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Immunohistochemical Alysis of Ctokeratin 7 and Cytokeratin 20 Expression in Esophageo-Gastrointestinal Epithelial Malignancies

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ABSTRACT

Gastrointestinal malignancies are commonest in the general population with higher incidence of adenocarcinomas. The primary site of origin of adenocarcinomas is difficult to determine, solely by histopathological examination. Thus understanding the site specific immunohistochemical expression of gastrointestinal adenocarcinomas is worthwhile. This study aims to analyse the expression of Cytokeratin 7 and 20 by immunohistochemistry in esophageal, gastric and intestinal carcinomas. This study included 90 specimen, comprising of 20 esophageal, 30 gastric and 40 colonic carcinomas. Routine histopathological examination was done and paraffin blocks were stained with Cytokeratin 7 and 20 immunohistochemistry. Correlation of the study results with anatomic site of origin of tumor was done. Out of 20 esophageal carcinomas, 15 were squamous cell carcinoma and 5 were esophageal adenocarcinoma. Cytokeratin 7 and 20 was negative(Score 0) in 100% cases of squamous cell carcinoma. Majority of the esophageal adenocarcinomas showed score 2+ positive staining for Cytokeratin7 and were negative (Score 0) for cytokeratin 20. 30 gastric carcinomas were included in the study and all the cases were adenocarcinomas. Cytokeratin 7 positivity was noted in 100% of cases of gastric adenocarcinoma and Cytokeratin 20 was found to be positive in 19/30 cases (63%). 39/40 cases (95%) of colonic carcinomas showed Cytokeratin 20 positivity. Esophageal adenocarcinoma showed CK7+/20-, dual positivity favored gastric carcinoma and CK7-/CK20+ expression in colonic adenocarcinoma. We conclude that the property of organ and site specificity of Cytokeratins can be utilised in diagnosing the primary esophageo-gastriointestinal carcinomasand highlights the combined utility of Cytokeratin 7 and 20 immunoexpression.

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Key Words
Cytokeratin 7, cytokeratin 20, colonic adenocarcinoma, esophageal carcinoma, gastric cancer, Immunohistochemistry

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INTRODUCTION

Gastrointestinal tract is a fertile soil for many malignancies, affecting the general population, worldwide[1]. Esophageal carcinomas constitute the 4th most common cause of deaths due to cancer and generally have poor prognosis[2]. Stomach cancer is the second most common cancer and leads to 20% of deaths within 5 years of diagnosis. These carcinomas usually present at advanced stage of the disease[3]. Colorectal malignancy is a major health burden which leads to increased morbidity and mortality[4]. Colorectal carcinoma has an incidence of 1.3 million cases each year. It is the fourth most common cancer worldwide, while it is the fifth common cancer affecting the Indian population. Compared to western world the incidence rates are low in India. It is thought that the westernized lifestyle of physical inactivity, poor diet, obesity and increased alcohol consumption contributes to the increased burden of the colorectal carcinoma[5]. Most commonly occurs after 6th to 7th decade of life. Colorectal malignancy in young people are located in distal colon and rectum, aggressive in behaviour and occur in cases of hereditary genetic mutation. Alcohol use, tobacco and obesity also contributes to the increased risk of incidence. With the advent and widespread use of colonoscopy, it is now possible to readily detect the precursor lesions such as adenoma, chronic inflammatory bowel disease and treat them accordingly. Colorectal carcinoma is the most curable carcinoma of gastrointestinal tract[5].

The most common malignancies of gastrointestinal tract are adenocarcinomas. Due to the late clinical presentation, metastatic deposits to the regional and distant lymph nodes is the common initial hint to the underlying advanced carcinoma. The primary site of origin of adenocarcinomas is difficult to determine, solely by histopathological examination. This poses an enigmatic situation to both the clinician and the pathologist. Thus understanding the site specific immunohistochemical expression of gastrointestinal adenocarcinomas is worthwhile[1].

Structurally, Cytokeratins are the intermediate filaments in the cytoplasm of epithelial cells[4]. Cytokeratin 7 belongs to high molecular weight (Type II) keratin and Cytokeratin 20 is a low molecular weight keratin (Type I)[6]. Interestingly, the distribution these cytokeratins is organ and site specific. This property can be used to determine thesite of origin of epithelial. Neoplasms[6]. This study aims to analyse the expression of Cytokeratin 7 and 20 by immunohistochemistry in the esophageal and gastrointestinal epithelial malignancies.

