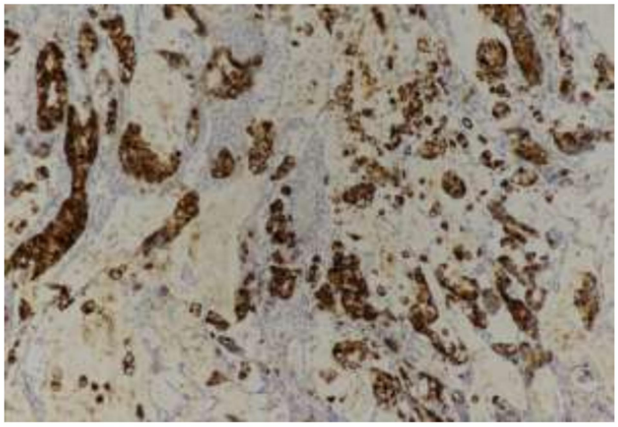


Figure 7: IHC for MUC1 showing intense membranous positivity, 100X

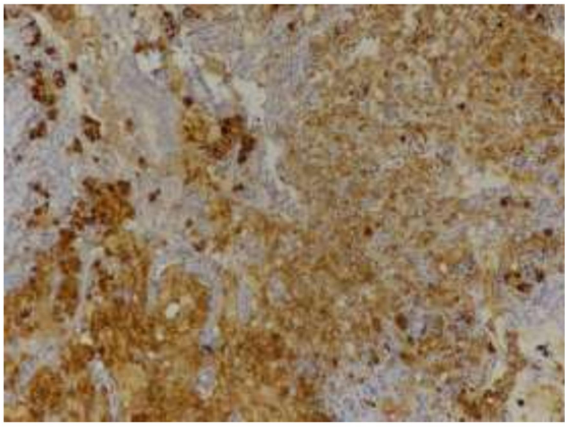


Figure 8: IHC for MUC2 showing intense membranous positivity, 100X

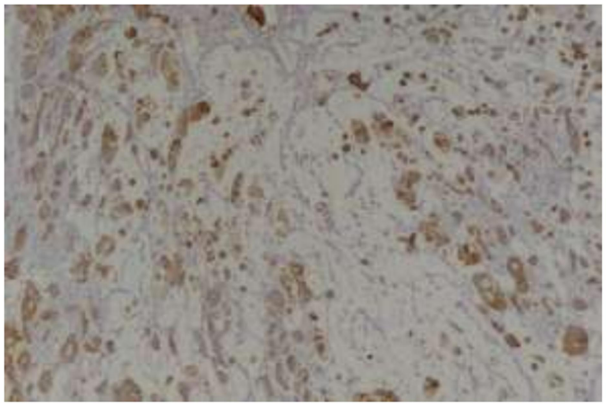


Figure 9: IHC for CK7 showing membranous/cytoplasmic positivity, 100X

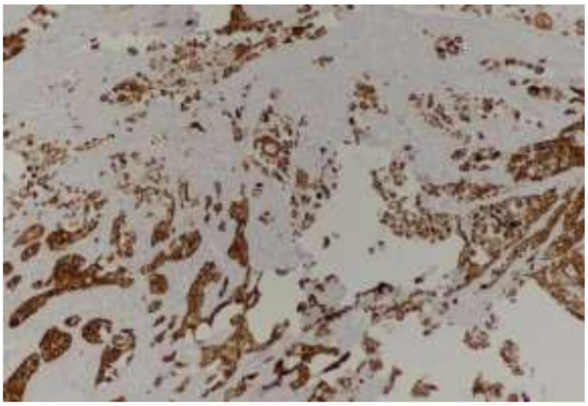


Figure 10: IHC for Cytokeratin 19 (CK19) showing intense membranous and cytoplasmic positivity, 100X

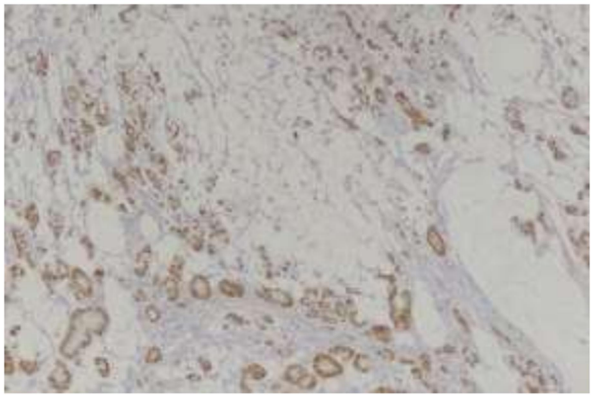


Figure 11: IHC for CDX2 showing nuclear positivity, 100X

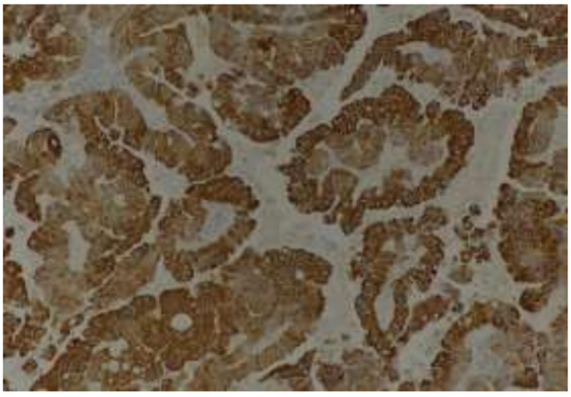


Figure 12: CK20 cytoplasmic positivity in tumour cells, 100X.

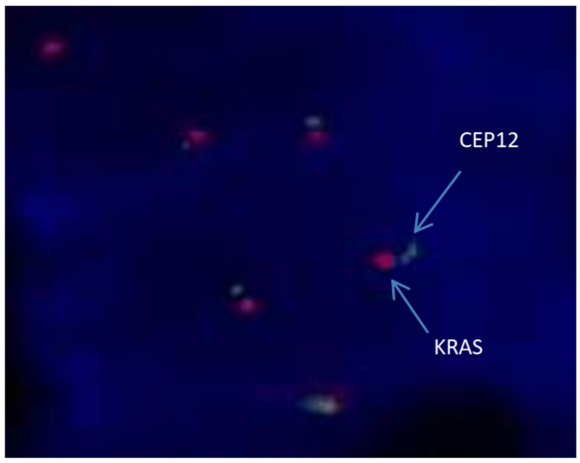


Figure 13: FISH for KRAS showing KRAS/CEP12 ratio of < 2. KRAS amplification absent.

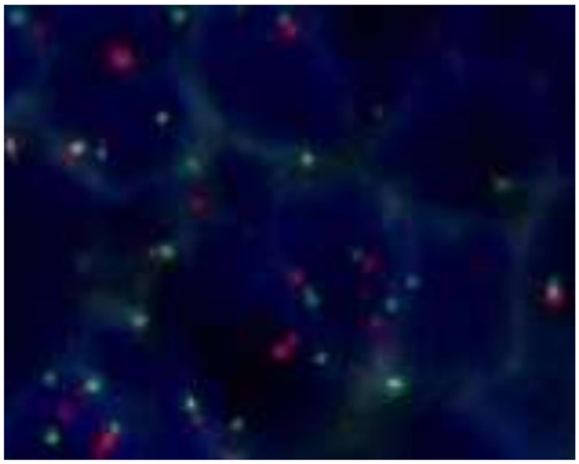


Figure 14: FISH for KRAS showing KRAS/CEP12 ratio of ≥ 2. KRAS amplification present.

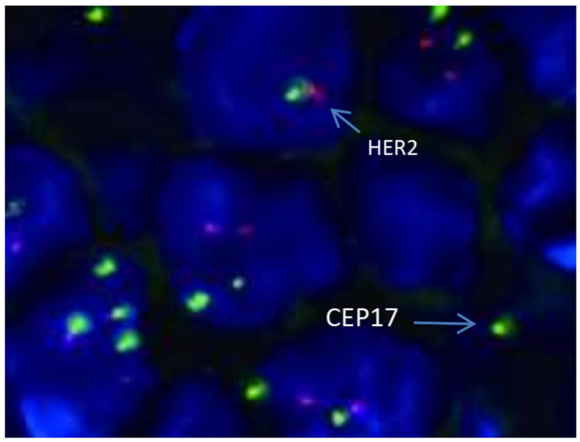


Figure 15: FISH for HER2 showing HER2/CEP17 ratio < 2.0. HER2 amplification absent.

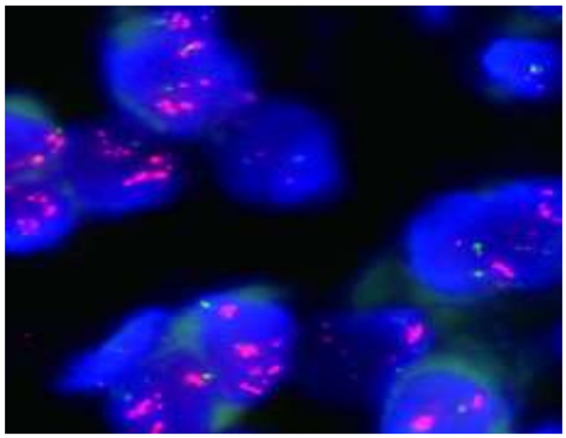


Figure 16: FISH for HER2 showing HER2/CEP17 ratio ≥ 2.0. HER2 amplification present.

5. Results

This study was undertaken in the Department of Surgical Gastroenterology at AIIMS, Jodhpur, from January 2020 to September 2021. A total of 30 patients gave consent and were included in the study. All the patients underwent pancreatoduodenectomy for periampullary carcinoma. Genetic mutational analysis of KRAS and HER2 mutation was done on post-operative specimens via the FISH technique.

(A) Demographic data of study population

Out of the 30 patients, 13 (43.3%) were females, and the female to male ratio was 0.76:1. The median age for the study population was 57.5 (37-83 years).

Table 1: Age distribution in the study population.

Age group (years)	Total number of patients (n)	Females (n)	Males (n)
31-40	1 (3.3%)	0	1 (5.8%)
41-50	8 (26.4%)	3 (23.1%)	5 (29.4%)
51-60	8 (26.6%)	3 (23.1%)	5 (29.4%)
61-70	10 (33.3%)	7 (53.8%)	3 (17.6%)
71-80	2 (6.6%)	0	2 (11.7%)
81-90	1 (3.3)	0	1 (5.8%)

Figure 17: Age distribution in the study population.

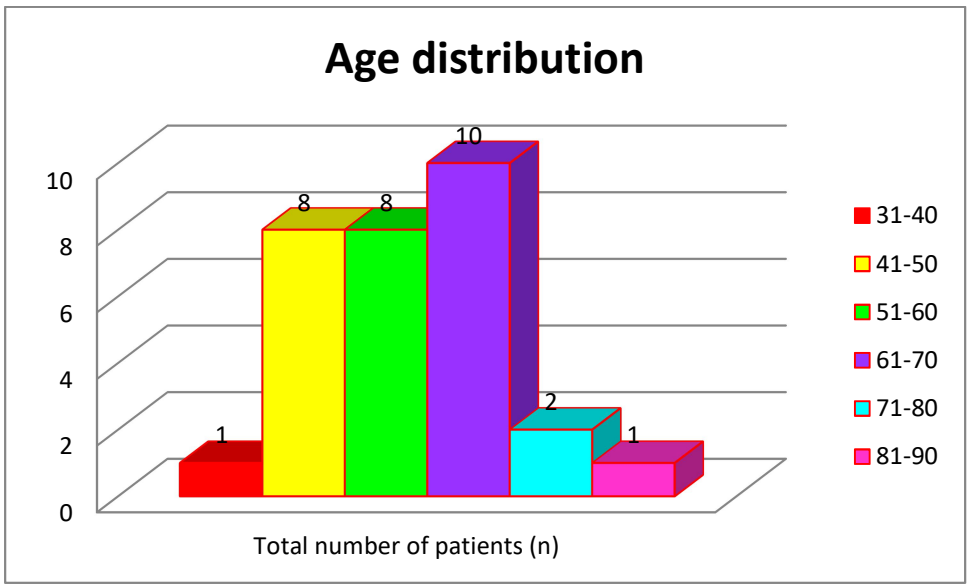
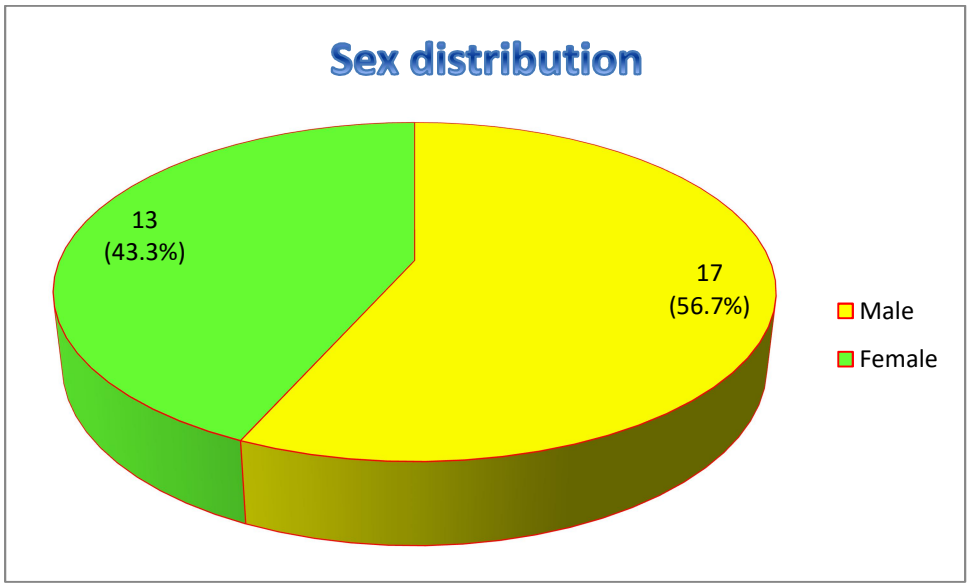


Figure 18: Sex distribution in the study population.



(B) Preoperative demographic profile of study population.

A total of 30 patients of PAC were included in the study. In the study population, jaundice (14/30, 46.7%) was the most common presenting complaint, followed by pain abdomen (30%) and cholangitis (23.3%). The median duration of the complaints was one month (0.4-12 months. Among the associated symptoms, 8 (26.7%) patients had nausea and vomiting, 3 (10%) had GI bleed. At the same time, 20 (66.6%) and 22 (73.3%) patients lost appetite and significant weight loss, respectively.

Out of 30 patients, 9 (30%) patients had co-morbidities. Nine (30%) had type 2 DM and hypertension each among these patients. The median weight of the study population was 55.07 (34-80) kgs, and the median BMI of the study population was 21 (14.7-26.6) kgs/m^2.

Out of the 30 patients, 19 (63.3%) patients had ECOG performance status (ECOG-PS) 1, followed by 8 (26.7%) patients who had ECOG-PS 0, 2 (6.7%) patients had ECOG-PS 3, and 1 (3.3%) patient had ECOG-PS 2.

Laboratory investigations: Median hemoglobin of study population was 11.0 (7.3-14.9) gm% whereas median total leukocyte count was 8.5 (4.2-18.9) x 103 cells/cumm. In liver function tests, median total bilirubin, direct bilirubin and alkaline phosphatase levels were 3.0 (0.3-14.6) mg%, 1.5 (0.06-9) mg%, 397 (45-1031) IU/L respectively. Median total protein and albumin were 7.0 (4.3-7.6) gm% and 3.0 (1.9-6.0) gm% respectively.

Among tumor markers, pre-operative CA 19-9 and CEA levels were 35.5 (0.8-2000) U/ml and 2.0 (0.2-29.4) ng/ml respectively.

For pre-operative imaging assessment, all patients underwent pancreatic protocol CT. The median maximum tumor size was 2.64mm. The median maximum diameter of CBD and PD were 16.1 mm and 5.6 mm, respectively. Double duct sign was present in 19 (63.3%) patients. Lymph node involvement was present in 16 (53.3%) patients. (Table 2)

Table 2: Pre-operative demographic profile of study population.

	N (%)
Chief complains	
a. Jaundice	14 (46.7%)
b. Abdominal pain	9 (30%)
c. Cholangitis	7 (23.3%)
Duration of chief complain (median, Range) (months)	1 (0.4-12)
+ Nausea and vomiting	8 (26.7%)
+ GI bleed	3 (10%)
+ Loss of appetite	20 (66.6%)
+ Significant loss of weight	22 (73.3%)
Co-morbidities	9 (30%)
a. Diabetes mellitus type 2	9 (30%)
b. Hypertension	9 (30%)
Weight (median, Range), (kgs)	55.07 (34-80)
BMI (median, Range), (kgs/m^2)	21 (14.7-26.6)
ECOG performance status	
0	8 (26.7%)
1	19 (63.3%)
2	1 (3.3%)
3	2 (6.7%)
Laboratory findings (median, Range)	
Hemoglobin (gm %)	11 (7.3-14.9)
Total leukocyte count (x 10^3 cells/ cumm)	8.5 (4.2-18.9)
Total bilirubin (Pre-operative) (mg %)	3 (0.3-14.6)
Direct bilirubin (Pre-operative) (mg %)	1.5 (0.06-9)
Alkaline phosphatase (IU/L)	397 (45-1031)
Total protein (gm %)	7.0 (4.3-7.6)
Albumin (gm%)	3.0 (1.9-6.0)
CA 19-9 (U/ml)	35.5 (0.8-2000)
CEA (ng/ml)	2.0 (0.2-29.4)
Imaging findings	
Maximum tumor size (median, range) (mm)	2.64 (1-5)
CBD diameter (median, range) (mm)	16.1 (6-31)
PD diameter (median, range) (mm)	5.6 (1-16)
Presence of double duct sign	19 (63.3%)
Lymph node involvement	16 (53.3%)

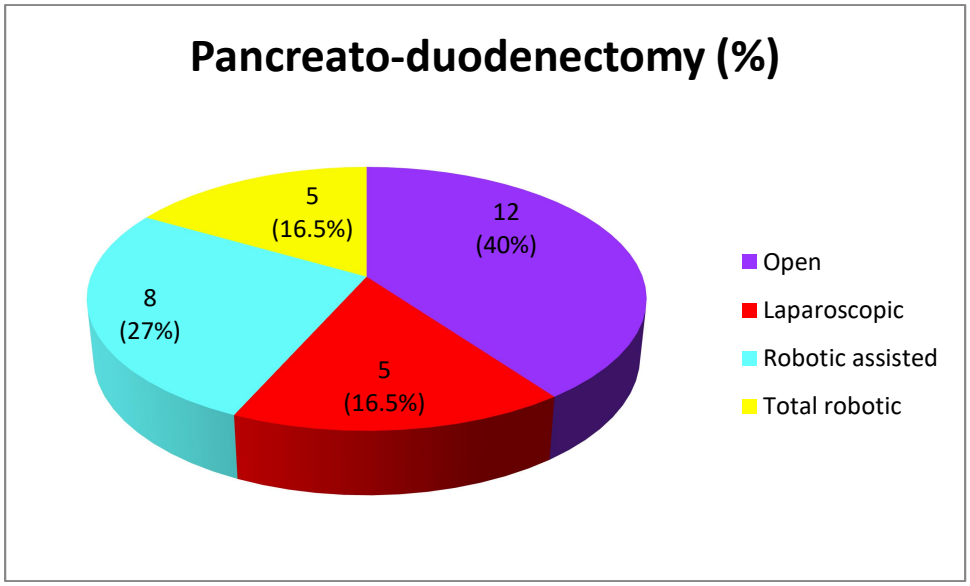
(C) Methods of pancreatoduodenectomy performed in the study population.

