Section: Pathology

Original Research Article

COMPARATIVE ANALYSIS OF EBV LMP-1 AND TUMOR-INFILTRATING LYMPHOCYTES IN GALLBLADDER CARCINOMA

Prachi Goel[1], Priya Sachan[1], Ajay Kumar[1], Sumaira Qayoom[1]

[1]Department of Pathology, King George's Medical University, Lucknow, Uttar Pradesh, India

Received : 10/03/2026
Received in revised form : 23/04/2026
Accepted : 12/05/2026

Keywords:
Gallbladder carcinoma, Epstein–Barr virus, LMP-1, Tumor-infiltrating lymphocytes, Immunohistochemistry, Prognostic marker.

Corresponding Author:
Dr. Prachi Goel
Email: dr.prachimittalgoel@gmail.com

DOI: 10.47009/jamp.2026.8.3.209

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2026; 8 (3); 1164-1173

ABSTRACT

Background: Gallbladder cancer (GBC) is the most common biliary tract malignancy, predominantly affecting females and often diagnosed at advanced stages. Chronic cholelithiasis, gallbladder wall thickening, and genetic or viral factors, including Epstein–Barr virus (EBV), have been implicated in its pathogenesis. Tumor-infiltrating lymphocytes (TILs) are emerging as potential prognostic markers in GBC. The aim and objective is to evaluate EBV expression using LMP-1 and assess TIL density in neoplastic and non-neoplastic gallbladder lesions and their clinicopathological correlations. **Materials and Methods:** This analytical study included 250 gallbladder cases, comprising 60 neoplastic and 190 non-neoplastic lesions. Histopathological examination was performed on formalin-fixed, paraffin-embedded tissues. Immunohistochemistry using LMP-1 antibody assessed EBV expression, and TIL density was quantified in neoplastic cases. Clinical data, gallstone status, gallbladder wall thickness, tumor site, and demographic details were recorded. Statistical analysis evaluated associations between TILs, clinicopathological features, and survival. **Result:** Adenocarcinoma constituted 88.3% of malignant cases, most commonly affecting the fundus and patients aged 41–60 years. High TIL density was associated with lower tumor invasion, fewer lymph node metastases, and improved survival (88.9% vs. 52.4%), though overall survival difference was not statistically significant. Cholelithiasis and gallbladder wall thickening were more frequent in malignant cases. EBV expressions were not detected in any neoplastic or non-neoplastic lesions. **Conclusion:** High TIL density may serve as a favorable prognostic indicator in GBC, whereas EBV does not appear to contribute to gallbladder carcinogenesis. Gallstones and wall thickening remain important clinicopathological markers.

INTRODUCTION

Gallbladder cancer (GBC) arises from the epithelial lining of the gallbladder and cystic duct and represents the most common malignancy of the biliary tract worldwide.[1] Clinically, it presents either as diffuse thickening of the gallbladder wall or as a mass arising from the fundus, body, or neck.[1] The incidence of GBC demonstrates significant geographical and ethnic variations. In India, the highest prevalence is reported from North, East, Northeast, and Central regions, with rates ranging from 10–22 per 100,000 population, while South and West India show comparatively lower incidence.[2,3] Internationally, high-incidence regions include South America, particularly Chile, Bolivia, and Colombia, while moderate incidence is observed in East Asia (Korea, Japan, China) and central Europe (Slovakia, Poland, Czech Republic).[4] Neighboring countries in the Indian subcontinent such as Pakistan, Nepal, Bangladesh, and Bhutan also report high rates of GBC.[5] Conversely, countries like Sri Lanka, Maldives, Yemen, Afghanistan, Tajikistan, Turkmenistan, and Uzbekistan demonstrate low prevalence. Ethnic predispositions have also been described, with Hispanics, American Indians, Mexican Indians, Alaskan natives, and Asian Indians demonstrating higher-than-average risk.

