DOI: 10.7860/JCDR/2025/80605.21551

Original Article

Pathology Section

Expression of CK20 and Ki-67 in Colon Carcinoma and their Association with Various Histopathological Parameters: A Cross-sectional Study

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ABSTRACT

Introduction: According to 2022 data, globally colon carcinoma ranks 4th. In India, colon carcinoma ranks 6th in incidence and 7th in mortality. It is the 4th most common cancer in males and 5th in females. Diagnosis of colon carcinoma in recent times has been largely depended on Immunohistochemical (IHC) markers such as Cytokeratin 20 (CK20), CDX2, Ki-67, Cadherin 17, etc., alongside histopathology.

Aim: To study the association of histological parameters with IHC expression of Ki-67 and CK20 in predicting the behaviour of colon carcinoma.

Materials and Methods: This was a cross-sectional study conducted in the Department of Pathology in association with the Department of General Surgery, Calcutta National Medical College and Hospital, Kolkata, West Bengal, India on patients clinically diagnosed with colon cancer who underwent surgery (n=44). Data were collected from October 2022 to March 2024 (17 months) and analysed in April 2024. Microscopic examination was performed using Haematoxylin and Eosin (H&E) to assess histological features such as tumour grading and staging, margin status, Lymphovascular Invasion (LVI) and lymph node metastasis. IHC staining was performed with ready-to-use antibodies against CK20 and Ki-67 and the staining was recorded. For statistical analysis, the Fisher-Freeman-Halton test and Fisher's exact test were used, with Statistical Package for the Social Sciences (SPSS) version 26.0. Statistical significance was set at p-value <0.05.

Results: In this study, there were 44 cases and the ages of the patients ranged from 12 to 89 years. The male-to-female ratio was 1.4:1. 20 (45.5%) of the tumours were moderately-differentiated adenocarcinomas, followed by 13 (29.6%) well-differentiated and 5 (11.4%) poorly differentiated. Cytokeratin 20 (CK20) expression was observed in 35 (79.6%) of cases. Ki-67 expression was categorised as grade 1 (≤25%), grade 2 (26-50%) and grade 3 (≥51%). A statistically significant association was found between CK20 expression and increased tumour differentiation (p-value <0.001), absence of LVI (p-value=0.006) and decreased margin involvement (p-value=0.007). Ki-67 expression showed association with decreased tumour differentiation (p-value <0.001), higher stage (p-value=0.032), increased LVI (p-value <0.001), lymph node metastasis (p-value=0.005) and increased margin involvement (p-value <0.001). CK20 and Ki-67 expression also showed a significant inverse association with each other (p-value <0.001) based on histological differentiation. CK20 expression was gradually lost in higher Ki-67-expressing tumours.

Conclusion: The most prevalent colon carcinoma was adenocarcinoma; the majority were moderately-differentiated adenocarcinomas. CK20 expression was higher in well-differentiated tumours and those without LVI, while Ki-67 expression increased with poorer differentiation, higher stage, more LVI and lymph node metastasis. An inverse relationship was observed between CK20 and Ki-67 expression based on histological parameters.

Keywords: Adenocarcinoma, Cytokeratin 20, Immunohistochemistry, Kiel 67

INTRODUCTION

According to GLOBOCAN, colon carcinoma ranks fourth worldwide, with an Age-Standardised Rate (ASR) of 10.7 per 100,000 in incidence. In India, colorectal carcinoma ranks sixth in incidence. It is the fourth most common cancer in males (6.3%) and the fifth most common in females (3.7%) [1]. Established risk factors include the consumption of processed and red meat, obesity, alcohol consumption, chronic inflammatory bowel disease and genetic predisposition [2]. The majority of colon carcinomas (approximately 90%) are adenocarcinomas [3]. Approximately 70% of colon carcinomas are moderately differentiated (50-95% gland formation), with well-differentiated (>95% gland formation) and poorly differentiated (<50% gland formation) carcinomas constituting about 10-20% [4]. Predictive biomarkers of colon carcinomas are divided into established markers such as RAS, BRAF and MSI and markers still under development, including cancer-immunity markers, PIK3CA and c-MET [2].