All 30 patients underwent pancreatoduodenectomy (PD) as a standard of care surgical management for PAC. Open PD (12/30, 40%) was the most common method in the study population, followed by Robotic-assisted (8/30, 27%), total robotic (5/30, 16.5%), and laparoscopic PD (5/30, 16.5%), respectively. (Table 3)

Table 3: Pancreatoduodenectomy methods performed study population.

Method	n (%)
Open	12 (40%)
Laparoscopic	5 (16.5%)
Robotic-assisted	8 (27%)
Total robotic	5 (16.5%)

Figure 19: Pancreatoduodenectomy methods performed study population.



(C) Histopathological outcomes of the study population.

All PD specimens were assessed as per standard protocol after H&E staining. 28 (23.4%) patients had firm masses on gross examination, whereas 1 (3.3%) patients had ulcero-proliferative and polypoidal mass each as gross appearance, respectively. Most common primary tumor site was ampulla (17/30, 53.1%) followed by Distal bile duct (5/30, 15.6%), Pancreas (4/30, 15.5%) and duodenum (4/30, 15.5%). Twenty-six (87.5%) patients had moderately differentiated tumors, whereas 3 (10%) and 1 (3.3%) patients had well and poorly differentiated tumors, respectively.

Out of 30 patients, two patients had pathological T1 stage, whereas 14 patients had pT2 and pT3 each. None of the patients had the pT4 stage. Median maximum tumor size was 2.0 cm (0.8-4.6 cm). Out of 30 patients, 16 (53.4%) patients had no pathological lymph nodes involvement (pN0). pN1 involvement was present in 9 (30%) patients, whereas pN2 involvement was 5 (16.6%). The median lymph node ratio was 20 (5.26-45)%. Fourteen (43.8%) patients had perineural invasion (PNI), whereas 12 (37.5%) patients had lymphovascular invasion (LVI). Nine (30%) patients had stage I disease, whereas 8 (26.7%) patients had stage II disease. 13 (43.3%) patients had stage III disease. None of the patients had stage IV disease. (Table 4)

Table 4: Histopathological parameters in the study population.

	N (%)
Tumor gross appearance	
Firm mass	28 (93.4%)
Ulcero-proliferative mass	1 (3.3%)
Polypoidal mass	1 (3.3%)
Primary tumor site	
Pancreas	4 (12.5%)
Duodenum	4 (12.5%)
Ampullary	17 (53.1%)
Distal bile duct	5 (15.6%)
Grade of tumor	
Well-differentiated	3 (10%)
Moderately differentiated	26 (86.7%)
Poor differentiated	1 (3.3%)
pT stage	
T1	2 (6.4%)
T2	14 (46.8%)
T3	14 (46.8%)
T4	0
Maximum tumor size (median, range), (cm)	2.0 (0.8-4.6)
Lymph nodal stage	
pN0	16 (53.4%)
pN1	9 (30%)
pN2	5 (16.6%)
Lymph node ratio (%)	20 (5.26-45)
Perineural invasion	14 (43.8%)
Lympho-vascular invasion	12 (37.5%)
Overall stage	
I	9 (30%)
II	8 (26.7%)
III	13 (43.3%)
IV	0

(II) Analysis of morphological subtypes differentiation in periampullary carcinoma.

IHC markers were used to validate the differentiation of morphological subtypes in PACs.

Among 21 patients with PB subtypes morphology, 20 (95.2%) and 1 (4.8%) patients had MUC1 present and MUC1 absent, respectively. MUC2 was present in 1 (4.8%) patients and absent in 20 (95.2%) patients. CK7 and CK20 markers were present in 20 (95.2%)) and 5 (23.8%), whereas these markers were absent in 1 (4.8%) and 16 (76.2%), respectively. CDX2 and CK19 markers were present in 8 (38.1%) and 11 (52.4%), whereas these markers were absent in 13 (61.9%) and 10 (47.6%), respectively. (Table 5, 6)

Table 5: IHC differentiation in pancreato-biliary morphological subtype (n=21).

	Present	Absent
MUC1	20 (95.2%)	1 (4.8%)
MUC2	1 (4.8%)	20 (95.2%)
CK7	20 (95.2%)	1 (4.8%)
CK20	5 (23.8%)	16 (76.2%)
CDX2	8 (38.1%)	13 (61.9%)
CK19	11 (52.4%)	10 (47.6%)

Table 6: IHC differentiation in Intestinal morphological subtype.(n=9)

	Present	Absent
MUC1	8 (88.9%)	1 (11.1%)
MUC2	7 (77.8%)	2 (22.2%)
CK7	7 (77.8%)	2 (22.2%)
CK20	4 (44.4%)	5 (55.6%)
CDX2	7 (77.8%)	2 (22.2%)
CK19	7 (77.8%)	2 (22.2%)

Figure20: IHC differentiation in pancreato-biliary morphological subtype (n=21).

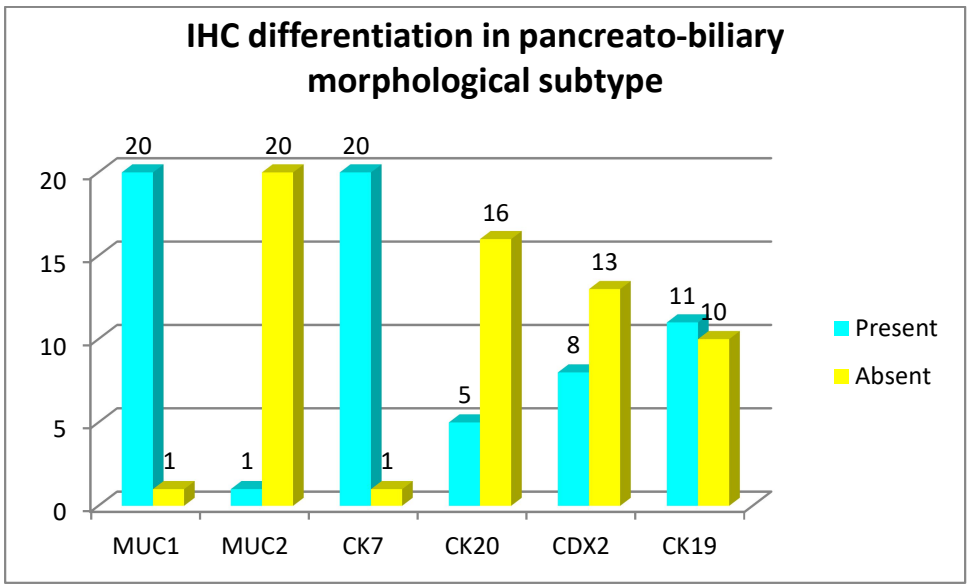
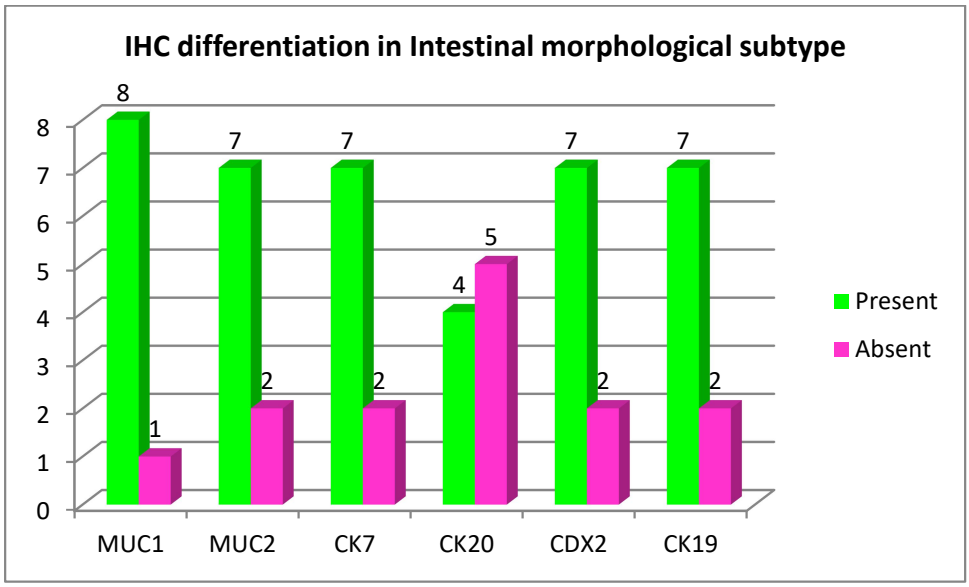


Figure 21: IHC differentiation in Intestinal morphological subtype.(n=9)



(III) KRAS and HER2 mutation analysis and their clinico-pathological correlation.

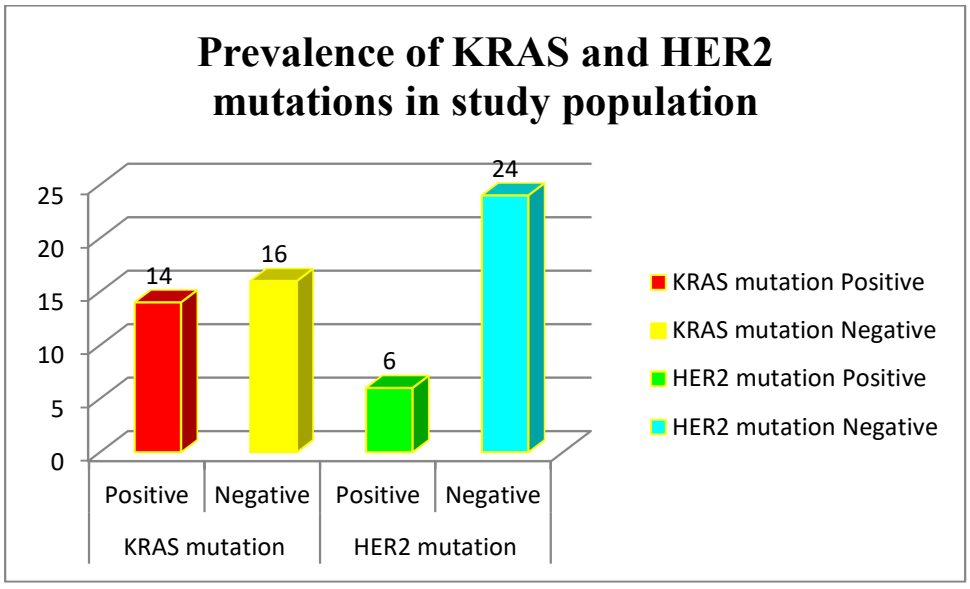
(A) Prevalence of KRAS and HER2 mutations in Periampullary carcinomas:

Out of 30 patients, 14 (46.66%) patients were KRAS mutation-positive, whereas 16 (53.33%) patients were KRAS mutation-negative. 6 (20%) patients were HER2 mutation-positive, whereas 24 (80%) were HER2 mutation-negative. (Table 7)

Table 7: Prevalence of KRAS and HER2 mutations in PACs in the study population.

		N (%)
KRAS mutation	Positive	14 (46.66%)
	Negative	16 (53.33%)
HER2 mutation	Positive	6 (20%)
	Negative	24 (80%)

Figure 22: Prevalence of KRAS and HER2 mutations in PACs in the study population



(B) Demographic profile.

Out of 14 KRAS-positive patients, 6 (35.3%) were male, and 8 (61.5%) were female. However, 11 (64.7%) were male among KRAS negative patients, and 5 (38.5%) were females. The median age of KRAS-positive patients was 58.5 years (37-75 years), whereas the median age of KRAS-negative patients was 56.5 years (41-83 years).

Of 6 HER2-positive patients, 5 (29.4%) were male, and 1 (7.7%) were female. Among HER2 negative patients, 12 (70.7%) were male, and 12 (92.3%) were females. The median age of HER2-positive patients was 50.5 years (41-60 years), whereas the median age of HER2-negative patients was 61.5 years (37-85 years).

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with sex distribution as depicted below: Table 8)

Table 8: Co-relation between KRAS and HER2 mutation with sex distribution.

		Median age (years)	Male	Female	p- value
KRAS mutation	Positive (n= 14)	58.5 (37-75)	6 (42.8%)	8 (57.2%)	0.15
	Negative (n= 16)	56.5 (41-83)	11 (68.7%)	5 (31.3%)	
HER2 mutation	Positive (n= 6)	50.5 (41-60)	5 (83.3%)	1 (16.7%)	0.14
	Negative (n= 24)	61.5 (37-85)	12 (50%)	12 (50%)	

Figure 23: Correlation between KRAS and HER2 mutation with sex distribution.

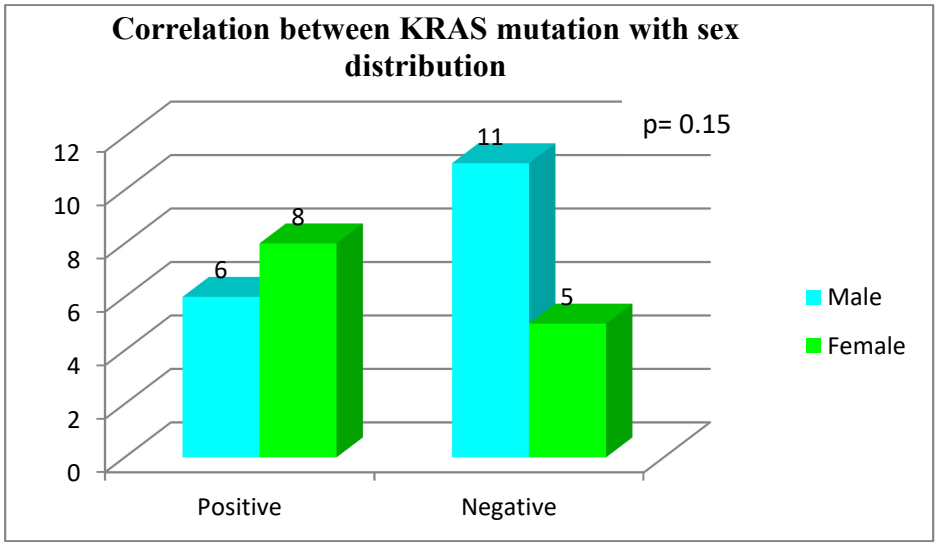
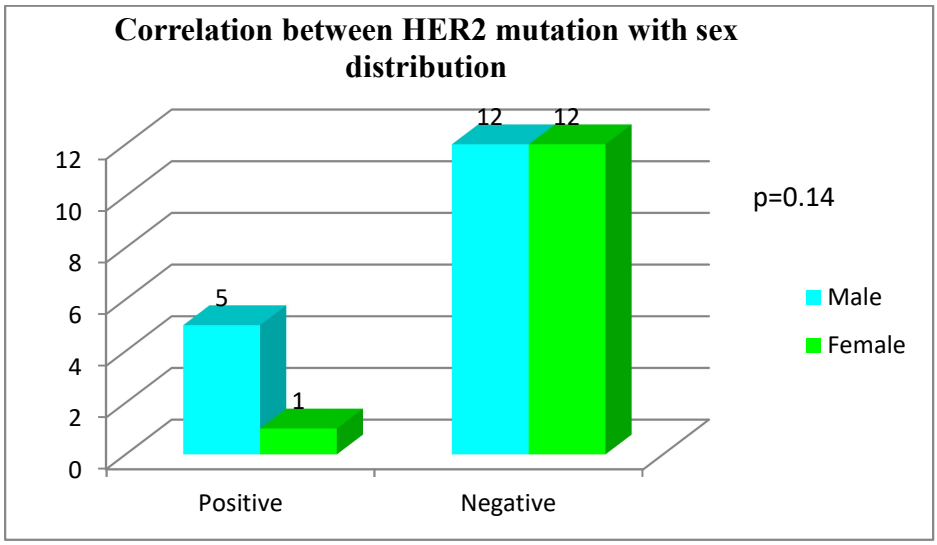


Figure 24: Correlation between HER2 mutation with sex distribution.



(C) CA 19-9 levels.

Among 30 patients, 16 (53.3%) patients had normal CA 19-9 levels (≤37 U/ml) whereas 14 (46.7%) patients had high CA 19-9 levels (>37U/ml). Out of the 14 KRAS-positive patients, normal CA 19-9 levels were present in 7 (50%) patients, whereas 7 (50%) had high CA 19-9 levels. Of the 16 KRAS negative patients, 9 (56.2%) patients had normal CA 19-9 levels, whereas 7 (43.8%) patients had high CA 19-9 levels.

Out of the 6 HER2 positive patients, normal CA 19-9 levels were present in 3 (50%) patients, whereas 3 (50%) had high CA 19-9 levels. Out of the 24 HER2 negative patients, 13 (54.2%) patients had normal CA 19-9 levels, whereas 11 (45.8%) patients had high CA 19-9 levels.

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with CA 19-9 levels as depicted below: (Table 9)

Table 9: Correlation between KRAS and HER2 mutation with CA 19-9 levels.

