Original Article

DOI: 10.7860/JCDR/2024/69311.19379

Clinicopathological and Immunohistochemical Association of CD55 and CD59 in Colorectal Carcinoma: A Cross-sectional Study

Pathology Section

RATHIN HAZRA[1], ANANYA KHAN[2], RAJIB KUMAR MONDAL[3], SARBARI KAR RAKSHIT[4]

ABSTRACT

Introduction: Colorectal Carcinoma (CRC) is one of the significant causes of fatality worldwide. High-grade and high-stage cancers contribute to this fatality. In most terminal cases, new immunomarkers (CD55 and CD59) are commonly detected as positive. The expression of these immunomarkers and their clinical relevance in CRC has not yet been firmly established. While their upregulation has been demonstrated in some CRC several groups have also reported that they are not highly expressed in CRC tissues.

Aim: To study the expression of CD55 and CD59 in CRC.

Materials and Methods: This was a cross-sectional study involving 90 patients with colorectal growth, conducted from February 2020 to July 2021 in the Department of Pathology in collaboration with the Department of General Surgery at Nil Ratan Sircar Medical College, Kolkata, West Bengal, India. Histopathological findings were evaluated, and associations of grading, staging, tumour type, tumour location, age, and sex were studied with immunomarkers (CD55 and CD59). Clinical parameters of the patients with CRC diagnosed by colonoscopic biopsy were also studied, including age, sex, clinical pictures, food habits, family history of CRC, and prior chemotherapy. The Chi-square test was used to determine the significance of the study. Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 27.0 (IBM, Illinois, US). A p-value of ≤0.05 was considered statistically significant.

Results: A total of 90 CRC patients were included in this study, with the majority being male 56 (62.2%) aged 51-60 years. Most cases 59 (65.6%) were moderately differentiated adenocarcinoma, followed by poorly differentiated adenocarcinoma 18 (20%) and well-differentiated adenocarcinoma 13 (14.4%). About 62 (68.9%) and 28 (31.1%) patients showed strong and weak CD55 expression, respectively. In total, 65 (72.2%) and 25 (27.8%) patients exhibited strong and weak CD59 expression, respectively. The association of CRC grade and stage with CD55 and CD59 was significant (p-value=0.0001, p-value=0.0013, p-value=0.0001, and p-value=0.0001, respectively).

Conclusion: There are several variables to consider during the histopathological reporting of CRC, with tumour differentiation, grade, and stage being among the most important. Particularly, high-grade (poorly differentiated) and high-stage adenocarcinomas demonstrate enhanced expression of CD55 and CD59, indicating a poor prognosis. It is essential for pathologists to meticulously perform grossing and reporting of CRC and dispatch the histopathology report after proper clinicopathological correlations. These immunomarkers can also be included in a routine IHC panel for prognostic and therapeutic purposes.

Keywords: Adenocarcinoma, Immunohistochemistry, Membrane bound complement regulatory proteins, Tumour grade, Tumour stage

INTRODUCTION

Cancer is undoubtedly a worrisome disease in our country, leading to increased morbidity and mortality worldwide. The main cause of mortality associated with this cancer is tumour invasion and metastasis. There are many factors that determine the prognosis of this cancer, with two important factors being the level of invasion and lymph node metastasis. Node-negative disease usually shows a favourable clinical course. It is also observed that approximately one-third of node-negative CRC cases show recurrence and disease progression. It is probably due to a failure to detect occult disease [1]. During the progression of the tumour, aberrant genetic changes occur early. To predict the potentiality of tumour metastasis, molecular profiling of specific tumour markers in the primary tumour may be helpful [2]. There are different complement proteins in our body that help fight against tumour formation. Among them, some are known as Decay Accelerating Factor (DAF) or membrane-bound Complement Regulatory Proteins (mCRPs), for example, CD55, CD59, and CD46. Their roles are currently being studied by immunohistochemistry. Recent studies report that aberrantly expressed mCRPs in cancer cells escape from complement-mediated cell lysis [1-3]. The aberrantly expressed or upregulated mCRPs in some cancer cells inhibit the activation of complements and the formation of the Membrane Attack Complex (MAC). They also block the activation of T cells, their proliferation, and differentiation. In this way, they help tumour cells escape from immune attack, leading to poor prognoses for patients with tumour [4].

It has been demonstrated that novel anti-CD55 and CD59 antibodies suppress colorectal tumorigenesis in subsets of colorectal cancer tissues. These antibodies activate different complements and cause cancer cell death through the production of proinflammatory cytokines [5]. Additionally, they can kill cancer cells by expanding natural killer cells and/or stimulating macrophage-mediated antibody-dependent cell-mediated cytotoxicity. The discovery of 5-fluorouracil (5-FU) in 1957 was a landmark advance for CRC patients and has since been used as one of the first-line treatments in advanced CRC [6]. However, many cases are facing resistance to it [5]. Importantly, combinational therapy with these novel anti-CD55 and anti-CD59 antibodies with 5-FU may enhance the therapeutic effect against CRC. Interestingly, the stools of CRC patients contain soluble CD55 and CD59, possibly mediated by metalloproteinase-7 [6]. This suggests that these immunomarkers may be used as a markers for CRC, and these antibodies could potentially be utilised for CRC diagnosis and therapeutics.

