RESEARCH ARTICLE

Histopathological study of tumor budding in colorectal carcinoma and its correlation with clinicopathological parameters

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ABSTRACT

Background: Colorectal cancer (CRC) is the third most common cancer worldwide. “Tumor budding,” defined by the presence of five or less tumor cell cluster in the invasive front of tumor, is a strong, reproducible, and independent prognostic marker of the biological aggressiveness of the tumor. Aim and Objective: The present study was conducted to assess the correlation of clinicopathological parameter with tumor budding in CRC. Materials and Methods: Sixty patients presenting with colectomy specimens with known histological diagnosis of colorectal adenocarcinoma were included in the study. Histological examination with hematoxyylene and eosin stain and immunohistochemistry with pancytokeratin (Pan-CK) was performed in equivocal cases. Tumor budding was counted and scored as per international tumor budding consensus conference, 2016, recommended criteria. Tumor budding was correlated with other relevant clinicopathological parameters. Results: The age distribution ranged from 19 to 78 years with a peak incidence in the age group of 41–50 years (31.7%). Low-grade tumor budding was seen in 20%, intermediate grade budding in 16.7%, and high-grade tumor budding in 63.3%. No correlation could be established between age, sex, site, size of tumor, lymphovascular invasion, histological grade, and budding intensity. However, association between tumor budding and nodal involvement, perineural invasion and higher American Joint Committee on Cancer stage has been found to be statistically significant in this study. Conclusions: Tumor budding is emerging to be a promising and powerful predictor of nodal metastasis and a higher stage of the tumor. Immunohistochemistry with Pan-CK can aid in the grading of tumor budding and build consensus.

KEY WORDS: Colorectal Carcinoma; Immunohistochemistry; International Tumor Budding Consensus Conference Criteria; Pancytokeratin; Tumor, Node, Metastasis Staging; Tumor Budding

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and one of the leading cause of cancer related deaths in the Western world, causing 655,000 deaths worldwide per year.[¹] In our country, the average annual incidence rate in men and women is 7.7 and 5.1 per million population, respectively, with the occurrence of 36,917 male and 27,415 female cases.[²] In a recent study from Chhattisgarh, CRC was rated third most common cancer among outpatient department attendance (10.6%) ranking right after oral cancers and breast.[³] The most important prognostic factor of CRC according to the “International Union against Cancer” is tumor, node, metastasis (TNM) staging, whereas lymphovascular invasion (LVI), tumor grade, perineural invasion (PNI), tumor budding, and tumor...
margin status are additional contributing factors. “Tumor budding,” defined by the presence of five or less tumor cell forming a cluster/chord that extends from the neoplastic glands into the adjacent stroma ahead of the invasive front is a strong, reproducible, and independent prognostic marker and represents the biological aggressiveness of the tumor.

About 20–40% of CRCs demonstrate this feature which is strongly correlated to local and distant metastases.

In cases of endoscopic biopsies also, it can be used as a criterion to identify patients with early-stage tumor requiring mucosal resection and thus helps deciding the management options. Some studies suggest that Stage II patients with tumor budding experience significantly worse outcomes, and adjuvant chemotherapy should be considered in these patients. The choice for neoadjuvant chemotherapy is also being considered in the high-risk patients who show moderate to high degree of tumor budding in mucosal biopsies. The present study was conducted to assess the correlation of tumor budding in CRC with its clinicopathological parameters.

MATERIALS AND METHODS

This institution based cross-sectional observational study was done over a period of 1 year to find any correlation between tumor budding density at the invasive front of tumor and clinicopathological parameters. After obtaining permission from Institutional Ethical Committee, 60 patients presenting to department of pathology or surgical oncology for colectomy with histological diagnosis of colorectal adenocarcinomas were included in the study. Secondary malignancies, melanomas, and squamous cell carcinomas were excluded from the study. Informed consent was taken along with the previous laboratory and colonoscopic findings were documented. Gross characteristics (size, distance from nearest margin, serosal involvement, and lymph node number) were documented and histological examinations were carried out. Tumor subtype, tumor characteristics, that is, size, morphology, the presence of tumor infiltrating immune cells, tumor grade, the presence of necrosis, LVI, nodal status, and margin status were documented. Immunohistochemistry with pan cytokeratin (Pan-CK) (Clone AE1/AE3, monoclonal, host-mouse, and Dako-agilent) was done only in equivocal cases following standard protocol (poly L lysine coating, alkaline TRIS-EDTA buffer mediated moist pressure cooker based antigen retrieval, and polymer detection kit mediated DAB chromogen based technique) where tumor budding is obscured by inflammation, tumor necrosis, and mucus. Tumor budding was reported according to the microscope

