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DIAGNOSTIC UTILITY OF IMMUNOHISTOCHEMISTRY IN VARIOUS GASTROINTESTINAL TRACT NEOPLASIA.

Pathology

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ABSTRACT

Among the most prevalent cancers in western nations, gastrointestinal tract tumors encompass a broad range of remarkably diverse tumor types. When these tumors are detected at a later stage, they frequently manifest as distant metastases that require biopsies and can be challenging to distinguish without the use of IHC stains. Most GI tumors may be distinguished from one another based on their distinct IHC profile. Immunohistochemical stains will play an ever more significant role in identifying the origin and differentiation of GIT malignancies when samples shrink in size. Concurrent improvements in imaging methods allowed for a wider and more supportive use of IHC in molecular research, which in turn made it easier to create new treatment approaches. Using IHC techniques, this work aims to discuss the various gastrointestinal tract neoplasm.

KEYWORDS

Immunohistochemistry(IHC), Gastrointestinal tract (GIT)neoplasm.

INTRODUCTION

Esophagus

Squamous cell carcinoma

Patients may present with dysphagia, odynophagia and weight loss. The IHC profile of SCC is CK7-, CD20-, CK5/6+, p63+. Additionally, most cases of esophageal SCC are also positive for p53, a finding not seen in normal esophageal mucosa. HPV cases have been found to be positive for p16 as well, much similar to cases of cervical SCC .(1)

- Intestinal Metaplasia : MUC2 IHC: de novo expression in the cytoplasm of goblet cells and CDX2: nuclear positivity of goblet cells and columnar mucosa.(2)
- Adenocarcinoma: These tumors typically have an immunohistochemical pattern resembling that of gastric adenocarcinoma, expressing CK7, variable CK20, AMACAR, and weak localized CDX-2. In contrast to SCC, P16 is negative in esophageal adenocarcinoma.(3)

Stomach

Most of the cases are adenocarcinoma either diffuse or intestinal type.

Adenocarcinoma

Intestinal type GA shows variable expression of CK7 ,CK20 , CDX-2, MUC1, and MUC5AC .Diffuse type of GA usually develops de novo, and is not associated with H. pylori induced IM. Over 70% of cases of the diffuse type of GA are positive for CDX-2 , CK7 , HepPar-1 and variable expression of CK20 ,MUC2 and MUC5AC, but negative for MUC1 and E-cadherin.(1)

The CK7/CK20 expression patterns of gastric carcinoma vary considerably; on the whole, approximately 70% of the cases are CK7+ and 20% are CK20+. (4)

Small Intestine

The small intestine includes the duodenum, jejunum and ileum extending from the pylorus to ileocecal valve.(1)

Adenocarcinoma: Small intestinal adenocarcinomas are CK7+ in more than half of all cases , unlike normal small intestinal mucosa which is CK7- and colorectal adenocarcinomas which are CK7-/CK20+ . Adenocarcinomas of the small bowel are also positive for CK20, CDX-2.(1)

Gastrointestinal Neuroendocrine Tumors:

The location of these carcinoid tumors can be divided based on their embryologic derivation into carcinoids of the foregut (esophagus, stomach and duodenum), midgut (jejunum, ileum, appendix and ascending colon) and hindgut (transverse colon, descending colon, sigmoid and rectum).(1)

Carcinoids from the foregut and midgut are generally positive for chromogranin A and CD56, while those from the hindgut are usually negative . Hindgut carcinoids on the other hand often express prostatic acid phosphatase . A less helpful marker is CDX-2, which although positive for most colorectal carcinomas has an immunoreactivity of about 40% in well differentiated carcinoids but has a reported 80% expression rate in poorly differentiated carcinoids.(1)

Colon and Rectum

Colorectal adenocarcinomas are invariably positive for cytokeratin (CK), with the most common pattern being positivity for CK20 and negativity for CK7.The reverse pattern is extremely rare, which is helpful in distinguishing colorectal adenocarcinoma from adenocarcinomas of other sites, such as lung and ovary. (5)

SATB2: Specific for colorectal carcinoma, differentiating from upper GI tract tumors.

Anal Tumors:

Immunohistochemically, most anal squamous carcinomas exhibit reactivity for keratins 4, 5/6, 13, 17, 18, and 19, with a minority expressing keratins 1, 7, and 10. p63 and SOX2 (an HMG-box embryonic stem cell transcription factor) are also expressed by the vast majority of anal SCC. (6)

Adenocarcinoma of the anal canal is much less common, accounting for about 10% of all anal cancers (1).