CK20 belongs to the cytokeratin family of intermediate filaments and is expressed in the epithelia of the gastrointestinal tract, urothelium and Merkel cells. CK20 expression is found in the vast majority of colon adenocarcinomas, urothelial carcinoma and Merkel cell carcinoma and may be expressed less frequently in the stomach, bile duct and pancreas [5]. Ki-67 is a nuclear antigen named after the antibody from which it was derived; the term "Ki" refers to Kiel, Germany, where it was first described. Ki-67 is expressed in the nucleus during all active phases of the cell cycle (G1, S, G2 and M) and is absent in quiescent cells (G0) [6]. The role of Ki-67 in colon carcinoma prognosis has been controversial. Some studies have shown that high Ki-67 expression is a poor prognostic factor in colorectal cancer; for example, Tong et al., used a 25% cut-off as a poor prognostic indicator [7]. However, other studies have associated high Ki-67 with better outcomes; for instance, Melling et al., found high Ki-67 expression significantly associated with favourable clinical outcomes [8]. Mulyawan IM, reported that Ki-67 expression increased with decreased differentiation and correlated with the presence of metastasis [9]. Conversely, some findings suggest that high Ki-67 may reflect greater tumour responsiveness to radiochemotherapy, complicating

its prognostic interpretation. Santos PMDD et al., reported a significant association between Ki-67 expression and tumour grade, with higher expression in poorly differentiated tumours and in undifferentiated colon carcinomas [10].

This study aimed to determine the association of CK20 and Ki-67 expression with histological differentiation and subtypes, tumour stage, lymph node metastasis, lymphovascular invasion and margin involvement in colon carcinoma and to examine the association between Ki-67 and CK20 expression with reference to histological differentiation. It will illuminate the roles of the epithelial marker CK20 and the proliferative marker Ki-67 in predicting tumour behaviour and prognosis, thereby aiding diagnosis and prognosis of colon carcinoma. The combined assessment of these two markers and their interplay in disease progression in colon carcinoma is not well studied and this study seeks to fill that gap and pave the way for new diagnostic approaches.

MATERIALS AND METHODS

This was a cross-sectional study conducted on patients attending the Surgical Outpatient Department (SOPD) or the Emergency Department of General Surgery at Calcutta National Medical College and Hospital, Kolkata, West Bengal, India, who were clinically diagnosed with colon cancer and had undergone surgery for the same in both elective and emergency settings (n=44). Institutional Ethical Committee (IEC) approval was obtained (IEC number: IEC-CNMC/70). The study was conducted from October 2022 to April 2024 (18 months). The sample size of 44 cases was determined based on the annual incidence rate calculated from cases observed over the past three years.

Inclusion criteria: All patients clinically and radiologically diagnosed with colon carcinoma who were admitted to the Department of Surgery and underwent surgery for colon carcinoma in either emergency or elective settings were included in the study.

Exclusion criteria: Patients with past history of colon carcinoma treated with chemotherapy and radiotherapy, malignancies other than colon carcinoma (e.g., lymphoma, GIST, malignant melanoma), patients not willing to give consent and grossly necrotic specimens were excluded from the study.

Study Procedure

After obtaining consent from the patient or guardian (in the case of a minor), relevant patient particulars were collected along with history and documentation from the clinical notes. Radiological investigations such as Ultrasound (USG), Computed Tomography (CT) and Magnetic Resonance Imaging (MRI), if available, were correlated with the clinical history. Gross examination of the specimens was performed and recorded, including whether partial or complete hemicolectomy was performed, the type of surgery, colour, consistency, haemorrhage, margins and any other gross abnormality.