Therefore, the primary aim was to assess the expression of CD55 and CD59 in colorectal cancer, while objectives were:

1. To determine the clinical parameters of the patients.
2. To identify the histological findings of different CRC
3. To determine the grading and staging (TNM) of CRC.
4. To evaluate the expression of CD55 and CD59 in histologically diagnosed carcinomatous colorectal tissue.
5. To examine the association of CD55 and CD59 expression with different clinical parameters (age, sex, tumour location, and tumour type) as well as the grade and stage of CRC.

MATERIALS AND METHODS

The present study was a prospective, cross-sectional, institution-based study conducted in the Department of Pathology in collaboration with the Department of General Surgery at Nil Ratan Sircar Medical College and Hospital (NRSMCH), Kolkata, West Bengal, India, for a period of 18 months (February 2020 to July 2021). The total number of study participants in this study was 90. The study was approved by the Institutional Ethical Committee (IEC) and was assigned the IEC number: Memo number- NO/NMC/693, Dated-10.02.2020.

Inclusion criteria: All specimens of colorectal growth obtained from all types of colectomy, abdominoperineal resection, and anterior resection, which were subsequently diagnosed histologically as CRC were included in the study.

Exclusion criteria: Benign and inflammatory (non malignant) lesions of the colon, malignant lesions not arising from the colon were excluded from the study.

Study Procedure

While undertaking this study, voluntary written informed consent was obtained from each patient, and full confidentiality of the patients was maintained. The study included all admitted indoor patients with a preoperative diagnosis of adenocarcinoma by colonoscopic biopsy. All types of colectomies, abdominoperineal resection, and anterior resection were performed in the Department of General Surgery at NRSMCH, and corresponding specimens were received in the Department of Pathology, NRSMCH, meeting the inclusion criteria during the study period. The grossing and reporting of the specimens were conducted according to the College of American Pathologists (CAP) Protocol [1].

The biopsy procedure involved removing the affected colorectal tissue from the patients under anaesthesia and sending it to the laboratory in an adequate amount of 10% Neutral Buffer Formalin (NBF) (1:10 dilution of a 37% formaldehyde stock solution) with proper labeling. The samples were kept for proper fixation for a minimum of 24 hours followed by grossing. Representative sections were taken from the tumour proper (3 sections-full thickness, including serosa), adjacent apparently uninvolved colonic mucosa (1 section), resection margins (proximal, distal, circumferential, if applicable), omental tissue (3 sections to look for any tumour deposit), and lymph nodes in the attached mesentery. Characteristics such as colour, consistency, necrosis, haemorrhage, calcification, etc., were also noted. A meticulous search was conducted for lymph nodes sent separately. After grossing, the tissue was dehydrated in graded ethyl alcohol (100%, 90%, 80%, 70%), cleared with xylene, and embedded in liquid paraffin (melting point 50-60°C) for proper block preparation in a Leuckart mold block using paraffin. sections were cut at a thickness of 4 µm using a Leica rotary microtome followed by Haematoxylin and Eosin (H&E) staining. Broders grading and staging (TNM) of CRC were performed through histopathological examination [1]. Grade-1 indicates a tumour with >95% gland formation, grade-2 indicates 50-95% gland formation, and grade-3 indicates 0-50% gland formation [1].

Immunohistochemical methods and analysis [1]: For the immunohistochemical analysis of CD55 and CD59, 3.0 µm paraffin sections were taken on poly-L-lysine-coated slides and deparaffinised in xylene, followed by hydration in descending grades of ethanol. Antigen retrieval was performed by heating sections at 95ºC (3 cycles of 5 minutes each for CD55 and CD59) in citrate buffer using an antigen retriever system (BioGenex, USA). Sections were then incubated with power block (Biogenex, USA) for 10 minutes to reduce non specific antibody binding, followed by incubation with primary antibodies (CD55-Rabbit polyclonal antibody and CD59-Rabbit polyclonal antibody) for one hour at 4ºC. After three washes with Trisphosphate Buffer Solution (TBS), a secondary antibody was added and incubated for 30 minutes. After a further three washes with TBS, 3,3´-diaminobenzidine substrate (DAB tetrahydrochloride) was applied to the sections for 10 minutes, and the sections were counterstained with Haematoxylin, dehydrated with ethanol and Xylene, and permanently mounted with DPX. In all the above immunostaining, cell counting was done in the areas where the tumour was most prominent. Areas of haemorrhage and necrosis were avoided. The immunohistochemical reports were mostly completed within three weeks after the clinical correlation of each case.

Internal control: Normal colonic mucosa.

Positive control: Previously known case of CRC (CD55 and CD59 positive case).

Negative control: Omitting the primary antibody.

The expression of CD55 and CD59 was observed at the membrane and cytoplasm.

A standard immunoreactivity scoring system was followed [7], based on: a) the percentage of stained cells (0 points-4 points); and b) the intensity of stained cells (0 points-3 points).

a) Percentage of stained cells (0 point- 4 points).

