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Correlation of PD-L1 Expression with CD8 Positive Tumor Infiltrating Lymphocytes and Tumor Budding in Adenocarcinoma Gallbladder

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HIGHLIGHTS

1. PD-L1 expression correlates with CD8+Tcells.
2. Increased tumor budding associates with PD-L1 levels.
3. CD8+lymphocytes indicate immune response strength.
4. Tumor budding reflects aggressive cancer behavior.
5. PD-L1 may guide immunotherapy strategies.

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ABSTRACT

Introduction:
Gallbladder cancer (GBC) is a prevalent malignancy of the biliary tract, characterized by its aggressive behavior and poor prognosis due to late-stage detection. Despite advancements in diagnostic and therapeutic strategies, the survival rates for GBC patients remain low. Identifying reliable prognostic markers is crucial for improving patient outcomes. Tumor budding, PD-L1 expression, and CD8+ tumor-infiltrating lymphocytes (TILs) have emerged as potential prognostic indicators in various cancers, but their roles in GBC are not well understood. **Objective:** This study aims to evaluate the prognostic significance of tumor budding, PD-L1 expression, and CD8+ TILs in patients with gallbladder adenocarcinoma. **Methods:** A two-year prospective study was conducted at the Department of Pathology, GSVM Medical College, Kanpur, involving histopathologically confirmed cases of gallbladder adenocarcinoma. Immunohistochemical analysis was performed to assess PD-L1 and CD8+ TILs, while tumor budding was evaluated as a histopathological parameter. The associations between these markers and patient outcomes were statistically analyzed. **Results:** The study included 51 participants, with a female predominance (82.4%). Tumor budding was prevalent in 54.9% of cases, with significant associations found between CD8+ TILs and tumor budding (p=0.0029). PD-L1 expression was observed in 21.6% of cases, with a significant correlation between PD-L1 and CD8+ TILs (p=0.034). However, no significant association was observed between PD-L1 expression and tumor budding (p=0.338). **Conclusion:** Tumor budding, PD-L1 expression, and CD8+ TILs are significant prognostic markers in gallbladder adenocarcinoma. The study highlights the importance of integrating these markers into routine clinical practice to improve the risk stratification and management of GBC patients. Further research is warranted to explore the therapeutic implications of these findings.

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INTRODUCTION

Gallbladder cancer (GBC) is the sixth most common gastrointestinal cancer and the leading malignancy of the biliary tract. Despite its prevalence, the origins of GBC remain largely unclear. Major risk factors include gallstones, gallbladder polyps, infections, and porcelain gallbladder, though the disease often presents asymptomatically in its early stages [1]. Consequently, GBC is frequently diagnosed at advanced or metastatic stages, contributing to its high mortality rate. The aggressive nature of GBC is due to its early dissemination through lymphatic channels, perineural spaces, and blood vessels, often leading to metastasis in distant organs like the liver, which complicates treatment and worsens prognosis [2].Prognostic. indicators for GBC include histopathological subtype, tumor size, differentiation degree, perineural and lymphovascular invasion, regional lymph node involvement, and the potential for complete surgical resection [3]. These factors are crucial in guiding clinical decisions and assessing patient prognosis, highlighting the importance of early diagnosis and effective surgical intervention. Despite advances in diagnostic imaging and treatment, managing GBC remains challenging due to its aggressive behavior and late-stage detection. Efforts to improve early detection and refine therapeutic strategies are essential for enhancing patient survival and quality of life [4].

GBC's clinical challenges are compounded by its complex etiology and aggressive metastatic behavior. Research into early detection and perso-nalized treatment strategies offers hope for better outcomes in the future. One area of focus is the role of mesenchymal-like cancer cells, which contribute to chemotherapy resistance and metastasis [5]. These cells, resembling mesenchymal stem cells, possess high migratory and invasive capabilities, as well as resistance to apoptosis induced by chemot-herapy drugs. Their epithelial mesenchymal transi-tion (EMT) enables detachment from the primary tumor, invasion of surrounding tissues, and establishment of metastatic sites. Targeting these cells is a critical research area for overcoming chemotherapy resistance and improving cancer management [6].