Microscopic examination was performed on H&E stained sections to evaluate histological features such as grade (by WHO 2019 classification), stage (by pathological TNM staging), margin status, lymphovascular invasion, depth of tumour penetration and lymph node status along with extranodal deposits for proper diagnosis. IHC staining for CK20 and Ki-67 expression and grading were performed.

Antibodies used:

- CK20: mouse monoclonal antibody (Ks20.8) (320M-15, 0.5 mL; 1:200, Cell Marque)
- Ki-67: monoclonal mouse anti-human, ready-to-use (clone MIB-1, Novocastra, Leica)

Ki-67: Nuclear staining in tumour cells was recorded as positive regardless of intensity. At least 1000 cells were observed under a microscope (40×objective). The Ki-67 labeling index was calculated as: (number of nuclei showing positive staining / total number of nuclei [1000])×100%. The percentage of immunostaining was grouped as: Grade 1 (≤25%), Grade 2 (26-50%) and Grade 3 (≥51%) [7]. Tonsil tissue was used as the positive control for Ki-67. Negative control was achieved by omitting the primary antibody.

CK20: Cytoplasmic and/or membranous staining was regarded as positive. Only cases showing >5% tumour cell positivity were regarded as CK20-positive [11]. Normal colon tissue was used as a positive control. The negative control consisted of sections of the study tissue with no primary antibody incubation.

STATISTICAL ANALYSIS

All data were thoroughly maintained in Microsoft Excel worksheets. For statistical analysis, SPSS version 26.0 was used. The Fisher-Freeman-Halton test and, Fisher's exact test were employed. Statistical significance was set at p-value <0.05.

RESULTS

The ages of patients ranged from 12 to 89 years, with a mean age of presentation of 52.1 years [Table/Fig-1]. Males accounted for 26 (59.1%) and females for 18 (40.9%), giving a male-to-female ratio of 1.4:1. Fifteen cases (34.09%) presented with abdominal pain and distension, followed by 12 cases (27.27%) with bleeding per rectum [Table/Fig-2]. 23 (52.3%) of tumours had sizes ranging between 3-6 cm. Left-sided tumours predominated (26 cases) [Table/Fig-3]. Grossly, 31 cases (70.45%) were polypoid; 11 cases (25%) were stricture-like and two cases (4.55%) presented as perforation [Table/Fig-4]. Microscopically, 20 cases (45.5%) were moderately differentiated adenocarcinomas, followed by 13 cases (29.6%) well-differentiated and 5 cases (11.4%) poorly differentiated adenocarcinomas. Mucinous adenocarcinomas comprised 3 cases (6.8%); 2 cases (4.6%) were signet-ring adenocarcinomas and 1 case (2.2%) was MANEC (mixed adeno-neuroendocrine carcinoma). Twenty cases (45.5%) belonged to the T3 stage. Fifteen cases (34.1%) had pN0 stage of lymph node involvement, followed by 10 cases (22.7%) at the pN1b stage.

Age (in years)	n (%)
11-30	5 (11.36)
31-50	14 (31.82)
51-70	19 (43.18)
>70	6 (13.64)

[Table/Fig-1]: Distribution of colon carcinoma according to age (n=44).

Chief complaint	n (%)
Abdominal pain and distension	15 (34.09)
Obstipation	10 (22.72)
Chronic anaemia	2 (4.54)
Intussusception	2 (4.54)
Abdominal lump	3 (6.81)
Bleeding per rectum	12 (27.27)

[Table/Fig-2]: Distribution of colon carcinoma according to chief complaint (n=44).

Laterality of tumour	n (%)
Left	26 (59.09)
Right	18 (40.91)

[Table/Fig-3]: Distribution of colon carcinoma according to laterality of tumour (n=44).

Gross nature of tumour	n (%)
Polypoidal	31 (70.45)
Stricture	11 (25)
Perforation	2 (4.55)

[Table/Fig-4]: Distribution of colon carcinoma according to gross nature of tumour (n=44).

