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Original Article

POORLY DIFFERENTIATED CLUSTERS IN COLORECTAL CARCINOMA: ASSOCIATION WITH OTHER HISTOPATHOLOGICAL PROGNOSTIC PARAMETERS INCLUDING TUMOUR BUDDING

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ABSTRACT

Objective: In India, colorectal carcinoma (CRC) ranks third in terms of cancer incidence. Pathologists are essential when it comes to determining the stage, examining surgical margins, and recording the histopathologic prognostic factors. A new prognostic grading system is proposed named poorly differentiated clusters (PDCs), which are defined by neoplastic clusters of 5 cells lacking glandular structure in the invasive front of the stroma. It is significant for cancer-specific and recurrence-free survival in CRC patients, reflecting the biological aggressiveness of the tumor. Aim of the study was to analyse of PDCs in colorectal carcinomas and association with other histopathological prognostic factors.

Methods: The Hematoxylin and Eosin (HandE)-stained slides of 76 histopathologically diagnosed CRC resection specimens were reviewed. Poorly differentiated clusters (PDC) were assessed into under 200x power. The correlation of PDC with other histopathological prognostic parameters like tumor size, site, grade, laterality, lymphovascular invasion, perineural invasion, T stage, N stage, and tumor budding was analyzed using descriptive statistics and the Chi-square test with SPSS version 26.0.

Results: There was no significant association between PDC and age, tumor location, tumor size, histological grade, LVI, PNI, or lymphnode status. Where a significant association was noted between the sex and PDC grade (P value = 0.035), T stage and PDC grade (P value = 0.045), and N stage and PDC grade is significant (P value = 0.001).

Conclusion: PDCs may be considered, along with other clinical-pathological parameters, a promising prognostic factor for the management of patients with CRC and should be included in pathological reports, but it still needs standardization and further validation. At the same time, tumor budding can become an irreplaceable histological indicator for identifying a high malignant potential carcinoma and poor prognosis in CRCS, thus indicating the need for aggressive postoperative management to improve the prognosis of the patient. PDC is important for the survival of CRC patients, indicating the aggressiveness of the tumor both in terms of cancer-specific survival and freedom from recurrence. PDC may help to identify patients who need a more intensive postoperative follow-up and the possibility of adjuvant therapy.

Keywords: Colorectal carcinoma, Outcome, Poorly differentiated clusters, Prognosis

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INTRODUCTION

Colorectal cancer (CRC) is the third most dangerous analyzed cancer in the world, as per 2018 information from GLOBOCAN. CRC arises when certain cells of the epithelium acquire a series of genetic and/or epigenetic events [1]. Currently, the pathological tumor node metastasis (pTNM) stage is considered the most relevant prognostic factor in CRC because of its strong, significant correlation with the patient's survival, thus aiding in making therapeutic decisions [2].

Another approach that accounts for the variability in survival of CRC patients is histopathologic tumor grading [3]. The World Health Organization (WHO) grades colorectal cancer into three grades based on the presence of a percentage of glandular structures forming the tumor on histological examination (Grade 1) (G1)>95%, grade 2 (G2)>= 50-95%, and grade 3 (G3)<50% gland formation) [4]. But, this grading system does not help in terms of stratification of patients by prognostic outcome [3]. However, Ueno H *et al.* (2012) proposed a new histological grading of CRC, which classifies CRC on the basis of the number of poorly differentiated clusters (PDC) of tumor cells identified in the tumorstroma or at its invasive front [5]. PDC are neoplastic clusters composed of five or more tumor cells; they lack a gland-like structure in the tumor border or stroma [6]. PDC grading has a significant prognostic factor for cancer-specific survival (CSS) and recurrence-free survival (RFS) in CRC patients and mostly reflects the biological aggressiveness of the tumor when compared to pTNM staging [3, 5].

This study is done to assess the expression of PDC in our study population and its association with other histopathological prognostic parameters like tumor size, site, grade, laterality, lymphovascular invasion, perineural invasion, T stage, N stage, and tumor budding.