MATERIALS AND METHODS

The present study was conducted in our institute during the period of January 2019 to May 2021. Approval to conduct the study was obtained from Institutional Ethics Committee.This is a prospective studyof 90 specimen which included biopsies and resected specimen of esophageal and gastrointestinal epithelial malignancies that were received in our department. Relevant clinical details were obtained from the patient case records. Specimen were grossed and histopathological examination was done according to the standard protocol. Formalin fixed paraffin blocks were obtained and sections were taken on charged poly-l-lysine slides for immunohistochemistry. TRIS EDTA buffer and citrate buffer were used for antigen retrieval for Cytokeratin 7 and 20, respectively. Poly Excel Stunn DAB was used for immunostaining and counterstained with hematoxylin.

Cells showing brown cytoplasmic stain were considered as positive and graded according to the three scaled scoring system (1+, 2+ and 3+) based on the intensity and completeness of staining in more than 10% of cells.

Ethics: Ethics Committee approval for the conduct of the study was obtained from Institutional Ethics Committe. Ref number-EC REG:ECR/134/Inst/KA/2013/RR-16

Statistics: The data was statistically represented in frequency distribution tables and tested using chi square/fischer test. $p<0.05$ was considered statistically significant.

RESULTS

Our study included 90 esophageo-gastrointestinal specimen, out of which 50 specimen belonged to upper gastrointestinal tract, including 20 esophageal cancers and 30 specimen from gastric origin. In our study, 40 cases of colonic carcinoma were included, among them 11 were from right colon and 29 specimen were from left colon, none from transverse colon. Demographic analysis revealed that majority of the cases were in 6th and 7th decade of life. 52/90 (58%) cases were males and 38/90 (42%) were females, which showed slight male preponderance.

Out of 20 esophageal carcinomas, 15 were squamous cell carcinoma and 5 were esophageal adenocarcinoma. These were graded according to three-tier system of grading based on the degree of differentiation. Majority of the squamous cell carcinomas (60%) were moderately differentiated (9/15 cases). Immunohistochemical analysis was done and Cytokeratin 7 and 20 was negative (Score 0) in all the cases (100%) of Squamous cell carcinoma (Fig. 1). Out of 5 adenocarcinomas, two were well differentiated and moderately differentiated each. One poorly differentiated carcinoma was included. Majority

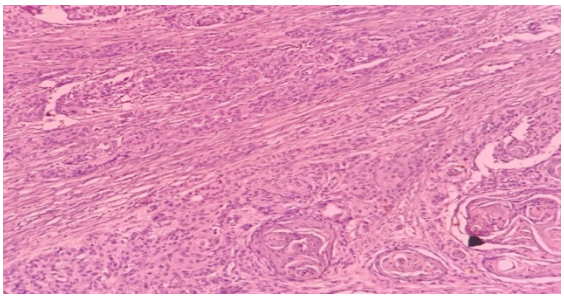

Fig. 1: Esophageal squamous cell carcinoma showing negative cytokeratin 7 expression
Score 0 (IHC;100x)

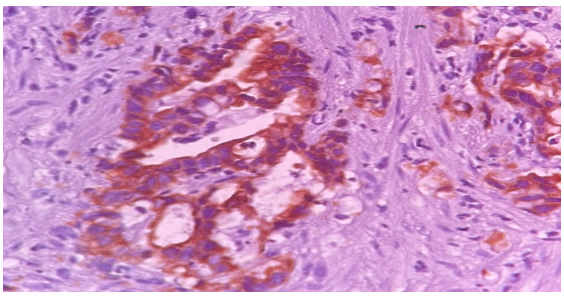

Fig. 2: Esophageal adenocarcinoma showing Cytokeratin 7 positivity
Score 2+ (IHC;400x)

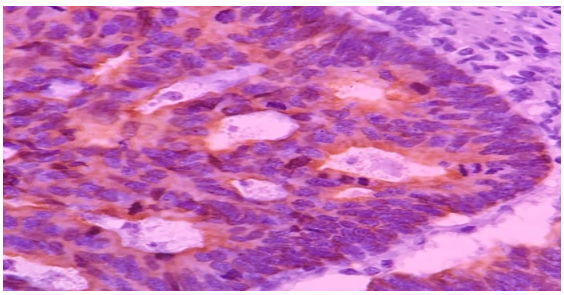

Fig. 3: Gastric carcinoma showing Cytokeratin 7 positivity
Score 2+ (IHC;400x)

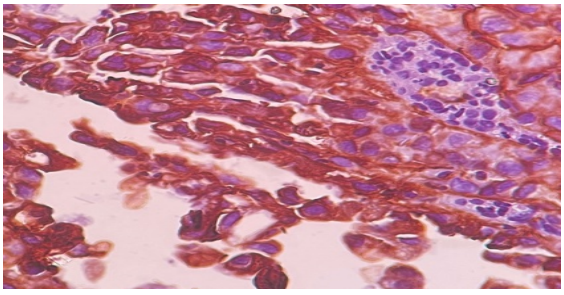

Fig. 4: Gastric biopsy specimen showing Cytokeratin 20 positivity
Score 3+ (IHC;400x)