0	5% or less
1	6% to 25%
2	26 % to 50%
3	51% to 75%
4	Greater than 75%

b) Intensity of stained cells (0 point-3 points).

0	No stain
1	Mild (buff)
2	Moderate (yellow)
3	Strong (brown)

a and b were multiplied together to obtain the final score. The staining score was stratified as weak (score range 0 to 4) or strong (score range 5 to 12) based on the proportion and intensity of positively stained cancer cells.

The following parameters were evaluated for association of immunomarkers (CD55 and CD59:

1) Clinical parameters (age, sex, clinical presentations, their duration, food habits, family history of CRC, prior chemotherapy, etc.).
2) Histopathological findings (type of colorectal cancer, their differentiation/grading, and staging) were evaluated.

STATISTICAL ANALYSIS

For statistical analysis, data were entered into Microsoft Excel (MS). The data were summarised as numbers, means±standard deviations (SD) for numerical variables and as percentages (%), and ranges for categorical variables. The Chi-square test was used to determine the significance of the study. Data analysis was conducted using SPSS version 27.0 (IBM, Illinois, US) and GraphPad Prism version 5. A p-value of ≤0.05 was considered statistically significant.

RESULTS

A total of 90 CRC patients were included in this study, with the maximum number of patients, 30 (33.30%), belonging to the 51-60 age group [Table/Fig-1]. The mean age was 52.32±14.007 years,

with the minimum and maximum ages being 16 years and 79 years, respectively.

Age in years	CD55		CD59	
	Strong	Weak	Strong	Weak
<30 (08)	06	02	04	04
31-40 (10)	09	01	09	01
41-50 (17)	13	04	11	06
51-60 (30)	20	10	22	08
61-70 (21)	10	11	16	05
71-80 (04)	04	00	03	01
Total (90)	62	28	65	25

[Table/Fig-1]: Frequency and expression of CD55 and CD59 with age.

Male preponderance was observed, with 56 (62.2%) cases being male. The number of male and female patients were 56 (62.2%) and 34 (37.8%), respectively [Table/Fig-2].

CD55	Strong	Weak	CD59	Strong	Weak
Male (56)	38	18	Male	37	19
Female (34)	24	10	Female	28	06
Total (90)	62	28		65	25

[Table/Fig-2]: Frequency and expression of CD55 and CD59 with gender.

Symptoms are shown in [Table/Fig-3]. Only 09 (10.%) patients had a positive family history of CRC. A total of 77 (85.6%) patients consumed a non vegetarian diet, while 13 (14.4%) patients consumed a vegetarian diet.

Clinical history	n (%)
Bleeding per rectum	44 (48.9)
Pain abdomen	28 (31.1)
Altered bowel habit	18 (20)

[Table/Fig-3]: Distribution of patients according to symptoms.

The rectum was the predominant site of involvement in 32 (34.4%) patients, while the descending colon (01) was the least involved site with only 01 (01.11%) patient [Table/Fig-4].

Tumour site	CD55		CD59	
	Strong	Weak	Strong	Weak
Ascending colon (11)	07	04	07	04
Caecum (27)	18	09	24	03
Descending colon (01)	01	00	01	00
Rectum (32)	21	11	24	08
Sigmoid colon (15)	11	04	08	07
Transverse colon (04)	04	00	01	03
Total (90)	62	28	65	25

[Table/Fig-4]: Frequency and expression of CD55 and CD59 with tumour site.

In terms of histological types, 77 (85.6%) patients were diagnosed with adenocarcinoma-No Special Type (NST), other types are shown in [Table/Fig-5].

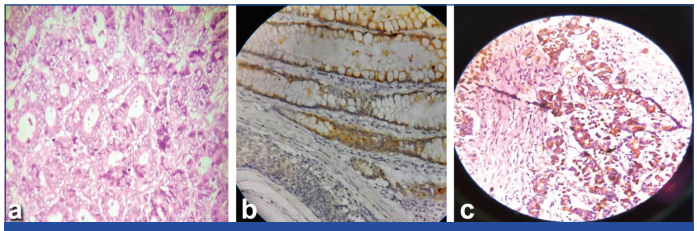
Tumour type	CD55		CD59	
	Strong	Weak	Strong	Weak
Adecocarcinoma –NST (77)	56	21	58	19
Mucinous adenocarcinoma (12)	05	07	06	06
Signet ring adenocarcinoma (1)	01	00	01	00
Total	62	28	65	25

[Table/Fig-5]: Frequency and expression of CD55 and CD59 with tumour types.

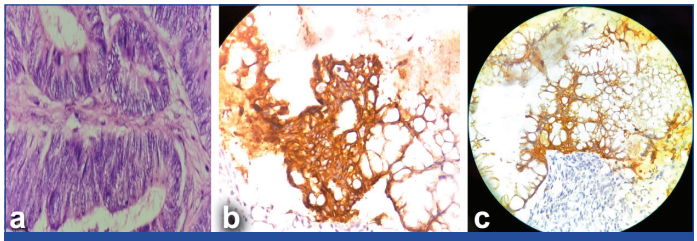
Among the adenocarcinomas, distribution of well, moderate and poorly differentiated is shown in [Table/Fig-6-9].

