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Immunohistochemical analysis of cytokeratin 7 and 20 expression in colorectal carcinoma and its correlation with histopathological grading of tumour

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ABSTRACT

Background: Colorectal carcinoma is a malignant epithelial tumor originating in the large bowel. More than 90% of colorectal carcinomas (CRC) are adenocarcinomas. This being graded morphologically at H&E stain in histopathology as well-differentiated, moderately differentiated, and poorly differentiated. **Aims and Objectives:** The aim of this study is to assess any association with different histopathological types and extent of differentiation of CRC with change in expression of CK 7 and Cytokeratin 20 (CK20). **Materials and Methods:** In the present study, total 59 patients has been selected by the predetermined sampling techniques. The specimen was collected and the necessary processing was done for H&E stain and for immunohistochemistry for CK 20 and CK 7 profile. **Results:** This study showed that most of the well-differentiated colorectal carcinoma are CK20 (+ve)/Cytokeratin 7 (CK7) (-ve) and poorly differentiated CRC are CK20 (+) and/CK7 (+) and also CK20 (−ve)/CK7 (+ve). **Conclusion:** Hence, from this study, it is evident that the expression of CK 20 positivity decreasing and CK7 positivity increasing in respect of progression of well differentiated to poorly differentiated colorectal carcinoma, that is, there is a relationship of expression of CK20 and CK7 profile with the histological grading of colorectal carcinoma. Furthermore, the expression profile of CK20 and CK 7 may be of helpful while searching for unknown primary in cases of metastatic deposit.

Key words: Colonic carcinoma; Cytokeratin 7; Cytokeratin 20

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INTRODUCTION

Colorectal carcinoma is a malignant epithelial tumor originating in the large bowel. More than 90% of colorectal carcinomas (CRC) are adenocarcinomas. Incidence increases with age and carcinomas are rare before the age of 40 years, except in individuals with genetic predisposition or predisposing conditions such as chronic inflammatory bowel diseases in high incidence countries. Rates of rectal cancer are about 50% higher and colon cancer rates about 20% higher in men than in women. Nonetheless, CRC is the most common cause of deaths from cancer that is not directly attributable to tobacco usage in some of these countries.[1]

Cytokeratin 7 (CK7) is a polypeptide with molecular weight 54 kDa and an isoelectric point at pH 6.0.[2,3] This protein is encoded by the KRT7 gene, located on chromosome 12q13. In normal tissues, this basic (Type II) keratin is found to be distributed in a wide variety of simple epithelia: in organs associated with the gastrointestinal tract (including only the gallbladder, hepatic ducts, and pancreatic ducts), female genital tract (the ovary, endometrium, fallopian tube, and cervix), breast, urinary tract (the cells of renal tubule and collecting ducts of kidney, as well as in the cells of the transitional epithelium of the mucosa of the renal pelvis, ureter and bladder), and respiratory tract (the sinonasal mucosa, trachea and lung).

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Cytokeratin 20 (CK20) is a polypeptide with molecular weight 48.5 kDa and an isoelectric point at pH 5.66.[2,3] This protein is encoded by the KRT20 gene, located on chromosome 17q21.2. CK20 was originally identified as protein "IT" in cytoskeletal extracts of intestinal epithelia. In normal human tissues, this acidic (Type I) keratin is found in more complex epithelia of the gastrointestinal tract (such as taste buds, gastric foveolar cells, and intestinal epithelium), urothelial umbrella cells, squamous epithelia from any site, and Merkel cells of the epidermis and hair follicle outer rootsheath.[4,5] In all of the colorectal lesions, high CK20 positivity was found, including 100% in hyperplastic polyps, 95% in serrated adenomas, 90% in conventional adenomas, and 92% in adenocarcinomas.[6]

Adenocarcinoma of the colon is being graded morphologically at H&E stain in histopathology as well-differentiated, moderately differentiated, and poorly differentiated.

In this study, the diverse expression patterns of CK7 and CK20 among CRC have been proposed to correlate with histopathological grading of tumor.

Aims and objectives

The purpose of this study is to assess any change in expression of CK 7 and CK20 in CRC and to assess any association with different histopathological types and extent of differentiation of CRC.

MATERIALS AND METHODS

It was an observational, descriptive, and cross-sectional study done in the Department of Pathology, in collaboration with Department of General Surgery in a tertiary care center of Eastern India for 18 months. Approval from an institutional review board was obtained at the initiation of the study.