		CA 19-9		p value
		Normal (<37 U/ml)	High (>37U/ml).	
KRAS mutation	Positive (n= 14)	7 (50%)	7 (50%)	0.73
	Negative (n= 16)	9 (56.2%)	7 (43.8%)	
HER2 mutation	Positive (n= 6)	3 (50%)	3 (50%)	0.85
	Negative (n= 24)	13 (54.2%)	11 (45.8%)	

Figure 25: Correlation between KRAS mutation with CA 19-9 levels.

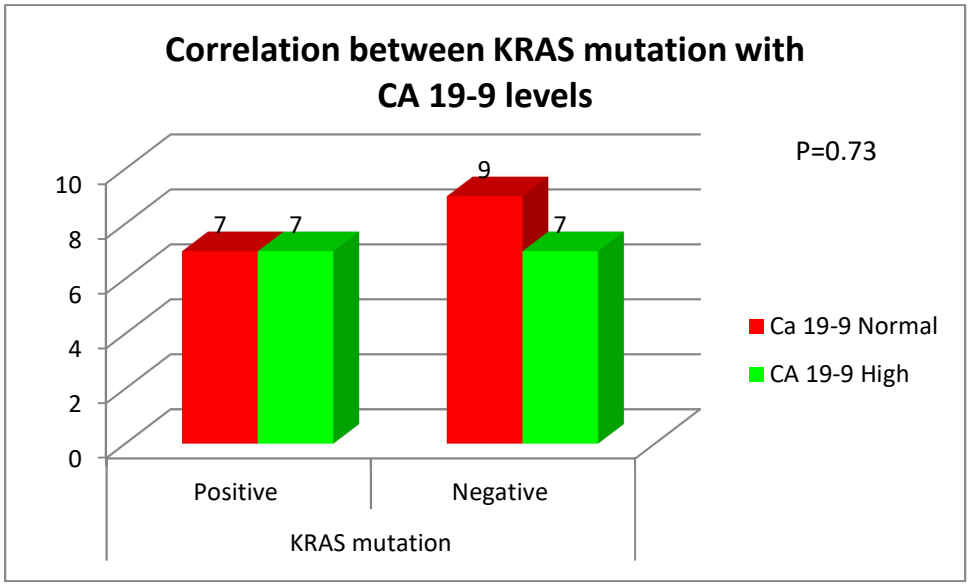
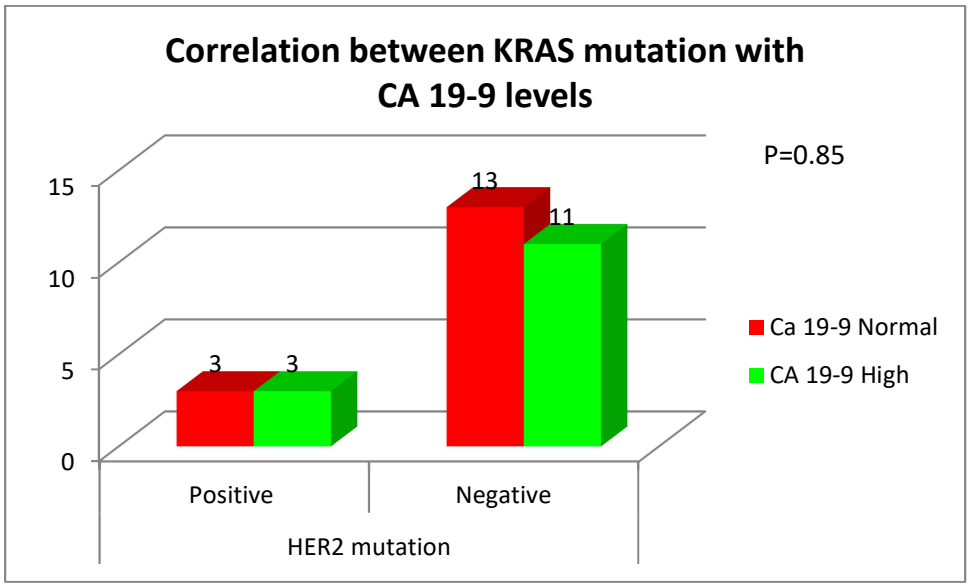


Figure 26: **Correlation between HER2 mutation with CA 19-9 levels.**



(D) CEA levels

Among 30 patients, 27 (90%) patients had normal CEA levels (≤ 5 ng/ml) whereas 3 (10%) patients had high CEA levels (> 5 ng/ml). Out of the 14 KRAS-positive patients, normal CEA levels were present in 12 (85.7%) patients, whereas 2 (14.3%) had high CEA levels. Out of the 16 KRAS negative patients, 15 (93.7%) patients had normal CA 19-9 levels, whereas 1 (6.3%) patients had CEA levels.

Out of the 6 HER2-positive patients, normal CEA levels were present in 4 (66.7%) patients, whereas 2 (33.3%) had high CEA levels. Out of the 24 HER2 negative patients, 23 (95.8%) patients had normal CEA levels, whereas 1 (4.2%) patients had high CEA levels. There was no statically significant co-relation between KRAS mutation on FISH with CEA levels. However, there was a statically significant correlation between HER2 mutation on FISH with CEA levels as depicted below: (Table 10)

Table 10: Correlation between KRAS and HER2 mutation with CEA levels.

		CEA level		p-value
		Normal	High	
KRAS mutation	Positive (n= 14)	12 (85.7%)	2 (14.3%)	0.46
	Negative (n= 16)	15 (93.7%)	1 (6.3%)	
HER2 mutation	Positive (n= 6)	4 (66.7%)	2 (33.3%)	0.03
	Negative (n= 24)	23 (95.8%)	1 (4.2%)	

Figure 27: Correlation between KRAS mutation with CEA levels.

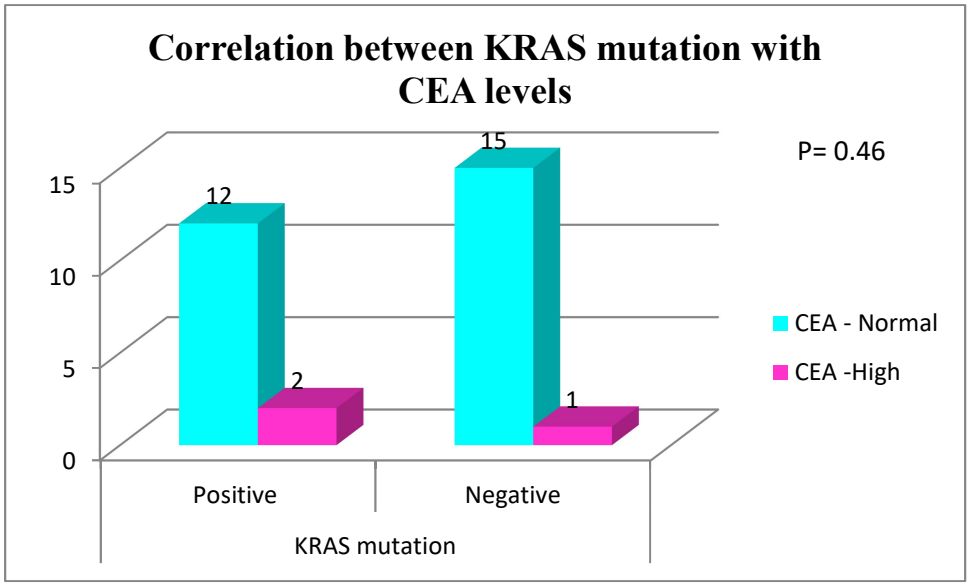
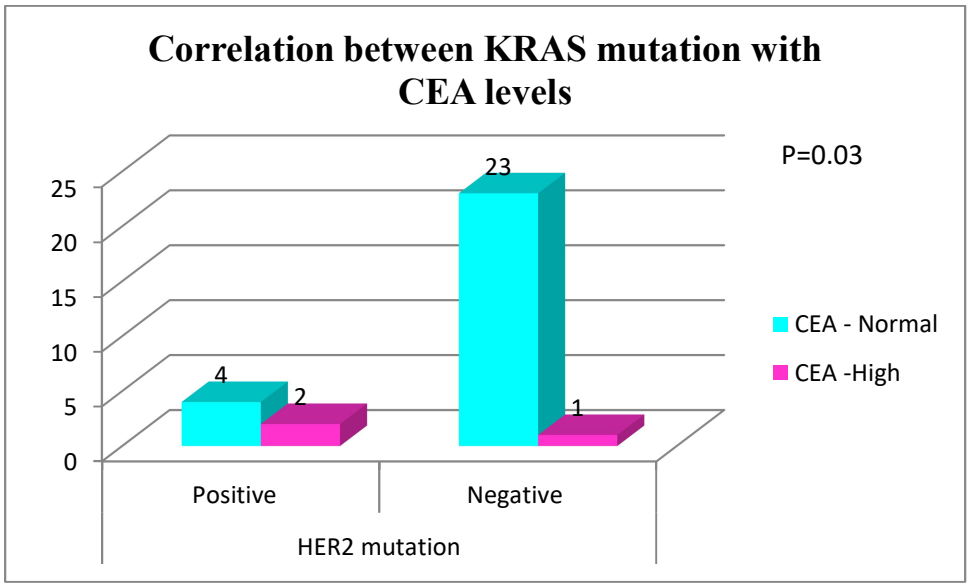


Figure 28: Correlation between HER2 mutation with CEA levels.



(E) Primary site of tumor origin.

Out of 14 KRAS-positive patients, the primary site of tumor origin was pancreas in 4 (28.5%) patients, duodenum in 1 (7.1%), ampulla in 6 (42.9%), and distal CBD in 3 (21.4%) patients. Among 16 KRAS negative patients, the primary site of the tumor was duodenum in 3 (18.7%), ampulla in 11(68.8%), and distal CBD in 2 (12.5%).

Out of 2 HER2 positive patients, the primary site of tumor origin was pancreas in 1 (16.7%) patients, duodenum in 1 (16.7%), and ampulla in 4 (66.6%) patients. Among 24 HER2 negative patients, the primary site of the tumor was the pancreas in 3 (12.5%), duodenum in 3 (12.5%), ampulla in 13(54.2%), and distal CBD in 5 (20.8%).

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with the primary site of tumor origin as depicted below: (Table 11)

Table 11: Correlation between KRAS and HER2 mutations with the primary site of tumor origin.

		Pancreas	Duodenum	Ampullary	Distal CBD	p-value
KRAS mutation	Positive (n= 14)	4 (28.5%)	1 (7.1%)	6 (42.9%)	3 (21.4%)	0.08
	Negative (n= 16)	0	3 (18.7%)	11 (68.8%)	2 (12.5%)	
HER2 mutation	Positive (n= 6)	1 (16.7%)	1 (16.7%)	4 (66.6%)	0	0.68
	Negative (n= 24)	3 (12.5%)	3 (12.5%)	13 (54.2%)	5 (20.8%)	

Figure 29: Correlation between KRAS mutation with Primary site of tumor origin.

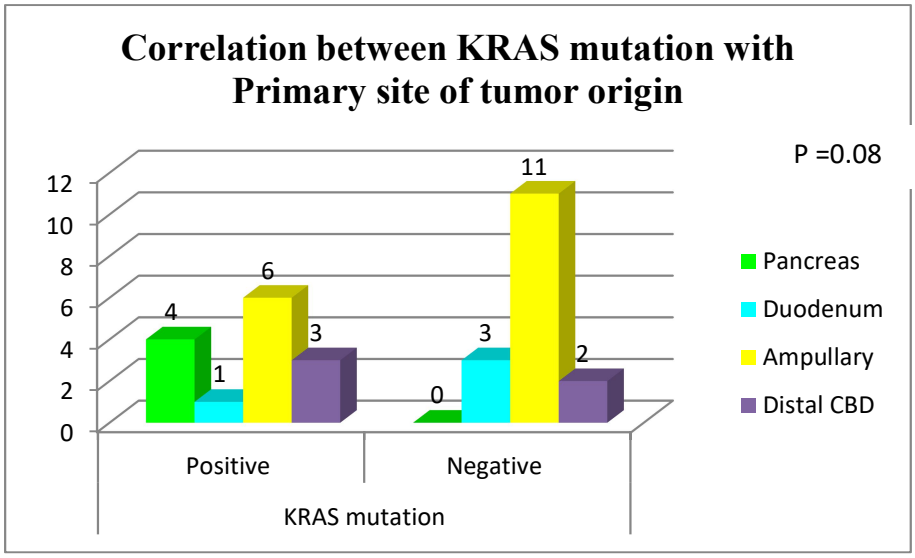
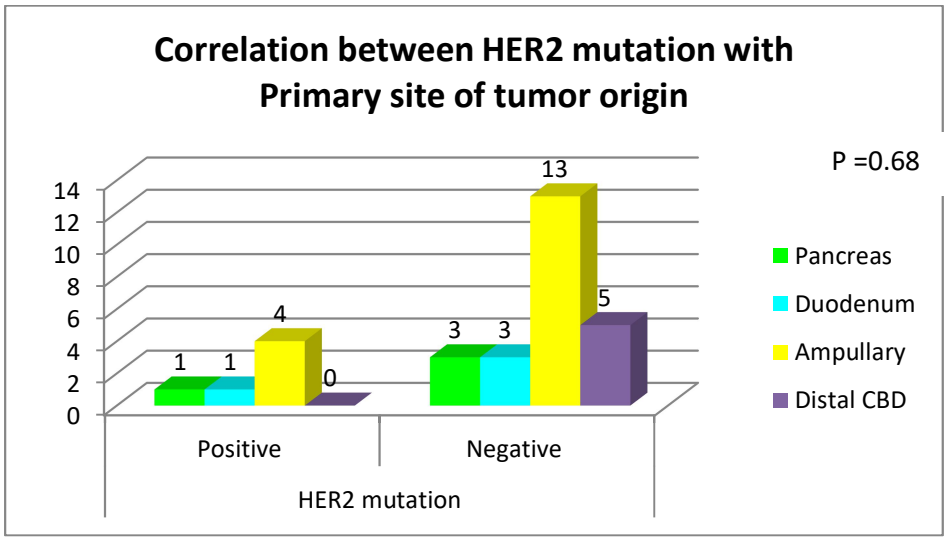


Figure 30: Correlation between HER2 mutation with Primary site of tumor origin.



(F) Morphological subtypes.

Among 14 KRAS-positive patients, 11 (78.6%) patients had PB morphological subtype, whereas 3 (21.4%) patients had I morphological subtype. Out of 16 KRAS negative patients, 10 (62.5%) patients had PB morphological subtype, whereas 6 (37.5%) patients had I morphological subtype.

Of 6 HER2-positive patients, 4 (66.7%) patients had PB morphological subtype, whereas 3 (33.3%) patients had I morphological subtype. Out of 24, KRAS negative patients, 17 (70.8%) patients had PB morphological subtype, whereas 7 (29.2%) patients had I morphological subtype.

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with morphological subtypes as depicted below: (Table 12)

Table 12: Correlation between KRAS and HER2 mutation with morphological subtypes.

		Pancreato-biliary	Intestinal	p-value
KRAS mutation	Positive (n= 14)	11 (78.6%)	3 (21.4%)	0.34
	Negative (n= 16)	10 (62.5%)	6 (37.5%)	
HER2 mutation	Positive (n= 6)	4 (66.7%)	2 (33.3%)	0.84
	Negative (n= 24)	17 (70.8 %)	7 (29.2%)	

Figure 31: Correlation between KRAS mutation with morphological subtypes

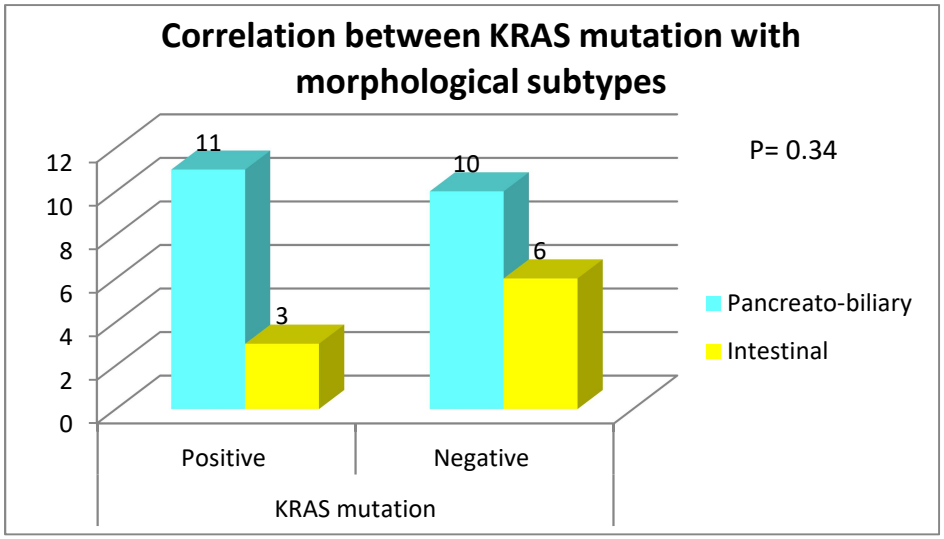
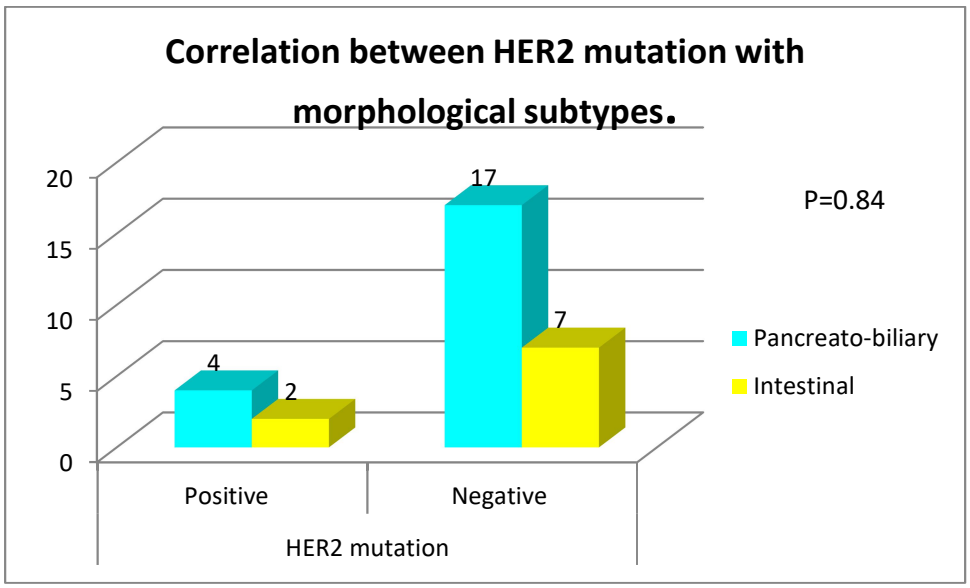


Figure 32: Correlation between HER2 mutation with morphological subtypes.