Lymphovascular invasion was present in 25 cases (56.8%). A total of 35 cases (79.6%) of tumours showed positive CK20 expression, while 9 cases (20.4%) were negative. Ki-67 expression was graded according to percentage expression as follows: Grade 1 (≤25%), Grade 2 (26–50%) and Grade 3 (≥51%), with 17, 19 and 8 cases in each group, respectively.

Association between CK20 and histological parameters [Table/Fig-5]: 100% of well-differentiated (13 cases) and moderately differentiated adenocarcinomas (20 cases) expressed CK20, whereas 100% of poorly differentiated adenocarcinomas (5 cases), signet-ring carcinomas (2 cases) and MANEC (1 case) were CK20 negative. Thus, CK20 expression showed a decreasing trend with decreasing differentiation of the tumour and this was statistically significant (p-value <0.001) (Fisher–Freeman–Halton test). However, no statistically significant association was found between tumour stage (p-value=0.24) and lymph node involvement (p-value=0.66) with CK20 expression (Fisher–Freeman–Halton test). Among CK20-positive tumours (n=19), all showed absence of LVI; the association was significant (p-value=0.006) using Fisher's exact test. A statistically significant association (p-value=0.007) was also noted between CK20 expression and absence of margin involvement, since 100% of tumours with no margin involvement expressed CK20.

Association between Ki-67 and histological parameters [Table/Fig-5]: Ki-67 expression showed a significant association with decreasing histological differentiation of tumours (p-value <0.001) (Fisher-Freeman-Halton test). A statistically significant association (p-value=0.032) was also noted between higher tumour stage and increased grade of Ki-67 expression. It was evident that tumours with pT2 and pT3 stage had grade 1 (≤25%) Ki-67 expression, whereas cases with pT4a stage tended to have grade 2 (26-50%) expression. Twelve (80%) of tumours with pN0 status had grade 1 (≤25%) Ki-67 expression. Also, 3 cases (100%) of pN1c and 60% (3 cases) of pN2a showed Ki-67 between 26-50% (grade 2). A 2 cases (40%) of pN2a had grade 3 (≥51%) Ki-67 expression. A statistically significant association (p-value=0.005) was found between higher Ki-67 grade and increased number of lymph node involvement (Fisher-Freeman-Halton test). A significant association has been found between Ki-67 expression and lymphovascular invasion (p-value <0.001) (Fisher-Freeman-Halton test). Tumours with grade 2 (26-50%) Ki-67 expression had higher chances of LVI, while cases having Ki-67 ≤25% (grade 1) had absence of LVI. Thirteen cases (76.47%) with no margin involvement showed grade 1 Ki-67 expression, while 15 cases (55.6%) with positive margin involvement showed grade 2 Ki-67 involvement. Thus, a statistically significant association was noted between them (p-value <0.001) (Fisher's exact test).