Inclusion criteria

The inclusion criteria being the newly diagnosed cases of primary CRC were included in the study.

Exclusion criteria

Patients with malignancy in other organ systems, recurrent gastrointestinal carcinoma, metastatic tumour to the colon and rectum with known primary, lesions of colon and rectum like Lymphomas and inflammatory bowel diseases etc., those having undergone operation after neoadjuvant chemoradiotherapy or specimens showing necrotic/gangrenous/autolytic changes were excluded from the study.

Total sample size was 59.

After gross examination, the part of the gastrointestinal tract (after either right or left hemicolectomy or Antero-posterior Resection) was sectioned. Sections from the apparently abnormal sites are taken. Figure 1 shows the gross appearance of a cut open colon with an annular growth.

Routine paraffin embedded sections were prepared and stained with Haematoxylin and Eosin stain (H&E stain.) The slides thus prepared then were examined under light microscope to detect the pathological lesion. After that, immunohistochemistry with CK 7 and CK 20 stain was done on CRC.

The tumor was given a histological grade based on the AJCC grading system as follows:

- Grade X- Grade cannot be assessed
- Grade 1 – Well differentiated (>95% of tumor composed of glands)
- Grade 2 – Moderately differentiated (50–95% of tumor composed of glands)
- Grade 3 – Poorly differentiated (49% or less of tumor composed of glands)
- Grade 4 – Undifferentiated.

CK 7 and CK 20 positivity was defined as cytoplasmic brown staining. Statistical analysis was done using SPSS software – version 20. Mean and standard deviations were determined for different variables. For statistical significance, $P<0.05$ was considered.

RESULTS

In this present study, the mean age of total 59 cases came to be 54.5 years.

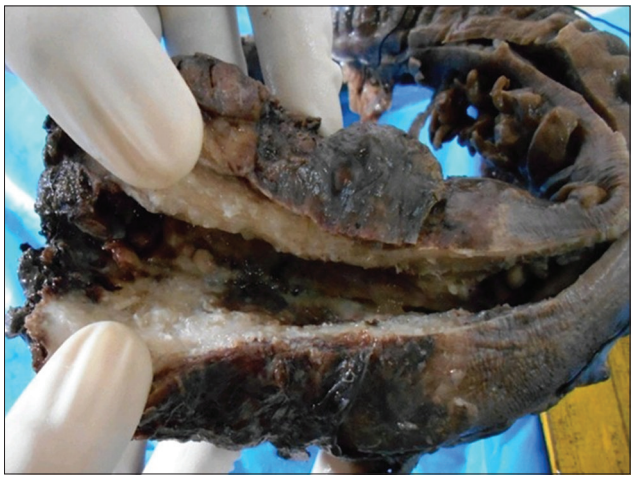

Figure 1: Gross appearance of annular growth in a cut open specimen of colon and rectum

The percentage of male patients is 40.7% (24 cases) and female patients is 59.3% (35 cases) (Table 1) which is statistically insignificant (P>0.05).

The highest percentage, that is, 52.5% (31 cases) of colorectal carcinoma was in the age group between 40 and 59 years, which is followed by age group of (60–80) years with 37.3% (22 cases) and then 20–39 years with 10.2% (6 cases) (Table 2).

In this study, well differentiated and moderately differentiated colorectal carcinoma is more in age group 40–59 years comprising 63.3% (19 cases) and 47.6% (10 cases), respectively. Poorly differentiated colorectal carcinoma is more in age group 60–80 years which is 62.5% (5 cases) (Table 3).

Furthermore, the incidence of colorectal carcinoma in lower age group is more common in right side of the colon (83.3% in 20–39 years and 64.5% in 40–59 years of age group) in contrast to older age group for which the left sided colonic growth is much higher (81.8% in 60–80 years of age group) (Table 4).

The females are affected (59.3%) more than the males (40.7%) (Table 5).

Furthermore, in the present study, among the total cases, 50.8% (30 cases) is well differentiated colorectal carcinoma, 35.6% (21 cases) is moderately differentiated carcinoma, and 13.6% (8 cases) is poorly differentiated colorectal carcinoma. Overall, well differentiated colorectal carcinoma is more common than other grades of carcinoma (Table 6).

Lymphovascular invasions (LVSI) and perineural invasion (PNI) (Figure 2) are highest among the poorly differentiated CRC cases (87.5% −7 cases) and 37.5% −3 cases respectively. Overall LVSI and PNI seen in 39.0% (23 cases) and 5.1% (3 cases), respectively (Table 7). This is statistically significant (P<0.05).