(G) Grade of the tumor.

All of the 14 KRAS-positive patients had a moderately differentiated tumor. Among 16 KRAS negative patients, three patients had well-differentiated tumors, whereas 12 (75%) and 1 (6.3%) patients had moderately and poorly differentiated tumors.

Out of 6 HER2-positive patients, 2 (33.3%) patients had well-differentiated tumors, and 4 (66.7%) patients had moderately differentiated tumors. Among 24 HER2 negative patients, 1 (4.2%) patient had a well-differentiated tumor, whereas 22 (91.2%) and 1 (4.2%) patients had moderately and poorly differentiated tumors, respectively.

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with tumor grade as depicted below: (Table 13)

Table 13: Correlation between KRAS and HER2 mutation with tumor grade.

		Grade of tumor			P-value
		Well	Moderate	Poor	
KRAS mutation	Positive (n= 14)	0	14 (100%)	0	0.13
	Negative (n= 16)	3 (18.7%)	12 (75%)	1 (6.3%)	
HER2 mutation	Positive (n= 6)	2 (33.3%)	4 (66.7%)	0	0.09
	Negative (n= 24)	1 (4.2%)	22 (91.6%)	1 (4.2%)	

Figure 33: Correlation between KRAS mutation with tumor grade.

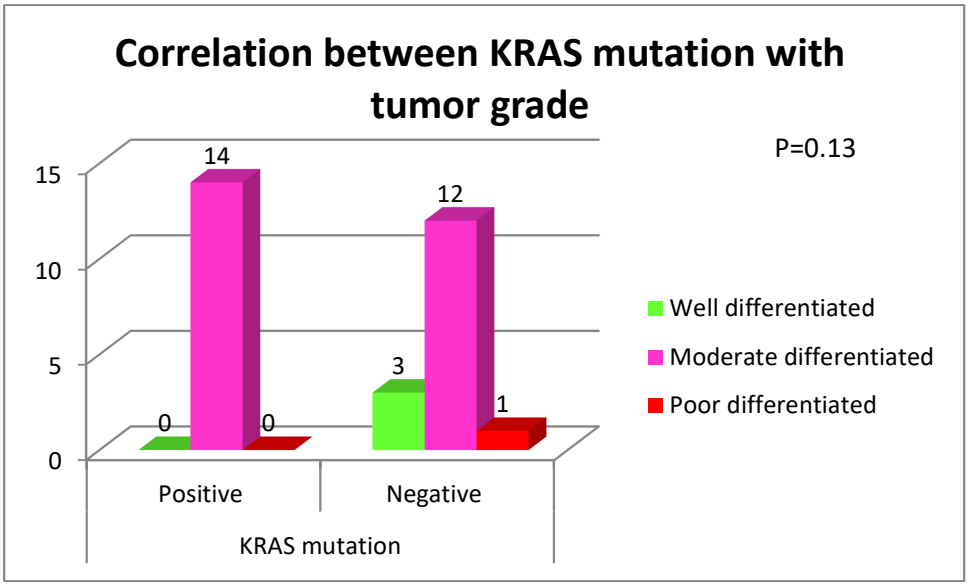
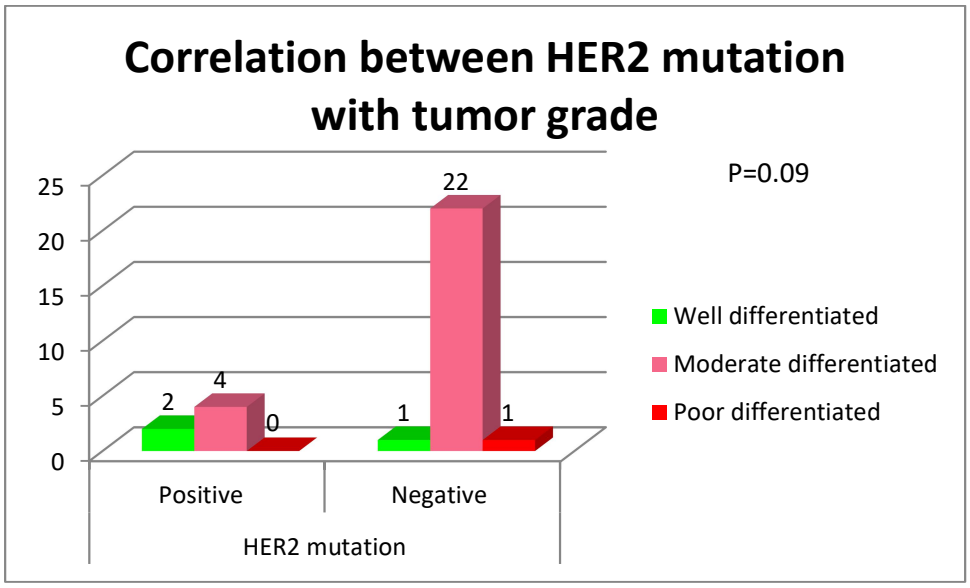


Figure 34: Correlation between HER2 mutation with tumor grade



(H) T stage

Out of 14 KRAS-positive patients, 2 (14.3%) patients had T1 tumor, whereas 7 (50%) and 5 (35.7%) patients had T2 and T3 tumor, respectively. None of KRAS positive patients had T4 tumor. Of the 16 KRAS negative patients, 7 (43.8%) and 9 (56.2%) patients had T2 and T3 tumor, respectively. None of the KRAS-negative patients had T1 and T4 tumor.

Of 6 HER2-positive patients, 2 (33.3%) and 4 (66.7%) patients had T2 and T3 tumor, respectively. None of the HER2-positive patients had T1 and T4 tumor. Out of the 24 HER2 negative patients, 2 (8.3%) patients had T1 tumor, whereas 12 (50%) and 10 (41.7%) patients had T2 and T3 tumor, respectively. None of the HER2-negative patients had T4 tumor.

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with T stage as depicted below: (Table 14)

Table 14: Correlation between KRAS and HER2 mutations with T stage.

		T stage				p-value
		T1	T2	T3	T4	
KRAS mutation	Positive (n= 14)	2 (14.3%)	7 (50%)	5 (35.7%)	0	0.22
	Negative (n= 16)	0	7 (43.8%)	9 (56.2%)	0	
HER2 mutation	Positive (n= 6)	0	2 (33.3%)	4 (66.7%)	0	0.5
	Negative (n= 24)	2 (8.3%)	12 (50%)	10 (41.7%)	0	

Figure35: Correlation between KRAS mutation with T stage.

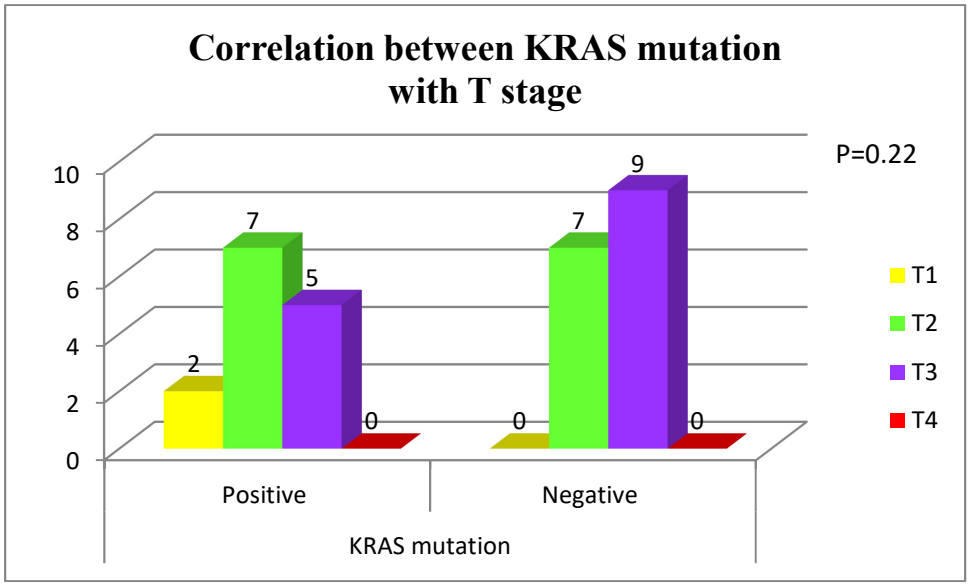
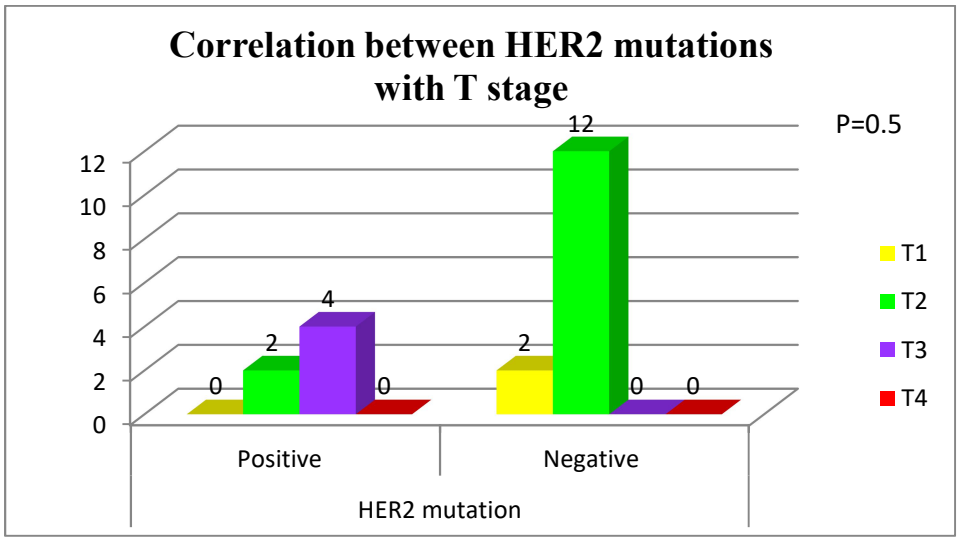


Figure 36: **Correlation between HER2 mutation with T stage.**



Among 30 patients, 16 (53.3%) patients had early T stages (T1/T2), whereas 14 (46.7%) patients had advanced T stages (T3/T4).

Out of the 14 KRAS-positive patients, early T stage were present in 9 (64.3%) patients, whereas 5 (35.7%) had advanced T stages. Out of the 16 KRAS negative patients, 7 (43.8%) patients had early T stages, whereas 9 (56.2%) patients had advanced T stages.

Out of the 6 HER2-positive patients, early T stage were present in 2 (33.3%) patients, whereas 4 (66.7%) had advanced T stages. Out of the 24 HER2 negative patients, 14 (58.3%) patients had early T stages, whereas 10 (41.7%) patients had advanced T stages.

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with early/advanced T stage as depicted below: (Table 15)

Table 15: Correlation between KRAS and HER2 mutations with early/advanced T stage.

		T stage		p-value
		T1/T2	T3/T4	
KRAS mutation	Positive (n= 14)	9 (64.3%)	5 (35.7%)	0.26
	Negative (n= 16)	7 (43.8%)	9 (56.2%)	
HER2 mutation	Positive (n= 6)	2 (33.3%)	4 (66.7%)	0.3
	Negative (n= 24)	14 (58.3%)	10 (41.6%)	

Figure 37: Correlation between KRAS mutation with early/advanced T stage

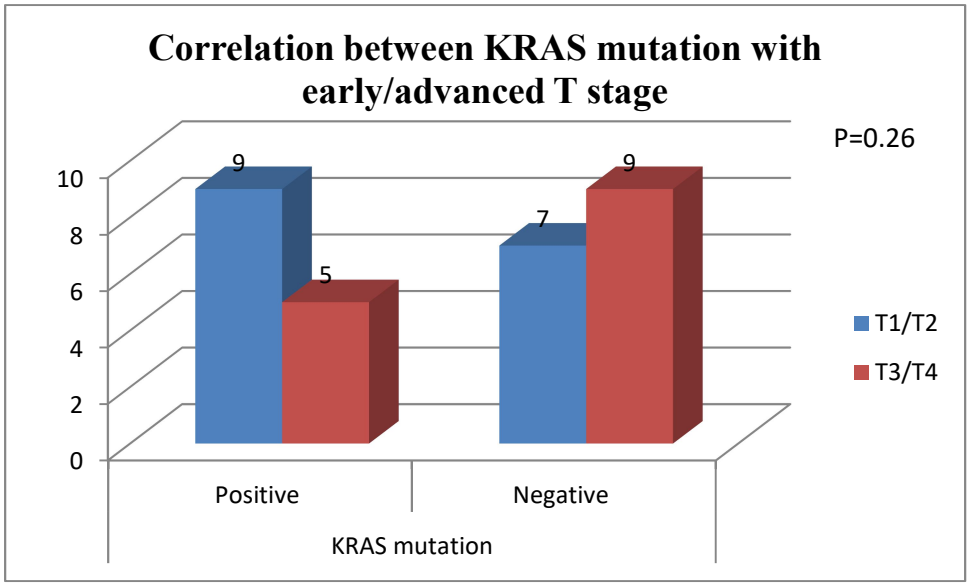
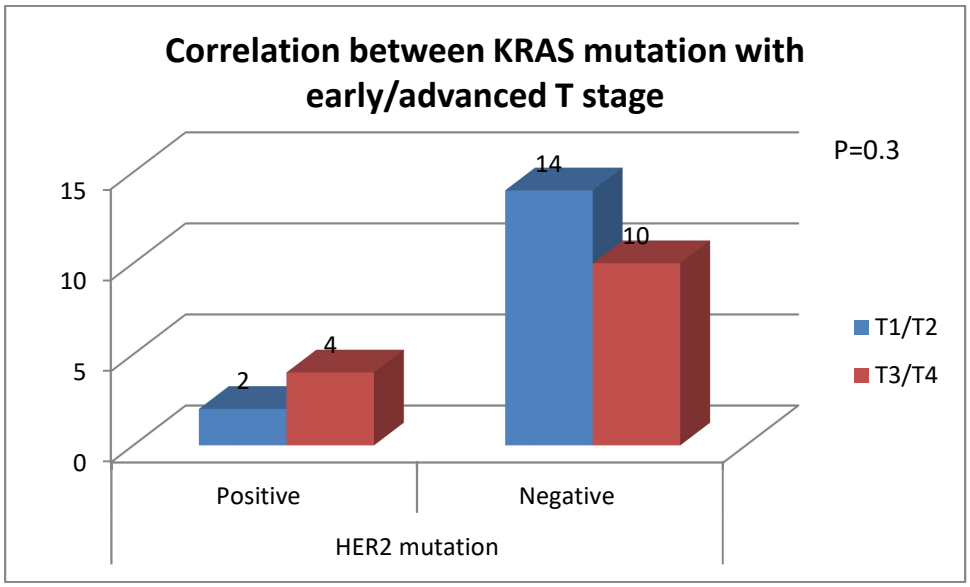


Figure 38: Correlation between HER2 mutation with early/advanced T stage



(I) Lymph node stage (N stage)

Among 30 patients, 16 (53.3%) patients had no lymph nodal involvement (N0), whereas 14 (46.7%) patients had lymph node involvement (N+).

Of the 14 KRAS-positive patients, 6 (42.8%) patients did not have lymph node involvement, whereas 8 (57.2%) had lymph node involvement present. Out of the 14 KRAS negative patients, 10 (62.5%) patients had no lymph node involvement, whereas 6 (42.9%) patients had lymph node involvement present.

Of the 6 HER2-positive patients, 2 (33.3%) patients had no lymph node involvement, whereas 4 (66.7%) had lymph nodal involvement present. Out of the 24 HER2 negative patients, 14 (58.3%) patients had no lymph node involvement, whereas 10 (41.7%) patients had lymph node involvement present.

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with lymph node involvement as depicted below: (Table 16)

Table 16: Correlation between KRAS and HER2 mutations with lymph node involvement.

		Lymph node involvement		p-value
		N0	N +	
KRAS mutation	Positive (n= 14)	6 (42.8%)	8 (57.2%)	0.36
	Negative (n= 16)	10 (62.5%)	6 (37.5%)	
HER2 mutation	Positive (n= 6)	2 (33.3%)	4 (66.7%)	0.24
	Negative (n= 24)	14 (58.3%)	10 (41.7%)	

Figure 39: Correlation between KRAS mutation with lymph node involvement.