Multiple factors contribute to the pathogenesis of GBC. Chronic cholelithiasis is the most widely recognized risk factor, while female gender, chronic inflammation, infections, and exposure to unknown mutagens act as neoplastic initiators or promoters.[5] Most GBCs (>80%) are adenocarcinomas arising predominantly from the fundus (60%), followed by the body (30%) and neck (10%).[6] A subset arises in the context of a "porcelain gallbladder," where diffuse calcification is present, with 12–21% showing malignancy.[7] Histologically, the majority

are well-to-moderately differentiated adenocarcinomas, with some papillary lesions exhibiting intraluminal growth.[7]

Hepatobiliary cancers, including hepatocellular carcinoma, cholangiocarcinoma, and GBC, have demonstrated rising incidence and mortality globally, often due to modifiable and non-modifiable risk factors.[8] Recent studies have proposed Epstein–Barr virus (EBV) as a potential contributor to hepatobiliary malignancies, although the precise mechanisms remain unclear.[8] Given the anatomical continuity of the biliary tract, cholangiocarcinoma and gallbladder carcinoma may share overlapping molecular pathways.[8] Intrahepatic cholangiocarcinoma (IHCC), accounting for 5–10% of primary liver cancers, is linked to chronic hepatitis B and C infections; however, EBV's role in its initiation or progression is still under investigation.[9–11]

Cholecystitis, an inflammatory condition of the gallbladder, occurs primarily due to cystic duct obstruction, with or without gallstones. Its prevalence is higher in the Indian population compared to Western countries. Although EBV-associated gallbladder involvement is rare, acute acalculous cholecystitis has been reported as an atypical presentation of primary EBV infection and may indicate severe disease.[12] Therefore, EBV infection should be considered in the differential diagnosis when ultrasonographic evidence of acute acalculous cholecystitis is observed.[12]

EBV is a double-stranded DNA virus belonging to the Herpesviridae family.[13] The virus is enveloped, containing a DNA core within an icosahedral nucleocapsid and tegument. Other family members include herpes simplex virus types I and II, varicella-zoster virus, cytomegalovirus, human herpesvirus 6 and 7, and human herpesvirus 8.[13] EBV infects over 90% of the adult population worldwide and can target multiple cell types, including epithelial cells, B, T, and natural killer (NK) lymphocytes, as well as mesenchymal cells such as smooth muscle cells.[14,15] Transmission occurs primarily through salivary contact, with primary replication in the stratified squamous epithelium of the oropharynx, followed by latent infection of B lymphocytes in the oropharyngeal lymphoid organs. The virus persists lifelong in circulating memory B cells of normal carriers.[16–18]

EBV expresses multiple proteins that facilitate cellular transformation and immortalization, including EBNA-1, EBNA-2, EBNA-LP, EBNA 3A, 3B, 3C, LMP-1, LMP-2A, LMP-2B, and non-coding RNAs EBER1 and EBER2. Among these, LMP-1 functions as a constitutively active CD40 receptor, activating key signaling pathways such as NF-κB, c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), and JAK/STAT, thereby promoting oncogenesis.[19]

Tumor progression is also influenced by the host immune response. Tumor-infiltrating lymphocytes, especially CD4+ and CD8+ T cells, are central to adaptive antitumor immunity. CD8+ T cells recognize tumor-associated antigens presented on major histocompatibility complex class I (MHC I) molecules, producing interferon-γ, which induces cell cycle arrest, apoptosis, angiostasis, and activation of antitumor macrophages. Immunohistochemical studies indicate that high infiltration of CD8+ T cells correlates with improved survival in multiple cancers, including colorectal, ovarian, breast, and pancreatic malignancies.[20,22] Understanding the interplay between EBV infection, LMP-1 expression, and tumor-infiltrating lymphocytes may provide insights into the pathogenesis and prognosis of gallbladder carcinoma.

MATERIALS AND METHODS

The study was conducted in the Department of Pathology, King George's Medical University, Lucknow, in collaboration with the Departments of Surgical Gastroenterology and Surgical Oncology, over two years (May 2018–April 2020) on 250 paraffin-embedded gallbladder specimens. Cases with histologically confirmed gallbladder disease, available clinical data, and patient consent were included, while poorly preserved tissue, insufficient tumor tissue, or non-consenting patients were excluded. Specimens were formalin-fixed, processed, paraffin-embedded, sectioned (3–5 µm), and stained with hematoxylin and eosin. Immunohistochemistry for LMP-1 was performed on 3–4 µm sections using a mouse monoclonal antibody (Clone EBV-LMP CS1-4, 1:200) following standard protocols including antigen retrieval, peroxidase and protein blocking, primary antibody incubation, post-primary link, streptavidin-HRP, DAB chromogen, and hematoxylin counterstaining. Hodgkin lymphoma served as positive control, and omission of primary antibody as negative control. Staining was evaluated in neoplastic cells and scored 0–3+ based on percentage positivity.