S. No.		CK 20 expression		p-value	Ki-67 expression			p-value
1.	Histological subtypes	Expressed (n=35)	Not expressed (n=9)		Grade 1 (≤25%) (n=17)	Grade 2 (26-50%) (n=19)	Grade 3 ≥51% (n=8)	
	Well differentiated adenocarcinoma (n=13)	13 (100%)	0	<0.001 (Fisher freeman Halton test)	13 (100%)	0	0	<0.001 (Fisher freeman Halton test)
	Moderately differentiated adenocarcinoma (n=20)	20 (100%)	0		2 (10%)	17 (85%)	1 (5%)	
	Poorly Differentiated adenocarcinoma (n=5)	0	5 (100%)		0	1 (20%)	4 (80%)	
	Mucinous adenocarcinoma (n=3)	2 (66.7%)	1 (33.3%)		2 (66.7%)	1 (33.3%)	0	
	Signet Ring carcinoma (n=2)	0	2 (100%)		0	0	2 (100%)	
	Mixed adenoneuroendocrine carcinoma (n=1)	0	1 (100%)		0	0	1 (100%)	
2	Tumour staging	Expressed (n=35)	Not expressed (n=9)	0.24 (Fisher freeman Halton test)	Grade 1 (≤25%) (n=17)	Grade 2 (26-50%) (n=19)	Grade 3 ≥51% (n=8)	0.032 (Fisher freeman Halton test)
	pT2 (n=5)	5 (100%)	0		4 (80%)	1 (20%)	0	
	pT3 (n=20)	17 (85%)	3 (15%)		11 (55%)	6 (30%)	3 (15%)	
	pT4a (n=17)	12 (70.6%)	5 (29.4%)		2 (11.8%)	11 (64.7%)	4 (23.5%)	
	pT4b (n=2)	1 (50%)	1 (50%)		0	1 (50%)	1 (50%)	
3.	Lymph node involvement status	Expressed (n=35)	Not expressed (n=9)	0.66 (Fisher freeman Halton test)	Grade1(≤25%) (n=17)	Grade 2 (26-50%) (n=19)	Grade 3 ≥51% (n=8)	0.005 (Fisher freeman Halton test)
	pNx (n=7)	5 (71.43%)	2 (28.57%)		3 (42.9%)	3 (42.9%)	1 (14.3%)	
	pN0 (n=15)	15 (100%)	0		12 (80%)	3 (20%)	0	
	pN1a (n=4)	3 (75%)	1 (25%)		1 (25%)	2 (50%)	1 (25%)	
	pN1b (n=10)	7 (70%)	3 (30%)		1 (10%)	5 (50%)	4 (40%)	
	pN1c (n=3)	3 (100%)	0		0	3 (100%)	0	
	pN2a (n=5)	2 (40%)	3 (60%)		0	3 (60%)	2 (40%)	
4.	Lymphovascular invasion	Expressed (n=35)	Not expressed (n=9)	0.006 (Fisher's exact test)	Grade1(≤25%) (n=17)	Grade 2 (26-50%) (n=19)	Grade 3 ≥51% (n=8)	<0.001 (Fisher's exact test)
	Present (n=25)	16 (64%)	9 (36%)		1 (4%)	16 (64%)	8 (32%)	
	Absent (n=19)	19 (100%)	0		16 (84.2%)	3 (15.8%)	0	
5.	Margin involvement	Expressed (n=35)	Not expressed (n=9)	0.007 (Fisher's exact test)	Grade1(≤25%) (n=17)	Grade 2 (26-50%) (n=19)	Grade 3 ≥51% (n=8)	<0.001 (Fisher's exact test)
	Involved (n=27)	18 (66.67%)	9 (33.33%)		4 (14.81%)	15 (55.56%)	8 (29.62%)	
	Not involved (n=17)	17 (100%)	0		13 (76.47%)	4 (23.52%)	0	

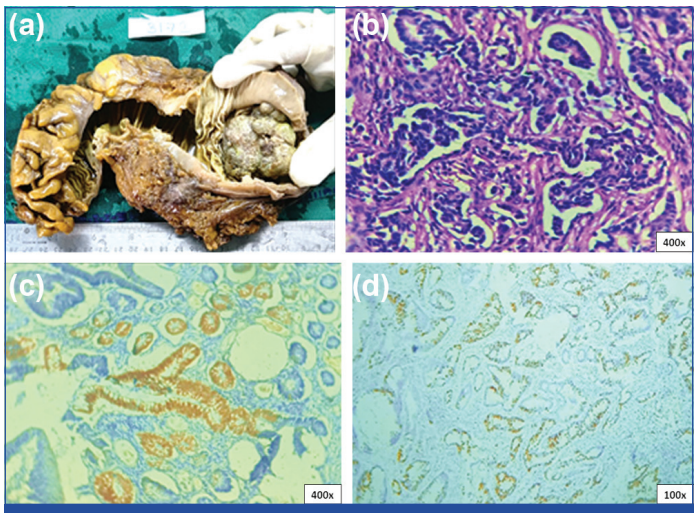
[Table/Fig-5]: Expression and association of CK20 and Ki67 in colon carcinoma differentiation, stage, Lymph node metastasis, LVI and margin involvement.

