Original Article

DOI: 10.7860/JCDR/2023/60715.17727

Pathology Section

Utility of Prognostic Scoring System Based on Histomorphological Parameters in Low-grade Colorectal Carcinoma

ANEESHA ASOK KUMAR[1], FASEELA KALAYAM KULATH[2], ASIQ SIDEEQUE[3], NISHA THATTAMPARAMBIL GOPALAKRISHNAN[4], AKHIL CHANDRAN[5]

ABSTRACT

Introduction: Colorectal Carcinoma (CRC) is the third most common cancer worldwide. Percentage of gland formation is the only valid parameter for histologic grading of CRC . Tumour budding and Tumour-infiltrating Lymphocytes (TILs) are emerging prognostic factors in CRC. In recent years high grade CRC has become subject to more precise molecular grading strategies. However low grade cases show in homogenous outcome due to still insufficient categorization. The focus of this study is to determine whether the combination of amount of gland formation, budding, and TILs will allow us to further characterize large in homogenous group of WHO low-grade cases into prognostically significant subgroups.

Aim: To estimate the significance of tumour budding and TILs in low-grade CRC and to categorise low-grade CRC into prognostic subgroups taking into account 3 histologic parameters-gland formation, tumour budding and TIL.

Materials and Methods: This was a descriptive cross-sectional study done in the Department of Pathology, MES Medical College, Malappuram, Kerala, India. It was an ambispective study (retrospective from January 2015 to December 2021 and prospective from December 2021 to March 2022) which analysed 105 World Health Organization (WHO) low-grade CRC cases. The demographic data of the patients was collected and histopathological assessment of tumour grade, pT, pN, lymphovascular invasion (LVI), Tumour-infiltrating lymphocytes (TIL) and tumour budding (TB) was done on Haematoxylin and Eosin (H&E) stained sections. A morphology-based risk score was developed taking into account 3 parameters- percentage of gland formation, budding, and TIL. For each parameter, 1 to 2 points were given, resulting in a sum score, dividing the CRC cases into a low-, an intermediate-, and a high-risk group. Statistical analysis was performed using SPSS 25. The results were expressed as numbers and percentage. Pearson Chi-square test was used to test the relationship.

Results: In the present study degree of budding significantly associated with pT stage (p=0.02), pN stage (p=0.042) and LVI (p=0.038). TIL also differed significantly with pT (p=0.001) pN (p=0.042) and LVI (p=0.004). Applying the prognostic scoring to 105 cases, 33 (31.4%) cases showed high score, 30 (28.6%) cases were of intermediate score and 42 (40%) cases showed low score. The 3 groups differs significantly with pT (0.027), pN (0.035) and LVI (0.015).

Conclusion: The present study showed combining different morphological parameters of tumour and tumour environment can help to further subdivide CRC into prognostically significant subgroups.

Keywords: Gland formation, Low grade colorectal carcinoma, Prognostic scoring, Tumour Budding, Tumour-infiltrating lymphocytes

INTRODUCTION

Colorectal carcinoma (CRC) is the third most common cancer worldwide, and second leading cause of cancer related death [1]. Tumour heterogeneity is a hot topic in cancer research now. Tumour heterogeneity in colorectal cancer is very well established [2]. The Tumour Node Metastasis (TNM) staging and histological tumour grading are the gold standard for classification of CRC patients into prognostic subgroups for the current treatment regimes. Despite advancements in the treatment of CRC, survival rates remain highly variable for different patients even within the same TNM staging [2]. CRC-grading according to the World Health Organization (WHO) classification is still only based on the percentage of gland formation which is subjected to high inter observer variability [3,4]. So, additional histomorphological parameters like tumour budding (TB) and tumour infiltrating lymphocytes (TIL) are recommended in the diagnostic work up protocols of CRC in addition to routine TNM staging and grading for better disease stratification and for more personalised treatment [5-8].