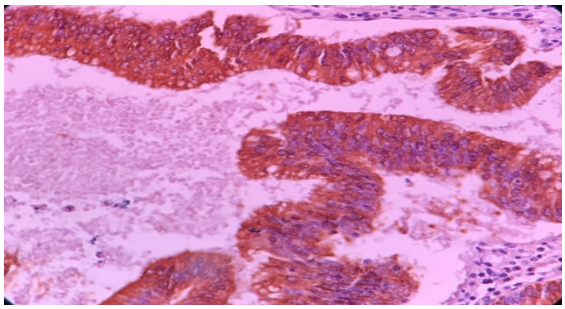

Fig. 5: Photomicrograph of colon adenocarcinoma showing Cytokeratin 20 positivity
Score 3+ (IHC;400x)

(4/5 cases) of the esophageal adenocarcinomas showed score 2+ positive staining for Cytokeratin 7 (Fig. 2) and were negative (Score 0) for cytokeratin 20 expression. There was no statistically significant correlation between the expression of Cytokeratin 7 and 20 with the grade of the tumor.

Thirty gastric carcinomas were included in the study and all the cases were adenocarcinomas. They weregraded as well, moderate and poorly differentiated carcinomas. 1 case of signet ring carcinoma was grouped under poorly differentiated carcinoma. Most of the cases were well differentiated adenocarcinomas (53%) of gastric origin. Cytokeratin 7 positivity was noted in 100 % of cases of gastric adenocarcinoma with majority of the cases showing intense and moderate positive staining (Fig. 3). Cytokeratin 20 was found to be positive in 19/30 cases(63%) with predominantly 2+ and 3+ immunoexpression (Fig. 4). Expression of Cytokeratin 7 and 20 did not show statistically significant correlation with the grade of the tumor (Table 1 and 2).

In this study, out of 40 colonic cancers, 39cases were histologically diagnosed as Adenocarcinoma, NOS and one case of Mucinous adenocarcinoma was reported. Three-tier system was used for grading. 17/40 cases were well-differentiated, 20/40 were moderately differentiated and 3 cases were poorly differentiated adenocarcinomas. Staging was done according to AJCC 7th edition criteria. 8/40, 12/40, 9/40 and 0/40 were staged as stage I, II, III and IV, respectively.

All the cases of colon cancer were stained with Cytokeratin 7 and Cytokeratin 20 immunohistochemistry. 39/40 (97.5%) cases were negatively stained (score 0) for Cytokeratin 7. There was no statistically significant correlation with the cytokeratin 7 expression and grade of the tumor.

Cytokeratin 20 staining was evaluated 39/40 cases (95%) showed positive immunostaining, with majority showing score 2+ and 3+ staining (Fig. 5 and 6).

Table 1: Cytokeratin 7 expression in gastric adenocarcinomas

Histologic grade (No of cases)	Score 0	Score 1+	Score 2+	Score 3+	Total Cytokeratin 7 positive (30/30; 100%)
I (16)	0	3	5	8	16 (100)
II (9)	0	2	2	5	9 (100)
III (5)	0	3	2	0	5 (100)

Table 2: Cytokeratin 20 expression in gastric adenocarcinomas

Histologic grade (No of cases)	Score 0	Score 1+	Score 2+	Score 3+	Total cytokeratin 20 positive (19/30; 63%)
I (16)	4	2	4	5	11/16 (69%)
II (9) (78%)	2	2	3	2	7/9
III (5) (40%)	3	0	2	0	2/5

Table 3: CK 20 expression in clorectal carcinoma

	CK 20 expression				
Histopathological diagnosis	Score 0/negative	Score 1+	Score 2+	Score 3+	Total no of cases -40
Well differentiated adenocarcinoma	-	2 (11.7%)	6 (35.3%)	9 (52.9%)	17
Moderately differentiated adenocarcino	10 (0.05%)	3 (15%)	7 (35%)	9 (45%)	20
Poorlydifferentiatedadenocarcinoma	1 (33.3%)	-	1(33.3%)	1 (33.3%)	3

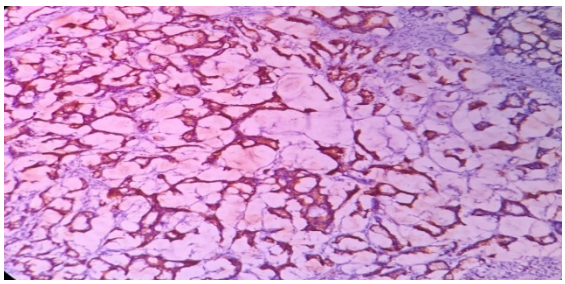

Fig. 6: Photomicrograph of mucinous adenocarcinoma of colon showing cytokeratin 20 positivity
Score 3+ (IHC;100x)

Correlation between cytokeratin 20 and grade of the tumor did not show statistically significant results. (Table 3).

