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Sub Classification of Periampullary Carcinoma in Whipple's Specimen Received Using Immunohistochemistry Markers Mucin1 (MUC1), Cytokeratin 20 (CK20) & Caudal Type Homeodomain Transcription Factor (CDX2) - A Tertiary Care Study

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Abstract: ***Background: Periampullary carcinoma (PAC) is a rare malignancy arising near the ampulla of Vater. Accurate histological subtyping into intestinal (INT), pancreatobiliary (PB), and mixed types is crucial for prognosis and therapeutic guidance. Morphological classification often lacks consistency, making immunohistochemistry (IHC) a vital tool. Objective: To subclassify PACs from Whipple's specimens using IHC markers-MUC1, CK20, and CDX2-and assess their clinicopathological significance. Methods: A retro-prospective observational study was conducted from April 2023 to October 2024 at G.R. Medical College, Gwalior. Thirty Whipple's specimens diagnosed with PAC were evaluated. IHC staining for MUC1, CK20, and CDX2 was performed. Tumors were subtyped as INT, PB, or mixed based on marker expression. Data were analyzed using SPSS v26 with p<0.05 as significant. Results: Most tumors were well-differentiated (56.67%) and G1 grade (60%), with significant p-values (0.0012, 0.0007). Vascular invasion was present in 23.34% (p = 0.0035). CDX2 and MUC1 positivity were observed in 66.67% and 80%, respectively. PB histological subtype predominated (53.34%). IHC-based classification showed PB in 46.67%, mixed in 33.33%, and INT in 20%. Conclusion: IHC markers MUC1, CK20, and CDX2 enhance the diagnostic precision of PAC subtyping, aiding prognosis and treatment decisions. PB subtype was most frequent and associated with aggressive features.***

Keywords: Periampullary carcinoma, Immunohistochemistry, MUC1, CDX2, CK20

1.Introduction

Periampullary carcinoma (PAC) is a rare but clinically significant malignancy that originates in the region where the distal bile duct, pancreatic duct, and duodenum converge at the ampulla of Vater. Although PACs account for only 0.5-2% of all gastrointestinal cancers, they comprise approximately 20% of all tumors of the extrahepatic biliary tract and often present earlier than pancreatic head cancers due to symptoms like obstructive jaundice, increasing their resectability rate [1].

Histologically, PACs are divided into two major subtypes: the intestinal (INT) type, which resembles colorectal adenocarcinoma, and the pancreatobiliary (PB) type, which mimics pancreatic ductal adenocarcinoma. This classification is not only morphologically distinct but also prognostically relevant, with intestinal-type carcinomas generally demonstrating better outcomes and longer survival [2]. However, this morphological classification is often hampered by overlapping histologic features and interobserver variability among pathologists [3]. Consequently, immunohistochemistry (IHC) has become an invaluable adjunct in enhancing diagnostic precision and reproducibility in subtyping periampullary carcinomas.

Immunohistochemical markers such as Mucin 1 (MUC1), Cytokeratin 20 (CK20), and Caudal Type Homeobox 2 (CDX2) have been widely used to distinguish between the intestinal and pancreatobiliary subtypes. MUC1 expression is typically associated with the pancreatobiliary phenotype, while CDX2 and CK20 are more frequently expressed in intestinal-type tumors [4]. The expression patterns of these markers, when used in a panel, can not only aid in diagnosis but also offer prognostic insight.

Manohar et al. conducted a comprehensive study on 94 PAC patients who underwent Whipple's procedure and showed that the combined use of MUC1, CK20, and CDX2 helped classify tumors into three IHC-based subtypes: IHC-PB, IHC-INT, and IHC-mixed. Their study reported better overall survival in the IHC-INT subtype (52.9 months) compared to the PB (27.9 months) and mixed subtypes (35.5 months), confirming the prognostic importance of immunophenotyping [5].

In another study, Sree et al. examined the diagnostic utility of IHC markers in 50 resected PACs and found that while CK7 had high sensitivity for pancreatobiliary type, CDX2 and CK20 showed strong correlation with the intestinal subtype. Specifically, CDX2 had a specificity of 96.4% and CK20+/CDX2+ co-expression yielded both high sensitivity (94.2%) and specificity (89.2%) in identifying intestinal-type PAC [6].