Association between CK20 and Ki-67 expression [Table/Fig-6]: CK20 and Ki-67 expression in colon carcinoma showed a negative, statistically significant (p-value <0.001) association (Fisher-Freeman-Halton test). It was noted that CK20-positive expression was found in tumours with Ki-67 expression of ≤25% (grade 1) and 26-50% (grade 2). CK20 expression was gradually lost in higher (grade 3) Ki-67 expressing tumours.

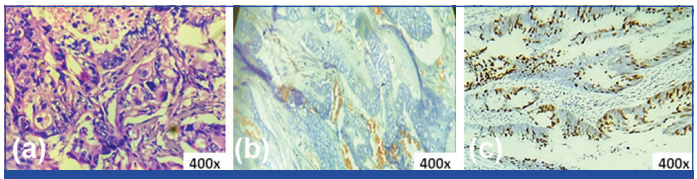
Ki67 (%) grade				p-value
CK20	Grade 1 (≤25%) (n=17)	Grade 2 (26-50%) (n=19)	Grade 3 [≥51%] (n=8)	
Expressed (n=35)	17 (48.6%)	17 (48.6%)	1 (2.9%)	<0.001 (Fisher freeman Halton test)
Not expressed (n=9)	0	2 (22.2%)	7 (77.8%)	

[Table/Fig-6]: Association of CK20 and Ki67 expression in colon carcinoma (n=44).

Gross, histology and IHC (CK20 and Ki-67) images of caecal mass [Table/Fig-7]. Histology and IHC (CK20 and Ki-67) images of growth in sigmoid colon [Table/Fig-8].



[Table/Fig-7]: a) Grossly shows a large polypoidal (cauliflower like) mass in the caecum; b) High power photomicrograph view (400x) showing moderately differentiated adenocarcinoma of caecum in Haematoxylin and eosin stain(H&E); c) High power photomicrograph view(400x) showing membranous and cytoplasmic Cytokeratin 20 expression; d) Low power photomicrograph view(100x) showing 35% Ki-67 (grade 2) nuclear positivity.



[Table/Fig-8]: a) High power view (400x) showing poorly differentiated adenocarcinoma on Haematoxylin eosin stain (H&E); b) High power view (400x) showing absence of Cytokeratin 20 expression; c) High power view (400x) showing high nuclear Ki-67 expression (45%) (grade 2).

DISCUSSION

Colon carcinoma occurs in both males and females with a slight male predominance; in India it is the 4th most common cancer in males and the 5th most common in females. Left-sided carcinomas are more common, but recently there is a declining trend due to early diagnosis by endoscopy. The molecular background shows location-specific features [2]. With recent advancements toward personalised medicine, the importance of immunohistochemistry using CK-20 and Ki-67 and molecular studies plays a significant role in diagnosis, prognosis and therapy in colon carcinomas. However, not many studies have been conducted on IHC analysis and its utility in colon carcinomas in the Indian subcontinent, especially in the eastern parts.

In this study, the patients' ages ranged from 12 to 89 years with a mean age of 52.1 years. The majority of patients (19 cases; 43.2%) ranged between 51-70 years. A study by Deori BJ et al., found that the highest incidence of colon carcinoma was between 46-60 years, representing 38.5%, with the mean age of presentation being 48.46±15.51 years, which nearly corroborates this study [12].

Males comprised 26 cases (59.1%) and females 18 cases (40.9%), establishing a male-to-female ratio of 1.4:1. Bhattacharya S et al., also found that colon carcinomas were more common in males than in females. Fifteen cases (34.1%) out of 44 presented with abdominal pain and distension, followed by 12 cases (27.3%) and obstipation (22.7%) [13].

