

## Original Research Article

**Tumour Budding As Prognostic Tool in Gastrointestinal Tract Malignancies: A Diagnostic Analytical Study at A Tertiary Care Hospital**Sanjay M<sup>1</sup>, Amita K<sup>2</sup>

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**Abstract**

*Background:* Gastrointestinal tract malignancies (GIT) are on the rise and it is imperative to identify individuals at risk of developing relapse or metastasis and decide on management protocol consequently. Accurate risk assessment is pivotal to balance benefit versus overtreatment. Recently the tumour bud scoring has been included as a marker in cancers. The present study was planned to determine the association of tumour budding with various clinicopathology parameters in GIT malignancies. *Material & Methods:* This was a retrospective study conducted over a duration of two years including 40 cases. Tumour budding was counted in the maximum invasive area. Tumour budding was defined as the presence of single tumour cells or small clusters of up to five cells in the tumour stroma, Correlation between tumour budding and various clinicopathological characteristics were tested by chi-square test, with  $p < 0.05$  significance. *Results:* There was a statistically significant association between grade of tumour budding and histologic type ( $p < 0.048$ ), histologic grade ( $p < 0.000$ ), lymph vascular invasion ( $p < 0.000$ ), TNM staging ( $p < 0.001$ ) and tumour interface (infiltrative versus expansile) ( $p < 0.004$ ). *Conclusion:* A standardized information about presence of tumour budding in routine histopathology reporting of GIT malignancies will help clinicians in adopting an effective modality of treatment for better patient care.

**Keywords:** Tumour budding; Gastrointestinal tract; Prognosis.

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**Introduction**

Gastrointestinal tract (GIT) carcinomas are the leading cause of malignancy in developing countries. In today's era of personalized oncomedicine, it

is imperative to identify individuals at risk of developing relapse or metastasis and decide on management protocol consequently. In traditional approach of tumour, lymph node and metastasis (TNM) system used for deciding treatment plan, early stage disease is subjected to a conservative

management protocol, while advanced stage diseases are taken up for multimodality therapy. Despite this, some early stage disease develops adverse outcomes.<sup>1,2</sup> Accurate risk assessment is pivotal to balance benefit versus overtreatment.<sup>3</sup> Hence, there is always a need to identify new prognostic factors that can predict such adverse events or identify high risk individuals. Recently, much emphasis has been placed on role of tumour host microenvironment, especially the epithelial mesenchymal transition in tumour progression. The epithelial mesenchymal transition refers to the dissociation of tumour cells, stromal lysis and migration of tumour cells.<sup>4-6</sup> Tumour budding is one such correlate of epithelial mesenchymal transition wherein small clusters of tumour cells less than five are seen at the invasive front of tumour with aggressive biologic potential.<sup>5</sup> Since its documentation for first time in 1950 by Gabbert H et al.,<sup>7</sup> several researchers have attempted to standardize the scoring of tumour budding. Recently the tumour bud scoring has been included as a marker in colorectal cancers. However, literature on the role of tumour budding in GIT malignancies is scarce. Hence the present study was planned to determine the association of tumour budding with various clinicopathology parameters in GIT malignancies.

## Materials and Methods

This was a retrospective study conducted over a duration of two years from June 2017 to May 2019. All the 40 consecutive cases of gastrointestinal tract (GIT) malignancies who had undergone surgical resection at Adichunchanagiri Institute of Medical Sciences, BG Nagara, were included in the study. Cases diagnosed of GIT malignancies at biopsy, those with concurrent malignancy at other sites or with previous history of malignancy were excluded from the study. The tumours were staged according to Tumour Node Metastasis staging system. Grading was performed as per organ specific standard guidelines. Computer system and case sheets from medical records department were screened for clinicodemographic information. Institutional ethical committee clearance was obtained.

All the surgically resected specimens were fixed in 10 % formalin, grossed as per the protocol and five-micron thin sections were obtained. The slides were stained with haematoxylin and eosin stain (H & E stain). Two senior Pathologist with more than eighty years experience in histopathology and unaware of the final diagnosis and clinical data, independently reviewed the slides. Histologic

typing was done as per WHO guidelines. Lymph vascular invasion was defined as presence of tumour cells in vessel wall, adherent to endothelium, covered by endothelium and protruding into the lumen. The pathologic staging was assigned as per the UICC guidelines.