Histopathological grade of lesion	n (%)
Well-Differentiated (WD)	13 (14.4)
Moderately Differentiated (MD)	59 (65.6)
Poorly Differentiated (PD)	18 (20)

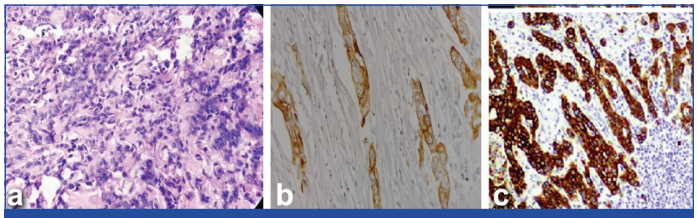
[Table/Fig-6]: Distribution of patients according to histological grade.



[Table/Fig-7]: a) Photomicrograph showing well differentiated adenocarcinoma (H&E, X400); b) Weak expression of CD55 in the cytoplasm and membrane (X400); c) Weak expression of CD59 in the cytoplasm and membrane (X400).



[Table/Fig-8]: a) Photomicrograph showing moderately differentiated adenocarcinoma (H&E, X400); b) Strong expression of CD55 in the cytoplasm and membrane (X400); c) Strong expression of CD59 in the cytoplasm and membrane (X400).



[Table/Fig-9]: a) Photomicrograph showing poorly differentiated adenocarcinoma (H&E, X400); b) Strong expression of CD55 in the cytoplasm and membrane (X400); c) Strong expression of CD59 in the cytoplasm and membrane (X400).

The majority of patients, 44 (48.9%), were in stage three [Table/Fig-10]. A total of 36 (40.00%) patients had stage pT3N0M0, followed by 26 (28.88%) with pT3N1M0, 05 (05.6%) with pT2N0M0, 04 (04.4%) with pT3N1bM0, pT3N1aM0, and pT4aN0M0 each, 03 (03.3%) with pT1N1M0, pT2N1aM0, and pT3N2aM0 each, and 02 (02.2%) with pT4N0M0.

TNM stage	n (%)
I	06 (06.7)
II	36 (40)
III	44 (48.9)
IV	04 (04.4)

[Table/Fig-10]: Distribution of patients according to TNM Staging.

Strong and weak intensity of CD55 and CD59 is shown in [Table/Fig-11].

Regarding the expression of CD55 with the grade of the tumour, mostly weak expression was observed in well-differentiated adenocarcinomas.

Immunomarkers	Intensity	n (%)
CD55	Strong	62 (68.9)
	Weak	28 (31.1)
	Total	90 (100)
CD59	Strong	65 (72.2)
	Weak	25 (27.8)
	Total	90 (100)

[Table/Fig-11]: Frequency and expression of CD55 and CD59 in colorectal cancer.

Among the 59 moderately differentiated carcinomas, 42 (71.18%) exhibited strong expression, and 17 (28.82%) showed weak expression. All 18 poorly differentiated carcinomas showed strong expression (100.00%) [Table/Fig-12].

Tumour grading	Strong	Weak	Total
Well-differentiated adenocarcinoma	02.0	11.0	13.0
Moderately differentiated adenocarcinoma	42.0	17.0	59.0
Poorly differentiated adenocarcinoma	18.0	0	18.0
Total	62.0	28.0	90.0

[Table/Fig-12]: Grading of CRC with expression of CD55.

Concerning the expression of CD59 with the grade of the tumour, predominantly weak expression of CD59 was noted in well-differentiated adenocarcinomas. Out of 59 cases of moderately differentiated adenocarcinomas, 46 cases (77.97%) showed strong expression, while 13 cases (22.03%) exhibited weak expression. Among the 18 poorly differentiated adenocarcinoma cases, 17 cases (94.44%) demonstrated strong expression, and 01 case (05.56%) showed weak expression [Table/Fig-13].

Tumour grading	Strong	Weak	Total
Well differentiated adenocarcinoma	02.0	11.0	13.0
Moderately differentiated adenocarcinoma	46.0	13.0	59.0
Poorly differentiated adenocarcinoma	17.0	1.0	18.0
Total	65.0	25.0	90.0

[Table/Fig-13]: Grading of CRC with expression of CD59.

Regarding the expression of CD55 with the stage of the tumour, Stage-III and Stage-IV cancers exhibited stronger expression of CD55 (44 cases, 91.66% out of 48 cases) compared to Stage-I and Stage-II cancers (18 cases, 42.85% out of 42 cases) [Table/Fig-14].

TNM stage	Strong n (%)	Weak n (%)	Total n (%)
I	02 (33.3)	04 (66.7)	06 (100)
II	16 (44.4)	20 (55.6)	36 (100)
III	40 (90.9)	04 (90.1)	44 (100)
IV	04 (100)	0	04 (100)
Total	62 (68.9)	28 (31.1)	90 (100)

[Table/Fig-14]: Distribution of patients according to TNM staging with expression of CD55.