Tumour budding was first recognized in the 1950s as “sprouting” at the invasive edge of carcinomas that may reflect a more rapid tumour growth rate [9]. Biologically tumour budding is closely related to epithelial mesenchymal transition. Tumour budding denotes single or small aggregates detached from the neoplastic gland at the invasive front. Recently, criteria for evaluating and reporting tumour budding in CRC have been well defined by the International Concensus Conference on Tumour Budding (ITBCC) [10]. Many studies showed, that high budding is associated with lymph node positivity, vascular and lymphatic infiltration, local tumour recurrence, distant metastases and higher tumour aggressiveness [11-16].

In all these years, for treatment stratification the main focus was on tumour cell component. Now, there is a shift of focus to tumour microenvironment (TME) [17]. From the recent advancements in the understanding of TME now it’s evident that the crosstalk between the tumour and TME plays an important role in the tumour progression. TIL is an important immunological biomarker of TME. Besides its prognostic value it also helps for personalized treatment with checkpoint blockage therapy which has been well established in cancers like melanoma [2,18,19]. Salgado et al had standardized the methodology for visual assessment of TIL in breast cancer on H&E sections [20].

Grading of CRC is based on the percentage of gland formation. Traditionally, it was 3-tiered: well-differentiated (grade 1) showing glandular differentiation in more than 95% of the tumor, moderately differentiated (grade 2) with 50% to 95% glandular differentiation and high-grade (grade 3) with less than 50% glandular differentiation [21]. In the current 5th edition of World Health Organization classification

of Gastrointestinal tumour, well- and moderately differentiated CRCs are summarized as one low-grade group because of similar behavior and better inter-observer agreement [22].

So, it is important to pay special attention to tumor morphology and TME for additional information on tumour behavior and prognosis.

The author focussed on whether current CRC grading system focusing only on gland formation or a grading system analogue to Elston and Ellis grading breast cancer or Federation Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading in sarcoma combining different histomorphological parameters is better for getting better information regarding tumour behaviour of each CRC-case. In this study, the authors aimed to determine if the combination of the percentage of gland formation, tumour budding, and TILs allows us to further characterize the large, inhomogeneous group of low-grade CRC into prognostically significant subgroups.

MATERIALS AND METHODS

This descriptive cross-sectional single institutional study was conducted in the Department of Pathology, MES Medical College, Malappuram, Kerala. The study was ambispective in nature (January 2015 to November 2021: retrospective, and December 2021 to March 2022: prospective), which analysed 105 WHO low-grade CRC cases. The study was approved by the Institutional Ethics Committee (Ref. No.IEC/MES/47/2021).

Inclusion criteria: All Haematoxylin and Eosin (H&E) stained slides of low-grade CRC diagnosed in resection specimens during the time period of January 2015 to November 2021 were retrieved from department archives and were studied.

Exclusion criteria: Cases with neo-adjuvant treatment , WHO high grade CRC, special subtypes, such as mucinous, serrated, medullary carcinoma, inflammatory bowel disease-related carcinoma were excluded.

Procedure

After applying inclusion and exclusion criteria, H&E slides of all histopathologically confirmed cases of low-grade CRC were retrieved and evaluated independently by two consultant pathologists in terms of gland formation, percentage of TIL, tumour budding and lymphovascular invasion (LVI). To receive higher interobserver concordance, a subset of the cases were viewed together by both observers on a multihead microscope.

The authors assessed tumour budding based on International Tumour Budding Consensus Conference (ITBCC) criteria. It defines tumour budding as a single tumour cell or a cluster of no more than 4 tumour cells and should be evaluated on H&E stained slides in 1 hotspot (in a field measuring 0.785 mm^2) at the invasive front [10]. In each case the authors selected slides with deepest invasion and then scanned 10 separate fields (20X objective) along the invasive front and a hotspot was identified. We counted the tumour bud in the hotspot (lens magnification 20X, ocular magnification 10X, eyepiece field number diameter 22) and adjusted it by dividing with normalisation factor (1.210) to get a field measuring 0.785mm2. ITBCC recommended three-tiered system is used for further risk stratification - low budding, 0-4 buds; intermediate budding 5 to 9 buds; and high budding ≥10 buds [10] .