1. The field area was defined for the 20x objective (low power) lens of microscope based on the eyepiece field number diameter from Table 1.
2. The H&E slide with greatest degree of budding at the invasive front was selected
3. Ten scanner field (4x/10x objective) was observed to identify the “hotspot” at the invasive front
4. Tumor buds in the selected “hotspot” were counted in the low power
5. The bud count was divided by the normalization factor from Table 1 to determine the tumor bud count per 0.785 mm²
6. Based on a scoring system bud count was divided into:
   - Bd1 (low) - (0–4 buds)
   - Bd2 (intermediate) - (5–9 buds)
   - Bd3 (high) - (10 or more buds).

Data were tabulated and analyzed using SPSS version 20 software. P < 0.05 was considered significant.

RESULTS

Age distribution of cases ranged from 19 to 78 years with a peak in the age group of 41–50 years (31.7%); 70% of them being male.

The most frequent site of involvement was the right side of colon (46.7%) (i.e., cecum and ascending colon) followed by the left colon (23.3%). Rectum is involved in 16.7% cases; rectosigmoid colon is involved in 6.7% cases, transverse colon in 3.3% cases followed by cecum and ileocecum (1.7%). Well differentiated tumor grade (G1) was observed in 60% patients whereas around 38.3% patients showed moderately differentiated tumor (G2). Only 1.7% tumors

<table>
<thead>
<tr>
<th>Eyepiece FN diameter (mm)</th>
<th>Specimen area (mm²)</th>
<th>Normalization factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>0.636</td>
<td>0.810</td>
</tr>
<tr>
<td>19</td>
<td>0.709</td>
<td>0.903</td>
</tr>
<tr>
<td>20</td>
<td>0.785</td>
<td>1.000</td>
</tr>
<tr>
<td>21</td>
<td>0.866</td>
<td>1.103</td>
</tr>
<tr>
<td>22</td>
<td>0.950</td>
<td>1.210</td>
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<tr>
<td>23</td>
<td>1.039</td>
<td>1.323</td>
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<tr>
<td>24</td>
<td>1.131</td>
<td>1.440</td>
</tr>
<tr>
<td>25</td>
<td>1.227</td>
<td>1.563</td>
</tr>
<tr>
<td>26</td>
<td>1.327</td>
<td>1.690</td>
</tr>
</tbody>
</table>

*Reproduced from ITBCC criteria 2000
were poorly differentiated (G3). The maximum number of cases were in PTN₃M₀ (26.7%)/Stage IIA American Joint Committee on Cancer (AJCC). Overall, Stage III tumor was reported in 48.3% patients, Stage II tumor seen in 46.7% patients, Stage I tumor seen in only 3.3% cases, and Stage IV tumor reported in 1.7% patients. LVI was present in 51.7% cases. PNI reported in 33.3% patients. Metastasis is reported in 3 (5%) patients. Local recurrences were reported in 4 (6.7%) patients. The number of patients who died within the study period was 3 (5%). About 23.6% cases had 1–3 lymph nodal involvement whereas 10% had 4–6 lymph nodes.

Low-grade tumor budding (0–4 buds/20× fields) was seen in 12 patients (20%). Intermediate grade budding (5–9 buds/20× fields) was reported in ten patients (16.7%). High-grade tumor budding (≥10/20× fields) was seen in 38 patients (63.3%) [Figure 1].