Adenocarcinoma of the gallbladder (GBC) is particularly challenging due to its aggressive behavior, late diagnosis, and poor prognosis. Despite advancements in diagnostic techniques and therapeutic strategies, patient outcomes remainmarkers and targeted treatments tailored to the tumor's characteristics. Tumor budding, a histopathological feature characterized by small clusters or single cells at the tumor's invasive front, has emerged as a significant prognostic indicator in various cancers [8]. Higher levels of tumor budding are associated with increased metastasis risk and poorer outcomes. This feature is now routinely assessed in cancers like colorectal and pancreatic, informing clinical decisions and therapeutic strategies [9].

In addition to tumor budding, the immune microenvironment of tumors is a key area of research. The interaction between tumor cells and immune cells, particularly programmed death-ligand 1 (PD-L1) and CD8+ tumor-infiltrating lymphocytes (TILs), plays a crucial role in tumor progression and treatment response [10]. PD-L1, expressed on tumor cells, interacts with the PD-1 receptor on T cells, leading to immune evasion and suppression of antitumor responses. Conversely, CD8+ TILs, representing cytotoxic T lymphocytes, infiltrate tumors and eliminate tumor cells. High levels of CD8+ TILs are associated with improved survival in several cancers, reflecting enhanced antitumor immunity [11].

The prognostic significance of PD-L1 expression and CD8+ TILs has been extensively studied in various cancers, serving as both predictors of clinical outcomes and potential immunotherapy targets. However, their role in GBC remains underexplored, despite emerging evidence suggesting that these markers could inform prognosis and guide treatment [12]. Integrating tumor budding assessment with immunological markers like PD-L1 and CD8+ TILs holds promise for refining risk stratification and guiding personalized treatment for GBC patients. As research advances, these integrated approaches could lead to better outcomes and improved quality of life for GBC patients [13].

The aim of this study is to investigate thee immunohistochemical (IHC) expression of PD-L1 and CD8+ TIL, along with tumor budding as a histopathological finding, and their prognostic significance in patients with gallbladder adenocarcinoma. The objectives are to analyze tumor budding as a histopathological finding in gallbladder adenocarcinoma and evaluate its prognostic significance, and to study the IHC expression of PD-L1 and CD8+ TIL and assess their prognostic significance in these patients.

MATERIALS AND METHODS

This two-year prospective study was conducted at the Department of Pathology in collaboration with the Postgraduate Department of Surgery, GSVM Medical

College, Kanpur. The study aimed to evaluate the prognostic significance of tumor budding, PD-L1 expression, and CD8+ tumor-infiltrating lymphocytes (TILs) in gallbladder adenocarcinoma.Objectives included examining tumor budding as a histopathological finding and assessing the immunohistochemical expression of PD-L1 and CD8+ TILs.

The study included histopathologically confirmed cases of gallbladder adenocarcinoma, excluding those with neuroendocrine differentiation, adeno-squamous carcinoma, and other rare tumors.

RESULTS

In this study, 82.4% of the participants were female, while 17.6% were male, indicating that gallbladder adenocarcinoma is more prevalent in females than

Table 1: Distribution of Age in the study

Group	Number	Percent
31-40	13	25.5
41-50	17	33.3
51-60	11	21.6
61-70	6	11.8
71-80	4	7.8
Total	51	100.0

In this study, approximately 59% of participants were under 50 years old, 21.6% were between 50 and 60 years, and about 19% were over 60. This distribution suggests that gallbladder adenocarcinoma is more common in younger individuals, with a higher prevalence observed in those under 50 compared to older age groups.

