ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 10, pp.74263-74267, October, 2018

DOI: https://doi.org/10.24941/ijcr.32659.10.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

TOPIC OF ARTICLE: A STUDY ON HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS OF RESECTED SPECIMEN OF COLORECTAL CARCINOMA

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ARTICLE INFO

Article History:
Received 30th July, 2018
Received in revised form
20 August, 2018
Accepted 25th September, 2018
Published online 30th October, 2018

Key Words:
Colorectal carcinoma,
Histomorphology,
Cytokeratin ,
Grade.

ABSTRACT

Background: Carcinoma in colon shows a wide range of morphological differentiation in spite of clinically being nondistinctive. Histomorphology is not enough for differentiating and categorising the type of colon carcinoma with confidence; immunohistochemistry acts as valuable adjunct to it.
Materials and Methods: Resected colon specimens were grossed and sections given from representative areas and stained by conventional H and E method and further evaluation done by immunohistochemical markers CK-7 and CK-20 with grading.
Results: 51 cases of colon carcinoma selected for study and elderly males dominated. Adenocarcinoma (NST) dominated in histopathology in 43 cases followed by 5cases of signet ring cell variant and 3 cases of mucinous type. In Adenocarcinoma-NST group, CK-7 was negative in all,13 showed grade2 CK-20 positivity, 26 showed grade3 and 4 showed grade4. All signet ring cell types showed CK-7 and grade 4 CK-20 positivity. Mucinous group showed CK-7 and grade2 CK-20 positivity.
Conclusion: Histology coupled with immunohistochemistry act as complete diagnostic tools for colon carcinoma and helpful for further therapeutic management. Histological typing shows statistically significant correlation with grade of CK-20 positivity (p=0.00).

Citation: ***Dr. Soma Ghosh and Dr. Snehasis Das*, 2018.** "Topic of article: a study on histopathological and immunohistochemical findings of resected specimen of colorectal carcinoma", *International Journal of Current Research*, 10, (10), 74263-74267.

INTRODUCTION

Colorectal carcinoma (CRC) is a major cause of morbidity and mortality worldwide. It is the third most commonly diagnosed cancer in males and the second in females (Mohandas, 2011; Gafà *et al.,* 2000). Over 12,35, 108 new CRC cases and 6,09,051 deaths are estimated to have occurred in 2008. It contributes to 15% of cancer related deaths. In recent years, high CRC rates have been reported in newly developed countries around the globe in which the risk was once low (Mohandas, 2011; Gafà *et al.,* 2000). The incidence of CRC is highest in Australia, New Zealand, Europe and North America, whereas it is lowest in Africa and South-Central Asia. All south Asian countries have low incidence of CRC, while it is high in all developed Asian countries like Japan, South Korea and Singapore (Mohandas, 2011; Gafà *et al.,* 2000; Jass, 2007; Mills AND Allen, 1979). Number of CRC cases in India in 2008 was 36,476 and number of deaths due to colorectal cancer was 25,690. The incidence of CRC in India is slowly rising. During a period of 32-years (1941-1972), 555 cases of CRC were recorded at the Tata Memorial Hospital, Mumbai. In contrast, a total of 560 cases of CRC were treated at the same institution in 2006 alone (Jass, 2007; Mills and Allen, 1979; Cooper and Slemmer, 1991).

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Life style and dietary habits are important in the causation of the CRC (Cooper and Slemmer, 1991; Nasir *et al.,* 2004). The development of colorectal adenocarcinoma is a multistep process which can arise due to accumulation of molecular alteration including chromosomal abnormalities, genetic mutations and epigenetic changes (Nasir *et al.,* 2004; Tada *et al.,* 1994). Most colorectal carcinomas are located in the sigmoid colon and rectum, but there is evidence of changing distribution in recent years, with an increasing proportion of more proximal carcinomas (Nasir *et al.,* 2004; Tada *et al.,* 1994; Yasuda *et al.,* 1997). Carcinomas may be exophytic/ fun gating with predominantly intraluminal growth, endophytic/ ulcerative with predominantly intramural growth, diffusely infiltrative/linitis plastica with subtle endophytic growth, and annular with circumferential involvement of the colorectal wall and constriction of the lumen (Younes *et al.,* 1993; Connelly *et al.,* 1991). Lesions with the morphological characteristics of adenocarcinoma that are confined to the epithelium or invade the lamina propria alone and lack invasion through the muscular is mucosae into the submucosa have virtually no risk of metastasis (Younes *et al.,* 1993; Connelly *et al.,* 1991; Kakar *et al.,* 2005). Most adenocarcinomas are diagnosed easily, the main problems being presented by the better differentiated tumors and paradoxically the highly malignant signet ring carcinomas in which only a few tumor cells may be present (Kakar *et al.,* 2005; Giacchero *et al.,* 1985). Adenocarcinomas

