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IMMUNOHISTOCHEMICAL EXPRESSION OF CYTOKERATIN 20 IN VARIOUS MALIGNANT CONDITIONS OF GASTROINTESTINAL TRACT

Pathology

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ABSTRACT

Cancer of gastrointestinal tract is one of the common types of malignancies occurring in human race. The diagnosis of GIT tumors can be made easily with haematoxylin and eosin stained sections but immunohistochemistry is needed in few cases as diagnostic and prognostic marker.

AIM: The aim of the study was to study the incidence of expression of cytokeratin 20 in various gastrointestinal malignancies of different histological types along with their metastases if any. With the above aim in mind 40 cases of gastrointestinal malignancies, primary and metastases in lymph node, submitted in the Department of Pathology, Government Medical College, Amritsar were included in the study.

MATERIAL AND METHOD: The formalin fixed and paraffin embedded sections were cut and stained with routine haematoxylin and eosin (H & E) for histological typing of tumor. These cases were further subjected to immunohistochemical staining for cytokeratin 20 using primary antibody: Ready to use mouse monclonal anti human cytokeratin 20 protein, clone Ks 20.8 (Novocastra).The immunostaining results were assessed semiquantitatively.

RESULTS: The Cytokeratin 20 seems to be a suitable adjunct marker to evaluate the primary origin of carcinomas in specific context, especially to separate metastatic adenocarcinomas of gastrointestinal versus non-gastrointestinal origin.

KEYWORDS

Carcinoma, Malignancies, Cytokeratin, CK20, Gastrointestinal.

INTRODUCTION

Since its inception in the 1940s, immunocytochemistry has gradually developed into a major diagnostic tool in diagnostic cellular pathology (1).Cancer of gastrointestinal tract is one of the common type of malignancies occurring in human race. Its distribution in different segments of tract exhibits variations from country to country (2). When an epithelium undergoes malignant transformation, its keratin profile usually remains constant. Therefore, since keratin expression varies so greatly among different epithelia, they have been widely used in the finger printing of various carcinomas. Demonstration of cytokeratin expression using immunohistochemical technique is used as a diagnostic marker to identify poorly differentiated carcinomas(3). CK 20 is valuable as a histodiagnostic tool for the subtyping of various carcinomas including adenocarcinomas(4). It is an aid for a more precise classification of many epithelial tumors whose differential diagnosis is otherwise difficult. Because of its restricted range of expression in humans, it has become an important tool for detecting and identifying metastatic cancer cells by immunohistochemistry and by PCR analysis. The high degree of stability of CK 20 expression during malignant transformation means that this CK is of value as a marker of malignancy(5). Thus, CK 20 as specifically detected by applying monoclonal antibodies to paraffin sections, represents a intermediate filament protein marker of promising diagnostic potential with regard to the histogenesis and typing of carcinomas(6). When applied in combination with other differentiation markers (including other cytokeratin polypeptides or intermediate filament proteins) and careful morphologic examination, this most recently identified CK should become a valuable aid for the precise classification of many epithelial tumors (e.g. adenocarcinoma of the endometrium, ovary (non mucinous), kidney, thyroid, breast, lung and salivary gland as well as mesothelioma) whose differential diagnosis is otherwise difficult. Hence, cytokeratin 20 seems to be a suitable adjunct marker to evaluate the primary origin of carcinomas in specific context, especially to separate metastatic adenocarcinomas of gastrointestinal versus non-gastrointestinal origin(7).

MATERIAL AND METHODS

The study was conducted in Department of Pathology, Government Medical College, Amritsar. The material for present study comprised of 40 cases of gastrointestinal malignancies, primary and metastases in lymph node, submitted in the Department of Pathology, Government Medical College, Amritsar. A detailed history and clinical profile were obtained from histopathological records along with gross examination of the specimen was done regarding site, size, growth pattern (polypoidal, infiltrative, ulcerative, fungating) etc. Areas of haemmorrhage, necrosis and lymph node involvement were looked for. These tissue were processed and finally sections of 3-5 m thickness were cut and stained with haematoxylin and eosin (H & E). The sections were studied microscopically for different histological types along with the involvement of muscle, serosa and lymph node. These cases were further subjected to immunohistochemical staining for cytokeratin 20 using primary antibody: Ready to use mouse monoclonal anti human cytokeratin 20 protein, clone Ks 20.8 (Novocastra).The slides were observed under light microscope and positive immunostaining for CK 20 was identified as brown staining in the cytoplasm, the cell membrane or both tumor cell components. The immunostaining result was assessed semi quantitatively. No staining or very exceptional positive cells were considered a negative result. Staining in <10% of the tumor cells was considered focally positive, and staining in >10% of tumor cells was considered diffusely positive(8).