MATERIALS AND METHODS

This is a cross-sectional study that included 76 resection cases of CRC from the pathology department of Hitech Medical College from January 2021 to December 2023. The clinicopathological data were archived from patient hospital records. The slides of only resection cases have been included. Ethical approval was obtained from the institutional review board.

Histopathological evaluation

The hematoxylin and eosin-stained sections were evaluated for histological type. Tumor grade was determined based on gland formation. Lymphovascular invasion, perineural invasion, and tumor budding (low grade, high grade, absent) were assessed. Pathologic staging of the tumor was performed according to the TNM system.

Microscopic assessment of PDC

PDC assessment in surgical resection specimens was done by examining the tumor-containing slides at a lower magnification (20x objective field), including both the center and the invasive front. The number of PDC was graded on the basis of the number of PDC clusters counted: G1:<5 PDC, G2: 5-9 PDC, and G3 ≥10 PDC. Further poorly differentiated clusters were calculated to assess the relationship to the stage and other histopathological factors.

Statistical analysis

Data were collected, tabulated, and statistically analyzed. The association between PDC grade and other categorical variables is studied using the Chi-Square test. The data analysis is carried out using SPSS (Version 26).

RESULTS

This study was conducted on 76 cases of CRC and includes only resection specimens. The study showed male preponderance in 50 (65.8%) cases with equal distribution in ages over 60 y and less than 60 y (50% each). 40 cases (52.6%) were located in the colon, with 30 cases (39.5**%**) located in the rectum, and 6 cases (7.9%%) were found in the recto-sigmoid region. In 52 cases (68.4%), tumors were of size more than 5 cm, and in 24 cases (31.6%), tumours were of size less than 5 cm. All cases were diagnosed as adenocarcinoma by histology. Grade I tumor was seen in 32 cases (42.1%), grade II tumor was seen in 40 cases (52.6%), and grade III tumor was seen in 4 (5.3%) cases. Lymphovascular invasion was noted in 30 cases (39.5%), with a slightly lower number (6 cases, 7.9%) depicting perineural invasion and nodal metastasis in 42 cases (55.3%). In T stage, 40 cases (52.6%) were of T3 stage, followed by T1 stage 2 cases (2.6%), T2 stage in 18 cases (23.7%), and T4 stage in 16 cases (21.1%) each. In 34 cases (44.7%) lymph nodes were involved. 36 cases (47.4%) were showing tumor budding, while 40 cases (52.6%) were not showing tumor budding.

PDC was not observed in 16 cases (21.1%), while we calculated PDCs in all these cases and found that 79% (60) cases belong in grade 1, followed by 10.5% (8) cases each in grade 2 and grade 3.

There was no significant association between PDC with age, tumor location, tumor size, histological grade, LVI, PNI, or lymphnode status, where a significant association was noted between the sex and PDC grade (P = 0.035), T stage and PDC grade (P = 0.045), and N stage and PDC grade is significant (P = 0.001).

There was no significant association between tumor budding and sex, tumor size, histological grade, LVI, PNI, T stage, or lymphnode status. However, a significant association was noted between age and tumor budding (P value = 0.006), tumor location and tumor budding (P value = 0.025), and histologic grade and tumor budding (P value = 0.095).

DISCUSSION

Poorly differentiated clusters (PDC) are formed by five neoplastic cells with no glandular formation. They are present in both the tumor stroma and the tumor-invasive front [5]. Tumor-budding (TB) foci are made of less than five cancer cells. As PDC are composed of a higher number of cells, that makes them easily identifiable under hematoxylin and eosin stain (HandE) [5]. Some hypotheses suggest that due to the similarity in morphology of PDC and tumor budding, they could represent sequential steps in tumor growth, as there is evidence regarding the presence of both PDC and tumor budding in the same tumor mass. So, there is a possibility of sequential transformation of the tumor budding into the PDC [6]. As suggested by Ueno H *et al.*, PDC assessment includes examination of the entire tumor in all the tumor-containing slides at a lower power magnification (20 x objective fields), including both the center and the invasive front [5]. The number of PDC in the hotspot is then graded on the basis of the number of PDC clusters counted: G1:<5 PDC, G2: 5-9 PDC, and G3: 10 PDC. This method provides better risk stratification in stage II and III CRC compared to other grading systems [5-7]. PDC grading of CRC can also be applied to endoscopic biopsies. But the probability to sample an area with a high number of PDCs is decreased in endoscopic biopsies as most of the PDCs are found at the invasive edge. Due to this reason, a different PDC grading scale was created for endoscopic biopsies in CRC patients: G1: 0, G2: 1-2, and G3: ≥ 3 (in 20x field) [8, 9].