In this study, Stage-III and Stage-IV cancers showed stronger expression of CD59 (46 cases, 95.83% out of 48 cases) than Stage-I and Stage-II cancers (19 cases, 45.23% out of 42 cases) [Table/Fig-15]. The association of both marker expressions with TNM staging and histological grade was found to be statistically significant [Table/Fig-16].

TNM stage	Strong n (%)	Weak n (%)	Total n (%)
I	01 (16.7)	05 (83.3)	06 (100)
II	18 (50)	18 (50)	36 (100)
III	42 (95.4)	02 (04.6)	44 (100)
IV	04 (100)	0	04 (100)
Total	65 (72.2)	25 (27.8)	90 (100)

[Table/Fig-15]: Distribution of patients according to TNM staging with expression of CD59.

Parameters	CD55 Chi-square value	CD55 p-value	CD59 Chi-square value	CD59 p-value
Histological grade of cancer	25.6386	0.0001 Significant	26.3348	0.0001 Significant
TNM staging	25.3372	0.0013 Significant	31.4685	0.0001 Significant
Age	8.9833	0.1097 Insignificant	4.2220	0.5179 Insignificant
Sex	0.0736	0.7861 Insignificant	2.7954	0.0945 Insignificant
Tumour site	2.7592	0.7371 Insignificant	4.7642	0.0582 Insignificant
Tumour type	5.1301	0.0769 Insignificant	3.7079	0.1566 Insignificant

[Table/Fig-16]: Association of tumour grade, stage, age, sex, tumour location and tumour type with the CD55 and CD59.

DISCUSSION

The CRCs are the third most common carcinoma worldwide [8]. As a result, many studies and research works have been done and are being conducted. More frequently, some newer parameters and markers are being used to detect and prognosticate these tumours as our knowledge regarding CRCs is continuously updated. Enhanced CD55 expression is found not only in CRC [9] but also in malignancies of the lung [10], stomach [11], breast [12], ovary [13], prostate [14], cervix [15], and leukemia [16]. High expression of CD55 and CD59 is a poor indicator of colorectal cancer [17,18]. One should also keep in mind that CD55 is also expressed in malaria, protein-losing enteropathy, and some autoimmune diseases [19-21].

Cancer is strongly age-related. The incidence rises progressively with age. Present study showed that the mean±SD age of the study population was 52.32±14.007 years. The majority belonged to the 50 to 60 years age group with a minimum age of 16 years and a maximum age of 79 years. Out of 90 cases, 18 (20.00%) cases were from the age group <40 years, while the remaining 72 (80.00%) cases were above 40 years of age similar to the study by Patil SP et al., [22]. In their study, the mean age of the patients was 47.20 years, and the age range was 11 years to 85 years. The mean age at diagnosis was 58.4 years, and the range was 23-85 years in the study of Peedikayil MC et al., [23].

The present study showed a slight increase in the incidence of CRC in males, 56 (62.2%), compared to females, 34 (37.8%), resulting in a male-to-female ratio of 1.64:1. Similar results were reported by Nayak SP et al., (Male-40, female-26, M: F ratio-1.53:1) [24], and Laishram RS et al., (Male-50, female-31, M: F ratio-1.61:1) [25].

In CRC, dietary factors play an important role; there is a close correlation between meat consumption and the incidence of large-bowel cancer. In this study, 13 patients (14.4%) were vegetarian, and the remaining 77 cases (85.6%) were non vegetarian, similar to the study by Laishram RS et al., (vegetarian-21, 15.75% and non-vegetarian-37, 84.25%) [25]. A positive family history was noted in only 09 cases (10.00%), similar to the study by Nayak SP et al., (20 cases, 14.56%) [24]. Symptomatically, 44 (48.9%) patients presented with rectal bleeding, 28 (31.1%) with abdominal pain, and 18 (20.00%) with altered bowel habits, similar to the findings of the study by Patil SP et al., (n=62, rectal bleeding 28 (45.16%), abdominal pain 20 (32.25%), and altered bowel habits in 10 cases (16.12%) [22]. The most common site of the lesion was the rectum 32 (35.55%), followed by the caecum 27 (30.00%), sigmoid colon 15 (16.66%), ascending colon 11 (12.22%), transverse colon 04 (04.44%), with the rarest being the descending colon 01 (01.11%) [Table/Fig-4]. Similar findings were observed in other studies as well [22,25-28]. Peedikayil MC et al., found that most of the tumours (N= 163, 74.00%) were located distal to the splenic flexure [23]. Nayak SP et al., found the commonest site of involvement to be the sigmoid colon {N=45, 25 (37.00%)}, followed by the caecum (10) and rectum (10) 18.50% each [24].