are graded predominantly on the basis of the extent of glandular appearances, and should be divided into well, moderately and poorly differentiated, or into low-grade (encompassing well and moderately differentiated adenocarcinomas) and high-grade (including poorly differentiated adenocarcinomas and undifferentiated carcinomas) (Connelly *et al.,* 1991; Kakar *et al.,* 2005; Giacchero *et al.,* 1985; Sugao *et al.,* 1997). When a carcinoma has heterogeneity in differentiation, grading should be based on the least differentiated component, not including the leading front of invasion (Connelly *et al.,* 1991; Kakar *et al.,* 2005). Metastatic adenocarcinoma from an unknown primary site is a common clinical problem that leads to extensive and costly clinical and radiological examinations, sometimes with discouraging results. It is often important to determine the site of origin of a metastatic carcinoma of unknown primary site, particularly because this may affect the choice of the treatment (Kakar *et al.,* 2005; Giacchero *et al.,* 1985). A more precise diagnosis leads to more effective treatment, substantially improving the overall outcome. The histological assessment is often very helpful, but may not differentiate adequately between various primary tumors (Younes *et al.,* 1993; Kakar *et al.,* 2005; Giacchero *et al.,* 1985). Immunohistochemistry is the most common adjunctive method used in the analysis of the patient with cancer of unknown primary site (Sugao *et al.,* 1997; Ulich *et al.,* 1983; Leong *et al.,* 1987).

Cytokeratins are expressed in the vast majority of epithelial-like sarcomas such as epithelioid and synovial sarcomas, in many rhabdoid tumors, and in mesotheliomas (Sugao *et al.,* 1997; Leong *et al.,* 1987; Jambhekar *et al.,* 2008). According to Battifora, cytokeratins are constituents of the intermediate filaments of epithelial cells and these are expressed in various combinations depending upon the epithelial type and the degree of differentiation (Battifora, 1988). Cytokeratins are most commonly studied determinants in immunohistochemistry to corroborate a diagnosis of carcinoma and typically ruling out the possibility of sarcoma, lymphoma or melanoma (Jambhekar *et al.,* 2008; Battifora, 1988; Shi *et al.,* 1991; Jaffer *et al.,* 2004). The most useful cytokeratins are CK7 and CK20 (Shi *et al.,* 1991; Jaffer *et al.,* 2004; Pecciarini *et al.,* 2001). CK7 is found in many ductal and glandular epithelia, including lung, breast, ovary, and endometrium. CK20 is expressed in the gastrointestinal (GI) epithelium, urothelium, and Merkel cells (Battifora, 1988; Pecciarini *et al.,* 2001; Park *et al.,* 2007). The combined expression patterns of CK7 and CK20 have been extensively studied in various primary and metastatic carcinomas. CK20 is expressed alone in the majority of intestinal adenocarcinoma and in Merkel cell carcinomas whereas CK7 is present without CK20 in most breast, lung and ovarian adenocarcinoma, and with CK20 in urothelial, pancreatic and gastric carcinomas (Park *et al.,* 2007; Moll *et al.,* 1982).

The CK7-/CK20+ expression pattern is known to be highly characteristic of colorectal carcinomas, however, not all colorectal carcinomas show the CK7-/CK20+ expression pattern (Park *et al.,* 2007; Campbell and Herrington, 2001). Occasionally colorectal carcinomas may show significant CK7 expression and conversely, expression of CK20 may be seen in a variety of non-colorectal adenocarcinomas such as urothelial, gastric and pancreatobiliary tract carcinomas (Moll *et al.,* 1982; Campbell and Herrington, 2001). For this reason, there is continued interest in the development of new and more specific markers of intestinal differentiation and CDX2 appears to be such a marker (Campbell and Herrington, 2001; Van Niekerk *et al.,* 1991; Ramaekers *et al.,* 1990).