Similarly, Kumari et al. evaluated over 100 periampullary tumors and demonstrated that CDX2 had a sensitivity of 89.5% and specificity of 100% for intestinal-type tumors.

Importantly, CDX2-positive tumors also had a longer median survival (44 months) compared to CDX2-negative cases (22 months), making CDX2 a useful prognostic as well as diagnostic marker [7].

The study by Goyal et al. emphasized that using both histomorphology and IHC markers like CK20 and CDX2 substantially increased diagnostic sensitivity and specificity, reaching above 90%. The diagnostic accuracy based on morphology alone was much lower, indicating the necessity of immunohistochemistry in routine pathological evaluation [8].

Moreover, Uğraş et al. explored the association of CDX2 and CK7 expression with clinicopathological parameters in 98 patients. While CDX2 was not directly correlated with overall survival in multivariate analysis, its presence was linked with lower perineural invasion and a less aggressive disease course, further supporting its role in risk stratification [9].

From a therapeutic standpoint, histologic subtyping using IHC markers may have implications for adjuvant chemotherapy selection. In a case study by Hu et al., a patient with a PB-type PAC identified through MUC1, CDX2, and CK20 expression failed to respond to 5-FU-based therapy but showed a marked response to gemcitabine-based treatment, underscoring the potential therapeutic relevance of accurate IHC-based subtyping [10].

The integration of immunohistochemical markers such as MUC1, CK20, and CDX2 into the diagnostic workflow of Whipple's specimens has markedly improved the precision of sub-classifying periampullary carcinomas. This subclassification is not only vital for prognostication but also for guiding appropriate postoperative therapeutic strategies in a tertiary care setting.

The study aimed to sub-classify periampullary carcinoma using immunohistochemical markers MUC1, CK20, and CDX2, and to correlate these subtypes with histopathological features for improved diagnosis and prognostic assessment.

2.Methodology

1. Study Design

This retro-prospective observational study aimed to sub-classify periampullary carcinoma using immunohistochemical markers MUC1, CK20, and CDX2. Both archival and newly received Whipple's specimens were analyzed to correlate IHC patterns with histopathological findings.

2. Study Setting

The study was conducted in the Department of Pathology, G.R. Medical College, Gwalior, a tertiary care center with facilities for histopathology and immunohistochemistry, receiving specimens from the associated surgical departments.

3. Study Duration

The study was carried out from April 2023 to October 2024, covering 18 months. Archival specimens from 2021-2022 and prospective cases from 2023-2024 were included for analysis.

4. Participants - Inclusion/Exclusion Criteria

All Whipple's specimens diagnosed as periampullary carcinoma between 2021 and 2024 were included. Cases were excluded if they had poor tissue preservation, non-adenocarcinoma pathology, or incomplete clinical or pathological data.

5. Study Sampling

Purposive sampling was used. All eligible Whipple's specimens within the study period were included consecutively based on availability of adequate tissue and complete data.

6. Study Sample Size

The study included 30 cases-15 retrospective and 15 prospective-selected based on case availability and feasibility, considering the low incidence of periampullary carcinoma.

7. Study Groups

Tumors were grouped into intestinal, pancreatobiliary, or mixed types based on IHC profiles: CK20/CDX2 positivity indicated intestinal, MUC1 indicated pancreatobiliary, and overlapping expression defined the mixed type.

8. Study Parameters

Key parameters included age, sex, tumor size, histological grade, lymphovascular and perineural invasion, lymph node status, and IHC expression of MUC1, CK20, and CDX2.

9. Study Procedure

Tissue sections were deparaffinized, rehydrated, antigen-retrieved, and stained using MUC1, CK20, and CDX2 antibodies. DAB was used for visualization, followed by counterstaining and mounting.

10. Study Data Collection

Clinical and pathological data were collected from hospital records and lab archives. IHC results were recorded and correlated with histopathology using structured data forms.

11. Data Analysis

Data were analyzed using SPSS version 26. Chi-square tests were used to evaluate associations between IHC

subtypes and clinicopathological variables, with $p<0.05$ considered significant.