RESULTS

Table 1: Groupwise distribution of study population

S. No.		Frequency (N=250)	Percentage
		No	%
1-	Neoplastic	60	24.0
2-	Non-neoplastic	190	76.0

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Specimens were collected from 250 cases including 60 neoplastic and 190 non-neoplastic lesions, fulfilling the inclusion criteria and giving their consent for enrollment in the study [Table 1].

Table 2: Distribution of study population according to diagnostic type in each group

S. No.		Frequency (N=250)	Percentage (%)
1-	Neoplastic (n=60)		
	Adeno WD	28	46.7
	Adneo MD	21	35.0
	Adeno PD	4	6.7
	ICPN	2	3.33
	Others	5	8.33
2-	Non-neoplastic (n=190)		
	Xanthogranulomatous cholecystitis	12	6.32
	Cc with antral metaplasia	6	3.16
	CC	172	90.53

In patients with neoplastic lesions, majority had WD lesions (46.7%), followed by MD (35.0%), other lesions (8.33%), and PD (6.7%). Smallest proportion of patients had ICPN (3.33%). On the other hand in patients with Non-neoplastic lesions, majority had CC (90.53%), followed by Xanthogranulomatous cholecystitis (6.32%) and the least proportion had chronic cholecystitis with antral metaplasia (3.16%) [Table 2].

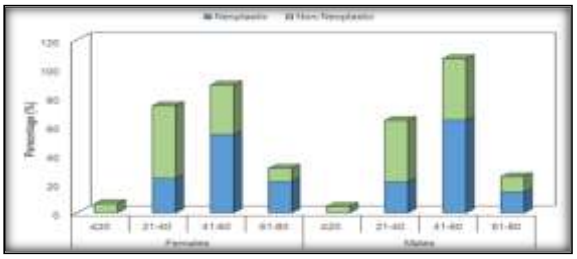

Figure 1: Demographic profile of the study population

Females predominated in the study (76.4%). In non-neoplastic lesions, most females were 21–40 years (50.3%), while in neoplastic lesions, the majority were 41–50 years (54.3%), with a significant age difference between groups (p<0.001). Most males in both groups were 21–60 years, with no significant age difference (p>0.05) [Figure 1].

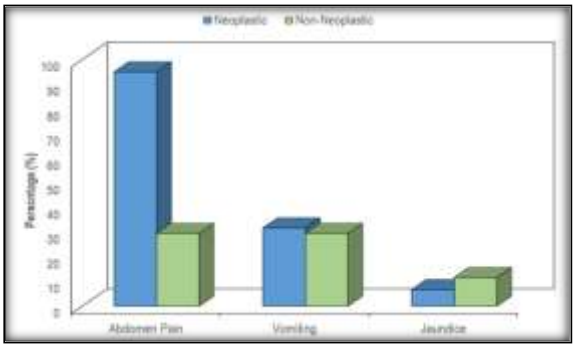

Figure 2: Comparison of symptoms between groups

A higher proportion of patients in neoplastic group as compared to non-neoplastic group had abdominal pain (95.0% vs. 29.5%) and vomiting (31.7% vs, 29.5%) while jaundice was found in higher proportion of non-neoplastic group as compared to neoplastic group (11.6% vs. 6.7%). On comparing statistically, a significant difference between the two groups was only found for presentation of abdominal pain (p<0.001) [Figure 2].

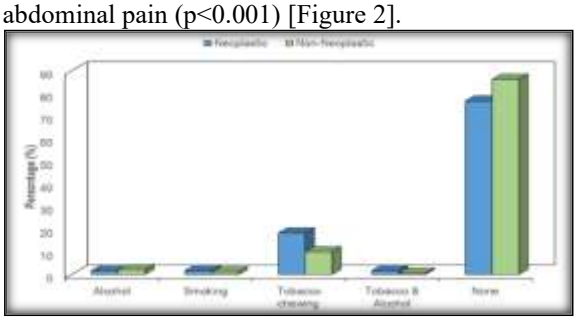

Figure 3: Comparison of personal habits/history between groups

Majority of patients in both the groups had no addiction history (Neoplastic:76.7%; Non-neoplastic:86.3%), followed by tobacco chewing (Neoplastic:18.3%; Non-neoplastic:10.0%). On comparing statistically, addiction history were not significantly different between the groups (p>0.05) [Figure 3].