Tumour budding was counted in the maximum invasive area. Tumour budding was defined as the presence of single tumour cells or small clusters of up to five cells in the tumour stroma, adjacent to large circumscribed areas of tumour cells. The buds were counted at 40x magnification in ten hotspots (densest area). The tumour budding was categorized as low, intermediate and high-grade tumour budding as follows; low grade - <4 tumour buds/10 HPF, intermediate 5 to 9/10 HPF and high grade - >10/10 HPF. Continuous variables were expressed as mean  $\pm$  standard deviation, median, and minimum and maximum, while categorical variables were expressed as frequency and percentage.

Correlation between tumour budding and various clinicopathological characteristics were tested by chi-square test, with  $p < 0.05$  significance.

## Results

Among the 40 cases of GIT malignancies, age range was from 25 to 88 years with maximum cases being more than 60 years old. Females outnumbered males, with female to male ratio being 4:1. Demographic details are shown in (Table 1). The most frequent site of involvement was colon (18/40), followed by stomach (16/40), appendix, oesophagus and tongue (two in each case). Most tumours were 1 to 3 cm in size (40%). Most common histologic type was adenocarcinoma in 55%, followed by signet ring cell carcinoma in 20%, intestinal type of adenocarcinoma 4%, squamous cell carcinoma 4% and tubular carcinoma 2% of cases. 50% of tumours were well differentiated whereas poorly differentiated and moderately differentiated tumours accounted for 30% and 20% of all cases respectively. Lymph vascular invasion was seen in 55% (22/40) cases. Median number of lymph nodes isolated was 12 (range 07-20). Low tumour budding was seen in (20/40) 50%, high tumour budding in 40% (16/40) and intermediate tumour budding in (4/40) 10% cases. Tumour infiltrating lymphocytes were seen in 70% (28/40) cases. Perineural invasion was noted in 30% (12/40) of cases.

Association of various demographic and morphologic characteristics with different grades of budding is depicted in (Table 1).

**Table 1:** Shows demographic and clinicopathologic variables

<b>Demographic characteristics</b>	<b>Frequency</b>	<b>Percentage (%)</b>
<b>Age in years</b>		
25-40	5	12.5
41-50	7	17.5
51-60	9	22.5
>60	19	47.5
<b>Sex</b>		
Male	8	20.0
Female	32	80.0
<b>Size of Tmour</b>		
1-3 cms	16	40.0
4-5 cms	10	25.0
≥6cms	14	35.0
<b>Histological Type</b>		
Adenocarcinoma	22	55.0
Signet ring adenocarcinoma	8	20.0
Tubular adenocarcinoma	2	5.0
Intestinal type adenocarcinoma	4	10.0
Squamous cell carcinoma	4	10.0
<b>Grading</b>		
Well differentiated	20	50.0
Moderately differentiated	8	20.0
Poorly differentiated	12	30.0
Lymph vascular invasion		
Absent	22	55.0
Present	18	45.0
<b>Tmour Budding</b>		
Low	20	50.0
Intermediate	4	10.0
High	16	40.0
<b>Tmour infiltrating lymphocytes</b>		
Present	28	70.0
Absent	12	30.0
<b>Perineural Invasion</b>		
Present	12	30.0
Absent	28	70.0
<b>TNM Staging</b>		
T1 & T2	18	45.0
T3 & T4	22	55.0
<b>Tmour Interface</b>		
Expansile	14	35.0
Infiltrating	26	65.0
<b>Proximal Margin</b>		
Involved	12	30.0
Uninvolved	28	70.0
<b>Distal Margin</b>		
Involved	14	35.0
Uninvolved	26	65.0

There was no statistically significant association between different grades of tumour budding and demographic and morphologic characteristics like age ( $p < 0.2$ ), gender ( $p < 0.859$ ), type of procedure ( $p < 0.124$ ), size of tumour ( $p < 0.381$ ), number of lymph nodes ( $p < 0.307$ ), tumour infiltrating lymphocytes ( $p < 0.48$ ) and perineural invasion ( $p < 0.327$ ). There was a statistically significant association between grade of tumour budding and histologic type ( $p < 0.048$ ), histologic grade ( $p < 0.000$ ), lymph vascular invasion ( $p < 0.000$ ), TNM staging ( $p < 0.001$ ) and tumour interface (infiltrative versus expansile) ( $p < 0.004$ ).