OBERVATIONS AND RESULTS

The present study was conducted on 40 cases of surgically resected biopsied specimens of gastro-intestinal malignancies, primary and the metastases in lymph node, received in the Department of Pathology, Government Medical College, Amritsar.

Detailed history of patients was obtained from histopathological records available and each specimen was processed and finally subjected to H & E staining. The cases diagnosed as malignant tumors were subjected to Cytokeratin 20 immunostaining.

TABLE I SHOWING AGE AND SEX

Age in years	No of cases of GIT cancers		Sex			
	No	%	Male		Female	
			No	%	No	%
0-10	0	0%	0	0%	0	0%
11-20	2	5%	2	100%	0	0%
21-30	4	10%	3	75%	1	25%
31-40	6	15%	5	83.3%	1	16.7%
41-50	4	10%	3	75%	1	25%
51-60	14	35%	8	57.1%	6	42.9%
61-70	8	20%	5	62.5%	3	37.5%

Maximum number of cases were observed in age group 51-60 years with total of 14(35%) which comprised 8(57.1%) males and 6(42.9%) females.

TABLE II SHOWING SITE OF LESION

Site of Lesion	No. of Cases	Percentage
Oesophagus	9	22.5%
Stomach	4	10%
Small Intestine	1	2.5%
Ileocaecal region	2	5%
Colorectal region	20	50%
Anal Canal	4	10%

Maximum number of cases i.e. 20(50%) involved colorectal region.

TABLE III GROSS EXAMINATION OF GIT MALIGNANCIES

Gross examn	Oesophagus	Stomach	Small Intestine	Ileocaecal region	Colorectal region	Anal
Ulcerative	-	-	1	-	2	-
Polypoidal	5	1	-	1	6	1
Infilterative	3	3	-	1	11	-
Ulceropolypoidal	-	-	-	-	-	1
Endoscopic biopsy	1	-	-	-	-	-
Colonoscopic biopsy	-	-	-	-	1	-
Biopsy	-	-	-	-	-	2
Total	9	4	1	2	20	4

Maximum number of malignancies were infilterative on gross examination.

TABLE IV HISTOPATHOLOGICAL EXAMINATION OF GIT MALIGNANCIES

HP	OES	Stomach	SI	ILEOCAECAL	COLORECTAL	ANAL
SCC						
WD	5	-	-	-	-	-
MD	2	-	-	-	-	-
PD	-	-	-	-	-	-
Adenoca.						
WD	2	-	-	1	12	2
Mucinous.	-	2		1	5	2
MD	-	2	-	-	3	-
PD	-	-	-	-	-	-
MALT -Lymphoma	-	-	1	-	-	-
T0TAL	9	4	1	2	20	4

WD-Well differentiated
MD-Moderately differentiated
PD-Poorly differentiated
Maximum cases were of colorectal carcinomas which were further histologically typed as Well differentiated Adenocarcinoma.

TABLE V LYMPH NODE, MUSCLE AND SEROSAL INVOLVEMENT IN GIT MALIGNANCIES

GIT Malignancies	Lymph node involvement			Muscle infilteration			Serosal involvement		
	+VE	-VE	NA	+VE	-VE	NA	+VE	-VE	NA
Oesophagus	2	6	1	6	2	1	-	-	-
Stomach	3	1	-	4	-	-	1	3	-
S.I	-	1	-	1	-	-	-	1	-
Ileocaecal region	-	2	-	1	1	-	-	2	-
Colorectal region	-	-	-	-	-	-	-	-	-
Anal	1	1	2	4	-	-	1	1	2

GIT malignancies when studied under microscope showed Muscle infiltration more as compared to Lymph node or Serosal involvement.

TABLE VI
CK 20 IMMUNOREACTIVITY IN GIT MALIGNANCIES

Site	Ck20 Immunostaining			Total
	Diffuse +ve	Focal +ve	-ve	
Oes	-	-	9(22.5%)	9
Stomach	-	2(5%)	2(5%)	4
S.I	-	-	1(2.5%)	1
Ileocaecal	1(2.5%)	1(2.5%)	-	2
Colorectal	17(42.5%)	1(2.5%)	2(5%)	20
Anal	-	-	4(10%)	4
Toatal	18(45%)	4(10%)	18(45%)	40

Out of the total 40 cases of GIT malignancies, 18(45%) cases were diffuse positive for CK 20 immunostaining whereas 4(10%) cases were focal positive and 18(45%) were negative.