The percentage of TILs was estimated according to the criteria defined by Salgado R et al in breast cancer [20]. The slide with the deepest invasion were scanned in a 200 fold magnification (ocular x10, objective x20) and the average percentage amount of stromal TILs within the border of invasion was assessed as high if >5% or low if ≤5% [23] .

The gland formation based on grading according to 3- tiered WHO grading system was done in each of these case. These parameters were then given score points of 1 to 2 to calculate a sum score and categorize them into 3 risk groups in a similar way Lang-Schwarz C, et al did by using Bayreuth scoring system in their study [23] [Table/Fig-1].

Gland formation [22]		Budding [10]		TILs [20]	
Percentage of gland formation	Score points	Amount of budding	Score points	Percentage of TILs	Score-points
>95% (WHO grade 1)	1	Low	1	>5%	1
95-50% (WHO grade 2)	2	Intermediate to high	2	≤5%	2

[Table/Fig-1]: Prognostic score based on 3 morphological parameters -Grading, budding, and TILs: 1 to 2 score points are given for each parameter resulting in a sum score that defines the risk group.
Total score 3 or 4 --- Low risk
Total score 5 --- Intermediate risk
Total score 6 --- high risk

STATISTICAL ANALYSIS

All the data collected were entered in Microsoft Excel and analysis was done with the help of Statistical Package for the Social Sciences software version 25.0 (SPSS Inc., Chicago, USA). The results were expressed as numbers and percentage and statistical analysis was done using Pearson chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

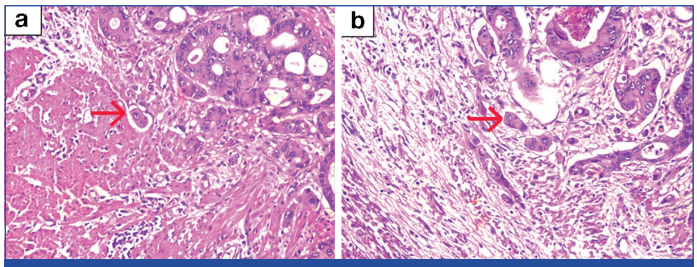
Summary of cases and tumour characteristics [Table/Fig-2].

Features	Frequency (%)
Age (years)	
Mean and Range	60.47 (32-87)
Gender	
Male	52 (49.5)
Female	53 (50.5)
Tumour location	
Left side	77 (73.3)
Right side	28 (26.7)
Differentiation	
Well	57 (54.3)
Moderate	48 (45.7)
T stage	
T1	15 (14.3)
T2	29 (27.6)
T3	43 (41.0)
T4	18 (17.1)
N stage	
N0	40 (38.1)
N1	48 (45.7)
N2	17 (16.2)
TIL	
≤5% (low)	35 (33.3)
>5% (high)	70 (66.7)
LVI	
Absent	32 (30.5)
Present	73 (69.5)
Tumour budding	
Low	43 (41.0)
Intermediate	31 (29.5)
High	31 (29.5)

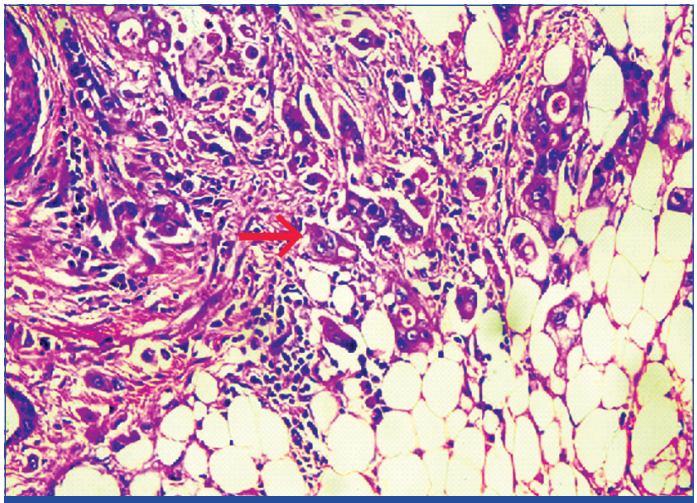
[Table/Fig-2]: Summary of cases and tumour characteristics.