Microscopically, adenocarcinoma- NST was the most common histological type, accounting for 77 (85.6%) cases, followed by mucinous adenocarcinoma 12 (13.6%) and signet ring cell carcinoma 1 (01.1%) in the present study. Among the adenocarcinoma NST category, moderately differentiated tumours were the most frequent,

with 59 (65.6%) cases, followed by poorly differentiated with 18 (20%) and well-differentiated tumours with 13 (14.4%) cases [Table/Fig-6]. This finding was similar to the study by Wong SCC et al., [29]. In the present study, the most common TNM staging was Stage-III, followed by Stage-II [Table/Fig-10]. This was in accordance with the observations of Wong SCC et al., {Stage-III-48 (52.76%), Stage-II-32 (42.56%)} [29] and Jass JR {Stage-III- 41 (42.76%), Stage-II-35 (40.96%)}, respectively [30]. Mesenteric lymph nodes were tested positive in 47 (52.22%) cases. The maximum number of lymph node involvements in this study was 09 in a single case. Out of 90 cases, only 05 (05.55%) patients had distant metastasis, with 03 patients having metastasis in the liver, 01 patient in the lung, and 01 female patient in the brain.

Immunohistochemically, CD55 was abundant in colorectal cancer tissue, but its intensity of expression was much lower in normal colonic tissue. Out of 90 histologically proven normal tissues, weak expression of CD55 was noted in 82 tissues (91.11%) and strong expression in 8 tissues (08.89%), while among 90 histologically proven cancerous tissues, 62 (68.9%) had strong expression and 28 (31.1%) had weak expression of CD55 [Table/Fig-11]. This result was similar to the study by Koretz K et al., {n=88, 57 (64.77%) strong and 31 (35.23%) weak} and Shang Y et al., {n=56, 43 (76.78%) strong and 13 (23.22%) weak} [9,31]. In this study, the association of the expression of CD55 with the age of the patient (p-value=0.1097), sex of the patient (p-value=0.7861), location of the tumour (p-value=0.7371), and histological type of the tumour (p-value=0.0769) were not significant. These findings were in concordance with the findings of the study done by Shang Y et al., (p-value=0.1207, p-value=0.5462, p-value=0.6545, p-value=0.0834) [31]. The association of the grade of the tumour with the expression of CD55 was statistically significant. The chi-square value and p-value were 25.6386 and 0.0001, respectively [Table/Fig-15]. These findings were consistent with the study conducted by Shang Y et al., (p-value=0.0054) [31]. While CD55 upregulation in CRC has been demonstrated in some studies [9,32,33], several groups have not found high expression of CD55 in colorectal tumour tissues [34]. Regarding the expression of CD55 with the stage of the tumour, a statistically significant association was found (p-value=0.0013) with a chi-square value of 25.3372 [Table/Fig-15], similar to the study by Shang Y et al., (p-value=0.0034) [31].

Immunohistochemically, the expression of CD59 was abundant in CRC tissue, but its intensity of expression was much lower in normal colon tissue. Out of 90 histologically proven normal tissues, weak expression of CD59 was found in 84 tissues (93.3%) and strong expression in 06 tissues (06.7%), while among 90 histologically proven cancerous tissues, 65 cases (72.22%) had strong expression and 25 cases (27.78%) had weak expression of CD59 [Table/Fig-11], similar to the study by Shang Y et al., {N-56, 41 (73.21%) strong, 15 weak (26.79%)} [31].

No significant association of the expression of CD59 with the age of the patient (p-value=0.5179), sex of the patient (p-value=0.0945), location of the tumour (p-value=0.0582), and histological type of the tumour (p-value=0.1566) was noted. These findings were consistent with the study conducted by Shang Y et al., (p-value=0.6712, p-value=0.1013, p-value=0.0654, p-value=0.1656) [31]. A significant association of the expression of CD59 with the grade of the tumour was found in this study. The association of the grade of the tumour with the expression of CD59 was statistically significant (p-value=0.0001) with a chi-square value of 26.3348 [Table/Fig-15], similar to the study conducted by Shang Y et al., (p-value=0.0002) [31]. In this study, Stage-III and Stage-IV cancer exhibited stronger expression than Stage-I and Stage-II cancer. A statistically significant association between the stage of the tumour and the expression of CD59 (p-value=0.0001) with a chi-square value of 31.4685 was found [Table/Fig-15], similar to the study by Shang Y et al., (p-value=0.0003) [31]. Watson NF et al., observed the expression of CD59 in 69 (15.00%) of cases overall, and it was significantly associated with tumour grade but not with tumour site, stage, or histological type [18]. Fonsatti E et al., had given major emphasis on CD59 extensively on solid malignancies, and clinical approaches of humoral immunotherapy also had been implemented by them [35].

Limitation(s)

Survival analysis of the patients (to understand the prognosis) as well as the follow-up of the patients was not conducted. The study did not focus on the association of CD46 (a type of DAF) with the parameters mentioned above. The stool of CRC patients was not examined for soluble CD55 due to limited resources in this study. All cancer patients were treated with 5-FU and not with novel anti-CD55 or CD59 antibody drugs. Since this study was conducted at a single centre in Kolkata, the results cannot be generalised to all regions.