12. Ethical Considerations

Ethical clearance was obtained. Informed consent was waived for retrospective cases and taken for prospective ones. Patient confidentiality and data security were strictly maintained.

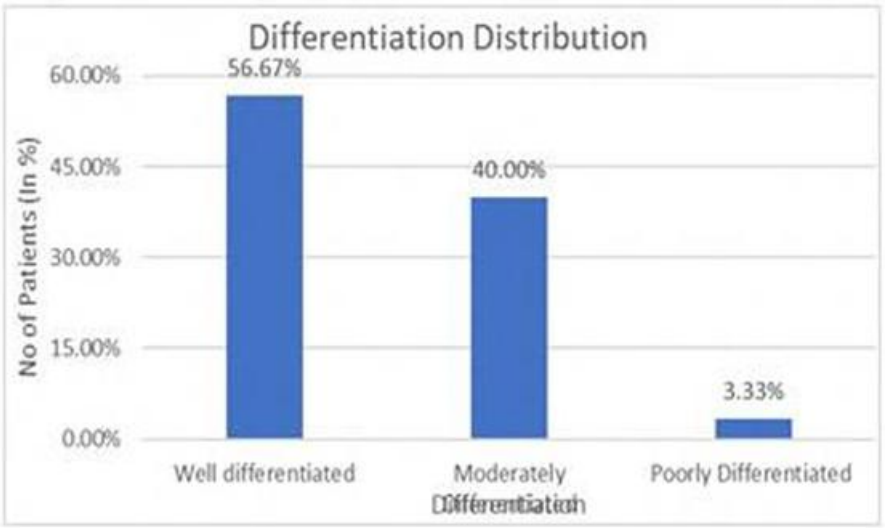
3.Results

1. Tumor Differentiation

Most periampullary carcinomas were well differentiated (56.67%), with a statistically significant p-value of 0.0012. This suggests tumor differentiation may impact prognosis and treatment outcomes (Table 1).

Table 1: Frequency Distribution of Tumor Differentiation

Differentiation	Frequency	Percentage	p-value
Well differentiated	17	56.67%	**0.0012**
Moderately Differentiated	12	40.00%	
Poorly Differentiated	1	3.33%	
Total	**30**	**100.00%**	



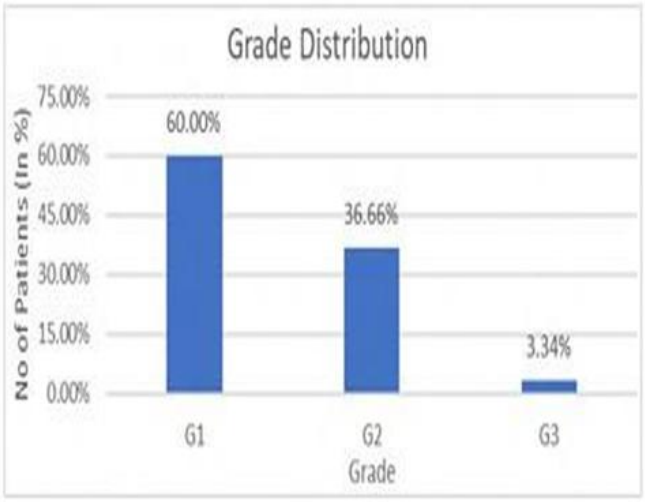
Graph 1: Frequency Distribution of Tumor Differentiation

2. Tumor Grade

Grade G1 tumors were most common (60%), with only 3.34% showing poor differentiation (G3), and this distribution was statistically significant ($p = 0.0007$) (Table 2).