Table 2: Distribution of Tumor Gradesin Adenocarcinoma

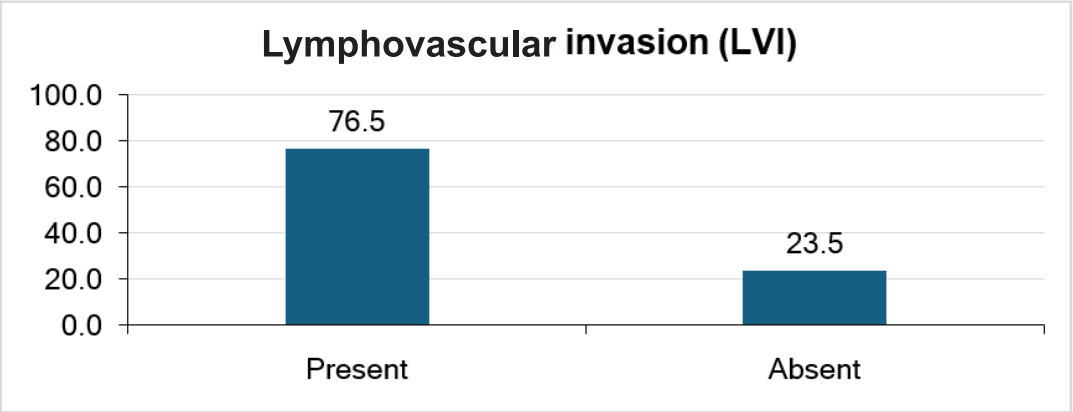
Tumor grade	Count	Percent
Grade-1	22	43.1
Grade-2	22	43.1
Grade-3	7	13.7
Total	51	100.0

The distribution of adenocarcinoma grades among participants showed that Grade 1 and Grade 2 each accounted for 43% of cases, while Grade 3, the higher-grade tumor associated with poorer survival, was present in only 14% of cases. The lower prevalence of Grade 3 tumors underscores the importance of early intervention in clinical management, as early stage tumors are more common and offer better chances for effective treatment.

Table 3: Distribution of pT Stagein Adenocarcinoma

pT Stage	Count	Percent
T1	13	25.5
T2	29	56.9
T3	7	13.7
T4	2	3.9
Total	51	100.0

The pT Stage in adenocarcinoma of the study participants. T2 pT stage is highest (56.9%) among them followed by 25.5% T1, 13.7% T3, and 3.9% is T4 stage.



Lymphovascular invasion (LVI) was observed in the majority of participants, with 76.5% showing its presence in tumor cells, while 23.5% did not exhibit LVI.This indicates that most gallbladder adenocarcinoma cases in the study had tumor cells with lymphovascular invasion, highlighting its prevalence in this cancer type.

Table 4: Distribution of Perineural Lnvasion (PNI) Presence in Adenocarcinoma

PNI	Count	Percent
Present	30	58.8
Absent	21	412
Total	51	100.0

Perineural invasion (PNI) was present in approxima - tely 59% of study participants, while 41% did not exhibit PNI. This suggests that perineural invasion is prevalent feature in gallbladder adenocarcinoma among the participants.

Table 5 : Comparison of PD-L1 Expression Verses TIL in Adenocarcinoma

PDL Expression	Absent (N)	Percent High (N)	Percent Low (N)	Absent (%)	Percent High (%)	Percent Low (%)
Negative	6	19	15	1.8	37.3	29.4
Positive	0	10	1	0	19.6	2.0
Total	6	29	16	1.8	56.9	31.4

In this study, 37.3% of cases showed negative PD-L1 expression with high CD8+ TILs, 29.4% had negative PD-L1 expression with low CD8+ TILs, and 11.8% had negative PD-L1 expression with absent CD8+ TILs. Among positive PD-L1 expression cases, 19.6% had high CD8+ TILs, and 2.0% had low CD8+ TILs, with no cases of positive PD-L1 expression and absent CD8+ TILs. This comparison was statistically significant, with a Chi-square value of 6.72 and a p-value of 0.034, indicating a meaningful association between PD-L1

expression and CD8+ TILs in gallbladder adenocarcinoma.