Similarly, cytokeratin 7 and 20 expression was correlated with the stage of the tumor of esophageo-gastrointestinal cancers. There was comparative positive scoring in stage I, II and III and the results were not statistically significant.

DISCUSSIONS

The incidence of upper gastrointestinal tract carcinomas is constantly rising due to sedentary lifestyle, changing food habits, increased alcohol and smoking habits among younger adults. Gastrointestinal tract carcinomas often metastasize to lymphnodes before they are detected at their primary site and it is difficult to determine their origin based on histopathological study. Immunohistochemistry is a boon to the modern day pathologists which can be used as a tool to diagnose the origin of malignant metastatic lymphnode of unknown primary tumour and undifferentiated tumors. The present study throws light on the utility of cytokeratin 7 and 20 in diagnosing upper gastrointestinal tract adenocarcinomas.

Chu *et al*.[7] in their study on esophageal squamous cell carcinomas showed that 11/14 cases (78.6%) were negative for cytokeratin 7 and 14/14 cases (100%) were negative for cytokeratin 20. This is in correlation with our study which showed 100% dual negativity for cytokeratin 7 and cytokeratin 20. Chu *et al*.[7] also showed that 50% of the gastric adenocarcinomas showed positive staining for cytokeratin 20. Similarly, our study showed 19/30 cases (63%) positive cytokeratin 20 immunostaining.

The study conducted by Wang *et al*.[10] included 29 cases of esophageal adenocarcinoma. Out of which, 21 cases (72.4%) showed positivity for cytokeratin 20 expression. This was in concordance with our study which showed 63% positivity for cytokeratin 20.

Kende *et al*.[8] studied cytokeratin 7 and 20 expression on adenocarcinoma of stomach and showed that 35/38 cases (92.1%) were positive for cytokeratin 7 and 30/38 cases (78.9%) showed cytokeratin 20 positivity. Cytokeratin 7 expression was comparable with our study which showed 100% positivity and cytokeratin 20 expression which showed 63% positive immunoexpression.

Bayrak *et al*.[9] conducted a study on 59 gastric adenocarcinomas and showed 47/59 (79.7) positivity for cytokeratin 7 and 31/59 (52.5%) positivity for cytokeratin 20. Our study showed similar results as comparable with their study.

In our study, 91.6% of colorectal cases were CK7-/CK 20+.This is in concordance with the studies done by Kummar *et al*[11[, Chu *et al*.[7] Kende *et al*.[8] Gheini *et al*.[12] and Wang *et al*.[10] which showed 100, 95, 79.3, 75 and 75%, respectively.

The grade of the tumor indicates the likelihood of tumor to spread and grow and hence indirectly determines the prognosis of the carcinoma. Therefore, it is essential to grade the tumor. In the present study, the tumors were graded according to WHO 2010 guidelines. Some of the authors have followed two-tier system of grading which groups well differentiated and moderately differentiated tumors under low grade and poorly differentiated under high grade tumors.

Cytokeratin 7 and 20 was variably expressed in well/moderately differentiated and poorly differentiated malignancies which was statistically insignificant. Similarly, the studies conducted by Oue *et al*.[14], Bayrak *et al*.[9], Gheini *et al*.[12] and Kende *et al*.[8] also showed statistically insignificant correlation.

Cytokeratin 7 and 20 was variably expressed in stage I, II and III. This was statistically insignificant. Similarly, the studies conducted by Yamada *et al*.[15]and Kim *et al*.[16] also showed statistically insignificant correlation with the stage of the tumor[17-21].

CONCLUSION

This study concludes that Cytokeratin 7 and 20 show distinct expression in esophageo- gastrointestinal carcinomas. Esophageal adenocarcinoma showed cytokeratin 7+/20- expression. Cytokeratin 7+/20+ expression favorsmajority of the gastric adenocarcinomas. Cytokeratin 7-/20- expression is a feature of esophageal squamous cell carcinoma. The findings of the study suggest that CK 20 positivity infers hindgut adenocarcinoma. CK7-/CK20+ immunoexpression suggests colorectal adenocarcinoma.

Thus, this study highlights the combined utility of Cytokeratin 7 and 20 which has superior benefit in diagnosing and determining the primary site of esophageal, gastric and colorectal carcinomas.

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