A total of 76 cases were included in our study. In the resection specimens, tumors were located in the colon, rectum, and recto-sigmoid. The predominant histologic type found was adenocarcinoma [52 (66.7%)], followed by mucinous adenocarcinoma [24 (29.6%)] and signet ring cell carcinoma [2]. 42.1% (32) cases were histologic grade I, followed by grade 2 (40, 52.6%) and grade 3 (5.3%).

We calculated PDCs in all these cases and found that 79% (60) cases belong in grade 1, followed by 10.5% (8) cases each in grade 2 and grade 3. Simultaneously, we tried to correlate the age and PDCs and found no significant correlation (p value>0.9). Although we observed male preponderance in our study, the maximum number of cases seemed to be grade 1 for both sexes. There was a statistically significant association between the sex and PDC grade (P value = 0.035). On correlating tumor location with the poorly differentiated clusters, we found 100%(6) rectosigmoidal tumors, 80% rectal tumors, and 75% colon tumors were observed to have Grade 1 PDC, although no statistical significance was observed in these two parameters. Irrespective of tumor size, the majority of colorectal carcinomas are high-grade in PDCs. Association between histological grade and PDC grade showed 100% of histological grade 3 colorectal carcinomas were found to have grade 1 in PDCs, although no statistical significance was observed in these two parameters.

In the 34 cases showing lymphovascular invasion (LVI), 86.7% (26 cases) were found to have grade 1 PDC; however, no statistical significance was observed in these two parameters. All cases with perineural invasion were Grade 1 PDCs. Here also no statistical significance was observed between PDC and PNI.

Table 1: Correlation between T stage and PDC

T stage	Grade 1PDC	Grade 2 PDC	Grade 3 PDC
T1	2	-	-
T2	14	-	-
T3	28	8	4
T4	16	-	-

On associating TNM stage T with grade of PDCs, our study showed 100% of cases of T1, T2, and T4 were given grade 1 in PDCs; however, T showed a variable distribution with 70% (28) cases in grade 1, 20% (8) cases in grade 2, and 10% (4) cases in grade 3. This association was found to be statistically significant (p value<0.04).

Table 2: Correlation between N stage and PDC

N stage	Grade 1 PDC	Grade 2 PDC	Grade 3 PDC
N0	30	6	-
N1	20	2	2
N2	8	-	2
Nx	2	-	4

Similarly, when we associated stage N of the TNM classification, we found a statistically significant correlation (p value<0.01). Out of the 70 cases assessed for nodal status>80%, cases were classified as grade 1 PDCs. These results encourage the presence and number of PDC as possible tools in risk assessment pertaining to nodal involvement.

Our results show that PDCs presented more important correlations with other clinicopathological parameters, with evidence that the PDCs represent an independent prognostic indicator in CRC patients, associated with other unfavorable parameters such as advanced stages of the disease, lymph node metastasis, and male sex.

Table 3: Correlation between tumour budding and age

Tumour budding	Present	Absent
<60 y	26	12
>60 y	14	24

Tumor budding was found more in the younger age group of this study (<60 y). Simultaneously, we evaluated the significance of tumor budding with age and found it to be statistically significant (p value>0.006). No significant correlation was found between sex and tumor budding (p value<o.4).