Tumour budding: In this study, the authors found low budding in 43 (41%),intermediate budding in 31 (29.5%) [Table/Fig-3] and high budding in 31 (29.5%) cases [Table/Fig-4]. In the present

study degree of budding significantly associated with pT stage (p=0.02),pN stage (p=0.042) and LVI (p=0.03. High budding was associated with higher pT stage, pN stage and more chance for lymphovascular invasion [Table/Fig-5].



[Table/Fig-3]: a) Low budding 0-4 buds, b) Intermediate budding 5-9 buds (20X, H&E).



[Table/Fig-4]: High budding ≥10 buds (20X, H&E).

T stage	Tumour budding			p-value
	High (%)	Intermediate (%)	Low (%)	
T1	7 (46.7)	2 (13.3)	6 (40.0)	0.022
T2	8 (27.6)	5 (17.2)	16 (55.2)	
T3	10 (23.3)	14 (32.6)	19 (44.2)	
T4	6 (33.3)	10 (55.6)	2 (11.1)	
Total	31	31	43	
N stage	**Tumour budding**			**p-value**
	High (%)	Intermediate (%)	Low (%)	
N0	11 (27.5)	6 (15.0)	23 (57.5)	0.042
N1	15 (31.3)	17 (35.4)	16 (33.3)	
N2	5 (29.4)	8 (47.1)	4 (23.5)	
Total	31	31	43	
LVI	**Tumour budding**			**p-value**
	High (%)	Intermediate (%)	Low (%)	
Absent	6 (18.8)	7 (21.9)	19 (59.4)	0.038
Present	25 (34.2)	24 (32.9)	24 (32.9)	
Total	31	31	43	

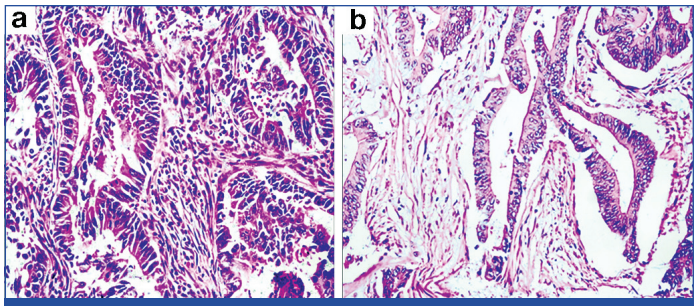
[Table/Fig-5]: Association of tumour budding with pT, pN and LVI.

Tumour infiltrating lymphocytes: Out of 105 cases 70 cases (66.7%) showed high TIL (>5%) and 35 cases (33.3%) showed low TIL (≤5%). TIL differed significantly with pT (p=0.001) pN (p=0.042) and LVI (p=0.004) High TIL was associated with higher pTstage, pN stage and more chance for lymphovascular invasion when compared to low TIL with a significant p-value [Table/Fig-6,7].

Gland formation: Based on the percentage of gland formation, out of 105 low grade cases we studied; 57 (54.5%) of cases were well differentiated and 48 (45.7%) of cases were of moderately differentiated.

T stage	TIL		p-value
	High (%)	Low (%)	
T1	3 (20.0)	12 (80.0)	0.001
T2	21 (72.4)	8 (27.6)	
T3	32 (74.4)	11 (25.6)	
T4	14 (77.8)	4 (22.2)	
Total	70	35	
N stage	**TIL**		**p-value**
	High (%)	Low (%)	
N0	21 (52.5)	19 (47.5)	0.042
N1	35 (72.9)	13 (27.1)	
N2	3 (82.4)	14 (17.6)	
Total	59	46	
LVI	**TIL**		**p-value**
	High (%)	Low (%)	
Absent	15 (46.9)	17 (53.1)	0.004
Present	55 (75.3)	18 (24.7)	
Total	70	35	

[Table/Fig-6]: Association of TIL with pT, pN and LVI.



[Table/Fig-7]: a) High TIL >5%, b) Low TIL ≤5% (20X, H&E).