Among the well differentiated cases, 86.7% (26cases) are CK20+/CK7− and 13.3% (4 cases) are CK20−/CK7− (Figure 3).

Among moderately differentiated carcinomas, CK20+/CK7−, CK20+/CK7+, CK20−/CK7+ and CK20−/CK7− cases are 71.4% (15 cases), 9.5% (2 cases), 4.8% (1 case), and 14.3% (3 cases), respectively (Figure 4).

Among poorly differentiated cases CK20+/CK7−, CK 20+/CK7+, CK20−/CK7+, and CK 20−/CK7− cases are 12.5% (1 case), 37.5% (3 cases), 25% (2 cases), and 25% (2 cases), respectively (Figure 5).

Table 1: Frequency distribution of colorectal carcinoma with sex

Sex	Frequency	Percentage
Female	35	59.3
Male	24	40.7
Total	59	100.0

Table 2: Frequency distribution of colorectal carcinoma in different age groups

Age (in years)	No. of cases	Percentage
20–39	6	10.2
40–59	31	52.5
60–80	22	37.3
Total	59	100.0

Table 3: Relationship between different grades of colorectal carcinoma with different age groups

Grade of colorectal CA	20–39 years (%)	40–59 years (%)	60–80 years (%)
WD (n=30)	3 (10)	19 (63.3)	8 (26.7)
MD (n=21)	2 (9.5)	10 (47.6)	9 (42.9)
PD (n=8)	1 (12.5)	2 (25)	5 (62.5)
Total (n=59)	6 (10.2)	31 (52.5)	22 (37.3)

WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated

Table 4: Relationship between side of growth in colon of colorectal carcinoma with different age groups

Age group	Side colon of growth	
	Left (%)	Right (%)
20–39 years (n=6)	1 (16.7)	5 (83.3)
40–59 years (n=31)	11 (35.5)	20 (64.5)
60–80 years (n=22)	18 (81.8)	4 (18.2)
Total (n=59)	30 (50.8)	29 (49.2)

Table 5: Sex distribution with different grades of colorectal carcinoma

Grade of colorectal CA	Male (%)	Female (%)
WD (n=30)	10 (33.3)	20 (66.7)
MD (n=21)	10 (47.6)	11 (52.4)
PD (n=8)	4 (50)	4 (50)
Total (n=59)	24 (40.7)	35 (59.3)

WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated

Table 6: Frequency distribution of different grades of colorectal carcinoma

Grade of colorectal CA	No. of cases	Percentage
WD	30	50.8
MD	21	35.6
PD	8	13.6
Total	59	100

WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated

Most (86%) of the well differentiated colorectal carcinoma are CK20+/CK7− and poorly differentiated CRC are CK20+/CK7+ and also CK20−/CK7+. Hence, as the histological grades progresses from well differentiated to poorly differentiated carcinoma, the expression of CK7 positivity is increasing and the expression of CK20 positivity is decreasing (Table 8).

DISCUSSION

Colorectal carcinoma is a malignant epithelial tumor originating in the large bowel. CRC ranks as the fourth most frequent cancer in men (after lung, prostate, and stomach cancer), and third in women (after cancers of the breast and uterine cervix).[1]

The present study results have been compared to different published studies over different parameters.

In the study of Kumari et al.,[7] showed that young patients were 24.17% of total 1096 colorectal cases. In young patients, 60.37% were male and 39.63% were female. About9% had family history and only 8% had history of alcohol intake.

In this present study, also it is showing that there are shifting of ages from older to middle age group and also in younger age group.

In this present study, the mean age of total 59 cases is 54.5 years and incidence of colorectal carcinoma in younger age group is 10.2%.

Localized disease is defined as that there is no sign that the cancer has spread outside of the colon or rectum.[8] As per study of Cheng et al.,[9] the percentage of localized disease, which increased from 31.9% among cancers in the proximal colon to 37.0% in the descending colon to 41.5% in the distal colorectum. Within the same subsite, dark skinned people were less likely than light skinned people to receive a diagnosis of localized disease and more likely to receive a diagnosis of distant disease whereas stage distributions were approximately the same for males and females. The male-to-female rate ratios progressively increased from the proximal colon to the distal colorectum. The ratios of proximal-to-distal colorectal cancer gradually increased with advancing age. From the Table 4 of this present study, it is showing that the incidence of colorectal carcinoma in lower age group is more common in right side of the colon (83.3% in 20–39 years and 64.5% in 40–59 years of age group) in contrast to older age group for which the left-sided colonic growth is much higher (81.8% in 60–80 years of age group).