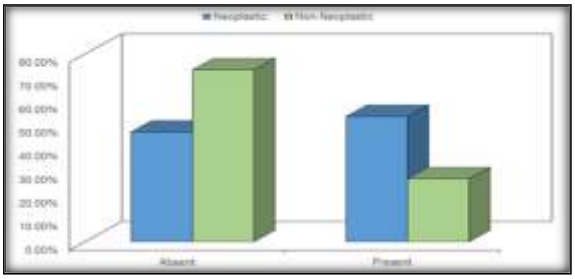

Figure 4: Between Group Comparison of Presentation of Cholelithiasis

Cholelithiasis was present in higher proportion of patients in neoplastic group as compared to non-neoplastic group (53.3% vs. 26.8%). On comparing statistically, this difference was found to be significant (p<0.001) [Figure 4].

ISSN (O): 2687-5365; ISSN (P): 2753-6556

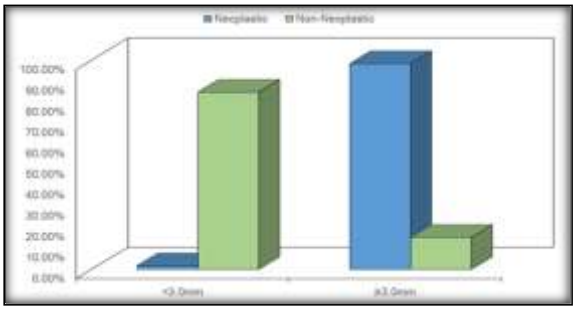

Figure 5: Between group comparison of Bladder Wall-Thickness

Higher proportions of patients in neoplastic group had bladder wall thickening as compared to non-neoplastic group (98.3% vs.15.3%). On comparing statistically, this difference was found to be significant (p<0.001) [Figure 5].

Table 3: Distribution of patient characteristics in neoplastic lesions

SN	Parameters	Frequency (n=60)	Percentage (%)
1-	Site (n=60)		
	Neck	5	8.3
	Body	15	25.0
	Fundus	26	43.33
	Whole of Gall Bladder	14	23.33
2-	Nodal Status		
	Negative (N0)	22	36.67
	1-3 lymph node positive (N1)	9	15.0
	≥4 lymph node positive (N2)	7	11.66
	Not sampled (NX)	22	36.67
3-	Depth of Invasion (n=48)		
	T0	1	2.08
	T1	18	37.50
	T2	10	20.84
	T3	18	37.50
	T4	1	2.08
4-	Liver Involvement (n=48)		
	Not involved	29	60.42
	Involved	19	39.58

Most common site of lesion was at Fundus (n=43.33%), followed by body (25.0%), whole of gall bladder (23.33%) and neck (8.3%). Similarly, the nodal status in most of the patients were Negative (36.67%) and Not Sampled (36.67%), followed by 1-3 positive lymph nodes (15.0%) and ≥4 positive lymph nodes (11.66%). Depth of invasion was measured in 48 cases (12 cases lost to follow-up could not be measured), of these patients majority had depth of invasion of T1 (37.50%) & T3 (37.50%), followed by T2 (20.84%) and least in T0 and T4 (2.08%). Similarly in these patients, liver involvement was found in 39.58% cases only [Table 3].

Tumor infiltrating lymphocytes ranged from 5-85 units. Mean±TIL was 35.69±26.16 units. Moreover Low TIL (units) was found in 58.33% cases, while the remaining 41.67% had High TIL [Figure 6].

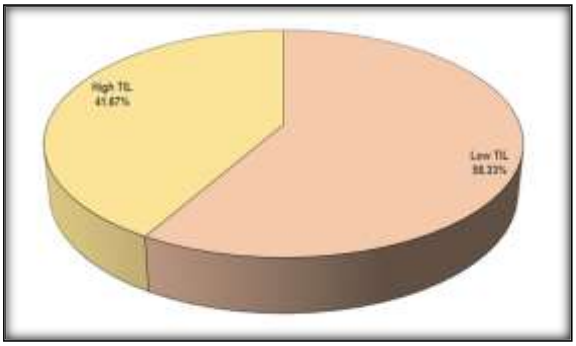

Figure 6: Tumor infiltrating lymphocytes in neoplastic lesions.