Table 2: Frequency Distribution of Tumor Grades

Grade	Frequency	Percentage	p-value
G1	18	60.00%	
G2	11	36.66%	
G3	1	3.34%	**0.0007**
Total	**30**	**100.00%**	



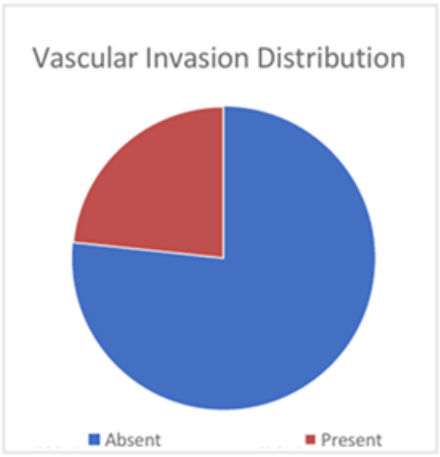
Graph 2: Frequency Distribution of Tumor Grades

3. Vascular Invasion

Vascular invasion was absent in 76.66% of cases, and its presence showed a statistically significant association (p = 0.0035) with periampullary carcinoma (Table 3).

Table 3: Frequency Distribution of Vascular Invasion

Vascular Invasion	Frequency	Percentage	p-value
Absent	23	76.66%	
Present	7	23.34%	0.0035
Total	30	100.00%	



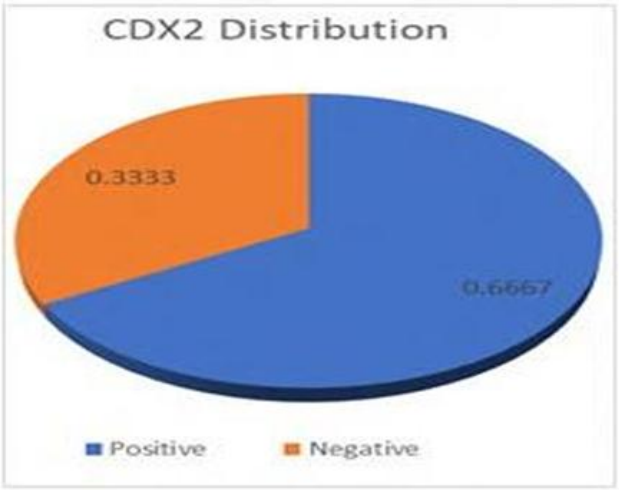
Graph 3: Frequency Distribution of Vascular Invasion

4. CDX2 Expression

CDX2 expression was positive in 66.67% of cases, indicating a dominant intestinal phenotype in periampullary carcinoma (Table 4).

Table 4: Frequency Distribution of CDX2 Expression

CDX2	Frequency	Percentage
Positive	20	66.67%
Negative	10	33.33%
Total	**30**	**100.00%**



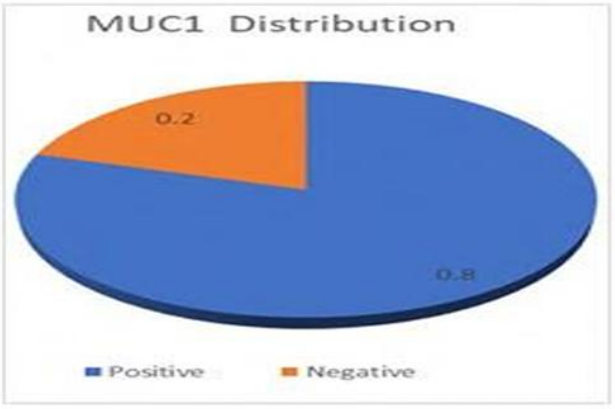
Graph 4: Frequency Distribution of CDX2 Expression

5. MUC1 Expression

MUC1 expression was seen in 80% of cases, supporting the predominance of a pancreaticobiliary or mixed phenotype in the study population (Table 5).

Table 5: Frequency Distribution of MUC1 Expression

MUC1	Frequency	Percentage
Positive	24	80.00%
Negative	6	20.00%
Total	**30**	**100.00%**