CONCLUSION(S)

The incidence of CRC is gradually increasing in our country. Since the clinical and histopathological presentations vary from case to case, clinicopathological association is crucial for prognostic purposes. Immunohistochemical associations of CD55 and CD59 can be performed in CRC, as high-grade (poorly differentiated) and high-staged adenocarcinoma commonly show increased expression of these new markers, ultimately leading to a poor prognosis for the patient. This may help in guiding the early initiation of treatment in the future.

Acknowledgement

Authors are indebted to Mr. Animesh Hazra (Assistant Professor, Computer Science and Engineering, Jalpaiguri Govt. Eng. College, West Bengal), Prof. Dr. Nibaran Das (Computer Science and Engineering, Jadavpur University, West Bengal) and Mr. Soumyajyoti Dey (PhD Scholar, Computer Science and Engineering, Jadavpur University, West Bengal) and Mr. Ranit Hazra (BA, Geography, Honours, Ashutosh College, Kolkata, West Bengal) for their technical support.

REFERENCES

[1] Gorter A, Meri S. Immune evasion of tumor cells using membrane-bound complement regulatory proteins. Immunol Today. 1999;20(12):576-82.
[2] Fishelson Z, Donin N, Zell S, Schultz S, Kirschfink M. Obstacles to cancer immunotherapy: Expression of membrane complement regulatory proteins (mCRPs) in tumors. Mol Immunol. 2003;40(2-4):109-23.
[3] Seya T, Matsumoto M, Hara T, Hatanaka M, Masaoka T. Distribution of C3-step regulatory proteins of the complement system, CD35 (CR1), CD46 (MCP), and CD55 (DAF), in hematological malignancies. Leuk Lymphoma. 1994;12(5-6):395-400.
[4] Wu J, Liu Q, Wang C, Tao M, Liu C. Targeting complement regulatory proteins in tumor immunotherapy. Int J Clin Exp Med. 2019;12(4):3083-94.
[5] Modest DP, Neumann UP, Pratschke J. FOLFOXIRI plus bevacizumab as conversion-therapy for liver metastases in colorectal cancer: A necessity? Eur J Cancer. 2017;73 (9):71-73.
[6] Ajona D, Ortiz-Espinosa S, Pio R. Complement anaphylatoxins C3a and C5a: Emerging roles in cancer progression and treatment. Semin Cell Dev Biol. 2019;85(5):153-63.
[7] Chu D, Zhao Z, Zhou Y. Matrix metalloproteinase-9 is associated with relapse and prognosis of patients with colorectal cancer. Ann Surg Oncol. 2012;19(1):318-25.
[8] Marisa L, de Reyniès A, Duval A, Selves J, Gaub MP. Gene expression classification of colon cancer into molecular subtypes: Characterization, validation, and prognostic value. PLoS Med. 2013;10(5):e1001453.
[9] Koretz K, Bruderlein S, Henne C, Moller P. Decay-accelerating factor (DAF, CD55) in normal colorectal mucosa, adenomas and carcinomas. Br J Cancer. 1992;66(5):810-14.
[10] Nakagawa M, Mizuno M, Kawada M, Uesu T, Nasu J. Polymorphic expression of decay-accelerating factor in human colorectal cancer. J Gastroenterol Hepatol. 2001;16(5):184-89.
[11] Inoue T, Yamakawa M, Takahashi T. Expression of complement regulating factors in gastric cancer cells. Mol Pathol. 2002;55(3):193-99.
[12] Keda J, Morii E, Liu Y, Qiu Y, Nakamichi N. Prognostic significance of CD55 expression in breast cancer. Clin Cancer Res. 2008;14(15):4780-86.
[13] Cocco E, Varughese J, Buza N, Bellone S, Lin KY. Tissue factor expression in ovarian cancer: Implications for immunotherapy with hI-con1, A factor VII-IgGF(c) chimeric protein targeting tissue factor. Clin Exp Metastasis. 2011;28(7):689-700.