Table 4: Association of TIL with histopathological findings in gall bladder cancer

SN	Histopathological findings	Low TIL (n=33)		High TIL (n=25)	
		No.	%	No.	%
1-	Depth of Invasion				
	T0	1	4.0%	0	0.0%
	T1	5	20.0%	13	56.5%
	T2	8	32.0%	2	8.7%
	T3	10	40.0%	8	34.8%
	T4	1	4.0%	0	0.0%
$\chi 2$=9.311 (df=4); p=0.054					
2-	Histopathological grading				
	WD	14	40.0%	14	56.0%
	MD	12	34.3%	9	36.0%
	PD	4	11.4%	0	0.0%
	others	5	14.3%	2	8.0%

ISSN (O): 2687-5365; ISSN (P): 2753-6556

$\chi2$=4.163 (df=3); p=0.244					
3-	P-stage Grouping				
	None	10	28.6%	3	12.0%
	I	5	14.3%	11	44.0%
	II	5	14.3%	0	0.0%
	III	10	28.6%	8	32.0%
	IV	5	14.3%	3	12.0%
$\chi2$=10.363 (df=4); p=0.035					
4-	Lymph Node Metastasis	Low TIL (n=20)		High TIL (n=18)	
		No.	%	No.	%
	Negative (N0)	12	34.3%	10	40.0%
	1-3 lymph node positive (N1)	4	11.4%	5	20.0%
	≥4 lymph node positive (N2)	4	11.4%	3	12.0%
$\chi2$=1.726 (df=3); p=0.631					

The histopathological findings in gall bladder cancer (Depth of invasion, Grading and Lymph Node Metastasis) had no significant association with the TIL category (p>0.05). However, on comparing P-stage grouping, a higher proportion of patients with high TIL as compared to Low TIL had P-stage none (28.6% vs. 12.0%), II (14.3% vs. 0.0%) & IV (14.3% vs. 12.0%), while higher proportion of Non-neoplastic patients had P-stage I (44.0% vs. 14.3%) and III (32.0% vs. 28.6%). On comparing statistically, this difference was found to be significant (p=0.035) [Table 4].

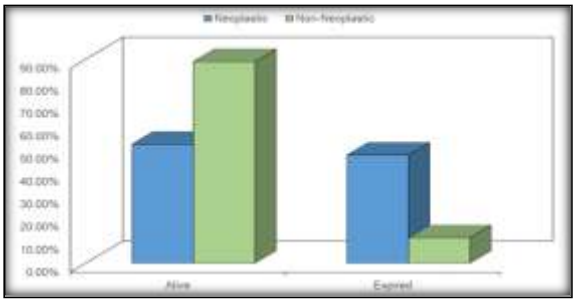

Figure 7: Association of TIL with Status at Follow-up

Majority of cases (65.0%) with neoplastic lesions completed the study, however 12 patients were lost to follow up. Of the remaining 48 patients, 21 (53.85%) had Low TIL and 18 (46.15%) had High TIL . On comparing the mortality of cases between TIL category, it was found that a higher proportion of patients with Low TIL expired as compared to patients with High TIL (47.6% vs. 11.11%). On comparing statistically, this difference was found to be significant (p=0.014) (Figure 7).

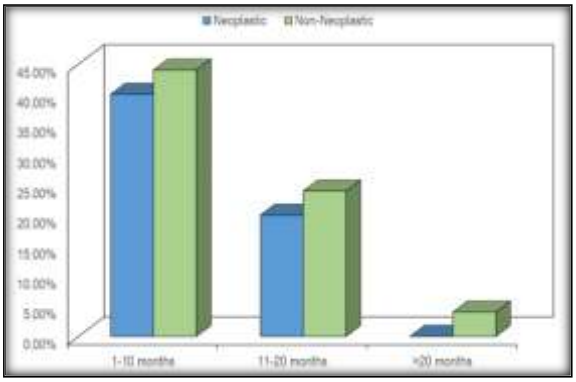

Figure 8: Association of TIL with overall survival

On comparing overall survival in patients with neoplastic lesions, no statistically significant difference was found (p>0.05) [Figure 8].

Table 5: Comparison of immunoexpression of LMP-1 between groups

SN	LMP-1 expression	Neoplastic (n=60)		Non-neoplastic (n=190)	
		No.	%	No.	%
	Negative	60	100.0	190	100.0
	Positive	0	0.0	0	0.0
$\chi2$=0; p=1.0					

Lmp-1 expression by immunohistochemistry was not observed in any of the neoplastic as well as non-neoplastic lesions [Table 5].