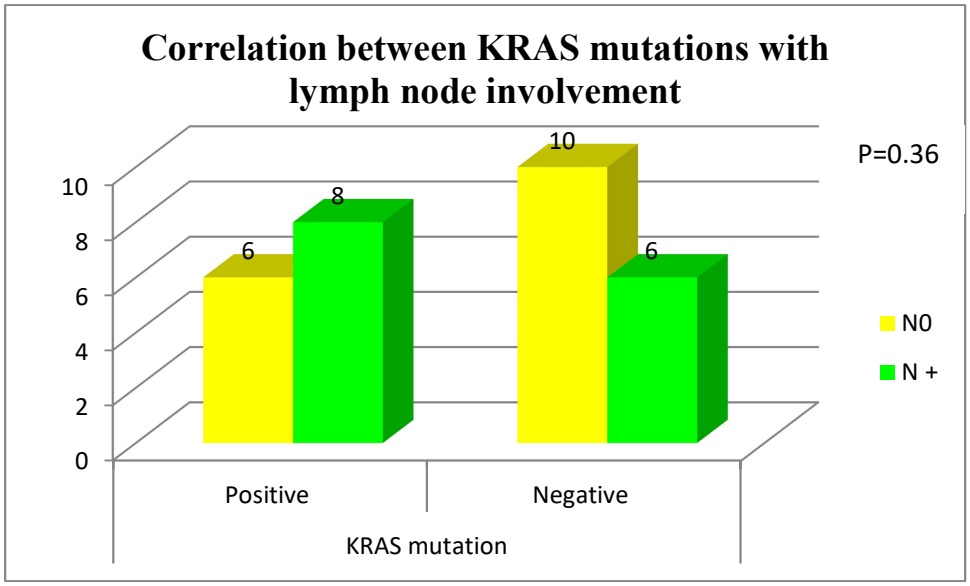
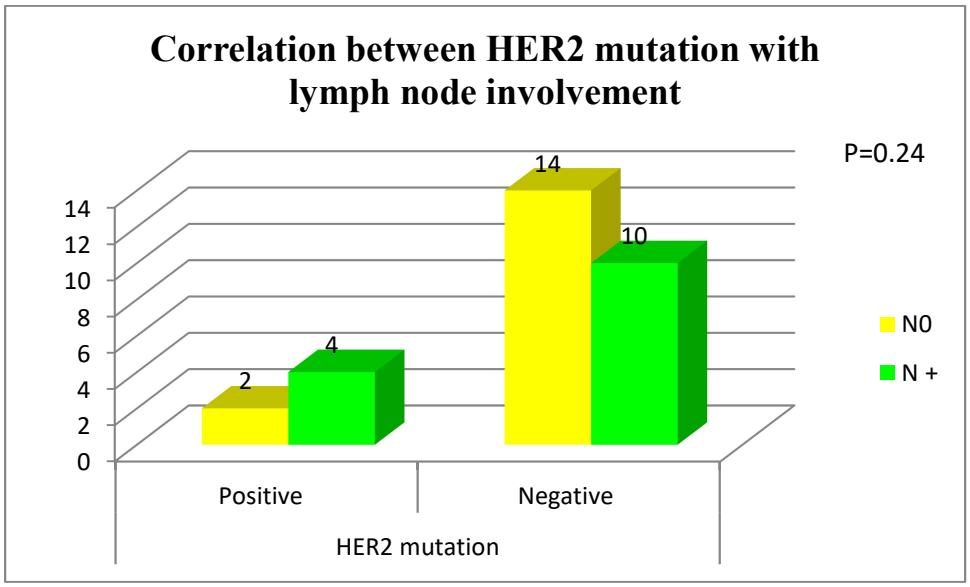


Figure 40: Correlation between HER2 mutation with lymph node involvement.



Of the 8 KRAS positive patients, 5 (62.5%) patients had N1 stage lymph node, whereas 3 (37.5%) had N2 stage lymph node. Out of the 5 KRAS negative patients, 3 (60%) patients had N1 stage lymph node, whereas 2 (40%) patients had N2 stage lymph node.

Of the 4 HER2-positive patients, 1 (25%) patients had N1 stage lymph node, whereas 3 (75%) had N2 lymph nodal involvement present. Out of the 9 HER2 negative patients, 7 (77.8%) patients had N1 stage lymph node, whereas 2 (22.2%) patients had N2 stage lymph node.

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with N+ stage as depicted below: (Table 17)

Table 17: Correlation between KRAS and HER2 mutations with N+ stage.

		N+ stage		p value
		N1	N2	
KRAS mutation	Positive (n= 8)	5 (62.5%)	3 (37.5%)	0.93
	Negative (n= 5)	3 (60%)	2 (40%)	
HER2 mutation	Positive (n= 4)	1 (25%)	3 (75%)	0.07
	Negative (n= 9)	7 (77.8%)	2 (22.2%)	

Figure 41: **Correlation between KRAS mutation with N+ stage.**

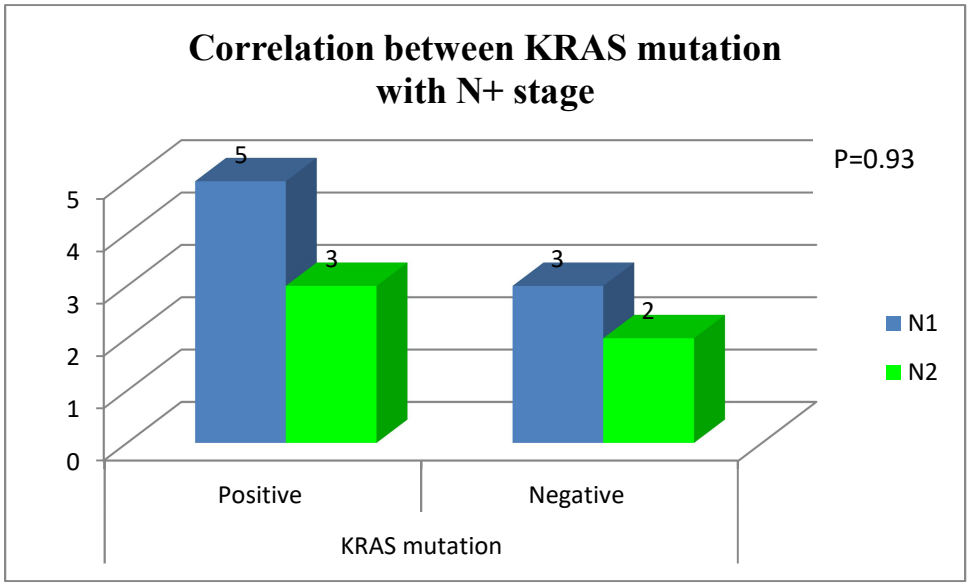
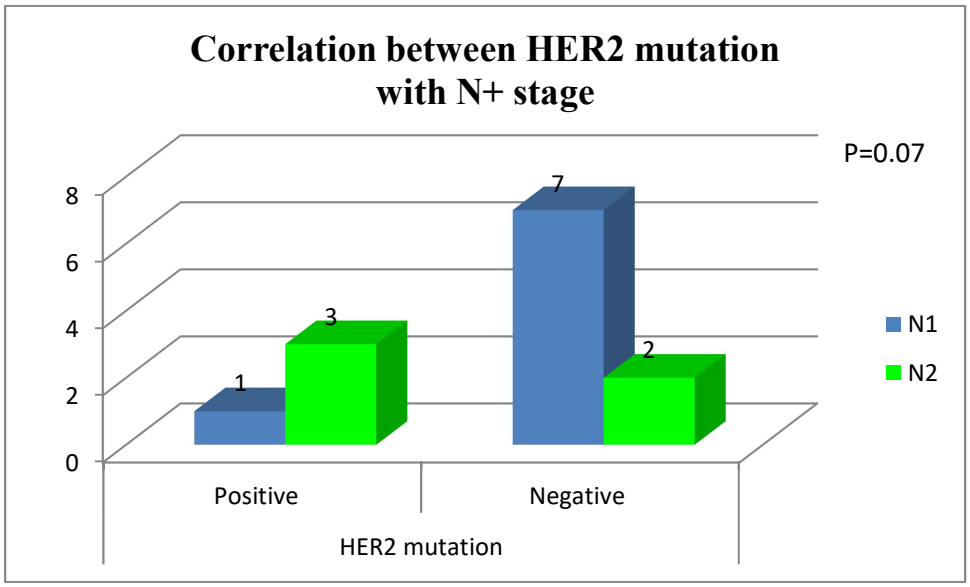


Figure 42: Correlation between HER2mutation with N+ stage.



(J) Perineural invasion (PNI)

Out of 14 KRAS mutation-positive patients, 7 (50%) and 7 (50%) patients had PNI involvement present and absent, respectively. Out of the 16, KRAS mutation-negative patients, 9 (56.2%) patients had PNI present, and 7 (43.8%) did not have PNI.

Out of 6 HER2 mutation-positive patients, 3 (50%) and 3 (50%) patients had PNI involvement present and absent, respectively. Out of the 24 HER2 mutation-negative patients, 11 (45.8%) patients had PNI present, and 13 (54.2%) did not have PNI.

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with PNI in the study population, as depicted below: (Table 18)

Table 18: Correlation between KRAS and HER2 mutation with perineural invasion.

		Perineural invasion		p-value
		Present	Absent	
KRAS mutation	Positive (n= 14)	7 (50%)	7 (50%)	0.73
	Negative (n= 16)	9 (56.2%)	7 (43.8%)	
HER2 mutation	Positive (n= 6)	3 (50%)	3 (50%)	0.85
	Negative (n= 24)	11 (45.8%)	13 (54.2%)	

Figure 43: Correlation between KRAS mutation with perineural invasion.

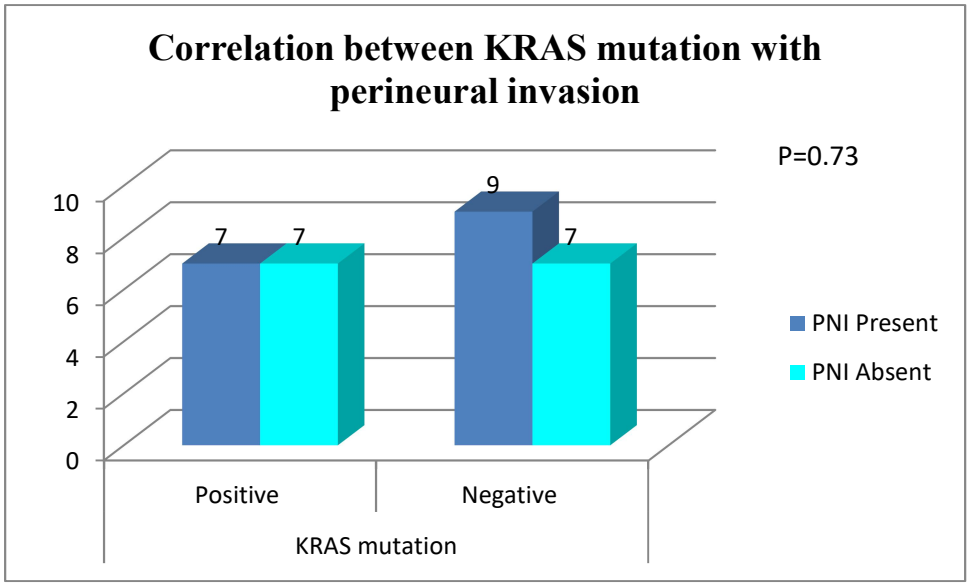
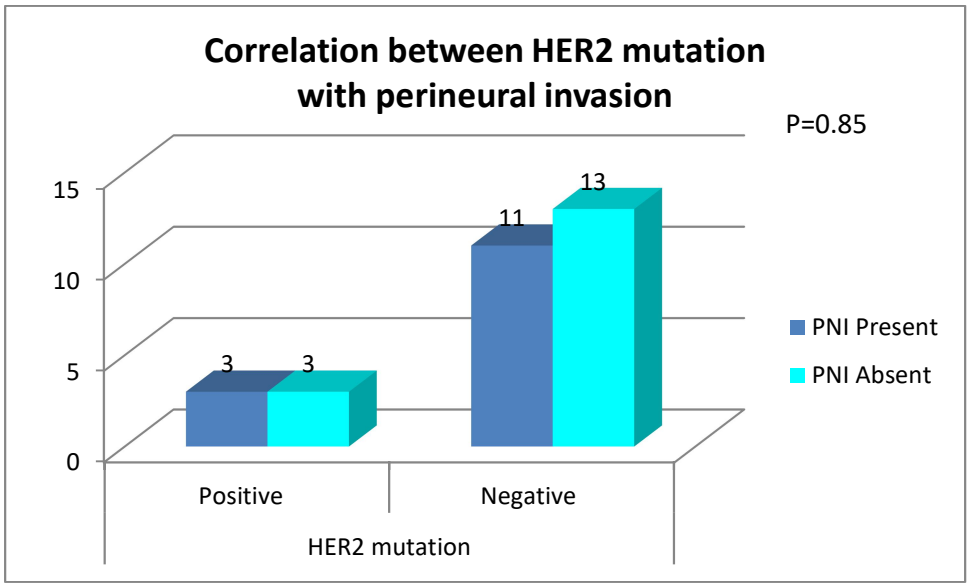


Figure 44: Correlation between HER2 mutation with perineural invasion.



(K) Lymphovascular invasion (LVI).

Out of 14 KRAS mutation-positive patients, 7 (50%) and 7 (50%) patients had LVI involvement present and absent, respectively. Out of the 16 KRAS mutation-negative patients, 11 (68.7%) patients had LVI present, and 5 (31.3%) did not have LVI.

Out of 6 HER2 mutation-positive patients, 4 (66.7%) and 2 (33.3%) patients had LVI involvement present and absent, respectively. Out of the 24 HER2 mutation-negative patients, 8 (33.3%) patients had PNI present, and 16 (66.7%) did not have LVI.

There was no statically significant co-relation between either KRAS or HER2 mutations on FISH with LVI in the study population, as depicted below: (Table 19)

Table 19: Correlation between KRAS and HER2 mutations with LVI.

		Lymphovascular invasion		
		Present	Absent	p-value
KRAS mutation	Present (n= 14)	7 (50%)	7 (50%)	0.29
	Absent (n= 16)	11 (68.7%)	5 (31.3%)	
HER2 mutation	Present (n= 6)	4 (67.7%)	2 (33.3%)	0.14
	Absent (n= 24)	8 (33.3%)	16 (66.7%)	

Figure 45: Correlation between KRAS mutation with LVI.

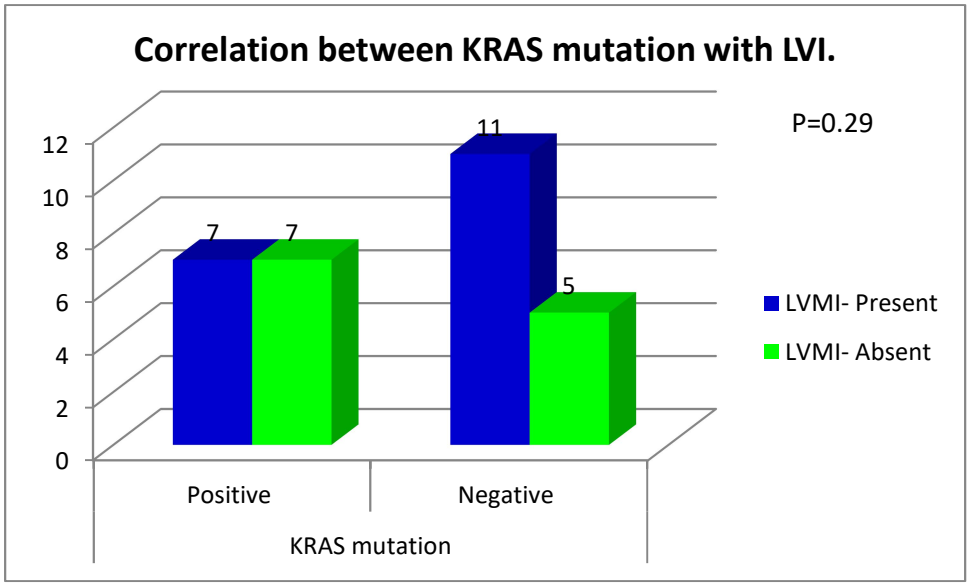
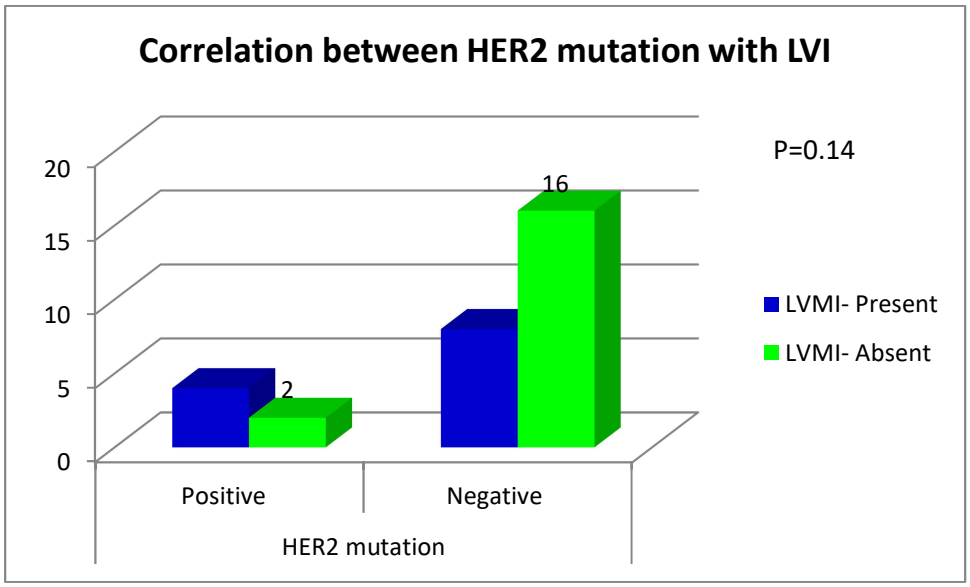


Figure 46:Correlation between HER2 mutation with LVI.



(L) Overall stage

Out of 14 KRAS-positive patients, 5 (35.8%) patients had stage I tumor, whereas 3 (21.4%) and 6 (42.8%) patients had stage II and III tumors, respectively. Out of the 16 KRAS negative patients, 4 (25%), 5 (31.3%), and 7 (43.7%) patients had stage I, II, and III tumors, respectively. None of the KRAS positive and negative patients had stage IV tumor.

Of 6 HER2-positive patients, 2 (33.3%) and 4 (66.7%) patients had stage II and III tumor, respectively. Out of the 24 HER2 negative patients, 9 (37.5%) patients had stage I tumor, whereas 6 (25%) and 9 (37.5%) patients had stage II and stage III tumor, respectively. None of the HER2 positive or negative patients had stage IV tumor.

There was no statically significant co-relation between either KRAS or HER2 mutation on FISH with overall tumor stage as depicted below: (Table 20)

Table 20: Correlation between KRAS mutation with overall stage.