MATERIALS AND METHODS

The specimens of colon specimen sent fixed in 10% formal saline subjected to thorough gross examination. Following fixation, bits taken from representative areas and are routinely processed with paraffin embedding.5µ thin multiple sections taken and stained with routine hematoxylin and eosin. Sections taken from tumor mass, surgically resected margins, resected lymph nodes, if any. These are then studied microscopically and evaluated. Special stains like CK-7 and CK-20 are done whenever required. It is a hospital based cross section observational study conducted after proper ethical clearance. All cases (51) selected for study were of colorectal carcinoma excluding carcinomas of anal canal and inoperable cases of colorectal carcinoma, non-neoplastic and inflammatory lesions. The operative details of the cases enquired with pre-operative blood parameters. The size of tumor mass assessed on gross examination. Special stains in immunohostochemistry needed 0.01M sodium citrate with pH of 6.0, primary antibodies. The tumors after routine hematoxylene and eosin staining categorised with respect to origin of lesion; involvement of resected margins; histopathologic variant of tumor; lymph node status (Lena *et al.,* 2008; Gamble, 2008).

The sections underwent immunohistochemistry for polymer detection system (Novocastra) where formalin used as fixative and poly L-lysine used as adhesive. The sections were deparaffinised in xylene, rehydrated through graded alcohol, antigen retrieval by citric anhydrous buffer followed by cooling and washed in Tris buffer. Peroxidase block (3%H2O2) added to neutralise endogenous peroxidase followed by washing in Tris buffer and the sections incubated shortly in protein block (0.4% casein in phosphate buffer saline), primary antibody for longer interval and washed. The subsequent incubation done with post-primary block (10% animal serum in triss buffered saline) followed by washing; incubation with Novolink polymer (Anti-mouse/rabbit IgG Poly HRP containing 10% animal serum in Tris buffered saline) and washing. Diluted DAB (chromogen -1.74% 3, 3‘-diaminobenzidine) applied and slide rinsed in water; stained with hematoxylene, again rinsed in water followed by dehydrating in graded alcohol and drying. The slides kept in xylene and mounted in DPX (Gamble, 2008; Moll *et al.,* 1992; Bayrak *et al.,* 2011; Zhang *et al.,* 2003).

Table 1. Grading of immunohistochemical staining pattern of CK-7 and CK-20

Staining pattern	Score	Assessment
No staining or nonreactive	0	Negative
Scattered spotty staining	1+	Weak positive
Up to 25% of tumour cells are positive	2+	Weak positive
25% to 50% tumour cells are positive	3+	Strong positive
More than 50% of tumour cells are positive	4+	Strong positive

RESULTS

In present study, 9.804% cases belong to 40 to 50 years age group, 37.25% cases belong to 51 to 60 years, 47.06% cases belong to 61 to 70 years and 5.882% cases belong to more than 70 years of age showing a range of 45-75 years. Females comprised of 23.53% cases and 76.47% males suffering from

CRC. 43.14% cases were Hindu in religion whereas 56.86% cases were muslims.47.06% cases presented with abdominal distention; 68.63% cases presented with palpable abdominal mass; only 3.922% cases presented with history of pain abdomen. 35.29% cases presented with history of altered bowel habit and72.55% cases of CRC presented with anemia. 29.41% cases presented with per-rectal bleeding; 60.78% patients had history of weight loss. In colonoscopy, a mere 7.843% cases showed mass in colon and 78.43% cases had ulceration in colon. Rectum and rectosigmoid areas had lesions in 72.55% cases; 21.57% cases had in sigmoid colon and 5.882% cases in ascending colon. Margins were involved in 9.80% cases. Histologically, 84.31% (43)cases belong to the group of adenocarcinoma NST, 9.804% (5)cases of signet ring cell carcinoma and rest 5.882%(3) are of mucinous adenocarcinoma type. 13 cases had size <4 cm and rest 38 cases size of >4 cm. Among the cases having size >4 cm, 25 cases had no history of lymph node metastasis.