While correlating with different variable with tumor budding [Table 2], we found no statistically significant correlation between tumor budding and age of the patient (P = 0.423) (the low bud count compared with the intermediate and high bud count). No statistical significant association was found between sex of the patient and tumor budding (P = 0.720). When correlating site of tumor with budding, we found no statistically significant correlation (P = 0.521). No significant correlation was found between budding and size of invasive tumor; P = 0.310. Histological grade of tumor (well differentiated/moderately differentiated/poorly differentiated) when correlated with grade of budding (low/intermediate/high) P = 0.265; which means no statistically significant correlation. Nodal involvement when correlated with tumor budding; high and intermediate grade budding seen in 65.79% and 60% cases of node involved cases. Which means intermediate and high-grade budding has significantly higher proportion of lymph node positive cases and there is statistically significant correlation (P = 0.001). LVI reported in 51.67% cases, among them high-grade budding seen in 60.53% cases and intermediate grade budding in 60.33% cases. When correlated we got P = 0.108; which means there is no statistically significant correlation between LVI and tumor budding. PNI reported in 33.33% cases and in 66.67% patients; there is no PNI. In PNI positive cases high-grade budding seen in 44.74%; intermediate budding seen in 20% cases. Among the PNI negative cases, high budding seen in 55.26%; intermediate budding in 80% cases. When correlated, it was found to be statistically significant (P = 0.049). AJCC stage when correlated with grade of budding it was found that Stage II tumors reported in 48.33% cases and Stage III tumors in 46.67% cases. About 60.53% of Stage III tumors showed high-grade budding and 40% showed intermediate grade budding. Whereas 36.48% of Stage II tumors showed high grade budding and 60% showed intermediate grade budding. There is statistically significant correlation between AJCC stage and high-grade budding (P = 0.004), that means high-grade budding has significantly higher stage.

**DISCUSSION**

In the present study, tumor budding was correlated with different clinicopathological parameters such as tumor site, tumor size, histologic type, histologic grade, nodal involvement, and AJCC stage. No correlation could be established between age, sex, site, size of tumor, LVI, histological grade, and budding intensity. However, association between tumor budding and nodal involvement, PNI and higher AJCC stage has been found to be statistically significant in this study. We also found that Pan-Ck; immunostaining is a useful adjunct to tumor bud counting in routine H&E and can provide consensus among observers.

Tumor budding is an emerging prognostic marker in addition to conventional TNM stage, lymphovascular embolization, indeterminate margin, and microsatellite instability.[10] Tumor buds are identifiable on H&E staining but their appreciation can be enhanced by immunohistochemistry, a Pan-Ck staining is a useful aid and eases the experience of identifying and counting tumor budding as described by Kai et al.[10] who proposed that immunohistochemistry by Pan-Ck may improve the interobserver variability in the evaluation of tumor budding. They observed it to be more evident in T1 CRC patients. Based on our experience, cytookeratin staining definitely improves the assessment of tumor budding and is recommended as an ancillary technique.
There is much controversy regarding where the buds should be counted and how many buds are considered significant. Ueno method is considered most standardized because it is practical, reproducible, has the well-standardized field size of 0.785 mm$^2$ and provides numerical scores and a three-tier system.\[6\]

In our study, 38 (63.3%) cases exhibited high-grade budding by implementing the hotspot counting method. This is in concordance with a study by El-Gendi and Al-Gendi who also used the hotspot method of bud count and recorded high-grade budding.

Conversely, only 10 (16.7%) cases showed intermediate-grade budding (5–9 buds), which is concordant with a study by Morodomi et al.\[18\] However, Tanaka et al. found significantly high number of intermediate grade as they did not follow the ITBCC protocol and depended on subjective assessment.\[19\]

Moreover, the use of cytokeratin staining helped us define the budding more effectively. No correlation was found between histological grade and budding intensity in our study and similar results have been reported by Morodomi et al., Ohtsuki et al., 2008; Wang et al., 2009; El-Gendi, and Al-Gendi.\[17-21\] However, study by Sevda et al. showed positive correlation between higher histologic grade and higher budding intensity, which is contrary to our findings. Zhang et al. have found positive correlation of with the high recurrence rate, lymph node metastasis, chemoresistance, and overall poor prognosis of CRC with the high tumor bud.

This required longer follow-up and preparation of survival curves which are outside our scope of study.\[22\]

When the tumor infiltration (pT) was correlated with tumor budding, it was found that 20% of intermediate grade tumors were pT$^3$ tumors whereas, 23.68% of high-grade tumors were of pT$^3$. El-Gendi and Al-Gendi and Sevda et al. both showed that the majority of the tumors were of pT$^3$ and there was no definite correlation with the intensity of budding and T stage of the tumor.\[17,23\]

Although there is no statistical correlation between T$^3$N$^0$M$^0$ tumors and grade of budding, Petrelli et al. in their recent study suggested that budding can be a deciding factor for administering adjuvant chemotherapy in node negative CRC tumors.\[24\]