As per study of Krasna et al.,[10] the incidence and significance of histologic vascular and/or neural invasion in 77 patients with colorectal carcinoma treated over a 6-year period were analyzed retrospectively. Vascular invasion was found in 37.6% of patients and neural invasion in 14.3%. The following three types of vascular invasion were identified: Tumor lining epithelium, tumor thrombi, and destruction of the vessel wall. The incidence of metastases in patients with vascular invasion was 60% as opposed to 17% in those without vascular invasions. Survival in these patients was 29.7% and 62.2%, respectively. Metastases were found in 72.7% of patients with neural invasion, as opposed to 27% of those without neural invasion. Survival was 29.6% as opposed to 57.7% in those without neural invasion. Examination of patients with colorectal carcinoma for the presence of vascular and neural invasion may provide

Table 7: Relationship of LVSI and PNI with different grades of colorectal carcinoma

Grade of colorectal CA	Lymphovascular space invasion		Perineural invasion	
	No. of cases	Percentage	No. of cases	Percentage
WD (n=30)	10	33.3	0	0
MD (n=21)	6	28.6	0	0
PD (n=8)	7	87.5	3	37.5
Total (n=59)	23	39	3	5.1

LVSI: Lymphovascular invasions, PNI: Perineural invasion, WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated

Table 8: Expression profile of CK7 and CK20 in different histological grades of colorectal carcinoma

Grade of colorectal CA	CK 20+, CK 7+		CK 20+, CK 7−		CK 20−, CK 7 +		CK 20−, CK 7−	
	No. of case	(%)	No. of case	(%)	No. of case	(%)	No. of case	(%)
WD (n=30)	26	86.7	0	0	0	0	4	13.3
MD (n=21)	15	71.4	2	9.5	1	4.8	3	14.3
PD (n=8)	01	12.5	3	37.5	2	25.0	2	25.0
Total (n=59)	42	71.2	5	8.5	3	5.1	9	15.3

WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated

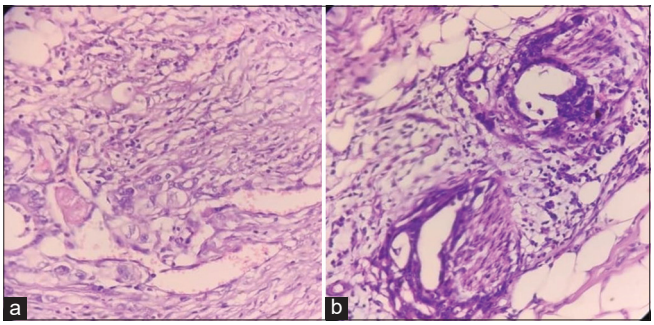

Figure 2: Photomicrograph showing (a) Lymphovascular invasion and (b) perineural invasion

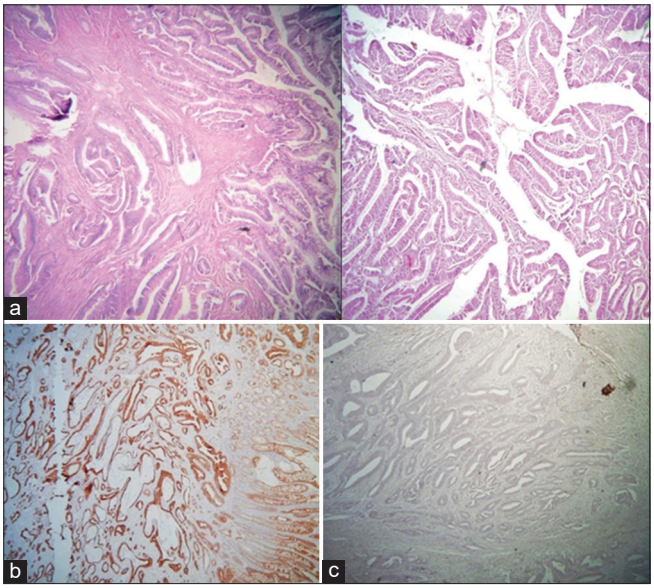

Figure 3: Photomicrograph showing well differentiated colorectal adenocarcinoma. (a) H&E ×400 (b) Cytokeratin 20 (c) Cytokeratin 7