Graph 5: Frequency Distribution of MUC1 Expression

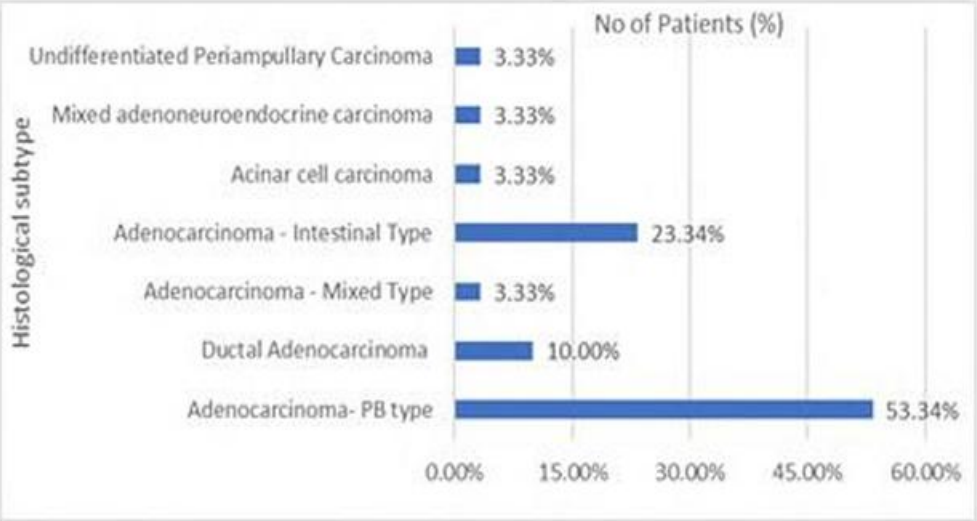
6. Histological Subtypes

The pancreaticobiliary type was the most common histological subtype (53.34%), and the subtype distribution was statistically significant ($p < 0.05$) (Table 6).

Table 6: Frequency Distribution of Histological Subtypes

Histological subtypes	Frequency	Percentage	p-value
Adenocarcinoma - Pancreaticobiliary Type	16	53.34%	
Ductal Adenocarcinoma	3	10.00%	
Adenocarcinoma - Mixed Type	1	3.33%	
Adenocarcinoma - Intestinal Type	7	23.34%	**<0.05**
Acinar cell carcinoma	1	3.33%	

Mixed adenoneuroendocrine carcinoma	1	3.33%	
Undifferentiated Periampullary Carcinoma	1	3.33%	
Total	**30**	**100.00%**	



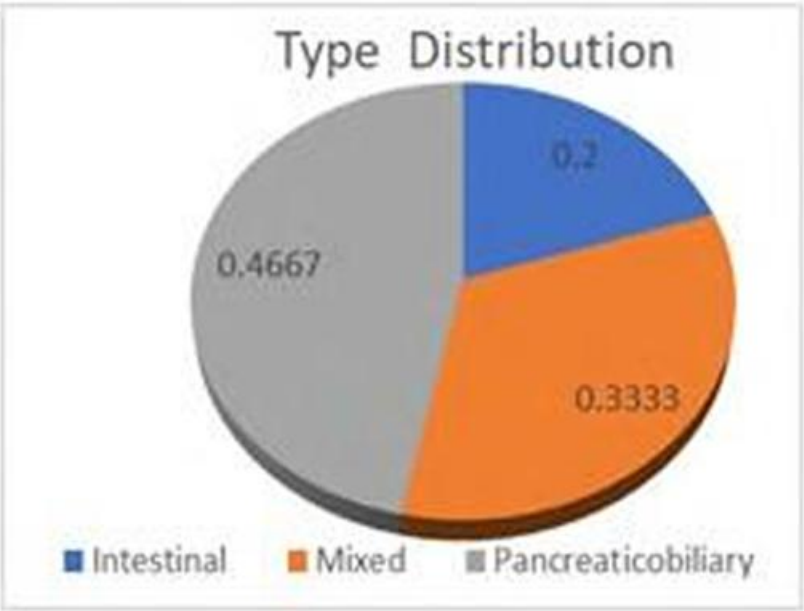
Graph 6: Frequency Distribution of Histological Subtypes