Out of the 36 cases with tumor budding, the maximum was found in the rectum (55%), followed by the colon (38.8%), and then recto-sigmoidal. This showed a statistically significant correlation (p value<0.02) between tumor budding and tumor location. No similar association was found between tumor size and tumor budding. Histological grade showed a significant association with tumor budding in our study, and tumor budding increased with an increase in the histological grade of the tumor. No similar correlation was found with lymphovascular invasion, perineural invasion, or lymph node status of the patient.

CONCLUSION

PDCs may be considered, along with other clinical-pathological parameters, a promising prognostic factor for the management of patients with CRC and should be included in pathological reports, but it still needs standardization and further validation. At the same time, tumor budding can become an irreplaceable histological indicator for identifying a high malignant potential carcinoma and poor prognosis in CRCS, thus indicating the need for aggressive postoperative management to improve the prognosis of the patient. PDC is important for the survival of CRC patients, indicating the aggressiveness of the tumor both in terms of cancer-specific survival and freedom from recurrence. PDC may help to identify patients who need more intensive postoperative follow-up and the possibility of adjuvant therapy.

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LIMITATIONS OF STUDY

Our study is of 2 y duration with a smaller sample size. If sample size had been larger, a better correlation would have been achieved. Also, correlation between tumour budding and PDC should be analysed and a longer follow up would provide a better picture.

AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Ewing I, Hurley JJ, Josephides E, Millar A. The molecular genetics of colorectal cancer. Frontline Gastroenterol. 2014;5(1):26-30. doi: 10.1136/flgastro-2013-100329, PMID 24416503.
2. Barresi V, Reggiani Bonetti L, Ieni A, Caruso RA, Tuccari G. Poorly differentiated clusters: clinical impact in colorectal cancer. Clin Colorectal Cancer. 2017 Mar 1;16(1):9-15. doi: 10.1016/j.clcc.2016.06.002, PMID 27444718.
3. Ueno H, Hase K, Hashiguchi Y, Shimazaki H, Tanaka M, Miyake O. Site specific tumor grading system in colorectal cancer: multicenter pathologic review of the value of quantifying poorly differentiated clusters. Am J Surg Pathol. 2014 Feb 1;38(2):197-204. doi: 10.1097/PAS.0000000000000113, PMID 24418853.
4. Bertoni L, Barresi V, Bonetti LR, Caramaschi S, Mangogna A, Lionti S. Poorly differentiated clusters (PDC) in colorectal cancer: does their localization in tumor matter? Ann Diagn Pathol. 2019 Aug 1;41:106-11. doi: 10.1016/j.anndiagpath.2019.06.008, PMID 31233902.
5. Ueno H, Kajiwara Y, Shimazaki H, Shinto E, Hashiguchi Y, Nakanishi K. New criteria for histologic grading of colorectal cancer. Am J Surg Pathol. 2012 Feb 1;36(2):193-201. doi: 10.1097/PAS.0b013e318235edee, PMID 22251938.
6. Reggiani Bonetti L, Barresi V, Bettelli S, Domati F, Palmiere C. Poorly differentiated clusters (PDC) in colorectal cancer: what is and ought to be known. Diagn Pathol. 2016 Dec 1;11(1):31. doi: 10.1186/s13000-016-0481-7, PMID 27004798.
7. Ueno H, Mochizuki H, Hashiguchi Y, Ishiguro M, Kajiwara Y, Sato T. Histological grading of colorectal cancer: a simple and objective method. Ann Surg. 2008 May 1;247(5):811-8. doi: 10.1097/SLA.0b013e318167580f, PMID 18438118.
8. Barresi V, Bonetti LR, Ieni A, Branca G, Baron L, Tuccari G. Histologic grading based on counting poorly differentiated clusters in preoperative biopsy predicts nodal involvement and pTNM stage in colorectal cancer patients. Hum Pathol. 2014 Feb 1;45(2):268-75. doi: 10.1016/j.humpath.2013.07.046, PMID 24289972.
9. Barresi V, Tuccari G. Colorectal carcinoma grading quantified by counting poorly differentiated clusters: is it feasible on endoscopic biopsies? Am J Surg Pathol. 2013 Jun;37(6):943-5. doi: 10.1097/PAS.0b013e31828a69e7, PMID 23629448.