Table 6: Survival analysis at follow up

Group	Total cases	No of expiry	% of expiry	Mean survival time	SE
Low TIL	21	10	47.6	9.114	1.417
High TIL	18	2	11.1	10.117	1.456
Overall	39	12	30.8	10.227	1.205
χ^2=0.064; p=0.800 (Log-Rank: Mantel-Cox)					

ISSN (O): 2687-5365; ISSN (P): 2753-6556

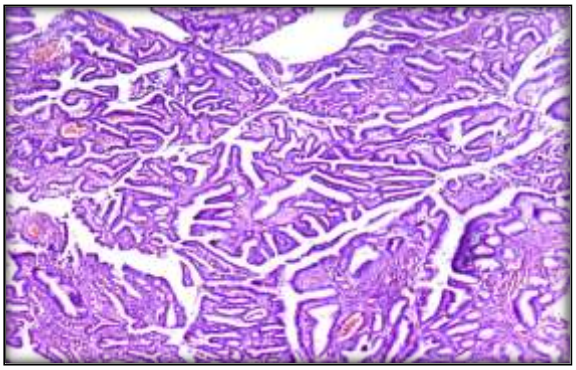

Figure 9: Adenocarcinoma-well differentiated, 40x, H&E, TIL=40%

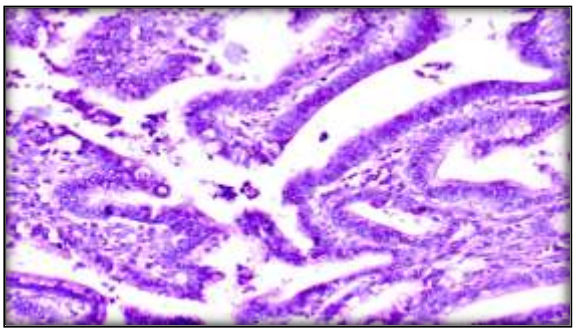

Figure 10: Adenocarcinoma- well differentiated, 200x, H&E. Inset shows no expression of LMP-1 by immunohistochemistry

Difference in duration of survival among cases with Low TIL (9.114±1.417 months) and High TIL (10.117±1.456 months) was not found to be significant [Table 6].

Well-differentiated adenocarcinoma of the gallbladder (H&E, 40×) showing approximately 40% tumor-infiltrating lymphocytes (TILs). High TIL density is highlighted within the stromal regions surrounding tumor glands, indicating potential immune activity [Figure 9].

Well-differentiated gallbladder adenocarcinoma (H&E, 200×). Inset shows negative immunohistochemical staining for EBV LMP-1, indicating absence of EBV in tumor cells [Figure 10].

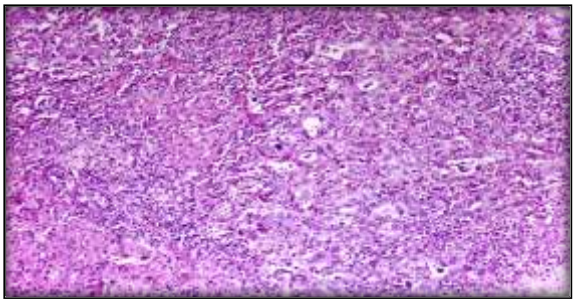

Figure 11: Adenocarcinoma- Moderately differentiated with dense TIL(85%), 40x magnification, H&E

Moderately differentiated gallbladder adenocarcinoma (H&E, 40×) showing dense tumor-infiltrating lymphocytes (TILs) comprising approximately 85% of the stromal area, indicating a pronounced anti-tumor immune response [Figure 11].

Figure 12: Immunohistochemistry for LMP-1 shows no expression

Immunohistochemistry for EBV latent membrane protein 1 (LMP-1) in gallbladder adenocarcinoma showing no detectable expression, indicating absence of EBV in the tumor cells [Figure 12].

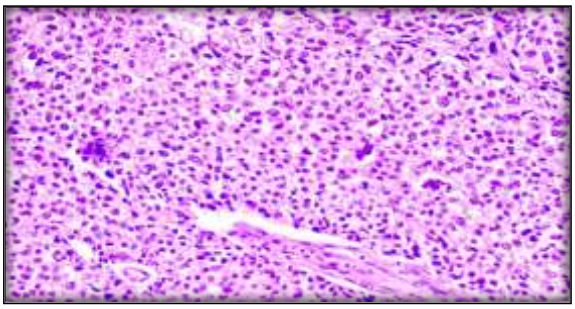

Figure 13: Adenocarcinoma- poorly differentiated, 200x, H&E, TIL=5%

Poorly differentiated gallbladder adenocarcinoma (H&E, 200×) with low tumor-infiltrating lymphocytes (TILs, ~5%), reflecting minimal immune cell infiltration [Figure 13].