7. IHC Subtypes

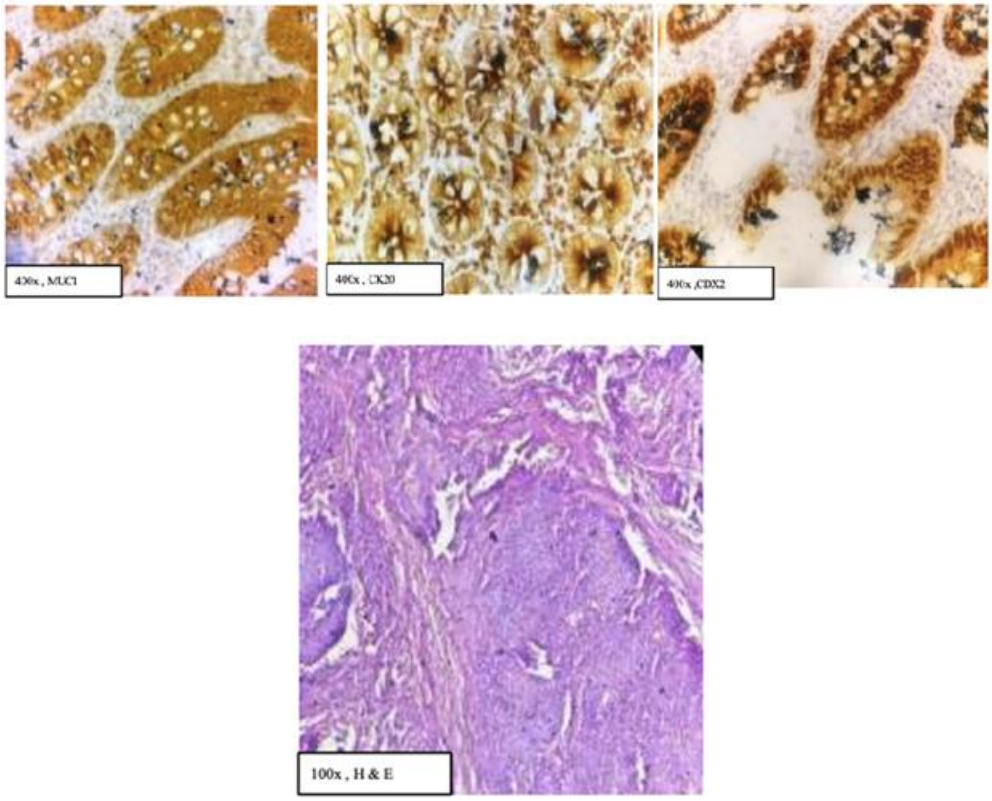
Pancreaticobiliary subtype was most frequent (46.67%), followed by mixed (33.33%) and intestinal (20%), though this distribution was not statistically significant (p = 0.202) (Table 7).

Table 7: Frequency Distribution of Periampullary Carcinoma Subtypes (IHC-Based)

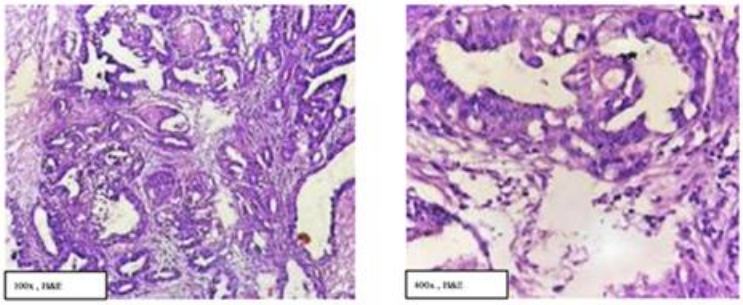
Type	Frequency	Percentage	p-value
Intestinal	6	20.00%	
Mixed	10	33.33%	
Pancreaticobiliary	14	46.67%	**0.202**
Total	**30**	**100.00%**	



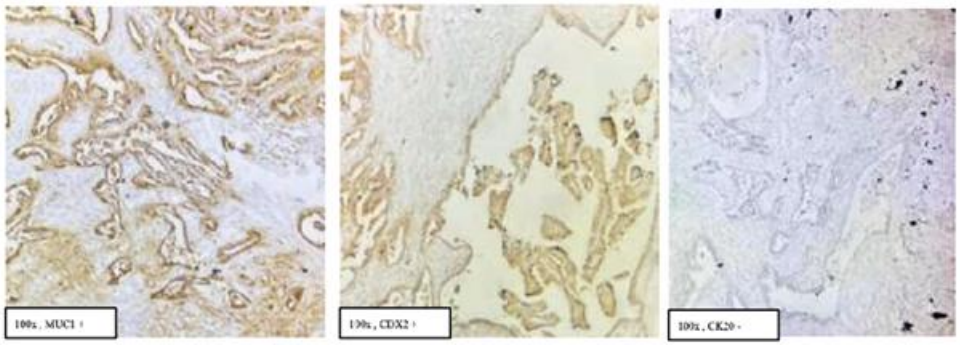
Graph 7: Frequency Distribution of Periampullary Carcinoma Subtypes (IHC-Based)