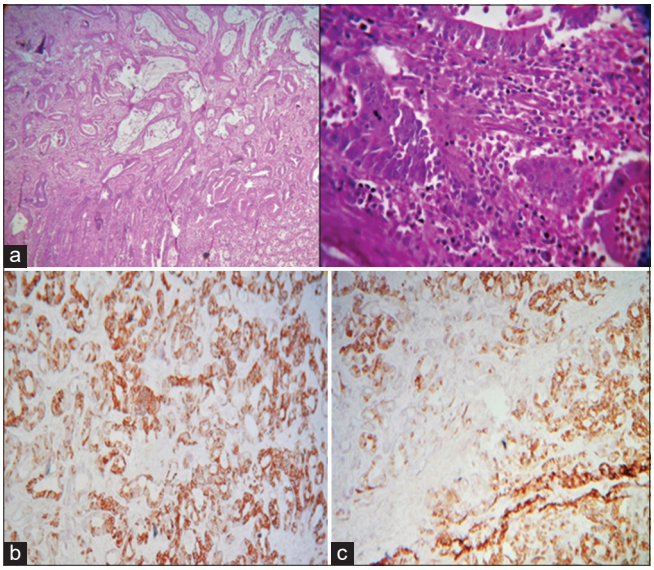

Figure 4: Photomicrograph showing moderately differentiated colorectal adenocarcinoma (a) H&E ×400 (b) Cytokeratin 20 (c) Cytokeratin 7

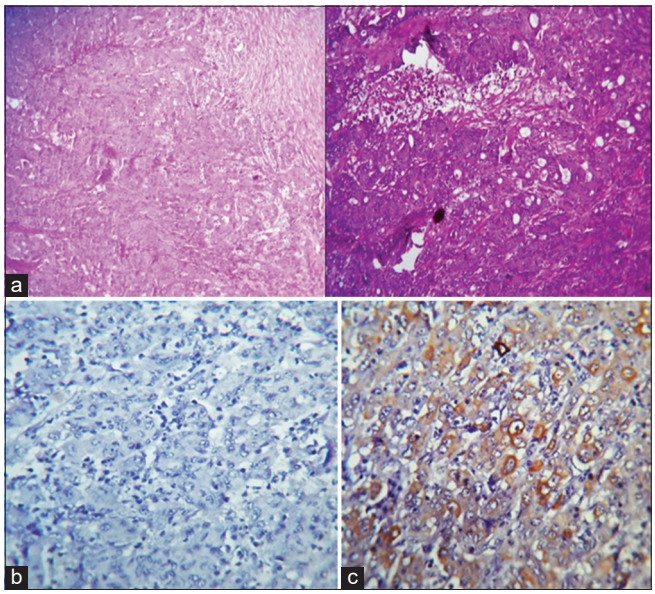

Figure 5: Photomicrograph showing poorly differentiated colorectal adenocarcinoma. (a) H&E ×400 (b) Cytokeratin 20 (c) Cytokeratin 7

useful information for determining future treatment and prognosis. The present study shows that LVSI and PNI are highest among the poorly differentiated CRC cases (87.5% and 37.5%) (7 cases and 3 cases, respectively) Overall LVSI and PNI seen in 39.0% (23 cases) and 5.1% (3 cases), respectively (Table 8) which corroborate with the above study of Krasna et al.

Kende et al.,[11] studied prospectively for immunohistochemical expression of CK7 and CK20. Well-differentiated and moderately differentiated adenocarcinomas of the large intestine, including appendix, were CK7–/CK20+ in the great majority of cases, as were goblet cell carcinoids, but half of the poorly differentiated adenocarcinomas exhibited aberrant expression, as did most of the mixed goblet cell carcinoid/adenocarcinomas. All five high-grade neuroendocrine carcinomas were negative for both CK7 and CK20. Not only the site but also the grade and histological type of a gastrointestinal carcinoma should be considered when assessing cytokeratin phenotype. From this data, it is showing that most of the well differentiated carcinomas are expressing CK20+/CK7− profile (86.7%) whereas in contrast to that poorly differentiated carcinomas are expressing mainly CK20+/CK7+ and CK20−/CK7+ profile (37.5% and 25.0%, respectively). In consideration of moderately differentiating carcinoma, although the main expression is CK20+/CK7− profile (71.4%), also expressing some profile of CK20+/CK7+and CK20−/CK7+ (9.5% and 4.8%, respectively) (Table 9). Hence, as the histological grades progresses from well differentiated to poorly differentiated carcinoma, the expression profile of CK7 positivity is increasing with the decreasing expression of CK20 profile.