TABLE VII
CK 20 IMMUNOREACTIVITY ADENOCARCINOMA, SQUAMOUS CELL CARCINOMAAND MALT LYMPHOMA

Histological type	No. (%)	CK+VE (Diffuse and Focal)	CK-VE
		No.(%)	No.(%)
Adenoca	28(70%)	22(78.6%)	6(21.4%)
SCC	11(27.5%)	-	11(100%)
MALToma	1(2.5%)	-	1(100%)
Total	40(100%)	22(55%)	18(45%)

Out of the total 40 cases, 28(70%) cases were of adenocarcinoma, 22(78.6%) cases of adenocarcinoma showed CK 20 positivity and 6(21.4%) cases were negative for CK 20 immunostaining.

Out of the total 40 cases, All the 11(100%) cases of Squamous cell carcinoma were negative for CK 20 immunoexpression. A single identified case was of MALT lymphoma did not show immunostaining for CK 20.

TABLE VIII
COMPARISION OF CK20 IMMUNOSTAINING IN ADENOCARCINOMA OF OESOPHAGUS, STOMACH, ILEOCAECAL REGION AND COLORECTAL REGION

Site	CK 20 Positive		CK 20 Negative	
	Diffuse Positive + Focal Positive			
	No.	%age	No.	%age
Oesophagus	-	-	2	100%
Stomach	2	50%	2	50%
Ileocaecal region	2	100%	-	-
Colorectal region	18	90%	2	10%
Total	22	78.6%	6	21.4%

Both the cases (100%) of Oesophageal adenocarcinoma did not express for CK 20.

Out of the total 4 cases of adenocarcinoma of stomach 2(50%) cases were positive for CK 20 immunostaining while 2(50%) cases were negative for CK 20 expression.

2(100%) cases of adenocarcinoma of ileocaecal region were positive for CK 20 immunoexpression.

Out of the total 20 cases of colorectal adenocarcinoma 18(90%) cases showed CK 20 staining pattern whereas 2(10%) cases were negative.

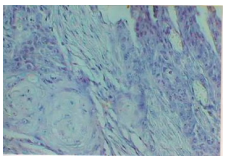
TABLE IX
SHOWING CYTOKERATIN CK 20 STAINING IN GIT MALIGNANCIES WITH LYMPH NODE METASTASES

GIT Malignancies with lymph node metastases		CK 20 Staining		
	TOTAL CASES	DIFFUSE POSITIVE	FOCAL POSITIVE	NEGATIVE
OESOPHAGUS	3	-	2(67%)	1(33%)
STOMACH	3	-	2(67%)	1(33%)
SMALL INTESTINE	-	-	-	-
ILEOCAECAL REGION	-	-	-	-
COLORECTAL REGION	5	4(80%)	-	1(20%)
ANAL	1	-	-	1(100%)

Out of all cases presenting with lymph node metastases maximum diffuse CK20 immunopositivity was seen in colorectal carcinoma .

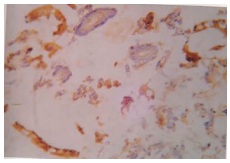
PHOTOGRAPHS

1)



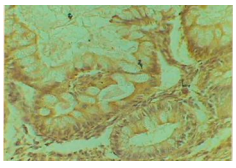
PHOTOGRAPH SHOWING NEGATIVE CK20 IMMUNO EXPRESSION IN WELL DIFFERENTIATED SCC OFOESOPHAGUS (400X)

2)



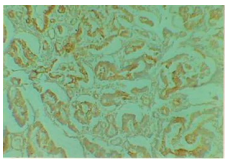
PHOTOGRAPH SHOWING FOCAL POSITIVE CK20 IMMUNOEXPRESSION IN MODERATELY DIFFEREN TIATED

3)



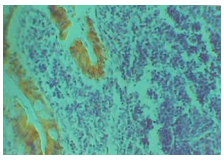
PHOTOGRAPH SHOWING DIFFUSE POSITIVE CK20 IMMUNOEXPRESSION IN WELL DIFFERENTIATED ADENOCARCINOMA OF ILEOCAECAL REGION(400X)

4)