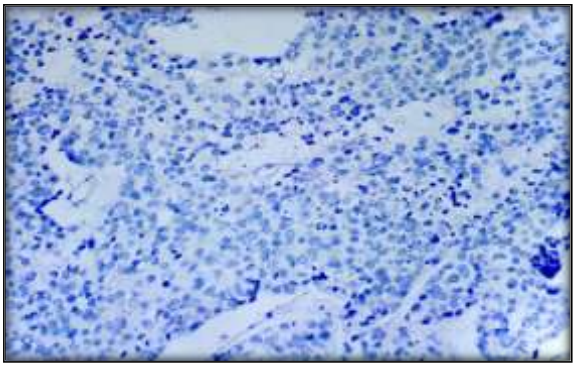

Figure 14: Immunohistochemistry for LMP-1 shows no expression, 200x

Immunohistochemistry for EBV latent membrane protein 1 (LMP-1) in poorly differentiated gallbladder adenocarcinoma (200×) showing no detectable expression, indicating absence of EBV in tumor cells [Figure 14].

ISSN (O): 2687-5365; ISSN (P): 2753-6556

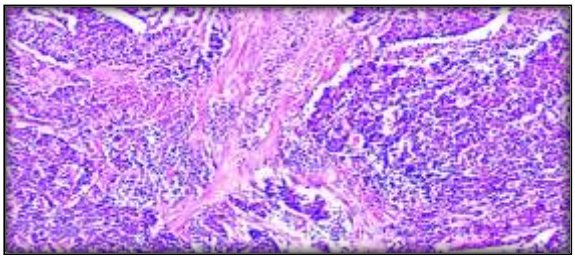

Figure 15: Mixed Neuroendocrine carcinoma, 40x magnification, H&E, TIL=40%. Inset shows positivity for Synaptophysin

Mixed neuroendocrine carcinoma of the gallbladder (H&E, 40×) with moderate tumor-infiltrating lymphocytes (TILs, ~40%). Inset shows positive immunohistochemical staining for Synaptophysin, confirming neuroendocrine differentiation [Figure 15].

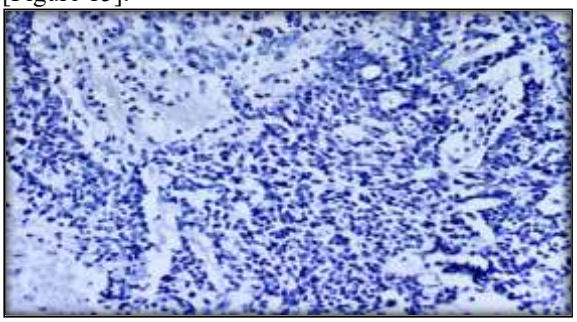

Figure 16: Immunohistochemistry for LMP-1 shows no expression, 100x magnification

Immunohistochemistry for EBV latent membrane protein 1 (LMP-1) in gallbladder carcinoma (100×) showing no detectable expression, indicating absence of EBV in tumor cells [Figure 16].

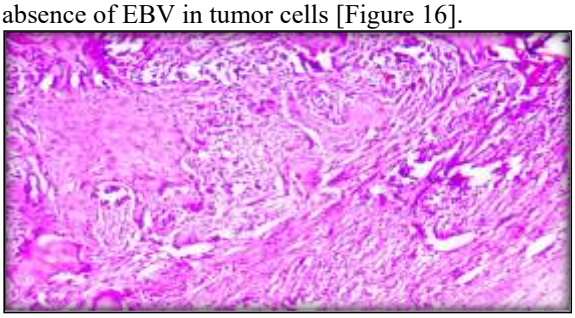

Figure 17: Squamous cell carcinoma, 100x magnification, H&E, TIL=20%

Squamous cell carcinoma of the gallbladder (H&E, 100×) showing low tumor-infiltrating lymphocytes (TILs, ~20%), indicating limited immune cell infiltration [Figure 17].

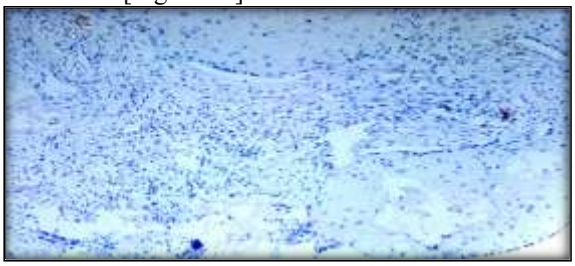

Figure 18: Squamous cell carcinoma, 200x. Immunohistochemistry for LMP-1 shows no expression

Squamous cell carcinoma of the gallbladder (200×) showing no detectable expression of EBV latent membrane protein 1 (LMP-1) by immunohistochemistry, indicating absence of EBV in tumor cells [Figure 18].