In cases of Stage 2 CRC, 15 cases showed histology of adenocarcinoma NST; but, in stage 3 CRC, 3 cases showed histology of mucinous adenocarcinoma, 5 cases of signet ring cell carcinoma and rest 28 of adenocarcinoma NST. 25.49% cases showed lymph node metastasis and all the cases of mucinous adenocarcinoma showed metastasis to regional lymph node; 23.26% cases of adenocarcinoma NST showed lymph node metastasis. But, all cases of signet ring cell carcinoma were devoid of lymph node metastasis. Thus, histological type of CRC has statistically significant correlation with lymph node metastasis. The p value is 0.005 ($p<0.05$/ Pearson Chi-square test). All cases of mucinous carcinoma showed grade 2 CK-20 positivity. Among the cases of adenocarcinoma NST, 30.23% cases showed grade 2 CK-20 positivity, 60.47% cases showed grade 3 CK-20 positivity and 9.302% cases showed grade 4 CK-20 positivity. All the cases of signet ring cell carcinoma showed grade 4 CK-20 positivity (Table-2). Grade 2 CK-20 was positive in 11, 54% cases of metastasising CRC and 38.46% showed grade 3 CK-20 positivity; while 73.33% cases of stage 2 CRC showed grade 3 CK-20 positivity and 26.67% showed grade 4 CK-20 positivity. 44.44% cases of stage 3 CRC showed grade 2 CK-20 positivity, 41.67% showed grade 3 CK-20 positivity and 13.89 % showed grade 4 CK-20 positivity. Thus, histological type of CRC had statistically significant correlation with grade of CK-20 positivity (p – 0.00). All cases of mucinous adenocarcinoma showed CK-7 positivity. All cases of signet ring cell carcinoma showed CK-7 positivity and all cases of adenocarcinoma NST showed CK-7 negativity (Table-3).

Table 2. Grading CK-20 expression in different histological types of CRC

Histological type	Grading CK-20			Total
	+2	+3	+4	
Mucinous	3	0	0	3
NST	13	26	4	43
Signet ring	0	0	5	5
	16	26	9	51

Table 3. CK-7 grading in different histological types of CRC

Histological type	CK-7		Total
	Negative	Positive	
Mucinous	0	3	3
NST	43	0	43
Signet ring	0	5	5
Total	43	8	51

DISCUSSION

In the present study, 51 cases of colorectal adenocarcinoma evaluated with respect to the clinical features and tumor morphology. The CK-7/CK-20 status was determined by immunohistochemistry in each case. Correlation between the CK-7/CK-20 studies and clinical features and tumour histopathology was done (Bayrak *et al.,* 2011; Zhang *et al.,* 2003). The present study shows that the maximum age is 75 years and the minimum age is 45 years with mean age of 60.51 ± 7.865 years; which correlates with study conducted by (Kressner *et al.,* 1999; Nasiri *et al.,* 2007; Mihalache, 2014) Elderly age group is thus considered a risk factor for the development of adenocarcinoma. The present study shows that, 23.53% cases are female patients and rest 76.47% are males suffering from CRC; which correlates with study by Peedikayil *et al.* 2009. In their study Peedikayil *et al.* found, 22.27% cases CRC presented with clinical feature of palpable abdominal mass and pain abdomen but present study shows only 3.922% cases with similar complaints (Peedikayil, 2009). Halder *et al.* shows in their study, 33.39% cases with altered bowel habit in CRC and Peedikayil *et al.* noted 33.18% cases with constipation; similar to present study. Peedikayil *et al.* found 25.9% cases with anemia; however present study noted 72.55% cases presented with anemia.Peedikayil *et al.* found 70.45% cases with per-rectal bleeding; Halder *et al.* found 71.27% cases with similar complaints,but present study found a mere 29.41% cases (Peedikayil, 2009; Halder *et al.,* 2008). Halder *et al.* found 79.80% cases with clinical feature of weight loss; Peedikayil *et al.* found 39% cases with similar complaints and present study found 60.78% cases. Peedikayil *et al.* found 24.54% cases with mass in ascending colon, 6.82% cases in descending colon and rest 68.64% cases in recto sigmoid colon. Halder *et al.* found 34.21% cases with mass in ascending colon, 26.31% cases in descending colon and rest 39.47% cases in recto sigmoid colon (Peedikayil, 2009; Halder *et al.,* 2008). Present study found 72.55% cases with lesion in rectum and rectosigmoid; 21.57% cases in sigmoid colon and 5.882% cases in ascending colon. Zhao *et al.* found 66% cases with lymph node metastasis in their study; Gurzu *et al.* found 47.3% cases with similar features (Gurzu, 2016; Zhao, 2017). In present study, 13 of 38 cases of CRC having size of >4cm showed metastasis in regional lymph nodes. All 13 cases of size <4cm were devoid of lymph node metastasis where P values is 0.023 ($p<0.05$; Fisher's exact test). So, the size of the tumor bears a statistically significant correlation with lymph node metastasis. Nabi U *et al.* found 59% cases presented with adenocarcinoma (NST) in their study (Nabi, 2018); Nasiri *et al.* shows 78% cases with NST (Nasiri, 2007); Omran *et al.* in their study shows 81.8% cases with NST (Omran *et al.,* 2018) and present study found 84.31% cases in the group of NST with 9.804% cases of signet ring cell carcinoma and 5.882% of mucinous adenocarcinoma type.