The majority of tumours are left-sided (59.1%) in location. Al-Maghrabi J et al., also found that left-sided colon carcinomas were more common than right-sided [14]. The gross presentation of most tumours was polypoid (70.45%), followed by stricture (25%), with only about 4.6% presenting with perforation. Deori BJ et al., also found the gross appearance to be mostly polypoid, which supported this study [12].

On microscopic examination, 100% of the tumours were adenocarcinomas. Of these, moderately differentiated adenocarcinoma was predominant (45.5%), followed by well-differentiated carcinoma (29.6%) and poorly differentiated adenocarcinoma (11.6%). Other subtypes—mucinous adenocarcinoma, signet-ring carcinomas and MANEC—comprised 6.8%, 4.6% and 2.3%, respectively. Patil PS et al., [15] and Deori BJ et al., [12] also found that 47.6% and 50% of the histological types were dominated by moderately differentiated adenocarcinomas, which supported this study. Most tumours in this study belong to the pT3 stage of pTNM staging, at 45.5%.

CK20 expression was positive in 79.6% of cases and 20.4% were negative. CK20 was expressed predominantly in colon carcinomas with greater differentiation (81.3%) compared with those with lesser differentiation (18.6%) and the association between CK20 positivity and tumour differentiation was significant (p-value <0.001). Studies by Bayrak R et al., showed CK20 expression in low-grade colon carcinoma (87.1%) compared with high-grade carcinomas [16]. Nandy S et al., also concluded that CK20 expression was mostly restricted to well-differentiated adenocarcinomas (87.6%) and all moderately and poorly differentiated adenocarcinomas showed a lack of CK20 expression [17].

However, there was no association between CK20 expression and tumour stage (p-value=0.24) or lymph node involvement (p-value=0.66). This finding was supported by Bayrak et al., and Ileiva/N et al., who also reported no significant association between tumour stage and CK20 expression [16,18]. Deori BJ et al., on the contrary, noted that CK20 expression was predominantly noted in the T2 stage, which did not corroborate the present study findings [12]. A 100% of colon carcinomas with lymphovascular invasion showed positive CK20 expression in this study, establishing a significant association (p-value=0.006) between them. A 100% (17 cases) of tumours with no margin involvement had positive CK20 expression, predicting a statistically significant association between CK20 expression and negative tumour margin (p-value=0.007).

Grade 2 (26-50%) Ki-67 expressing tumours were the most frequent, with 19 cases out of 44. In contrast, 38.6% of total tumours had Ki-67 expression ≤25% (Grade 1) and 18.18% had ≥51% (Grade 3). There was a statistically significant correlation between Ki-67 expression and lower histological differentiation of the tumour (p-value <0.001). Similarly, a significant association (p-value=0.032) was found between increasing tumour stage and Ki-67 expression. Most tumours with stage pT2 and pT3 tended to have Ki-67 Grade 1 expression, while tumours with stage pT4a and pT4b mostly had Ki-67 expression in Grades 2 or 3. Hence, this supports the conclusion that, with rising tumour stage, the Ki-67 proliferation index shows an increasing trend in this study.

Study by Nayak J et al., stated that a high Ki-67 index (2+ and 3+) was found in higher-grade and higher-stage tumours, which supported this study [19]. Tong G et al., also reported that the Ki-67 proliferation index (Ki-67 PI) was higher in poorly differentiated (grade III) tumours than in well-differentiated (grade I) tumours and this relationship was statistically significant (p-value <0.01). They found that patients with T4 tumours expressed significantly higher Ki-67 PI compared with patients with T2 and T3 tumours [7].

Lymphovascular invasion has been noted in tumours with Ki-67 expression in the 26-50% range (Grade 2), while tumours with Ki-67 ≤25% (Grade 1) largely lacked lymphovascular invasion. Lymphovascular invasion has also been found to be associated with higher Ki-67 expression (p-value <0.001). Fifty-five point six percent of tumours with positive margins had Ki-67 Grade 2 expression and 100% of tumours with negative margins had Ki-67 Grade 1 expression. A statistically significant association was found between higher Ki-67 expression and margin positivity (p-value <0.001).