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Association between tumor budding and nodal involvement ($P = 0.001$) has been found to be statistically significant in this study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Tumor budding grade</th>
<th>Total</th>
<th>$P$ value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJCC stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2 (16.67)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (3.33)</td>
</tr>
<tr>
<td>II</td>
<td>9 (75)</td>
<td>6 (60)</td>
<td>14 (36.84)</td>
<td>29 (48.33)</td>
</tr>
<tr>
<td>III</td>
<td>1 (8.33)</td>
<td>4 (40)</td>
<td>23 (60.53)</td>
<td>28 (46.67)</td>
</tr>
<tr>
<td>IV</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (2.63)</td>
<td>1 (1.67)</td>
</tr>
<tr>
<td>PNI</td>
<td>No</td>
<td>11 (91.67)</td>
<td>8 (80)</td>
<td>21 (55.26)</td>
</tr>
<tr>
<td>Yes</td>
<td>1 (8.33)</td>
<td>2 (20)</td>
<td>17 (44.74)</td>
<td>20 (33.33)</td>
</tr>
<tr>
<td>LVI</td>
<td>No</td>
<td>9 (75)</td>
<td>5 (50)</td>
<td>15 (39.47)</td>
</tr>
<tr>
<td>Yes</td>
<td>3 (25)</td>
<td>5 (50)</td>
<td>23 (60.53)</td>
<td>31 (51.67)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>Negative</td>
<td>11 (91.67)</td>
<td>4 (40)</td>
<td>13 (34.21)</td>
</tr>
<tr>
<td>Positive</td>
<td>1 (8.33)</td>
<td>6 (60)</td>
<td>25 (65.79)</td>
<td>32 (53.33)</td>
</tr>
<tr>
<td>Tumor infiltration</td>
<td>T3 or higher</td>
<td>8 (66.67)</td>
<td>8 (80)</td>
<td>29 (76.32)</td>
</tr>
<tr>
<td>T2 or lower</td>
<td>4 (33.33)</td>
<td>2 (20)</td>
<td>9 (23.68)</td>
<td>15 (25)</td>
</tr>
<tr>
<td>Histological grade</td>
<td>G1</td>
<td>8 (66.67)</td>
<td>4 (40)</td>
<td>24 (63.16)</td>
</tr>
<tr>
<td>G2</td>
<td>4 (33.33)</td>
<td>5 (50)</td>
<td>14 (36.84)</td>
<td>23(38.33)</td>
</tr>
<tr>
<td>G3</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>1(1.67)</td>
</tr>
<tr>
<td>Tumor size(cm)</td>
<td>5±1.48</td>
<td>6+1.91</td>
<td>5.63±2.39</td>
<td>0.310 Not significant</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>3 (25)</td>
<td>2 (20)</td>
<td>13 (34.21)</td>
</tr>
<tr>
<td>Male</td>
<td>9 (75)</td>
<td>8 (80)</td>
<td>25 (65.79)</td>
<td>42 (70)</td>
</tr>
<tr>
<td>Age</td>
<td>56.4±10.14</td>
<td>50.2±6.92</td>
<td>51.89±15.36</td>
<td>0.423 Not significant</td>
</tr>
</tbody>
</table>

AJCC: American Joint Committee on Cancer, PNI: Perineural invasion, LVI: Lymphovascular invasion
similar to Ueno et al., 2004; Guzinska-Ustymowicz, 2005; and Kanazawa et al., 2008. No statistically significant correlation was found among tumor budding and distant metastasis. Tanaka et al., 2003; Guzinska-Ustymowicz, 2005; and Ohtsuki et al., 2008, have found statistically significant correlation between tumor budding and distant metastasis. The reason for this contradictory result is hard to explain but may be related to the unique biology of Indian CRC or different stage distribution of study population.

Limitations of our study were that patients were not followed up for recurrence and larger sample size is needed for significant correlation.

CONCLUSIONS

Now is the era of targeted therapies and individualized cancer treatment. We are slowly understanding cancer biology more and more and it is getting clearer that not two cancers are same. Hence, risk stratification and selection of high-risk prioritized patients among the cancer pool are of utmost importance. Besides the time-tested prognostic factors such as stage, grade, and nodal status, newer prognostic markers such as high proliferative index, microsatellite instability, and tumor budding are gaining popularity. The major constraint in assessment is lack of uniformity and interobserver variation which can largely be reduced by adopting an uniform criteria such as ITBCC and taking the aid of immunohistochemistry. Conflicting data are emerging all over the globe regarding interplay of tumor budding with various clinicopathological parameters; a systematic review and randomized controlled trials will be helpful to consolidate the knowledge available.

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