Scoring: gland formation, budding and TIL

Applying the prognostic score described in [Table/Fig-1] to the 105 cases showed the following results: 33 (31.4%) cases showed high score, 30 (28.6%) cases showed intermediate score and 42 (40%) showed low score. High and intermediate scores correlated significantly with high pT (0.027), high pN (0.035) and had more chance for lymphovascular invasion (0.015) [Table/Fig-8].

T stage	Prognostic score			p-value
	H (%)	I (%)	L (%)	
T1	6 (40.0)	3 (20.0)	6 (40.0)	0.027
T2	9 (31.0)	5 (17.2)	15 (51.7)	
T3	11 (25.6)	12 (27.9)	20 (46.5)	
T4	7 (38.9)	10 (55.6)	1 (5.6)	
N stage	**Prognostic score**			**p-value**
	H (%)	I (%)	L (%)	
N0	10 (25.0)	7 (17.5)	23 (57.5)	0.035
N1	15 (31.3)	17 (35.4)	16 (33.3)	
N2	8 (47.0)	6 (35.3)	3 (17.6)	
LVI	**Prognostic score**			**p-value**
	H (%)	I (%)	L (%)	
Absent	12 (37.5)	3 (9.4)	17 (53.1)	0.015
Present	21 (28.8)	27 (37.0)	25 (34.2)	

[Table/Fig-8]: Association between prognostic score and pT, pN and LVI.

DISCUSSION

Cancer is not a single disease. It is a heterogenous disease which involves complex interplay between the tumour and TME. Due to intertumoural heterogeneity CRC differs on various levels resulting in differences in prognosis and therapeutic response even for patients

with the same stage and grade. So there is a need for a robust classification system for CRC which includes both molecular and histopathological parameters [2].

Tumour budding has been a hot topic in cancer research for many years. In the recent (June 2022) CAP (College of American Pathologist) protocol for reporting primary carcinomas of colon and rectum recommends reporting of tumour budding in stage I and II cases and for cancers arising from polyp, but it's not considered as a required element. In a study conducted by Hase K et al., in 1993 they found out that more severe budding in CRC is associated with worst outcome and also suggested meticulous follow up and possibly neoadjuvant chemotherapy for such patients irrespective of the stage [24]. Sadek SA et al., in 2020 assessed TB in H&E and cytokeratin (CK) stained sections and found it is significantly associated with adverse prognostic variables including vascular invasion, lymph node metastasis, advanced Dukes and TNM stages and inversely associated with TIL which is known to be a good prognostic indicator [25]. The present study also found that high budding is associated with high pT, pN and more chance for LVI.

Cancer immunoediting is a dynamic process that consists of immunosurveillance and tumour progression. TIL is an important factor in cancer immunoediting and has not only prognostic significance but also emerging as an important biomarker in predicting the efficacy and treatment outcome [26]. In the study conducted by Lang-Schwarz C et al., in 2019, TILs ≤5% versus >5% showed significant advantages for the higher TILs group concerning the parameters pT stage , pN stage, M stage, TNM stage, lymphatic vessel invasion and also venous invasion [8]. In 2020 Fuchs TL et al., studied the prognostic significance of TIL in 1034 CRC patients and found out that TIL is a powerful predictor of survival in CRC [27]. In the present study also TIL differed significantly with pT, pN and LVI.

There are a very few studies which combines different aspects of tumour and TME to create a prognostically significant grading system in CRC. In 2009, Lugli A et al., proposed a CD8+ lymphocytes/ tumour budding index which they found to have prognostic significance in CRC [28].

Lang-Schwarz C, et al., in 2018 studied 501CRC cases and found Budding/TIL –score correlates with most clinicopathological parameters [8]. In 2019 the same group of pathologist combined budding, TIL, and gland formation in low grade CRCs and proposed Bayreuth score that enables separating the large group of WHO low-grade CRC cases into subgroups, which differ significantly in outcome and survival [23]. This study was first of its kind that integrates budding and TILs with traditional grading parameter-gland formation.

Combining different histomorphological parameters along with molecular markers for therapeutic stratification is nothing new in tumour diagnostics. We have been using Ellston-Ellis grading system which combines three different histomorphological parameters along with molecular markers for patient stratification in breast cancer [29].