		Overall stage				
		I	II	III	IV	p-value
KRAS mutation	Present (n= 14)	5 (35.8%)	3 (21.4%)	6 (42.8%)	0	0.75
	Absent (n= 16)	4 (25%)	5 (31.3%)	7 (43.7%)	0	
HER2 mutation	Present (n= 6)	0	2 (33.3%)	4 (66.7%)	0	0.19
	Absent (n= 24)	9 (37.5%)	6 (25%)	9 (37.5%)	0	

Figure 47: **Correlation between KRAS mutation with overall stage.**

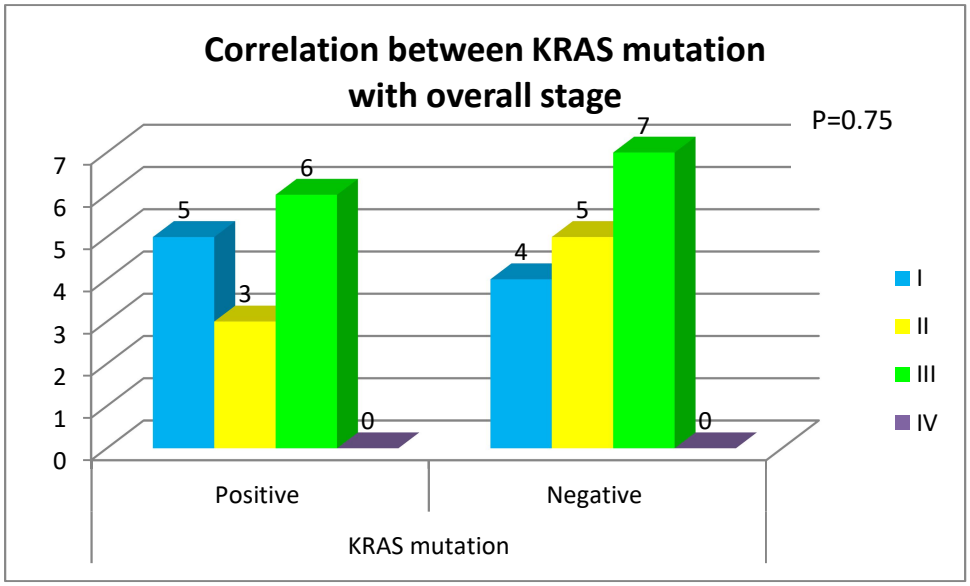
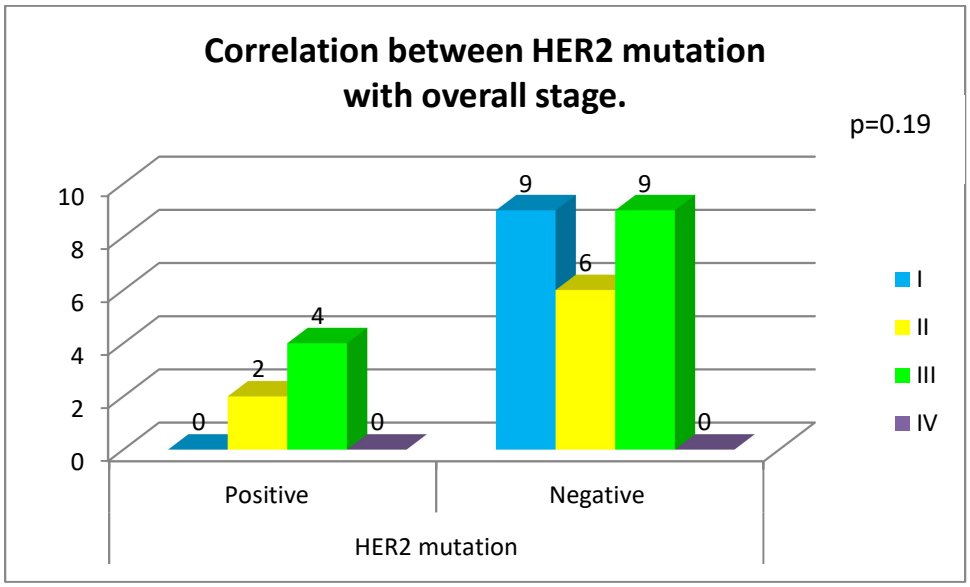


Figure 48: **Correlation between HER2 mutation with overall stage.**



6. Discussion

Recent advances have been made in the molecular characterization of PACs. More and more genetic alteration studies are evaluating the mutations in gastrointestinal malignancies, which has opened up a new paradigm to target specific gene mutations. In colo-rectal carcinomas, the role of biological target therapies is now well established. In colorectal cancer, KRAS mutations have been shown to reliably predict which patients will not respond to an EGFR inhibitor. (67,68) Similarly, studies in lung cancer have also shown that patients with mutations in KRAS are less likely to respond to small molecule EGFR inhibitors. (69,70)

PACs are a heterogeneous group of tumors with a wide range of genetic alterations, including KRAS, SMAD4, TP53 mutations, as well as a high rate of microsatellite instability, WNT pathway, etc. (4) Among them, KRAS and HER2 mutation are one of the earliest mutations present in PACs and also have target therapies available.

Chung et al. (1996) are one of the earliest descriptions in literature to study the frequency of KRAS mutation in PAC. (57) However, its frequency range is wide in worldwide literature. KRAS mutation has been found in 30-80% cases of PACs, whereas HER2 is found in 2-80% cases in the literature. These wide ranges are attributed not only to the heterogeneity of tumors in PACs but also to the geographic and demographic profile of the study population, along with a difference in the method of genetic assessment. We aimed to assess the frequencies of KRAS and HER2 mutations in PACs in our study population and also assessed the relation of KRAS and HER2 mutation with clinico-pathological outcomes in the study population.

(A) Prevalence of KRAS mutations in the study population:

In an initial study published by Chung et al., KRAS mutation was reported in 39% (11 out of 28) of PAC patients. (57) Schultz et al. (2012) evaluated the frequency of KRAS mutation in both PDAC and PAC and reported that KRAS mutation was 67% in PAC and 80% in PDAC. Though the 2/3rd of the patients had KRAS mutation, the low frequency in PAC compared to PDAC was attributed to the heterogeneity of the group of tumors in PAC. (55) Similarly, Swain et al. also showed that KARS mutation was present in 32 (64%) of the patients in the study population. (76) Further, Mikhitarian et al. (2014) studied the role of Epidermal Growth Factor Receptor

Signaling Pathway genetic mutation in PAC and found that KRAS was the most common genetic mutation (47%) in PAC. (60)

Kwon et al. analyzed the genetic mutations in the RAS-RAF-MAPK pathway in PAC. The study reported that the KRAS mutation was present in nearly one-third of patients in PAC, similar to Valsangka et al. (74, 75) Kim et al. in their meta-analysis of the KRAS mutations in PACs, showed that 175 (45%) patients had KRAS mutation. (58) In a recent review article by Lundgren et al., KRAS mutation was present in 47 (46.1%) patients in the study population of PAC patients. (59) Similarly, the frequency of KRAS mutation in our study (46.7%) was comparable to other studies in the literature. This indicates that nearly half of the patients of PAC have KRAS mutation, which can be a target for therapeutic management. (Table 21)

Table 21: Prevalence of KRAS mutation in literature.

	Year	Total number of patients	KRAS positive patients (%)
Chung et al	1996	28	11(39%)
Howe et al	1997	27	9 (37%)
Schönleben et al	2009	25	7 (28%)
Schultz et al	2012	87	58 (67%)
Cunha et al	2012	68	28 (41.2%)
Sitthideatphaiboon et al	2014	63	29 (43%)
Mikhitarian et al	2014	52	25 (47%)
Valsangka et al	2015	75	9 (12%)
Kwon et al	2016	62	20 (30.6%)
Kim et al (meta-analysis)	2017	396	175 (45%)
Swain et al	2018	49	32 (64%)
Lundgren et al	2019	102	47 (46%)
Our study	2022	30	14 (46.7)

(B) KRAS mutation and clinico-pathological parameters.

In literature, KRAS has been reported to be associated with advanced age, with most of the studies reporting median age of presentation in the sixth decade of life. (74, 75) In our study, the median age of KRAS mutation-positive patients was 58.5 (37-75) years which corresponds to the literature. In contrast, most retrospective studies showed no predilection for sex distribution, with few studies showing either slight male or female predominance. Female predominance was present among KRAS-positive patients in our study population, as seen in Kwon et al. (2016). (75) This suggested that the PAC is more common in the sixth decade of life with slight female predominance.

Tumor markers like CA19-9 and CEA are helpful in monitoring and prognostication of PAC tumors. Only one study, Cunha et al. (2012), reported the relation between CA 19-9 with KARS mutation in patients with PAC. (76) The study reported that normal CA 19-9 levels were more common in both KRAS positive and negative groups. We evaluated the relation of both the tumor markers with KRAS mutation. CA19-9 levels did not relate to normal or high levels; however, there was a non-significantly higher trend for normal CA19-9 levels in KRAS negative patients. In contrast, normal CEA levels were more common in both KRAS positive and negative groups. However, the trends were not statistically significant in our analysis; none of the studies in our knowledge has evaluated the impact of KRAS mutation on CEA levels. So KRAS mutation-positive patients may have higher CEA levels with no CA 19-9 levels predilection.

Among various histopathological parameters, T stage, N stage, morphological subtype, PNI, LVI, and overall stage have been evaluated. Evaluation of morphological subtype is of prime importance in PAC as PB subtype has been reported to be associated with poorer OS compared to intestinal subtype. In literature, most of the studies have reported a strong correlation between KRAS mutation and PB subtype in patients of PDAC. (37) However, most studies reported mixed results for morphological assessment in PAC with slight intestinal subtype predominance in KRAS-positive patients. Mikhitarian et al. (2014) evaluated the relationship between morphological subtypes and KRAS mutation. Of the 15 KRAS positive patients, 46.6% and 53.4% had PB and intestinal subtypes, respectively, whereas out of 12 KRAS negative patients, 41.7% and 58.3% had PB and intestinal subtypes, respectively. (60)

Similar results have been reported by Valsangkar et al (2015) and Kwon (2016). (74,75) Both studies reported slightly more prevalence of intestinal subtype in KRAS-positive patients. In contrast, our study reported that 78.6% and 21.4% KRAS-positive patients had PB and intestinal subtypes, respectively. Similarly, 62.5% of patients had PB morphological subtype among KRAS negative patients, whereas 37.5% patients had intestinal subtype. The KRAS analysis did not significantly correlate with the prediction of morphological subtype. Nevertheless, the PB subtype was more common in KRAS positive patients than KRAS negative patients in our analysis. This may translate into the poor OS in survival analysis among KRAS-positive patients, as explained in PDAC literature.

In the T stage, most of the studies have reported predominance of advanced T stage (T3/T4) in KRAS positive patients, which tends to correlate with the poor OS in KRAS positive patients. Schönleben et al. (2009) showed that 28.5% and 71.5% had T1/T2 and T3T4 tumors among KRAS mutation-positive patients, respectively. (72) Valsangkar et al. (2015) and Kwon (2016) also reported that around two-thirds of the patients had advanced T stage in KRAS positive group in PAC. (74,75)

In contrast, our study showed non-significantly higher T1/T2 tumor patients in the KRAS mutation-positive group compared to other studies in the literature. However, Among KRAS mutation-negative patients, the T1/T2 and T3/T4 tumor frequencies were comparable to other studies in the literature. Our contrary results in KRAS-positive patients may be explained by the difference in the study population, with most of the other study populations having advanced disease tumors in their analysis. Also, our result indicated that the KRAS-positive patients show a non-significant trend towards the early T stage, which in some specific subgroups of the early tumor may show a good prognosis.

Schönleben et al. (2006) reported higher LN positivity among KRAS positive and negative patients. Among KRAS-positive patients, three-fourths of the patients had LN involvement present. (72) In contrast, Valsangkar et al. (2015) and Kwon et al. (2016) had reported that LN involvement was absent in KRAS-positive patients. In Valsangkar et al., around two-thirds of KRAS-positive patients did not have LN involvement, whereas Kwon et al. reported that three-fourths of the patients had LN involvement absent. (74,75)

In our study population, 57.2% had lymph node involvement present in KRAS-positive patients. Also, 62.5% of patients had lymph node involvement absent in KRAS negative patients. Our finding correlates with Schönleben et al. (2006), which reported similar results. (72) This suggests that KRAS-positive patients tend to have higher LN positivity in KRAS-positive patients. LN involvement is a critical predictor of OS in PAC patients, and this result may indicate that the KRAS patients may have poor OS due to the higher prevalence of LN involvement.

Most of the studies in the literature have reported PNI involvement absent in patients with KRAS mutation. Valsangkar et al. (2015) and Kwon et al. (2016) had reported that 66% and 79% of the KRAS-positive patients had PNI involvement absent. (74) However, we did not also find any relation between PNI involvement and KRAS mutation. Nevertheless, half of the patients had PNI involvement in KRS positive patients. Similarly, 56.2% of patients had PNI involvement in KRAS-negative patients. As PNI involvement is a poor prognostic parameter, our finding of higher PNI involvement than other studies in the literature is of prime importance. This indicates that patients with KARS mutation harbor a 50% risk for PNI involvement.

Similar to PNI, LVI is also a poor prognostic factor for OS in PAC. Most of the studies in the literature have reported LVI involvement absent in patients with KRAS mutation. Valsangkar et al. (2015) and Kwon et al. (2016) had reported that 77.8% and 57.9% of the KRAS-positive patients had LVI involvement absent. (74, 75) Our results for LVI involvement were similar to other reported studies. With 58.3% patients of LNI involvement absent were KRAS-mutation positive. This suggests that the KRAS mutation positivity is associated with lower LVI involvement.

Valsangkar et al. (2015) reported that 44.4% and 55.6% of patients had stage I and II/III tumors among KRAS-positive patients, respectively. (74) Similarly, Schönleben et al. (2006) reported that more than half of the KRAS-positive patients had stage II/III present. (72) Our study showed similar results to the above-mentioned studies with a higher prevalence of stage II/III tumors in KRAS-positive patients. Around two-thirds of our KRAS-positive patients had an advanced overall stage, and there was a non-significant trend for the advanced overall stage in PAC among KRAS-positive patients. This finding is clinically significant as these patients will have a poorer OS. (Table 22)

Table 22: Correlation between KRAS positive mutation with clinico-pathological parameters.

	Schönleben et al	Valsangkar et al	Kwon et al.	Our study
Year	2006	2015	2016	2022
Type of study	Retrospective	Retrospective	Retrospective	Propspective
Prevalence (%)	7/25 (28%)	9/75 (33.3%)	19/62 (30.6%)	14/30 (47.2%)
Median age (year)	67 (43-70)	68 (34-92)	63	58.5 (37-75)
M:F	4 (57.1%): 3 (47.8%)	14 (56%): 11 (44%)	6 (31.6%): 13 (68.4%)	6 (42.8%): 8 (57.2%)
PB	N	4/9 (44.4%)	9/29 (47.4%)	11/14 (78.6%)
Intestinal	N	5/9 (55.6%)	10/33 (52.6%)	3/14 (21.4%)
T1/T2	2/7 (28.5%)	3/9 (33.3%)	7/18 (36.8%)	9/14 (64.3%)
T3/T4	5/7 (71.4%)	6/9 (66.7%)	12/44 (63.2%)	5/14 (35.7%)
LN +ve	5/7 (71.4%)	3/9 (33.3%)	5/14 (26.3%)	8/14 (57.2%)
LN –ve	2/7 (28.6%)	6/9 (66.7%)	14/48 (73.7%)	6/14 (42.8%)
PNI +	N	3/9 (33.3%)	4/12 (21%)	7/14 (50%)
PNI -	N	6/9 (66.7%)	15/50 (79%)	7/14 (50%)
LVI +	N	2/9 (22.2%)	8/32 (42.11%)	7/14 (50%)
LVI -	N	7/9 (77.8%)	11/30 (57.9%)	7/14 (50%)
Overall stage I	1/7 (8%)	4/9 (44.4%)	10/ 19 (52.6%)	5/14 (35.7%)
Overall stage II/III	6/7 (57.1%)	5/9 (55.6%)	9/ 19 (47.3%)	9/14 (64.3%)

(C) Frequency of HER2 mutation in literature:

Only three studies have evaluated the prevalence of HER2 in PAC patients. Ajiki et al. (2001) reported the frequency of 23% in the study population of 30 PAC patients. (78) Hechtmann et al. (2015) reported that 14 (13%) of the 106 PAC patients had HER2 mutation present. (61) Wheras Elebro et al. (2016) mentioned that only 2% of patients had HER2 mutation in PACs. (79)

Some studies have reported the prevalence of HER2 in PDAC patients. Safran et al. (2001) reported a frequency of 21%. (65) Stoecklein et al. (2004) reported that the frequency of HER2 mutation in PDAC was 24%. Harder et al. (2012) reported that 17 (7.42%) of the 229 PDAC cases had harbored HER2 mutation. (80) Chou et al. (2013) reported that 2.1% of patients had HER2 mutation present. (81) Han et al. (2021) showed the prevalence of 42% out of the 55 PDAC patients. (82)

In our study, 20% of patients were HER2 mutation-positive. The frequency in our study is not only within the range of available literature (2-85%) but also closely similar to most of the studies in the literature. (Table 23)

Table 23: Frequency of HER2 mutation in literature:

Study	Primary malignancy	Year	N	HER2 mutation-positive (%)
Hechtman et al	PAC	2015	106	14 (13)
Elebro et al	PAC	2016	175	4 (2%)
Ajiki et al	PAC	2001	30	7 (23%)
Safran et al	PDAC	2001	154	34 (21%)
Stoecklein et al	PDAC	2004	50	12 (24%)
Harder et al	PDAC	2012	229	17 (7.42%)
Chou et al	PDAC	2013	469	10 (2.1%)
Han et al	PDAC	2021	74	55 (85%)
Our study	PAC	2022	30	6 (20%)

(D) HER2 mutation and clinico-pathological outcomes:

Though less prevalent (compared to KRAS mutation), HER2 mutation analysis may play a pivotal role in managing PACs as targeted therapy for this mutation is available and widely used in other malignancies with successful results. In literature for both PDAC and PAC tumors, HER2 mutations are commonly present in the sixth decade of life. Hechtmann et al. (2015), in their study population of HER2 positive PAC patients, showed a median age of 62 (37-83) years. (61) Similarly, Chou et al. (2013) and Han et al. (2021) reported the median age of 69.7 (47-73) and 64 (42-77) years in the study population of HER2 positive PDAC patients. (81, 82) However, in our study, the median age was 50.5 (41-60) years. This suggests that the HER2 mutation may occur in slightly younger patients than the reported frequencies in the literature.