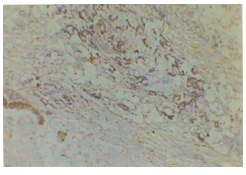
PHOTOGRAPH SHOWING DIFFUSE POSITIVE CK20 IMMUNOEXPRESSION IN WELL DIFFERENTIATE MUCINOUS ADENOCARCINOMA OF COLON(100X)

5)



PHOTOGRAPH SHOWING NEGATIVE CK20 IMMUNOEXPRESSION IN MALT LYMPHOMA OF SMALL INTESTINE(400X)

6)



PHOTOGRAPH SHOWING DIFFUSE POSITIVE CK20 IMMUNOEXPRESSION IN METASTATIC DEPOSITS IN LYMPH NODES (I00X)

DISCUSSION

CK 20 is valuable as a histodiagnostic tool for the subtyping of various carcinomas including adenocarcinomas(4). It is an aid for a more precise classification *of* many epithelial tumors whose differential diagnosis is otherwise difficult. Because of its restricted range of expression in humans, it has become an important tool for detecting and identifying metastatic cancer cells by immunohistochemistry(5).

In the present study it was observed that squamous cell carcinoma involving GIT either in the oesophagus or in the anal canal, primary as well as metastatic, lack expression for CK 20. This was in congruence with the two different studies conducted by Roland Moll(1992) and Chu PG(2000) along with their colleagues, where all the 5(100%) cases of oesophageal squamous cell carcinoma (primary tumors and metastases) and all the 14 cases (100%) of squamous cell carcinoma were respectively negative for CK 20 immunostaining(4,9)

In a study done by Chu PG and Weiss LM it was observed that anal squamous cell carcinomas are negative for CK 20 expression(10).

Adenocarcinomas, primary as well as metastatic were found to be positive for CK 20 immunostaining. Maximum immunopositivity was seen in adenocarcinomas involving ileocaecel and colorectal region simulating a study by Roland Moll and associates (1992) of 711 cases of primary and metastatic cancers, CK 20 positivity was consistently expressed in 95.6% (n=89/93) cases of adenocarcinomas of the colon. (11) Whereas moderately differentiated adenocarcinomas of stomach had focal positive expression for CK 20. Poorly differentiated adenocarcinomas involving stomach were negative for the same. In a study conducted by Chu PG and colleagues (2000) 50% cases of gastric adenocarcinoma were CK 20 positive(11).

Oesophageal adenocarcinomas included in the current study were also negative for CK 20 expression contradicting the previous study, by Chu PG and Weiss LM (2002) where 16% cases of adenocarcinoma of oesophagus were observed to be positive for CK 20 expression(5). This variation in the result could be attributable *to* small sample size. Hence, it is concluded that CK 20 expression is less marked in fore gut adenocarcinomas as compared to those of hind gut. Majority of the cases of well differentiated mucinous adenocarcinoma involving ileocaecal and colorectal region had diffuse positive CK 20 immunostaining whereas few cases were observed to be focally positive.

Also lymph node metastases from majority of the colorectal adenocarcinomas had a diffuse positive CK 20 immunostaining.

Hence, it is concluded that most consistent and extensive CK 20 immunoexpression is seen in primary and metastatic colorectal adenocarcinomas. Other tumors involving gastrointestinal tract such as squamous cell carcinoma and MALT lymphoma was observed to be negative for CK 20 expression.

Metastases to gut from adenocarcinomas located elsewhere such as those from endometrium, ovary (non-mucinous), kidney, thyroid breast, lung and salivary glands as well as mesothelioma lack CK 20 immunoexpresion. Hence CK 20 is a useful marker in the differential diagnosis of poorly differentiated tumors of gastrointestinal tract.

SUMMARY AND CONCLUSIONS

It was concluded that CK 20 immune reactivity was found to have wide range of variation in different segments of gastrointestinal tract malignancies and noted CK 20 immunopositivity was more marked and consistent in hind gut adenocarcinomas as compared to those of foregut.

In comparison to adenocarcinomas of gut, squamous cell carcinomas and MALT lymphoma lack immunostaining for CK 20.

Diagnostic Applications

The present study has shown that high degree of stability of CK 20 expression during malignant transformation in both the primary tumor and corresponding metastases of a clearly defined subset of carcinomas marks it as valuable marker of malignancy. The Cytokeratin 20 seems to be a suitable adjunct marker to evaluate the primary origin of carcinomas in specific context, especially to separate metastatic adenocarcinomas of gastrointestinal versus non-gastrointestinal origin.

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