Appendix:

Mucinous neoplasms of the appendix

Mucinous neoplasms of the appendix are the most common type of epithelial neoplasms in the appendix.(1) Appendiceal mucinous neoplasms and pseudomyxoma peritonei typically express cytokeratin 20, CDX-2, and MUC2; cytokeratin 7 is variably positive. (7)

Pancreas (1)

Ductal adenocarcinoma of the exocrine pancreas comprises about 90% of all cases of pancreatic malignancy. Most pancreatic ductal adenocarcinomas express cell surface associated mucins, including MUC1, MUC3, MUC4, and MUC5AC.

The keratins expressed are those of simple epithelia such as 7, 8,18, and 19 (as normal ductal cells),208 but also 20, 17, and occasionally cytokeratin 5/6.Cytokeratin 20 is expressed less frequently in pancreatic ductal than in ampullary adenocarcinomas

Ampullary carcinoma : (1)
The tumors with pancreatobiliary appearance are CK7+/CK20–, whereas those with an intestinal morphology are usually CK7–/CK20+.Furthermore, the combination of MUC2 and CDX2 positivity is typical of the intestinal type tumors but not pancreatobiliary type .

Gall Bladder:
Microscopically, the large majority of bile duct malignancies are well-differentiated pancreatobiliary-type adenocarcinomas similar to their counterparts in the gallbladder and pancreas.(8)

The usual keratin profile for carcinoma of the gallbladder and extrahepatic bile ducts is CK7+/CK20+, although the CK20 staining is often focal. In contrast, peripheral (intrahepatic) cholangiocarcinoma is usually CK7+/CK20−. Other markers found in gallbladder adenocarcinoma include CK19, claudin-4, MUC1, CA19-9, and carcinoembryonic antigen (CEA).A proportion of cases show CDX2 expression but usually not as extensively and intensely as in colorectal carcinoma, and gallbladder adenocarcinomas may also express hepatocyte antigen. Of note, the mucinous variant of gallbladder carcinoma is more likely to express CK7−, MUC5AC+, and MUC1−. (8)

Liver:
Hepatocellular carcinoma (HCC)
HCC is the sixth most common malignancy and third most common cause of mortality from cancer worldwide.(2)

Hep Par-1 (also known as hepatocyte-specific antibody, or HSA) is a monoclonal antibody that is believed to react with urea cycle enzyme carbamoyl phosphate synthetase 1 (CPS1), a rate-limiting enzyme in the urea cycle that is present in hepatocyte mitochondria. Because of its good (although not absolute) specificity, it has become one of the most reliable markers of hepatocytic differentiation. (9)

Glyican-3, a marker which is exclusively expressed in neoplastic processes and not normal tissue in humans , and CD34 a vascular marker which highlights the increased vascularity seen in HCC .

Cholangiocarcinoma(CC) arises from the intrahepatic bile duct epithelial cells and can be divided based on the location of origin, intrahepatic/peripheral CC arise at the confluence of the right and left hepatic ducts, while the extrahepatic CC arise between the ampulla of Vater and the hepatic hilium. Depending on their location the presenting symptoms may also differ. Histologically, CC is similar to ductal adenocarcinoma of the pancreas with tumor cells arranged in tubules and glands which may be cribriform or form nests, solid cords and papillary structures . CC is positive for CK7, CK17, mucin, CEA (cytoplasmic and luminal), CAM 5.2, CK19, EMA and CK20 (30-70%). (2)

Metastasis:
Metastasis to GIT from other sites are also included in which primaries are detected by using IHC markers.

Aims and Objectives
- Evaluate the role of immunohistochemistry (IHC) in diagnosing and differentiating gastrointestinal neoplasms.
- Assess the utility of IHC markers in tumor classification and subtyping.
- Explore the role of IHC in determining the site of origin of tumors.
- To know the diagnostic utility of IHC markers in GIT neoplasm.

Table 1 : No. of Cases with IHC Marker in GIT.

Location	No. of cases	Neoplasm Type	IHC markers	Diagnostic Implications
Esophagus	10	Dysplasia	P63 Negative	Useful to rule out doubtful cases
	20	Squamous cell carcinoma	P63+	P63+ supports Squamous cell carcinoma and rule out
	02	Adenocarcinoma	CDX2 +	adenocarcinoma if the histopathology is not clear.

Material and Methods
Place of Study: At the department of pathology, in Histopathology and IHC section of Shri M.P. Shah Government Medical College, Jamnagar.

Design of Study: A diagnostic Prospective and Retrospective Study.

Duration of Study: 2 Years .