Table 9: Expression profile of CK7 versus grades of carcinoma

CK 7	WD (%)	MD (%)	PD (%)
Positive (n=8)	0 (0)	3 (37.5)	5 (62.5)
Negative (n=51)	30 (58.8)	18 (35.3)	3 (5.9)

WD: Well differentiated, MD: Moderately differentiated, PD: Poorly differentiated

As per study of Al-Maghrabi et al.,[12] CK20 was expressed in a higher percentage of CRC and nodal metastasis than CK7. No difference in CK7 and CK20 immunostaining in primary and metastasis carcinomas was found. Four patterns of CK20/CK7 were identified; CK20+/CK7− (60.4%), CK20+/CK7+ (2.1%), CK20−/CK7− (35.4%), and CK20-/CK7+ (2.1%). Our study corroborate with this study.

Ramalingam et al.,[13] in their study concluded that immunostains for CK7, CK20, and gross cystic disease fluid protein 15 are useful in identifying cases associated with rectal adenocarcinoma. Twenty-six of 30 cases of rectal adenocarcinoma (87%) had a CK7−/CK20+, similar to the cases of non-rectal large intestine adenocarcinoma. In 4 cases (13%), neoplastic cells coexpressed cytokeratins 7 and 20. Hence, from this study, it is evident that the expression of CK 20 positivity decreasing and CK7 positivity increasing in respect of progression of well differentiated to poorly differentiated colorectal carcinoma i.e. there is a relationship of expression of CK20 and CK7 profile with the histological grading of colorectal carcinoma.

Limitations of the study

The colonoscopic biopsies could not be included in the study as the samples were inadequate and staging cannot be done. A wide spectrum of cases was not available for study as the study period was of one and half year. Subjective bias has taken into account in reporting the histological grade of the CRC and expression of the CK20 and CK7 immunostains.

CONCLUSION

Colorectal carcinoma is a malignant epithelial tumor originating in the large bowel. An estimated 1.23 million new cases of CRC occurred worldwide in 2008, representing about 9.7% of all new cancers. The defining feature of CRC is invasion through the muscularis mucosae into the submucosa. More than 90% of CRCs are adenocarcinomas.

In the present study, total 59 patients has been selected by the predetermined sampling techniques. The specimen was collected and the necessary processing was done for H&E stain and for immunohistochemistry for CK 20 and CK 7 profile. Moreover, the expression profile of CK7 and CK 20 in colorectal carcinoma has been compared with histological grading. The mean age of colorectal carcinoma is 54.5 years and well differentiated colorectal carcinoma is more common than other grades of carcinoma. While considering the relationship between age group and histological grades this is showing that overall colorectal carcinoma is more common in age group between 40 and 59 years among which well differentiated and moderately differentiated colorectal carcinoma is more in age group 40–59 years and poorly differentiated colorectal carcinoma is more on age group 60–80 years. In context of relationship between gender and histological grades, it was found that well differentiated carcinomas are more common in female age group than male but in both moderately and poorly differentiated carcinomas the incidence rate in both males and females are more or less same.

In this study, it was found a statistically significant relationship between the World Health Organization (WHO) pTNM staging with the histological grading. The well differentiated carcinomas are ≤WHO stage pT2 and poorly differentiated carcinoms are ≥WHO stagepT3. Furthermore, there is a statistically significant relationship between the LVSI and PNI according to the histological grading. LVSI is considerably high in poorly differentiated colorectal carcinoma. This study also showed that most of the well differentiated colorectal carcinoma are CK20 (+ve)/CK7 (−ve) and poorly differentiated CRC are CK20 (+) and/CK7 (+) and also CK20 (−ve)/CK7 (+ve). So from this study it is evident that the expression of CK 20 positivity decreasing and CK7 positivity increasing in respect of progression of well differentiated to poorly differentiated colorectal carcinoma i.e. there is a relationship of expression of CK20 and CK7 profile with the histological grading of colorectal carcinoma. Furthermore, the expression profile of CK20 and CK 7 may be of helpful while searching for unknown primary in cases of metastatic deposit.

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Authors' Contributions:
SN- Design of study, literature review statistical analysis and interpretation and drafting manuscript; **GB**- Concept, coordination, statistical analysis and interpretation, Interpretation of results; **VM**- Prepared first draft of manuscript, manuscript preparation.

Work attributed to:
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