A study by Zhabagin KT, found that tumours at stages II (2.63×), III (3.16×) and IV (3.97×) had a higher likelihood of high Ki-67 expression than cases at stage I [20]. The presence of four or more regional lymph node metastases and higher rates of metastasis to distant sites also had higher Ki-67 expression. Tumours with lower differentiation had 5.1 times higher odds of having higher Ki-67 expression than tumours with higher differentiation. Studies by Mulyawan IM and Santos PMDD et al., had similar findings; their colon carcinoma studies also showed that increased Ki-67 expression was associated with lower tumour differentiation [9,10].

However, the study by Melling N et al., found that high Ki-67 expression was associated with lower tumour stage (p-value <0.0001) and nodal status (p-value=0.0315) but not with tumour grade (p-value=0.8639), histological tumour type (p-value=0.1542), or tumour localisation [8]. Thus, their findings suggest that high Ki-67 expression in colon carcinomas is associated with a favourable outcome, which does not support the present study's findings.

In the present study, the association between CK20 and Ki-67 with respect to histological differentiation of the tumour was statistically significant (p-value <0.001). CK20 expression was higher when Ki-67 was in the ≤25% (Grade 1) and 26-50% (Grade 2) ranges. Conversely, 77.8% of tumours with negative CK20 expression showed Ki-67 expression of ≥51% (Grade 3). This indicates an inverse association between CK20 and Ki-67 expression in colon carcinomas. The combined analysis of CK20 and Ki-67 may be valuable in the comprehensive assessment of tumour behaviour. For example, co-expression of a higher Ki-67 grade with abnormal CK20 has indicated a more aggressive tumour with higher recurrence and metastasis. However, there are not many studies available on this particular combination of markers in colon carcinoma to corroborate these findings.

Limitation(s)

The study was single-institution based and the number of cases was small, limiting generalisability. The study period was short, so survival analysis could not be appropriately predicted. IHC approaches have technical reproducibility issues and interpretations can be subjective with qualitative readouts, although all IHC was performed and interpreted in a single laboratory by the same core group of pathologists to minimise these issues as much as possible. Corroboration with molecular analyses could not be performed due to unavailability, limiting confirmation of potential immunohistochemical aberrancies.

CONCLUSION(S)

The most prevalent histology of colon carcinoma is adenocarcinoma. The majority are moderately differentiated adenocarcinomas. CK20 expression has been found to be higher in moderately and well-differentiated carcinomas and lower in poorly differentiated carcinomas and signet-ring cell carcinomas; thus CK20 expression decreases as differentiation worsens. However, no association was observed between CK20 expression and tumour stage or lymph node status. There has been a significant association between positive CK20 expression and the absence of margin involvement and the absence of lymphovascular invasion. Ki-67, on the other hand, has been found to be expressed more in moderately differentiated and poorly differentiated carcinomas, in mixed adenoneuroendocrine carcinomas and in higher tumour stages. Thus, Ki-67 expression increases with rising tumour grade and stage and also correlates with increased lymph node metastasis, lymphovascular invasion and positive margin involvement. An association between Ki-67 and CK20 expression was also statistically significant. Thus, CK20 and Ki-67 can be used as biomarkers for the grading and staging of colon carcinomas and for predicting prognosis.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 02, 2025
- Manual Googling: Aug 16, 2025
- iThenticate Software: Aug 18, 2025 (3%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: IHC kits were funded partly by Dr. Sarmila Sen and partly by the Department of Pathology, CNMCH.
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **May 13, 2025**
Date of Peer Review: **Jul 03, 2025**
Date of Acceptance: **Aug 20, 2025**
Date of Publishing: **Sep 01, 2025**