Sample Size: A total of 120 patients were enrolled for this study presented with various GI lesions .

Sample Types: GIT specimens (Biopsy or excised Lesion) received in histopathology section of the pathology department.

Inclusion Criteria
- All age groups of both sexes.
- Various investigations (Endoscopy, Colonoscopy, CT scan) suggestive of GIT lesion.

Exclusion Criteria
- Lesions with only inflammatory etiology are excluded.

Procedure
For resected specimen and biopsy: For histopathological analysis, the specimen's representative sections—or, in the case of biopsies, the entire specimen—was submitted. Blocks of tumour tissue of 2cm x 2cm x 0.5cm were gathered, fixed for a 24 hours period in a 10% neutral buffered formalin solution, and then processed histologically and paraffin embedded. Three to five um thick histological paraffin slices were cut. Light microscopy was used to assess the H&E staining. Morphology was used to make the diagnosis.

IHC Panel: IHC using relevant antibodies was done according to histomorphological features wherever needed. Immunohistochemical studies were carried out with 3-4um thick sections . The antibodies included are p63, CK7,CK20, CDX 2, SMA, Desmin, DOG-1, Caldesmon, CD 117, CD34, S100,Vimentin, Pan CK,WT1, EMA, SATB2, MUC-2, p40, Glypican-3, AFP, CEA, Ki67, ER, PR, Her2Neu, GATA3, TTF-1, Napsin A, Chromogranin.

The sections will be taken on poly L lysine coated glass slides. The secondary antibody kit of Dygnova will be used for the study. The primary monoclonal ready to use antibodies will be of DAKO, Thermofischer, Biogenex, Diagnostic Biosystems and Biocare Medical company. Tris-EDTA solution of high pH (8.4) will be used for the antigen retrieval, i.e Heat Induced Epitope Retrieval (HIER), in pressure cooker for up to one whistle. Thereafter the slides will be brought down to room temperature and taken through the steps of the immunostaining protocol using Tris buffered saline as the wash buffer. The peroxide block will be freshly prepared for each use. The sections will be covered with one to two drops of the primary antibody for incubation for 30 minutes to 2 hours according to company manual in humid chamber. The slides will be thoroughly washed with tris buffered saline in between each step. After this the polymer HRP provided in the kit will be used. Finally DAB chromogen will be used in the specific concentration as specified by the company. The slides will be counter stained by haematoxylin and the mounted by DPX. After drying the test slides will be examined under the light microscopy simultaneously with the positive control slides. Negative external control slides will be treated similarly except that the primary antibody was omitted. The positive control section for respective IHC marker will be selected appropriately.

OBSERVATIONS AND RESULTS :

Fundus	05 z 05 and 03	Gastric polyp Signet cell adenocarcinoma Intestinal type of adenocarcinoma	CK20,CDX 2 :Negative CK7,CK20 + CDX 2+	Useful to rule out doubtful cases IHC is supportive to the histopathology findings.
Pylorus	04	Adenocarcinoma (Diffuse and intestinal type)	CK7,CK20 CDX2 +	IHC is supportive to the histopathology findings.
Duodenum	02 01	Polyp Adenocarcinoma	CK20 - CDX 2- CK7 – CK20 + CDX 2+	Useful to rule out doubtful cases
Jejunum	02	Leiomyoma	SMA: + Desmin: + DOG1: -	
Ileum	01 01	Leiomyoma GIST	Caldesmon: + SMA: + Desmin: + DOG1,CD117,CD34: - DOG 1: + CD117: + S100: -	Supports leiomyoma and negative markers rules out GIST IHC is supportive to the histopathology findings.
Ileocaecal junction	05 05 01	Adenocarcioma (Intestinal type) Mucinous adenocarcinoma GIST	CK20: + CDX2: + CK 7: - CK7- CK20+ CDX2+ MUC2+ DOG 1: + CD117: +	
Colon	06 01	Adenocarcinoma Desmoplastic small round cell tumor(01)	CK20: + CDX2: + CK 7: - SATB2 + PAN-CK,Vimentin: + (Dot like cytoplasmic) Desmin: + (Dot lke perinuclear cytoplasmic) WT-1(6F-H2): + (cytoplasmic nuclear) EMA: + (cytoplamic)	
Rectum	02 02	Rectal polyp Dysplasia	CK7: - CDX2: -	IHC is supportive to the histopathology findings.
Colorectal	05 03	Diffuse Colorectal adenocarcinoma Mucinous adenocarcinoma of colon	CK7: - CDX2: + CK20: + CK20 + CDX2+ MUC2+	
Rectosigmoid	06	Adenocarcinoma	CK7: - CDX2: + CK20: +	
Anal	04	Squamous cell carcinoma	p63: + p40: + CK7: - CDX2: - CK20: -	
Appendix	01	Low grade appendiceal mucinous neoplasia	CK20,CDX2,MUC2 +	
Liver	02 01	Intra hepatic cholangiocarcinoma Hepatocellular carcinoma(01)	CK 7+ CK20- Glypican-3 – AFP+ Glypican-3 +	