HER2 mutation strongly predicts male predominance in literature in PAC and PDAC patients. Both Chou et al. (2013) and Han et al. (2021), in their analysis of PDAC tumors, showed a predilection for male sex distribution. (81, 82) Although there was no significant association between sex distribution and HER2 mutation, our analysis showed that strong male predominance (83.3%) was present among HER2-positive patients, as seen in Hechtmann et al. (2015). (61)

Further, Harder et al. (2022) reported the relation of CA 19-9 with HER2 mutation in PDAC patients. 85% of patients with HER2 mutation had high CA 19-9 levels. (83) In our study, an equal association was present for both normal and high CA 19-9 levels in HER2 positive patients, and no statically significant correlation was found between HER2 mutation with CA 19-9 levels. Compared to the only study with such analysis, our study did not show any trend for CA 19-9 levels associated with HER2 mutation. However, its clinical use cannot be ruled out in practice, even in patients with HER2-positive patients.

No study in our knowledge has evaluated the relation of CEA levels with HER2 mutation in PAC and PDAC patients. In our study, one-third of patients with HER2 mutation had high CEA levels. However, only 4.2% of HER2-negative patients had high CEA levels, and 95.8% HER2 negative patients had normal CEA levels. There was a statically significant correlation between HER2 mutation on FISH with CEA levels. It indicated that HER2 mutation is associated with high CEA levels.

HER2 mutation in PAC has a slight predilection for PB morphology. In our study population, two-thirds of HER2-positive patients had PB morphological subtype, whereas one-third of patients had intestinal morphological subtype. Our study results were similar to the other studies in literature like Hechtmann et al. (2015) and Elebro et al. (2015). (78, 82) There was a non-statically significant co-relation between HER2 mutation on FISH with PB subtype. As PB morphology is associated with poorer OS, even a non-significant trend towards PB morphology is of great clinical importance.

Most of the retrospective studies in PDAC showed no predilection for the T stage with HER2 mutation. Chou et al. (2013) showed that 70% of patients with HER2 mutation in PDAC had T3/T4 stage. However, among HER2 mutation-negative patients, 80% of patients had T3/T4 stage. (81) In contrast to this, Han et al. (2021) showed that 80% and 84% of patients with HER2 mutation present and absent had early T stage (T1/T2), respectively. (82) Our study results were similar to Hectmann et al. (2015) in the HER2 positive group. Two-thirds of our HER2 patients had T3/T4 stage. (61) However, in the HER2 negative group, 58% of patients had the T1/T2 stage, as seen in Han et al. (2021). (82) Although statically non-significant, our results showed HER2 mutation patients had advanced T stage whereas HER2 mutation-negative patients have early T stage.

Chou et al. (2013), in their study population of PDAC patients, reported that 40% of patients had LN involvement present, whereas 60% of patients did not have LN involvement in the HER2 mutation-positive group. (81) On the contrary, Han et al. (2021) have shown that 66.7% of the HER2 mutation-positive patients had LN involvement, and in HER2 negative patients, only one-third of the patients had LN involvement. (82) Our study findings were comparable to Han et al. (2021). Among HER2-positive patients in our study, two-thirds had LN involvement, whereas, in HER2-negative patients, 58% of the patients did not have LN involvement. Although statically non-significant, our results show that HER2-positive patients had a trend toward lymph node positivity which may translate into the poor OS.

The few available retrospective studies in PAC have shown no association between PNI and HER2 mutation. Hechtmann et al. (2015) reported that half of the HER2 mutation-positive patients had PNI involvement present. (61) In contrast, Chou et al. (2013) had reported that 80%

of HER2 mutation-positive patients with PDAC tumors had PNI involvement present, whereas 72% of HER2 mutation-negative patients had PNI involvement. (82)

Our study results were similar to Hechtmann et al. (2015). (61) Among HER2 mutation-positive, half of the patients had PNI involvement present. Among HER2-negative patients, 54.2% did not have PNI involvement present. Our study did not find any relation between PNI and HER2 mutation. Nevertheless, 50% PNI involvement among HER2-positive patients is still a warning sign as these patients will have a poor OS.

Chou et al. (2013), in their study population of PDAC, showed that 80% of patients of HER2 mutation did not have LVI involvement present. (81) On the contrary, Hechtmann et al. (2015) reported higher LVI positivity among HER2 mutation-positive patients. In 64% of the patients with HER2 mutation had LVI involvement present whereas, among HER2 mutation-negative patients, half of the patients had LVI involvement absent. (61) In HER2 positive group, our study results had similar findings as Hechtmann et al. (2015). (61) Two-thirds of the patients with HER2 mutation had LVI involvement present. However, among HER2 mutation-negative patients, two-thirds of patients had LVI involvement absent. This implies that HER2 mutation patients have higher LVI involvement rates which may translate into the poor OS in these patients.

Chou et al. (2013) reported that among HER2-positive patients, 20%, 70%, and 10% of patients had stage I, II, and III/IV tumors, respectively. (81) In contrast, Han et al. (2021) reported that more than half of the HER2 positive PDAC patients had stage I tumors, whereas 41.8% and 16.4% of patients had stage II and II/IV tumors. (82) Our study showed similar results to those mentioned above, with a higher prevalence of stage III/IV tumors in HER2-positive patients. Around two-thirds of our HER2-positive patients had stage III/IV tumors, whereas one-third had stage II tumors. There was a non-significant trend for the advanced overall stage in PAC among HER2-positive patients. These are findings of great clinical significance as HER2-positive patients had either stage II or stage III/IV tumors, which indicates that these patients may have a poor OS.

Table 24: Correlation between KRAS positive mutation with clinico-pathological parameters.	Hechtman et al	Chou et al	Han et al	Our study
Year	2015	2013	2021	2022
Type of study	Retrospective	Retrospective	Retrospective	Propspective
Pri. malignancy	PAC	PDAC	PDAC	PAC
Prevalence (%)	14/106 (13%)	10/440 (2.1)	55/75 (85%)	6/30 (20%)
Median age	62, 37–83	69.5 (47-73)	64 (42–77)	50.5 (41-60)
M:F	10 (71.4%): 4 (28.6%)	6 (60%) : 4 (40%)	9 (52.9%) : 8 (47.1)	5 (83.3%): 1 (16.7%)
PB	6 (60%)	N	N	4 (66.7%)
Intestinal	4 (40%)	N	N	2 (33.3%)
T1/T2	N	3 (30%)	39 (79.5%)	2 (33.3%)
T3/T4	N	7 (70%)	16 (21.5%)	4 (66.7%)
LN +ve	N	4 (40%)	4 (66.7%)	4 (66.7%)
LN –ve	N	6 (60%)	2 (33.3%)	2 (33.3%)
PNI +	7 (50%)	8 (80%)	N	3 (50%)
PNI -	7 (50%)	2 (20%)	N	3 (50%)
LVI +	9 (64%)	2 (20%)	N	4 (67.7%)
LVI -	5 (36%)	8 (80%)	N	2 (33.3%)
Overall stage I	N	2 (20%)	11/20 (55%)	0/6
Overall stage II	N	7 (70%)	23 (41.8%)	2 (33.3%)
Overall stage III/IV	N	1 (10%)	9 (16.4%)	4 (66.7%)

STRENGTHS AND LIMITATIONS

- **Advantages:**

  - First Indian study with genetic analysis for periampullary carcinoma.

  - Prospective analysis.

  - Single-center analysis: Good compliance to the protocol

- **Limitations:**

  - Observational study

  - Limited genetic analysis

  - Low sample size

7. CONCLUSIONS

1. The prevalence of KRAS and HER2 mutations were 46.7% and 20% in the study population, respectively.
2. KRAS mutation was associated with a non-significant trend towards the early T stage, PB subtype, lymph node-negative with equivocal association with PNI and LVI disease.
3. HER2 mutation was associated with a non-significant trend towards advanced T stage, PB subtype, lymph node-positive disease, and LVI with equivocal association with PNI disease.
4. Further prospective studies with a large sample size, more genetic mutational assessments, and their impact from targeted therapy are required.

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Summary

Background: Periampullary adenocarcinomas (PAC) is a heterogeneous group of tumors with a wide range of genetic alterations. KRAS mutation and HER2 overexpression are one of the earliest mutations in the pathogenesis of PAC. To our knowledge, no study has evaluated genomic alteration in PAC in the Indian population. Our study aims to evaluate KRAS and HER2 mutations' frequencies and their relation with clinico-pathological outcomes in PAC in patients undergoing pancreatoduodenectomy (PD).

Objectives: To study the prevalence of KRAS and HER2 mutations in PAC and their relation with clinico-pathological outcomes post PD.

Method and materials: This was a single-center prospective cohort study conducted in the Department of Surgical Gastroenterology of a tertiary care hospital of Jodhpur from January 2020 to August 2021. Patients of age > 18 years who underwent PD for PAC were included in the study. Histopathological assessment was done via H & E staining as per standardized protocol. Genetic mutational analysis of KRAS and HER2 mutation on a post-operative specimen of pancreatoduodenectomy for PAC was done via the FISH technique.

Results: A total of 30 PAC patients were included in the study. Out of the 30 patients, 13 (43.3%) were females, and the median age for the study population was 57.5 years (37-83 years). KRAS and HER2 mutations were positive in 14/30 (47.2%) and 6/30 (20%) patients in the study population. Out of 14 (47.2%) KRAS mutation-positive patients, pancreatobiliary (PB) and intestinal subtypes were present in 11 (79%) and 3 (21%) patients, respectively. Five (35%) and 8 (57%) patients had advanced T stage and lymph node (LN) positive disease, respectively. Perineural invasion (PNI) and lymphovascular invasion (LVI) were present in 7 (50%) and 7 (50%) patients, respectively. Out of 6 (20%) HER2 mutation-positive patients, PBand intestinal subtypes were present in 4 (66%) and 2 (34%) patients. Four (66%) and 4 (66%) patients had advanced T stage and LN positive disease. PNI and LVI were present in 3 (50%) and 6 (66.6%) patients.

Conclusion: Prevalence of KRAS and HER2 mutations were 46.7% and 20% in the study population, respectively. KRAS mutation was a/w non-significant trend towards early T stage, PB subtype, LN negative with equivocal association with PNI and LVI disease. HER2 mutation was a/w non-significant trend towards advanced T stage, PB subtype, LN positive disease, and more LVI with equivocal association with PNI and LVI disease

10. Annexure

10.1 Ethical clearance

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/2668 **Date: 01/01/2020**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/952**

Project title: **"Analysis of KRAS and HER2 mutations with their clinico-pathological relation in periampullary carcinoma undergoing pancraetoduodenectomy"**

Nature of Project:	**Research Project**
Submitted as:	**M.Ch. Dissertation**
Student Name:	**Dr.Ashish Swami**
Guide:	**Dr.Vaibhav Kumar Varshney**
Co-Guide:	**Dr.Subash Chandra Soni, Dr.Poonam Elhence, Dr.Shilpi Gupta Dixit & Dr.Ashok Kumar Puranik**

This is to inform that members of Institutional Ethics Committee (Annexure attached) met on **23-12-2019** and after through consideration accorded its approval on above project. Further, should any other methodology be used, would require separate authorization.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Enclose:
1. Annexure 1

Page 1 of 2

Basni Phase-2, Jodhpur, Rajasthan-342005, **Website:** www.aiimsjodhpur.edu.in, **Phone:** 0291-2740741 Extn. 3109
Email: ethicscommittee@aiimsjodhpur.edu.in

10.2 PARTICIPANT INFORMED CONSENT FORM (PICF)

Participant identification number for this trial: _____

Title of project: Analysis of KRAS and HER2 mutations with their clinico-pathological relation in periampullary carcinoma undergoing pancreatoduodenectomy.

Name of Principal Investigator: Dr. Ashish Swami Tel. No(s). 9413265876

The contents of the information sheet dated …….. That was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

The nature and purpose of the study and its potential risks/benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from AIIMS. I give permission for these individuals to have access to my records.

I agree to take part in the above study.

---------- Date:

(Signatures / Left Thumb Impression) Place:

Name of the Participant: _____

Son / Daughter / Spouse of: _____

Complete postal address: _____

This is to certify that the above consent has been obtained in my presence.

Signatures of the Principal Investigator Date: Place:

1) Witness – 1

Signatures

Name:

Address:

2) Witness – 2

Signatures

Name:

Address:

10.3 सहभागी सुचित सहमति प्रपत्र

इस जाचं के लिए सहभागी पहचान नमबर_____

अनुसन्धान शीर्षक : Analysis of KRAS and HER2 mutations with their clinico-pathological relation in periampullary carcinoma undergoing pancraetoduodenectomy.
मुख्य अन्वेषक का नाम : Dr Ashish Swami फोन नंबर: 9413265876
मैंने दिनांक_____ के सूचना पत्र में दिये गए सभी तथ्यो को पड़ लिया हैं| मुझे समझ आने वालीं भाषा मैं विस्तारपूर्वक बत्ता दिया है और मैनें तथ्यो को भली भांति समझ लिया है| मैं पुष्टि करता हूँ कि मुझे प्रशन पुछने का अवसर दिया गया है|

मुझे अध्ययन की प्रकृति, उद्देश्य और इसके सम्भावित लाभ/जोखिमों और अध्ययन की सम्भावित अवधि अन्य प्रासंगिक जानकारी के बारे में विस्तार पुर्वक समझा दिया गया है | में समझाता हूँ कि इस अध्ययन में मेरी भागिधारी स्वेछिक है और इस अध्ययन से किसी भी समय बिना कोई कारण बताए, बिना मेरी चिकित्सा देखभाल या कानूनी अधिकारों के प्रभावित हए अपना नाम वापिस ले सकता/सकती हूँ

मैं समझता हूँ कि इस अनुसन्धान में मेरी सहभागिता से मेरे बारे में एकत्र जानकारी और चिकित्सीय नोटों को एम्स अस्पताल के जिम्मेदार लोगो द्वारा देखा जायेगा| मैं इन व्यक्तियों को अपने रिकोर्ड देखने कि अनुमति प्रदान करता/करती हूँ |

मैं उपयुर्क्त अध्यन में भाग लेने के लिए अपनी सहमति प्रदान करता /करती हूँ |

सहभागी के हस्ताक्षर / बाएं अंगूठे का निशान दिनांक: स्थान:

सहभागी का नाम

पिता/पति का नाम पूरा पता

यह प्रमाणित किया जाता हे कि उपयुर्क्त सहमति मेरी उपस्थति में ली गईं हैं |

मुख्य अन्वेषक के हस्ताक्षर दिनाक: स्थान:

१) गवाह के हस्ताक्षर
नाम
पता

२) गवाह के हस्ताक्षर
नाम
पता

10.4 Information to participants:

Title: Analysis of KRAS and HER2 mutations with their clinico-pathological relation in periampullary carcinoma undergoing pancreatoduodenectomy.

Name of Participant: .....

You are invited to take part in this research study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

You are being asked to participate in this study being conducted in AIIMS, Jodhpur, because you satisfy our eligibility criteria.

What is the purpose of research?

This study looks into the mutational status of 2 selected genes (KRAS and HER2neu) in periampullary carcinoma. If you enroll in it, you will be benefitted from the genetic and mutational status of the carcinoma with possible early prognostic stratification. We have obtained permission from the Institutional Ethics Committee to conduct this study.

The study design

The study will be a single-center prospective observational study, and patients will be recruited from the Department of Surgical gastroenterology.

Study Procedures

The study involves evaluation of the correlation of mutational status detected by FISH technique with histopathology specimen (including subtype detection via IHC) of post pancreatoduodenectomy in periampullary carcinoma. You will be informed about procedures to be done on the histopathology specimen.

Possible risks to you.

There is no added risk other than the risk involved due to surgery and disease.

Possible benefits to you

Identification of genetic alterations in the periampullary carcinoma with possible early prognostic stratification of the tumor.

Compensation

Nil

Possible benefits to other people

The results of the research may provide benefits to society in terms of advancement of medical knowledge and/or therapeutic benefit to future patients.

The alternatives you have

If you do not wish to participate, you still will get the histopathology report of your histopathology specimen.

Reimbursement

You will not be paid to participate in this research study.

What should you do in case of injury or a medical problem during this research study?

Your safety is the prime concern of the research. If you are injured or have a medical problem as a result of being in this study, you should contact one of the people listed at the end of the consent form. You will be provided the necessary care/treatment.

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, sponsors, institutional ethics committee, and any person or agency required by law like the Drug Controller General of India to view your data, if required. The results of clinical tests and therapy performed as part of this research may be included in your medical record. The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

How will your decision not to participate in the study affect you?

Your decision not to participate in this research study will not affect your medical care or your relationship with the investigator or the institution. Your doctor will still take care of you, and you will not lose any benefits to which you are entitled.

Can you decide to stop participating in the study once you start?

Participation in this research is purely voluntary, and you have the right to withdraw from this study at any time during the course of the study without giving any reasons.

Can the investigator take you off the study?

You may be taken off the study without your consent.

Right to new information

If the research team gets any new information during this research study that may affect your decision to continue participating in the study or raise some doubts, you will be told about that information.

Contact persons

For further information/questions, you can contact us at the following address:

Principal Investigator:

Dr. Ashish Swami

Senior resident Ph: 9413265876

Dept. of Surgical Gastroenterology email: drashishswami10@gmail.com

Principal guide and Co-Investigator

Dr Vaibhav Kumar Varshney Ph: 9968223072

Associate Professor email: drvarshney09@gmail.com

Dept. of Surgical Gastroenterology

10.5 भागीदारोंकेलिएसूचना

शीर्षक: Analysis of KRAS and HER2 mutations with their clinico-pathological relation in periampullary carcinoma undergoing pancreatoduodenectomy.