Limitation(s)

Impact of prognostic scoring system in long term survival is not analysed in this study. Molecular biomarkers were not used in this study.

CONCLUSION(S)

In our study we found out that budding and TIL are independent prognostic factors in CRC and combining these parameters along with the well established prognostic factor tumour grade could be a clue to better understanding of tumour behaviour. Assessing budding and TIL is a simple, cost effective, time saving and reproducible method for routine practice. Even in the age of molecular pathology it is still worthy to pay attention to H&E based tumour morphology. For better disease stratification, in addition to TNM staging and grading, additional histomorphological factors like budding and TIL along with Microsatellite Instability Analysis (MSI), KRAS, BRAF and NRAS mutation analysis are recommended in CRC. More studies are required to analyse whether a prognostic scoring system combining different histopathological parameters will be effective in CRC which will lead to more unified and simplified reporting system in CRC.

Acknowledgement

We are extremely thankful to Dr Jane Mary, Soumya Shinu, Menshi Joseph, Jisha K.

REFERENCES

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A cancer journal for clinicians. 2021;71(3):209-49.
[2] Sagaert X, Vanstapel A, Verbeek S. Tumor heterogeneity in colorectal cancer: What do we know so far?. Pathobiology. 2018;85(1-2):72-84.
[3] Brierly JD GM, Wittekind C. Tnm classification of malignant tumors. 8th ed. Oxford, UK: Wiley Blackwell, 2017.
[4] Hamilton SR, Bosman FT, Boffetta P et al. Carcinoma of the colon and rectum. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumours of the digestive system. International Agency for Research on Cancer, Lyon, 4th edition. 2010;134-46.
[5] Compton CC, Tanabe KK, Savarese DMF. Pathology and prognostic determinants of colorectal cancer. 2012.
[6] Pages F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, et al. International validation of the consensus immunoscore for the classification of colon cancer: A prognostic and accuracy study. Lancet. 2018;391(10135):2128-39.
[7] Dawson H, Christe L, Eichmann M, Reinhard S, Zlobec I, Blank A, et al. Tumour budding/T cell infiltrates in colorectal cancer: proposal of a novel combined score. Histopathology. 2020;76(4):572-80. doi: 10.1111/his.14006. Epub 2020 Feb 3. PMID: 31560788.
[8] Lang-Schwarz C, Melcher B, Haumaier F, Lang-Schwarz K, Rupprecht T, Vieth M, et al. Budding and tumor-infiltrating lymphocytes - combination of both parameters predicts survival in colorectal cancer and leads to new prognostic subgroups. Hum Pathol. 2018;79:160-67. Doi: 10.1016/j.humpath.2018.05.010.
[9] Imai T. The growth of human carcinoma: a morphological analysis. Fukuoka Igaku Zasshi. 1954;45:13-43.
[10] Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol. 2017;30(9):1299-11.
[11] Lugli A, Karamitopoulou E, Zlobec I. Tumour budding: A promising parameter in colorectal cancer. Br J Cancer. 2012;106(11):1713-17.
[12] Van Wyk HC, Park J, Roxburgh C, Horgan P, Foulis A, McMillan DC, et al. The role of tumour budding in predicting survival in patients with primary operable colorectal cancer: A systematic review. Cancer Treat Rev. 2015;41(2):151-59.
[13] Rogers AC, Winter DC, Heeney A , Gibbons D, Lugli A, Puppa G, et al. Systematic review and meta-analysis of the impact of tumour budding in colorectal cancer. Br J Cancer.2016;115(7);831-40.
[14] Ueno H, Murphy J, Jass JR, Mochizuki H, Talbot IC. Tumour budding as an index to estimate the potential of aggressiveness in rectal cancer. Histopathology. 2002;40(2):127-32.
[15] Ueno H, Mochizuki H, Hashiguchi Y, Shimazaki H, Aida S, Hase K, et al. Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterology. 2004;127(2):385-94.
[16] Kazama S, Watanabe T, Ajioka Y, Kanazawa T, Nagawa H. Tumour budding at the deepest invasive margin correlates with lymph node metastasis in submucosal colorectal cancer detected by anticytokeratin antibody CAM5.2. Br J Cancer. 2006;94(2):293-98.
[17] Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Ebrahimi-Kalan A, Jaymand M, et al. Tumor microenvironment complexity and therapeutic implications at a glance. Cell Communication and Signaling. 2020;18(1):01-09.
[18] De Smedt L, Lemahieu J, Palmans S, Govaere O, Tousseyn T, Van Cutsem E, et al. Microsatellite instable vs stable colon carcinomas: Analysis of tumour heterogeneity, inflammation and angiogenesis. Br J Cancer. 2015;113(3);500509.
[19] Roxburgh CS, McMillan DC. The role of the in situ local inflammatory response in predicting recurrence and survival in patients with primary operable colorectal cancer. Cancer Treat Rev. 2012;38(5):451-66.
[20] Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: Recommendations by an International TILs Working Group. 2015.
[21] Hamilton SR, Vogelstein B, Kudo S. In: Hamilton SR, Aaltonen LA, editors. Carcinoma of the Colon and Rectum. Lyon: International Agency for Research on Cancer. 2000;110-11.
[22] WHO Classification of Tumours Editorial Board. WHO Classification of Tumors: Digestive System Tumours. 5th ed. Lyon, France: International Agency for Research on Cancer. 2019.