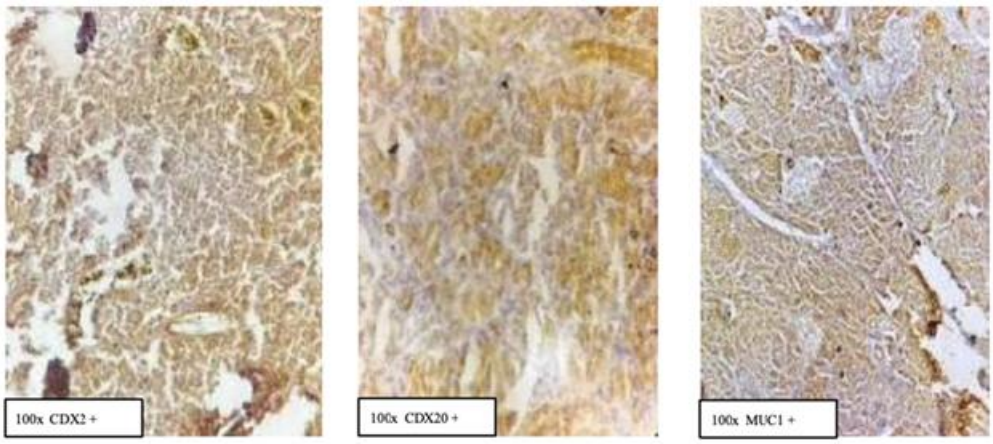
COLOUR PLATE 1 - CONTROL SLIDES - COLON (MUC1, CK20 &CDX2)



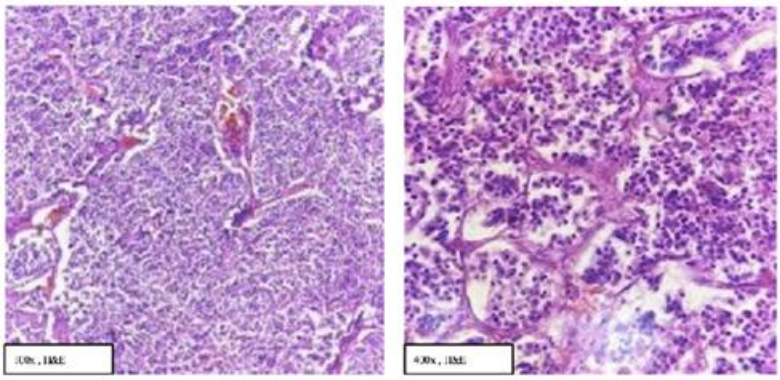
COLOUR PLATE 2A - PANCREATIC ADENOCARCINOMA (H & E STAIN)



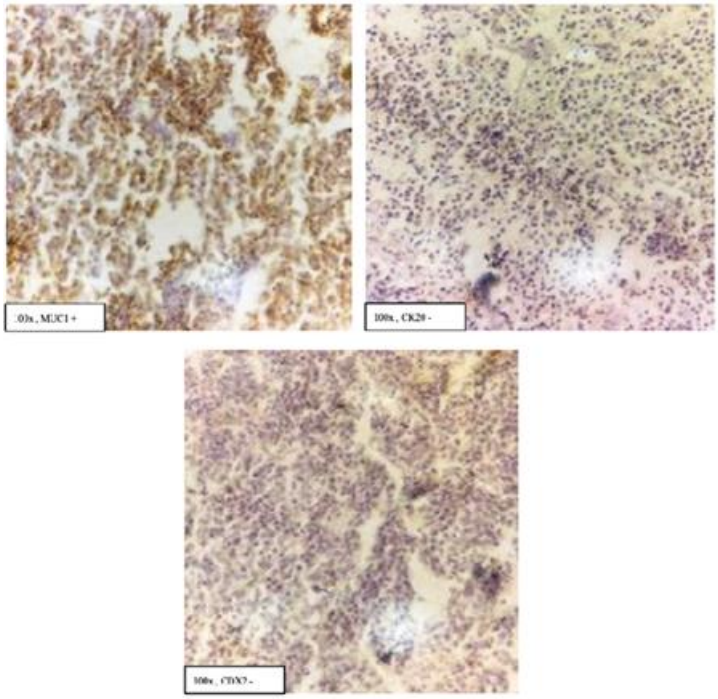
COLOUR PLATE 2B - PANCREATIC ADENOCARCINOMA- MIXED SUBTYPE (IHC RECLASSIFICATION)