प्रतिभागी का नाम:

……

आपको इस शोध अध्ययन में भाग लेने के लिए आमंत्रित किया जाता है। इस दस्तावेज़ में दी गई जानकारी यह तय करने में आपकी सहायता करने के लिए है कि भाग लेना है या नहीं। कृपया पूछें कि क्या आपके पास कोई प्रश्न या चिंता है या नहीं। आपको एम्स, जोधपुर में आयोजित इस अध्ययन में भाग लेने के लिए कहा जा रहा है क्योंकि आप हमारे योग्यता मानदंडों को पूरा करते हैं।

शोध का उद्देश्य क्या है?

यह अध्ययन पेरीएम्पुलरी कार्सिनोमा में 2 चयनित जीनों (KRAS तथा HER2neu) की उत्परिवर्तन स्थिति को देखता है। यदि आप इसमें दाखिला लेते हैं तो आप संभावित प्रारंभिक रोगनिरोधी स्तरीकरण के साथ कार्सिनोमा की आनुवंशिक और उत्परिवर्तन स्थिति जानने से लाभान्वित होंगे। हमने इस अध्ययन के संचालन के लिए संस्थागत आचार समिति से अनुमति प्राप्त की है।

अध्ययन डिजाइन

अध्ययन एक एकल केंद्र संभावित अवलोकन अध्ययन होगा और रोगियों को सर्जिकल गैस्ट्रोएंटेरोलॉजी विभाग से भर्ती
कराया जाएगा।

अध्ययन प्रक्रियाएं

अध्ययन में पेरिअम्पुलेरी कार्सिनोमा में पोस्ट पैन्क्रियाटिको-डुओडेनेक्टॉमी के माध्यम से हिस्टोपैथोलॉजी नमूना (IHC के माध्यम से उपप्रकार का पता लगाने सहित) के साथ जीनों की उत्परिवर्तन की स्थिति का पता लगाया गया है। आपको हिस्टोपैथोलॉजी नमूने पर की जाने वाली प्रक्रियाओं के बारे में सूचित किया जाएगा। सभी घटनाओं को रिकॉर्ड किया जाएगा।

आपके लिए संभावित जोखिम

शल्य चिकित्सा और बीमारी के कारण जोखिम के अलावा कोई अतिरिक्त जोखिम नहीं है।

आपके लिए संभावित लाभ

ट्यूमर के संभावित प्रारंभिक रोगाणुरोधी स्तरीकरण के साथ पेरीएम्पुलरी कार्सिनोमा में आनुवंशिक परिवर्तन की पहचान।

नुकसान भरपाई- शून्य

अन्य लोगों के लिए संभावित लाभ

शोध के नतीजे भविष्य के मरीजों को चिकित्सा ज्ञान और / या चिकित्सकीय लाभ के उन्नयन के मामले में समाज को लाभ प्रदान कर सकते हैं।

आपके पास विकल्प हैं

यदि आप भाग लेना नहीं चाहते हैं, तो भी आपको अपनी हालत के लिए मानक उपचार मिलेगा।

अदायगी

इस शोध अध्ययन में भाग लेने के लिए आपको भुगतान नहीं किया जाएगा।

इस शोध अध्ययन के दौरान चोट या चिकित्सा समस्या के मामले में आपको क्या करना चाहिए?

आपकी सुरक्षा अनुसंधान की प्रमुख चिंता है। यदि आप इस अध्ययन में होने के परिणामस्वरूप घायल हो गए हैं या चिकित्सा समस्या है, तो आपको सहमति फॉर्म के अंत में सूचीबद्ध लोगों में से एक से संपर्क करना चाहिए। आपको आवश्यक देखभाल / उपचार प्रदान किया जाएगा।

आप से प्राप्त जानकारी की गोपनीयता

आपको अपनी चिकित्सा जानकारी (व्यक्तिगत विवरण, शारीरिक परीक्षाओं के परिणाम, जांच, और आपके चिकित्सा इतिहास) की गोपनीयता के संबंध में गोपनीयता का अधिकार है। इस दस्तावेज़ पर हस्ताक्षर करके, आप अनुसंधान टीम जांचकर्ताओं, अन्य अध्ययन कर्मियों, प्रायोजकों, संस्थागत नैतिकता समिति और कानून के अनुसार आवश्यक किसी भी व्यक्ति या एजेंसी को भारत के ड्रग कंट्रोलर जनरल की आवश्यकता होगी, यदि आवश्यक हो तो आपका डेटा देखने के लिए। इस शोध के हिस्से के रूप में किए गए नैदानिक परीक्षण और चिकित्सा के परिणाम आपके मेडिकल रिकॉर्ड में शामिल किए जा सकते हैं। इस अध्ययन की जानकारी, यदि वैज्ञानिक पत्रिकाओं में प्रकाशित या वैज्ञानिक बैठकों में प्रस्तुत की गई है, तो आपकी पहचान प्रकट नहीं होगी।

अध्ययन में भाग लेने का आपका निर्णय आपको कैसे प्रभावित करेगा?

इस शोध अध्ययन में भाग लेने के आपके निर्णय से आपकी चिकित्सा देखभाल या जांचकर्ता या संस्थान के साथ आपके संबंध प्रभावित नहीं होंगे। आपका डॉक्टर अभी भी आपकी देखभाल करेगा और आप किसी भी लाभ को खो देंगे नहीं जिसके लिए आप हकदार हैं।

क्या आप शुरू करने के बाद अध्ययन में भाग लेने से रोकने का फैसला कर सकते हैं?

इस शोध में भागीदारी पूरी तरह से स्वैच्छिक है और आपको बिना किसी कारण बताए अध्ययन के दौरान किसी भी समय इस अध्ययन से वापस लेने का अधिकार है।

क्या जांचकर्ता आपको अध्ययन से बाहर ले जा सकता है?

आपको अपनी सहमति के बिना अध्ययन से बाहर ले जाया जा सकता है

नई जानकारी का अधिकार

यदि इस शोध अध्ययन के दौरान शोध दल को कोई नई जानकारी मिलती है जो अध्ययन में भाग लेने के आपके फैसले को प्रभावित कर सकती है, या कुछ संदेह उठा सकती है, तो आपको उस जानकारी के बारे में बताया जाएगा।

संपर्क करें

अधिक जानकारी / प्रश्नों के लिए, आप निम्नलिखित पते पर हमसे संपर्क कर सकते हैं:

मुख्य जाँचकर्ता:

Dr. Ashish Swami
Senior resident Ph: 9413265876
Dept. of Surgical Gastroenterology email:
drashishswami10@gmail.com

प्रिंसिपल गाइड और सह-जांचकर्ता
Dr Vaibhav Kumar Varshney Ph: 9968223072
Associate Professor email: drvarshney09@gmail.com
Dept. of Surgical Gastroenterology

10.6 PROFORMA Patient ID:

BASIC INFORMATION OF PATIENT

Name	
Age (in years)	
Sex	
Hospital No.	
Address	
Phone number	
Index Diagnosis	

CHIEF COMPLAINTS

RISK FACTORS

NATURE	YES	NO	DURATION	ABSTINENCE

Pre-op CECT (STAGE):

PREOPERATIVE PERIOD

	YES	**NO**
Neoadjuvant therapy(CT/RT/CRT)		
Preoperative counseling		

Type of neoadjuvant therapy (CT/RT/CTRT):

Procedure:
Date of Surgery:
Intra operative:
Findings
Vascular involvement: **Yes/ No**
Histopathological analysis:

	Finding
T stage	
Size of tumor	

	Finding
N Stage	
No. of lymph node-positive	
Lymph node yield	

	Finding
Grade	
Vascular invasion	
Lymphatic invasion	
Perineural invasion	

Final Pathological stage :

Morphology	**Finding**

Pancreatobiliary	**Yes/ No**
Intestinal	**Yes/ No**
Mucin	

Genetic analysis:

Mutation	
KRAS mutation	**Yes / No**
Her 2 overexpression	**Yes/ No** **+1, +2, +3**

Final Findings: **Patient Id:**

10.7 Abbreviations

CBD	Common bile duct
CK	Cytokeratin
HER2	Human epidermal growth factor receptor 2
H&E	Hematoxylin and Eosin
IHC	Immuno-histochemistry
KRAS	Kirsten rat sarcoma virus
LN	Lymph node
LVI	Lymphovascular invasion
MUC	Mucin
PAC	Periampullary carcinoma
PB	Pancreatobiliary
PDAC	Pancreatic adenocarcinoma
PD	Pancreatoduodenectomy

PNI Perineural invasion

Patient datasheet

Name	Age	Sex	UHID	HPE no.	on (Present	tion(Prese	es (Pancreatol
Uda Ram	83	M	19/08/0107	H/6557/19	0	0	0
Raj Kumar	50	M	19/08/0085	H/6516/19	1	1	1
Sayri Devi	67	F	19/09/0157	H/7303 /19	0	0	1
Sumitra	66	F	19/09/0171	H/7516 /19	1	0	1
Moola Ram	60	M	19/11/0007	H/8808 /19	0	1	1
Guman singh	75	M	19/12/0039	H/9314 /19	1	0	1
Muli Devi	48	F	20/01/0224	H/0512 /20	0	0	1
Jawara Ram	67	M	20/02/0034	H/1092/2020	1	0	1
Anisha Bano	62	F	20/02/0158	H/1902 /20	1	0	1
Mangi lal	72	M	20/02/0165	H/1826 /2020	0	0	1
Surta Ram	51	M	20/01/0210	H/2270/20	0	1	0
Gopi	37	M	20/04/0008	H/2558 /20	1	0	1
Om Prakash	45	M	20/06/0058	H/3032/20	0	0	0
Keli Devi	55	F	20/02/0164	H/2598/20	1	0	0
Maffta Devi	49	F	20/07/0004	H/3199/2020	1	1	1
Ajaram	54	M	20/08/0054	H/3633/20	1	0	1
Bhagawana Ram	58	M	20/07/0092	H/3783/20	0	0	1
Sotki	50	F	20/09/0099	H/4183/20	0	0	0
Parvati devi	63	M	20/09/0026	H/4427/2020	0	0	1
Mohani Devi	63	F	21/01/0155	H/0513 /21	1	0	1
Bachla	55	F	21/02/0002	H/747/21	0	0	1
Surendra singh	44	M	21/02/0084	H/1242 /21	0	0	1
Jjavari lal	51	M	21/06/0044	H/3286/21	0	1	0
Premlata	64	F	21/07/0048	H/4128 /21	1	0	0
Varadi devi	63	F	21/07/0125	H/4244/21	0	0	1
Meena Kumari	70	F	21/07/0119	H/4722 /21	1	0	0
Chagan Lal	41	M	21/08/0109	H/5245/21	0	1	1
Jagadish	61	M	21/08/0199	H/5303/21	0	0	0
Punma Ram	48	M	21/04/0137	H/2973/21	1	0	1
Gawri Devi	55	F	21/09/0196	H/6693 /21	1	0	1

Present=1, Ab	2 (Present=1, Abse	7 (Present=1, Absent	sent=1,	(Present=1, Abse	(Present=1, Abse	**CA19-9**
1	0	0	0	0	0	618
1	0	1	0	1	1	15.46
1	0	1	0	0	0	495
1	0	1	0	0	0	39.7
1	0	1	0	1	0	148.9
1	0	1	1	1	0	13.4
1	0	1	1	0	0	222.6
1	0	0	1	1	0	211
1	0	1	0	0	0	210.5
1	0	1	0	1	1	6.8
1	1	1	1	1	1	2.1
1	0	1	0	0	0	7.52
0	0	0	0	0	0	30.6
1	1	1	0	1	1	2.31
0	0	1	0	1	0	1000
1	0	1	0	0	1	9.6
1	0	1	0	0	1	502
1	1	1	0	1	1	0.8
1	0	1	0	0	0	31
1	0	1	0	0	1	92
1	0	0	0	0	1	22.8
1	0	1	0	0	1	16.9
1	1	1	1	1	1	18.8
1	1	1	0	1	1	10.1
1	1	1	1	1	1	24.2
1	1	1	1	1	1	59.9
1	0	1	1	1	1	70.5
1	1	1	1	1	1	114
1	0	1	0	0	1	2000
1	0	0	0	0	1	156

(normal=1,	CEA	lormal=1, HJM=2, AMF	SIGN (PRES	F CBD (PR	METER OF	entins (Not	=1, high bil
2	3.53	1	5	1	20	11	2	1
1	4.15	1	3	0	12	2	1	4
2	1.8	1	5	1	13	3.5	0	
1	1.2	1	5	1	22	5	1	4
2	5.2	2	4	0	15	3	1	2
1	3.15	1	5	1	20	NA	1	2
2	1.07	1	5	1	10	5	1	2
2	1.5	1	3	1	21	5	1	2
2	1.46	1	5	1	18	5	1	2
1	1.72	1	3	1	12	10	1	2
1	2.47	1	5	1	23	12	1	1
1	2.52	1	1	1	22	16	1 (PTBD)	1
1	0.26	1	5	1	31	6	1 (PTBD)	2
1	1.77	1	3	1	15	8.5	1	4
2	29.44	2	5	1	10	4	1	2
1	1.2	1	5	1	15	6	1	2
2	1.8	1	1	0	26	6	2	1
1	3.8	1	5	0	12	3	0	NA
1	0.55	1	5	1	12	9	1	2
2	0.5	1	5	1	15	7	1	4
1	2.26	1	4	0	15	3	0	NA
1	1.46	1	5	0	11.5	4	1	4
1	1.75	1	2	1	18	10	1	1
1	1.23	1	4	0	10	1	1	1
1	3.3	1	3	0	15	1	2	1
2	3	1	4	1	15	4.6	0	NA
2	1.09	1	5	1	18	6	0	NA
2	3.37	1	3	0	10	1	0	NA
2	1.5	1	1	0	6	6	0	NA
2	9.1	2	4	0	20	4	1	2

Hospital sta	operative	ED/convert	DUODENUI	OR APPEAR	1, ADENOS	MODERATE	TUMOR SIZ	AX DIAMET
21	9	1	2	Ulcerated	1	2	1.2x1x0.8	1.2
13	6	3	3	FIRM	1	2	1.2x1x0.8	1.2
39	36	4	3	FIRM	1	2	3.5x3.5x3	3.5
45	38	4	4	FIRM	1	2	3x1.5x2.6	3
13	6	3	3	FIRM	1	1	3.5x1x1	3.5
13	9	4	3	FIRM	1	2	2x1.3x1.5	2
13	6	3	3	Firm	1	2	1.5x1x0.9	1.5
82	81	1	4	Polypoidal	1	2	2x0.9x0.7	2
11	2	3	1	Firm	1	2	2.4x2.1x1.4	2.4
16	10	1	3	Firm	1	2	1x0.7x0.8	1
14	12	1	2	Firm	1	2	4.5x3x5	4.5
22	8	1	1	Firm	1	2	3x2.5x1.8	3
19	6	1	2	Firm	1	2	2x1.8x1.5	2
12	8	1	3	Firm	1	2	1x1x1.3	1.3
28	9	1	1	Firm	1	2	4.6x4.2x4.3	4.6
29	20	2	4	Firm	1	2	3x2x2	3
17	7	1	4	Firm	1	2	3.5x2.8x1.5	3.5
20	12	1	3	Firm	1	2	1.5x1x0.5	1.5
16	5	2	3	Firm	1	1	1.3x1.3x2	1.3
27	15	2	3	Firm	1	2	1 x 0.8x 0.8	1
23	15	1	3	Firm	1	2	2x1.5x0.8	2
25	21	3	4	Firm	1	3	0.8x06x0.5	0.8
26	22	3	3	Firm	1	2	1x0.8x0.4	1
33	29	3	2	Firm	1	2	1.7x 1x 0.6	1.7
18	14	3	3	Firm	1	2	3x2x2	3
28	15	2	3	Firm	1	2	1.7x1.5x1.5	1.7
23	12	4	3	Firm	1	1	1.1x0.8x 0	1.1
11	10	4	3	Firm	1	2	1.4x1.1x1	1.4
21	8	1	1	Firm	1	2	4x4x3.5	4
36	27	2	3	Firm	1	2	2.2x1.8x1.5	2.2

T1=1, T2=2,	=1, Advanc	N EXAMINE	NT (PRESE	(N0=0, N1	PH NODE R	EMENT (PR	PERINEUR/	Lymphova
3	2	11	0	0	0	0	1	0
2	1	23	1	2	17.23	0	1	1
3	2	9	1	1	22.22	0	1	0
2	1	9	0	0	0	0	0	0
3	2	19	1	1	5.26	1	1	1
3	2	65	0	0	0	0	1	1
3	2	9	0	0	0	0	0	0
1	1	14	0	0	0	0	0	0
1	1	14	0	0	0	0	0	0
2	1	8	1	1	12.5	0	1	1
3	2	12	0	0	0	0	0	0
2	1	11	1	1	4.54	0	1	0
3	2	24	0	0	0	0	0	0
2	1	9	0	0	0	0	0	0
3	2	46	1	2	10.8	1	1	1
2	1	10	1	1	10	0	0	0
3	2	14	1	2	50	0	1	1
2	1	20	0	0	0	0	1	0
2	1	24	0	0	0	0	0	0
3	2	9	1	2	45	0	1	1
2	1	15	0	0	0	0	0	0
2	1	8	0	0	0	0	0	0
3	2	13	0	0	0	0	0	0
2	1	19	1	1	5.26	0	0	1
3	2	16	1	0	18.75	0	1	1
2	1	12	0	0	0		0	0
2	1	28	1	2	21.4	0	0	1
2	1	13	0	0	0	0	0	0
3	2	11	1	1	20	0	1	1
3	2	37	1	1	40	0	1	1

OVERALL p	SUBSTAGE	RESECTION	Chief Complain (jaundice=1, pain abdomen=2, cholagitis=3)
3	3b	1	3
3	3b	0	1
3	3A	0	2
1	1B	0	1
3	3A	1	3
2	2A	0	1
2	2B	0	1
1	1A	0	1
1	1A	1	1
3	3A	0	1
2	2A	0	2
2	2B	0	3
2	2B	0	1
1	1B	0	1
3	3B	1	1
2	2A	0	1
3	3B	0	3
2	2A	0	2
1	1B	0	1
3	3A	0	2
1	1B	0	2
1	1B	0	2
2	2A	0	3
3	3A	0	3
3	3A	0	3
1	1B	0	2
3	3B	0	2
1	1B	0	1
3	3A	0	2
3	3B	0	1

10.9 Plagiarism

Thesis

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