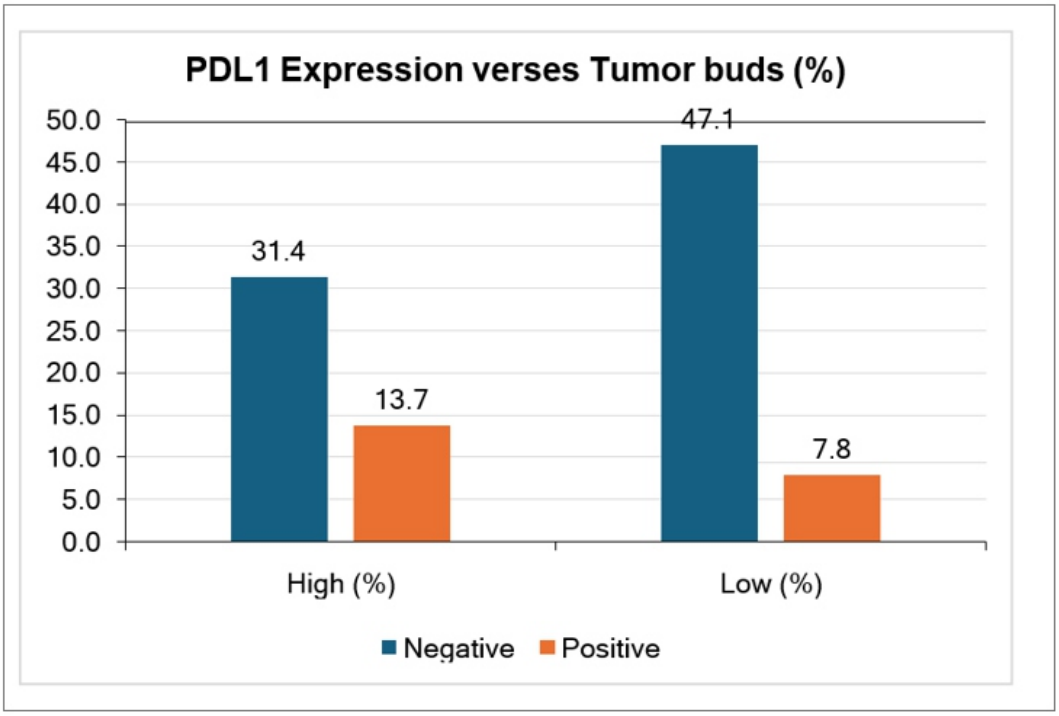


Figure 2: Comparison of PDL1 Expression Verses Tumour Buds in Adenocarcinoma

In this study, 47.1% of cases with negative PD-L1 expression had low tumor buds, while 31.4% had high tumor buds. Among those with positive PD-L1 expression, 13.7% had high tumor buds, and 7.8% had low tumor buds. The comparison between PD-L1 expression and tumor budding was not statistically significant, with a Chi-square value of 0.74 and a p-value of 0.338, indicating no strong association between these variables in gallbladder adenocarcinoma.

Table 6: Comparison of TIL Verses Tumor Buds in Adenocarcinoma

TL	High (N)	Low (N)	High (%)	Low (%)
Present (low)	9	7	17.6	13.7
Present (high)	9	20	17.6	39.2
Absent	5	1	9.8	2.0
Total	23	28	45.1	54.9

In this study, 39.2% of cases had high CD8+ TILs with low tumor buds, 13.7% had low CD8+ TILs with low tumor buds, and 2% had absent CD8+ TILs with low tumor buds. Additionally, 17.6% of cases with low CD8+ TILs and 17.6% with high CD8+ TILs had high tumor buds, while 9.8% of cases with absent CD8+ TILs had high tumor buds. This comparison was highly significant, with a Chi-square value of 11.62 and a p-value of 0.0029, indicating a strong association between CD8+ TILs and tumor budding in gallbladder adenocarcinoma.

DISCUSSION

Gallbladder cancer (GBC) is the most common malignant biliary tract disease and the sixth most prevalent gastrointestinal cancer. Its early stages are typically asymptomatic, leading to late diagnoses and high mortality. Key prognostic factors include tumor size, differentiation, and lymphovascular invasion. Recent studies focus on tumor budding and PD-L1 expression, exploring their roles in prognosis and potential immunotherapy effectiveness in GBC patients [14].