Gall bladder	04 02	Adenocarcinoma Adenomyomatosis	P53+ CK7+ CK20- EMA+ CEA+ Ki67-63% Negative	IHC is supportive to the histopathology findings.
Pancreas	01	Ductal Adenocarcinoma	CK7+ CK20- CDX2- MUC-1+	
Ampulla	03 03	Pancreaticobiliary adenocarcinoma Intestinal type periampullary carcinoma	EMA(MUC1): + CDX2,CK20: - CDX2,CK20, MUC2: + MUC1: -	

Table 1: Shows the no. of cases with the IHC marker details of various areas of GIT.

Table 2: Cases of Metastasis into GIT from Various Primaries.

Sr. No.	Metastasis	Primary organ	IHC markers	Diagnostic implication
1.	Liver	Breast	PanCK + EMA+ ER,PR- Her2Neu+ Ki67-60%	IHC is supportive to the histopathological and radiological findings.
2.	Liver	Breast	ER+ PR+ Her2Neu – Ki67-76%	
3.	Liver	Breast	GATA3 + PanCK + Ki67-15%	
4.	Liver	Adenocarcinoma of GIT	CDX2+ CK7+ CK20-	
5.	Liver	Small cell carcinoma of lung	TTF-1+ CK7+ Chromogranin + Napsin A-	
6.	Soft tissue near stomach	Adenocarcinoma of sigmoid colon	CK7+ CDX2+	
7.	Mass over Gluteal region	Colorectal Adenocarcinoma	CK7+ CK20+ CDX2+	

RESULT

Out of 120 cases of GIT over period of 2 years , 113 are of primary origin in which 26 are benign and rest are malignant. Remaining 7 cases are secondaries to GIT from other body tissue such as breast, lung or part of GIT other than original lesion site.

IHC markers were very useful to detect the origin of lesion and for subtyping.

DISCUSSION

Wong HH et.al. published the various IHC markers of GIT in their publication as follows:

Summary of immunohistochemical profiles of tumors of the gastrointestinal tract

Tumor types	Keratin 7	CK20	MUC1	MUC2
Esophageal adenocarcinoma	92%	62%	36%	19%
Gastric adenocarcinoma, intestinal	63%	32%	0%	27%
Gastric adenocarcinoma, diffuse	75%	42%	33%	11%
Small intestinal adenocarcinoma*	92%	59%	52%	43%
Colorectal adenocarcinoma	9%	85%	35%	40%
Appendiceal adenocarcinoma	28%	100%	17%	100%
Pancreatic ductal adenocarcinoma	96%	40%	87%	9%
Cholangiocarcinoma	91%	35%	60%	10%
Adenocarcinoma of Ampulla of Vater	58%	44%	58%	21%

Image1: showing the summary of IHC profile of various GIT tumors published by Wong HH et.al.[1]

In my present study, we have taken the 120 cases of various regions of GIT and performed the IHC markers after doing the histopathological examination.

Table3: Percentage of various adenocarcinoma cases showing IHC marker positivity in GIT.

Tumors	CK7	CK20	MUC1	MUC2
Esophageal Adenocarcinoma	100%	-----	-----	-----
Gastric adenocarcinoma (intestinal)	100%	100%	-----	-----
Gastric adenocarcinoma (Diffuse)	100%	100%	-----	-----
Small intestine adenocarcinoma	0%	100%	-----	50%
Colorectal adenocarcinoma	0%	100%	-----	50%
Appendiceal adenocarcinoma	-----	100%	-----	100%
Pancreatic ductal Carcinoma	100%	-----	100%	-----
Cholangiocarcinoma	100%	0%	-----	-----
Ampullary adenocarcinoma	50%	50%	50%	50%

CONCLUSION

IHC is a crucial tool for diagnosing GI neoplasms, helping in tumor type identification, origin determination of any metastatic lesion and differentiation of neoplastic lesions through specific antibodies. This enhances diagnostic accuracy and informs treatment strategies.

Ongoing research into novel IHC markers will further refine practice and improve patient outcomes in gastrointestinal oncology.

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