[23] Lang-Schwarz C, Melcher B, Haumaier F, Schneider-Fuchs A, Lang-Schwarz K, Krugmann J, et al. Budding, tumor-infiltrating lymphocytes, gland formation: Scoring leads to new prognostic groups in World Health Organization low-grade colorectal cancer with impact on survival. Hum Pathol. 2019;89:81-89. Doi: 10.1016/j.humpath.2019.04.006.
[24] Hase K. Shatney C, Johnson D, Trollope H, Vierra M. Prognostic value of tumor "budding" in patients with colorectal cancer. Dis Colon Rectum. 1993;36:627-35.
[25] Sadek SA, Rehim DM, Fatima S. The role of tumor budding in colorectal adenocarcinoma: Possible involvement of the intestinal cancer stem cell marker Lgr5. Indian Journal of Pathology and Microbiology. 2020;63(1):32.
[26] Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Ebrahimi-Kalan A, Jaymand M, et al. Tumor microenvironment complexity and therapeutic implications at a glance. Cell Communication and Signaling. 2020;18(1):01-09.
[27] Fuchs TL, Sioson L, Sheen A, Jafari-Nejad K, Renaud CJ, Andrici J, et al. Assessment of tumor-infiltrating lymphocytes using International TILs Working Group (ITWG) system is a strong predictor of overall survival in colorectal carcinoma: A study of 1034 patients. The American Journal of Surgical Pathology. 2020;44(4):536-44.
[28] Lugli A, Karamitopoulou E, Panayiotides I, Karakitsos P, Rallis G, Peros G, et al. CD8+ lymphocytes/tumour-budding index: An independent prognostic factor representing a 'pro-/anti-tumour'approach to tumour host interaction in colorectal cancer. British Journal of Cancer. 2009;101(8):1382-92.
[29] Elston CW, Ellis IO. experience from a large study with long-term followup. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer. Histopathology. 1991;19(5):403-10.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, MES Medical College, Malapuram, Kerala, India.
2. Assistant Professor, Department of Pathology, Malabar Medical College and Research Center, Kozhikode, Kerala, India.
3. Professor, Department of Pathology, MES Medical College, Malapuram, Kerala, India.
4. Associate Professor, Department of Pathology, MES Medical College, Malapuram, Kerala, India.
5. Senior Resident, Department of Pathology, MES Medical College, Malapuram, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Nisha Thattamparambil Gopalakrishnan,
Associate Professor, Department of Pathology, MES Medical College,
Malapuram, Kerala, India.
E-mail: nishatg33@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Oct 15, 2022
- Manual Googling: Nov 22, 2022
- iThenticate Software: Dec 08, 2022 (22%)

ETYMOLOGY: Author Origin

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: **Oct 13, 2022**
Date of Peer Review: **Nov 22, 2022**
Date of Acceptance: **Dec 19, 2022**
Date of Publishing: **Apr 01, 2023**