Our study found that 82.4% of participants were female and 17.6% were male, highlighting a higher prevalence of gallbladder adenocarcinoma in females. This aligns with findings from studies by Oz O et al. (2022), Ahuja S et al. (2024), and Seçinti İE et al. (2022), which reported similar female-to-male ratios of 80%-20%, 85%-15%, and 78%-22%, respectively, underscoring the gender disparity in this cancer [15,16,17].

Our study found that 59% of participants were under 50 years old, 21.6% were aged 50-60, and 19% were over 60, suggesting a higher incidence of gallbladder adenocarcinoma in younger individuals. This aligns with findings by Ahuja S et al. (2024) and Oz O et al. (2022), who reported similar age distributions, with around 60% of cases occurring in those under 50, further supporting the trend of younger age prevalence [16,15].

Our study found that adenocarcinoma grades among participants were distributed as 43% for Grade 1, 43% for Grade 2, and 14% for Grade 3. The lower prevalence of Grade 3, a higher-grade tumor with poorer survival rates, underscores the need for early intervention. These findings align with studies by Oz O et al. (2022), Ahuja S et al. (2024), and Xiao B et al. (2020), who reported similar grade distributions [15,16,18].

Our findings reveal that the pT stage in adenocarcinoma among participants is most common at T2 (56.9%), followed by T1 (25.5%), T3 (13.7%), and T4 (3.9%), indicating a predominance of early-stage tumors. This distribution aligns with studies by Seçinti İE et al. (2022), Oz O et al. (2022), and Ahuja S et al. (2024), who reported similar pT stage distributions, further supporting the prevalence of early-stage gallbladder adenocarcinoma [17,15,16]

Our findings indicate that lymphovascular invasion (LVI) is present in 76.5% of gallbladder adenocarcinoma cases and absent in 23.5%, suggesting LVI is common in these tumors. This aligns with studies by Oz O et al. (2022), Ahuja S et al. (2024), and Xiao B et al. (2020), which reported similar LVI distributions: Oz O et al.

(76% present, 24% absent), Ahuja S et al. (77% present, 23% absent), and Xiao B et al. (75% present, 25% absent) [15,16,18].

Our study compares PD-L1 expression and CD8+ TILs in adenocarcinoma cases, finding 37.3% of cases with PD-L1 negative/high CD8+ TILs, 29.4% with PD-L1 negative/low CD8+ TILs, and 11.8% with PD-L1 negative/absent CD8+ TILs. Additionally, 19.6% of PD-L1 positive cases had high CD8+ TILs, and 2.0% (Chi-square: 6.72, p-value: 0.034) aligns with findings by Xiao B et al. (2020) and Lin J et al. (2018) [18,20].

Our study compares CD8+ TILs and tumor buds in adenocarcinoma cases, showing 39.2% with high CD8+ TILs/low tumor buds, 13.7% with low CD8+ TILs/low tumor buds, and 2% with absent CD8+ TILs/low tumor buds. Additionally, 17.6% of cases with low or high CD8+ TILs and 9.8% with ab sent Cd8+ TILs had high tumorbuds. This significant association (Chi-square: 11.62, p-value: 0.0029) aligns with findings by Xiao B et al. (2020), Lin J et al. (2018), and Ahuja S et al. (2024) [18,20].

CONCLUSION

The study highlights the prognostic importance of tumor budding, PD-L1 expression, and CD8+ TIL density in gallbladder adenocarcinoma, offering critical insights into tumor biology and patient prognosis. These markers could play a key role in guiding clinical decisions and personalized treatment strategies. The significant correlation between these factors and patient outcomes underscores the necessity for further research to confirm these findings and investigate their therapeutic potential. Incorporating these markers into routine diagnostic and prognostic assessments could enhance the management and outcomes for patients with gallbladder adenocarcinoma.

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