COLOUR PLATE 3A - PANCREATIC ADENOCARCINOMA - INTESTINAL TYPE (H&E STAIN)



COLOUR PLATE 3B - PANCREATIC ADENOCARCINOMA- MIXED SUBTYPE (IHC RECLASSIFICATION)



COLOUR PLATE 4A - MIXEDADENO-NEUROENDOCRINE CARCINOMA (H & E STAIN)

COLOUR PLATE 4B - PANCREATIC ADENOCARCINOMA- PANCREATICOBILIARY SUBTYPE (IHC RECLASSIFICATION)

4.Discussion

Periampullary carcinoma represents a heterogeneous group of tumors with variable histological features and prognostic outcomes. In our study, the majority of tumors were well-differentiated (56.67%) and Grade G1 (60%), both of which were statistically significant, aligning with findings by Kumari et al. (2013) and Manohar et al.

(2021), who also reported a predominance of well-differentiated tumors, suggesting a better prognosis in early-presenting cases [2, 5]. The significant association of tumor differentiation and grade with clinical outcomes emphasizes the importance of early diagnosis.

Vascular invasion was present in 23.34% of cases and showed a significant correlation ($p = 0.0035$), which is consistent with studies by Sree et al. (2022) and Bakshi et al. (2019), indicating its role as a marker for aggressive disease and poor prognosis [6, 3]. Perineural invasion, while not included in this summary, is similarly reported in literature as a significant prognostic factor.

Our study found CDX2 positivity in 66.67% of cases, suggesting an intestinal differentiation in a substantial number of tumors. This aligns with the findings of Perysinakis et al. (2017) and Uğraş et al. (2017), who highlighted CDX2 as a reliable marker for intestinal subtype classification [4, 9]. On the other hand, MUC1 was positive in 80% of cases, indicating a predominance of pancreaticobiliary or mixed phenotype, which supports previous research by Manohar et al. (2021) and Kumari et al. (2013) suggesting that MUC1 expression correlates with more aggressive tumor behavior [5, 2].

Histologically, the pancreaticobiliary subtype was most common (53.34%), in concordance with Bakshi et al. (2019), where this subtype was associated with a worse prognosis [3]. Interestingly, 33.33% of cases showed mixed immunophenotypic expression, underscoring the complexity of subclassification and the limitations of relying solely on morphology.

Finally, when stratified by immunohistochemical profile, the pancreaticobiliary subtype remained most frequent (46.67%), followed by mixed (33.33%) and intestinal (20%). Although the IHC-based subtype distribution was not statistically significant ($p = 0.202$), these patterns resemble earlier studies and suggest potential clinical relevance when considered with other histological and clinical features. Our findings corroborate past research and reinforce the role of IHC markers in refining periampullary carcinoma classification for better diagnostic and therapeutic decisions.

5.Conclusion

This study underscores the clinical value of immunohistochemical markers in subclassifying periampullary carcinoma. MUC1, CK20, and CDX2 provided reliable stratification into intestinal, pancreatobiliary, and mixed types, correlating with histopathological parameters. The predominance of the pancreaticobiliary subtype highlights its aggressive nature and poorer prognosis. Integration of IHC into diagnostic workflows enhances subclassification accuracy, facilitates tailored therapeutic approaches, and supports better prognostic assessment in tertiary care pathology practice.

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