[14] Loberg RD, Day LL, Dunn R, Kalikin LM, Pienta KJ. Inhibition of decay-accelerating factor (CD55) attenuates prostate cancer growth and survival in vivo. Neoplasia. 2006;8(1):69-78.
[15] He Y, Wang W, Xu L, Li L, Liu J. Immunohistochemical expression and prognostic significance of CD97 and its ligand DAF in human cervical squamous cell carcinoma. Int J Gynecol Pathol. 2015;34(5):473-79.
[16] Hara T, Kojima A, Fukuda H, Masaoka T, Fukumori Y. Levels of complement regulatory proteins, CD35 (CR1), CD46 (MCP) and CD55 (DAF) in human haematological malignancies. Br J Haematol.1992;82(2):368-73.
[17] Durrant LG, Chapman MA, Buckley DJ, Spendlove I, Robins RA. Enhanced expression of the complement regulatory protein CD55 predicts a poor prognosis in colorectal cancer patients. Cancer Immunol Immunother. 2003;52(9):638-42.
[18] Watson NF, Durrant LG, Madjd Z, Ellis IO, Scholefield JH. Expression of the membrane complement regulatory protein CD59 (protectin) is associated with reduced survival in colorectal cancer patients. Cancer Immunol Immunother. 2006;55(2):973-80.
[19] Egan ES, Jiang RH, Moechtar MA, Barteneva NS, Weekes MP. Malaria-A forward genetic screen identifies erythrocyte CD55 as essential for *Plasmodium falciparum* invasion. Science. 2015;348(6235):711-14.
[20] Ozen A, Comrie WA, Ardy RC, DomínguezConde C, Dalgic B. CD55 deficiency, early-onset protein-losing enteropathy, and thrombosis. N Engl J Med. 2017;377(1):52-61.
[21] Visser L, de Vos AF, Hamann J, Melief MJ, van Meurs M. Expression of the pEGF-TM7 receptor CD97 and its ligand CD55 (DAF) in multiple sclerosis. J Neuro immunol. 2002;132(1-2):156-63.
[22] Patil SP, Saklani A, Gambhire P, Meheta S, Engineer R. Colorectal cancer in India: An audit from a tertiary centre in a low prevalence area. Indian J Surg Oncol. 2017;8(4):484-90.
[23] Peedikayil MC, Nair P, Seena SM, Radhakrishnan L, Sadasivan S. Colorectal cancer distribution in 220 Indian patients undergoing colonoscopy. Indian J Gastroenterol. 2009;28(6):212-15.
[24] Nayak SP, Sasi MP, Sreejayan MP, Mandal S. A case-control study of roles of diet in colorectal carcinoma in a south Indian population. Asian Pac J Cancer Prev. 2009;10(4):565-68.
[25] Laishram RS, Kaiho N, Shimray R. Histopathological evaluation of colorectal carcinomas status in Manipur, India. Int J Pathol. 2010;8(1):05-08.
[26] Singh JP, Maini VK, Bhatnagar A. Large bowel malignancy epidemiology and gut motility studies in South Asia. Diseases of Colon and Rectum.1984;27(1):10-15.
[27] Gupta S, Bhattacharya D, Acharya AN, Majumdar S, Ranjan P. Colorectal carcinoma in young adults: A retrospective study on Indian patients: 2000–2008. Colorectal Disease. 2010;12(10):182-89.
[28] Cora DC, Onime GD, Coppa GF, Valentine IS, Rivera L. Anatomic, age, and sex distribution of colorectal cancer in a New York City hispanic population. J Nat Med Asso. 1998;90(1):19-24.
[29] Wong SCC, Lo ESF, Lee KC, Chan JKC, Hsiao WLW. Prognostic and diagnostic significance of beta-catenin nuclear immunostaining in colorectal cancer. Clin Cancer Res. 2004;10(4):1401-08.
[30] Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology. 2007;50(1):113-30.
[31] Shang Y, Na C, Yong G, Li D, Yan Y. Systematic Immunohistochemical analysis of the expression of CD46, CD55, CD59 in colon cancer. Arch Patho Lab Med. 2014;138(9):910-19.
[32] Mikihiro K, Motowo M, Junichirou N, Tokurou U, Hiroaki O, Hiroyuki O, et al. Release of decay-accelerating factor into stools of patients with colorectal cancer by means of cleavage at the site of glycosylphosphatidyl inositol anchor. J Lab Clin Med. 2003;142(5):306-12.
[33] Niehans GA, Cherwitz DL, Staley NA, Knapp DJ, Dalmasso AP. Human carcinomas variably express the complement inhibitory proteins CD46 (membrane cofactor protein), CD55 (decay-accelerating factor), and CD59 (protectin). Am J Pathol. 1996;149(1):129-42.
[34] Thorsteinsson L, O'Dowd GM, Harrington PM, Johnson PM. The complement regulatory proteins CD46 and CD59, but not CD55, are highly expressed by glandular epithelium of human breast and colorectal tumour tissues. APMIS. 1998;106(9):869-78.
[35] Fonsatti E, Altomonte M, Coral S, De Nardo C, Lamaj E, Sigalotti L, et al. Emerging role of protectin (CD59) in humoral immunotherapy of solid malignancies. Clin Ter. 2000;151(3):187-93.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Pathology, Diamond Harbour Government Medical College, Diamond Harbour, West Bengal, India.
2. Senior Resident, Department of Pathology, Nil Ratan Sircar Medical College, Kolkata, West Bengal, India.
3. Associate Professor, Department of Pathology, Nil Ratan Sircar Medical College, Kolkata, West Bengal, India.
4. Assistant Professor, Department of Pathology, Nil Ratan Sircar Medical College, Kolkata, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rathin Hazra,
Village Hasanpur, Town Joynagar, Joynagar Majilpur Municipality, Ward Number-13, Block-Jaynagar 1, PO-Ramakanta Nagar, PS-Jaynagar, Dist-South 24 Parganas, Jaynagar-74339, West Bengal, India.
E-mail: hazra_rathin@rediffmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 28, 2023
- Manual Googling: Feb 26, 2024
- iThenticate Software: Mar 01, 2024 (10%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- 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: **Dec 28, 2023**
Date of Peer Review: **Jan 31, 2024**
Date of Acceptance: **Mar 05, 2024**
Date of Publishing: **May 01, 2024**