In their study; Imai Y *et al.* found CK7 expression detected in 22% (26/118) of colorectal, 80% (47/59) of gastric, and 97% (31/32) of pancreatic adenocarcinoma. CK20 reactivity found in 84% (99/118) of colorectal, in 53% (31/59) of gastric, and in 22% (7/32) of pancreatic adenocarcinoma (Imai, 2014). The CK7-/CK20+ immunophenotype was expressed in 64% (75/118) colorectal and 5% (3/59) gastric tumors and was not observed in any pancreatic adenocarcinomas ($c2 = 79.992$; $p < 0.001$). The CK7+/CK20+ immunophenotype was expressed in 20% (24/118) of colon, 48% (28/59) of gastric and 22% (7/32) of pancreatic adenocarcinomas, which was not helpful in the

differential diagnosis. However, among the CK20 positive cases, CK20 reactivity was diffuse (more than 50% of cells were positive) in the majority of CRC in 64% (63/99) of the cases and mainly focal (< 50% of cells were positive) in gastric and pancreatic adenocarcinomas in 71% (22/31) and 100% (7/7) of cases respectively ($c2 = 19.509$; $p < 0.001$) (Imai, 2014). Conversely, among the CK7 positive cases, CK7 reactivity was diffuse in the majority of gastric and pancreatic adenocarcinomas in 74% (35/47) and 94% (29/31) of cases respectively, and this reactivity was focal in 54% (14/26)of CRC ($c2 = 16.228$;$p < 0.001$). The CK7+/CK20- expression pattern was observed in only 2% (2 of118) of CRC, although it was expressed in 32% (19/59) of gastric and 75% (24/32)of pancreatic adenocarcinomas ($c2 = 85.607$; $p < 0.001$) (Imai, 2014). No association between CK7 expression and anatomical location of carcinomas, tumor type, stage, and grade was found. No association was observed among CK20 expression and tumor type, tumor stage (pT), or nodal status. Among the colorectal tumors, CK20 positivity was more common in rectal carcinomas than in nonrectal colon carcinomas (89% versus 70%, $c2 = 6.839$; $p = 0.009$) and in low grade carcinomas than in high grade carcinomas (91% versus 55%, $c2 = 17,247$; $p < 0.001$) (Imai, 2014). Present study found that all the cases of mucinous adenocarcinoma showed CK-7 positivity. All the 5 cases of signet ring cell carcinoma showed CK-7 positivity and all 43 cases of adenocarcinoma NST showed CK-7 negativity. All 3 cases of mucinous adenocarcinoma showed grade 2 CK-20 positivity. Among 43 cases of adenocarcinoma NST, 13 cases showed grade 2 CK-20 positivity, 26 cases showed grade 3 CK-20 positivity and rest 4 cases showed grade 4 CK-20 positivity. All the 5 cases of signet ring cell carcinoma showed grade 4 CK-20 positivity. Thus, histological type of colorectal carcinoma bears statistically significant correlation with grade of CK-20 positivity ($p = 0.00$).

Conclusion

Colorectal carcinoma can present with a wide range of morphological variations, frequently confusing and overlapping and an accurate diagnosis can be made in majority of the cases by using immunohistochemistry as an adjunct to histomorphological evaluation. This study supports the result of the previous studies and states the significant association of CK-20 positivity with different histological types of colorectal carcinoma.

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