## Discussion

Carcinogenesis is a multistep process with several molecular changes occurring at each step. One of the critical and earliest steps defining tumour progression is the epithelial mesenchymal transition, i.e., invasion of tumour cells into the surrounding stroma which is reflected at morphology as tumour budding. Studies have linked tumour budding to epithelial mesenchymal transition wherein the TB acquire fibroblastic morphology capable of undergoing migration. At the molecular level, both tumour budding and EMT phenotype show reduced expression of Beta catenin and E Cadherin.<sup>8</sup>

Early in 1950's, pathologists for the first time recognized "sprouting" seen at the invasive front of the tumour and presumed that it reflected an aggressive behaviour.<sup>9</sup> Later in 1980's, researchers described the presence of dissociated cells at invasive front.<sup>10</sup> Since then few studies have attempted to analyse the role of tumour budding as a prognostic marker, albeit with discrepancies in the criteria's used. It was only in 2016, when ITBC laid down guidelines for standard reporting of tumour budding.<sup>11,12</sup> Prognostic potential of tumour budding has been reported in colorectal, oesophageal, pancreatic, gastric, breast, lung and laryngeal carcinoma.<sup>13-16</sup>

GIT malignancies are one of the leading causes of cancer related mortality in India. Several prognostic factors have been identified that directs management and overall outcome of the disease. Identification of additional prognostic markers with potential clinical impact is imperative for delivering personalized treatment. To have a clinical realm, the prognostic markers should be easy to assess, cost effective, less time consuming and reproducible. Counting and grading of tumour budding used in the present study, as per ITBC guidelines, met all the above criteria.

In the present study, there was significant association between grades of TB and histologic types. There are few studies investigating tumour budding in gastric adenocarcinoma. Chek et al., in their study, included all cases of gastric adenocarcinoma, irrespective of the histologic type and observed a significant association between tumour budding and other prognostic factors.<sup>17</sup> Some authors opine that signet ring carcinomas are primarily high-grade tumours and hence tumour budding should not be applied to these subtypes. However, in a study by Gabbert et al, involving 445 patients with gastric carcinoma.<sup>13</sup> Tumour budding emerged as an independent prognostic marker in all the histologic subtypes. Yama et al. reported tumour budding to be significantly associated with histologic subtypes of lung cancer.<sup>15</sup> These findings suggest a variation in pathogenetic process of tumour budding in different histologic subtypes.

In the present study, the analysis as per grade revealed that tumour budding was significantly associated with tumour grade. 100% of Grade 3 tumours showed high grade tumour budding, 90% of Grade 1 tumours showed low grade tumour budding. Sevda et al. reported significant correlation between tumour grade and tumour budding.<sup>18</sup> Contrary to this, Mehta et al. did not find any such correlation. They proposed that such correlation may be false, since, in high grade tumours, single cells may be falsely counted as tumour budding.<sup>19</sup>

Gendi S et al. and Mehta et al. did not report any correlation between tumour budding and T stage of the disease.<sup>19,20</sup> However, Fukumoto et al. observed that tumour budding is an important prognostic factor for predicting prognosis in stage.<sup>21</sup> In stage pT1 colorectal carcinomas, presence of tumour budding has been incorporated as an important risk factor, with presence of high-grade tumour budding, in addition to other high-risk factors, necessitating surgical intervention.<sup>22</sup> Similarly, in Stage II colorectal cancers, high grade tumour budding implicates initiation of adjuvant therapy. Promisingly, in colorectal and oesophageal carcinomas, TB has emerged as a potential predictive marker of response to neoadjuvant therapy.<sup>23</sup> Tumours with low grade tumour budding respond to EGFR therapy and have non progressive disease, while presence of high grade tumour budding predicts non responsiveness to EGFR therapy.

Roh et al. reported an association between tumour size and tumour budding. In a cohort of 56 patients with oesophageal carcinoma.<sup>24</sup> In the present study, there was no significant association

between tumour size and grade of tumour budding.

In the present study, analysis of tumour budding and TIL did not reveal any significant association. This was in contrast to the findings reported by Lang-Schwarz C et al., who observed that the presence of tumour infiltrating lymphocytes correlated with low tumour budding and consequently good prognosis.<sup>25</sup>

Since tumour budding has been reported to be a significant prognostic marker in colorectal, gastric, oesophageal, lung and laryngeal carcinoma, it can be considered as an unequivocal prognostic marker, irrespective of the cell type. 18 out of 22 (81.81%) tumours with low grade tumour budding did not show LV invasion whereas, 88.8% of tumours with high-grade tumour budding showed LV invasion. Roh et al. reported significant association between LV invasion, high stage and high tumour budding.<sup>23</sup> Similar association of tumour budding with LV invasion, lymph node metastasis and survival has been documented by various authors.

## Conclusion

Assessment of tumour budding can be easily done under H & E stain which is very cost effective and reproducible. A standardized information about presence of tumour budding in routine histopathology reporting of GIT malignancies will help clinicians in adopting an effective modality of treatment for better patient care.

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