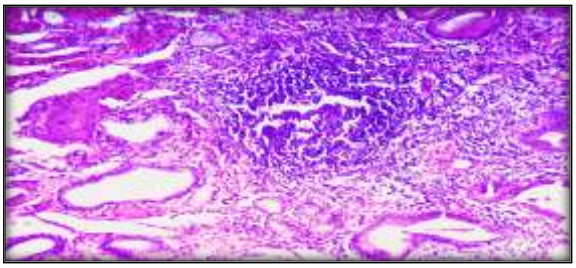

Figure 19: Chronic cholecystitis with adenomatous hyperplasia, H&E, 100x magnification

Chronic cholecystitis with adenomatous hyperplasia of the gallbladder (H&E, 100×) showing reactive epithelial changes without evidence of malignancy [Figure 19].

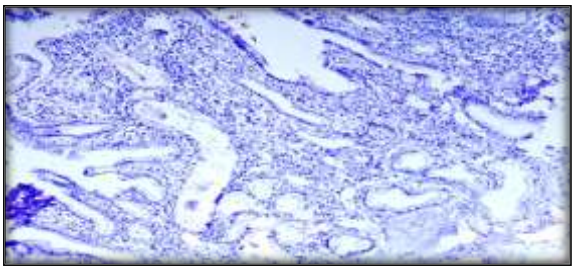

Figure 20: Immunohistochemistry for LMP-1 shows no expression, 100x magnification

Immunohistochemistry for EBV latent membrane protein 1 (LMP-1) in gallbladder tissue (100×) showing no detectable expression, indicating absence of EBV in non-neoplastic lesions [Figure 20].

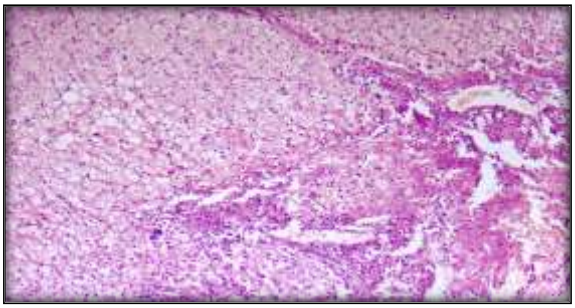

Figure 21: Xanthogranulomatous cholecystitis, 40x, H&E.

Xanthogranulomatous cholecystitis of the gallbladder (H&E, 40×) showing dense inflammatory infiltrate and foamy histiocytes [Figure 21].

ISSN (O): 2687-5365; ISSN (P): 2753-6556

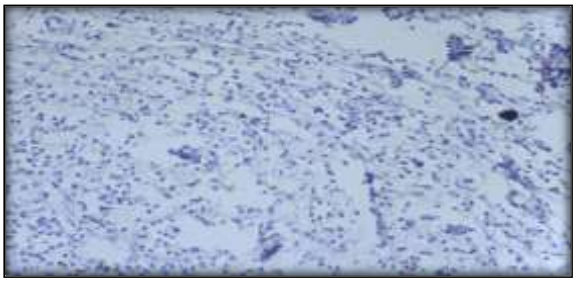

Figure 22: Immunohistochemistry for LMP-1 shows no expression

Immunohistochemistry for EBV latent membrane protein 1 (LMP-1) in xanthogranulomatous cholecystitis showing no detectable expression, indicating absence of EBV in the lesion [Figure 22].

DISCUSSION

Gallbladder cancer (GBC) is a relatively rare but highly aggressive malignancy, first described in 1777.[23] Despite centuries of study, late presentation, rapid progression, and limited therapeutic options continue to define its clinical course.[23,24] Prognosis remains poor, with 5-year survival rates of approximately 32% for lesions confined to the mucosa, and a dismal 1-year survival of 10% in advanced stages.[25,26] These survival statistics underscore the need for improved diagnostic markers and prognostic indicators. India, particularly the northern region, is a high-incidence area, reflecting both environmental and genetic risk factors, with higher rates observed compared to southern states.[27,28]

In the present study, adenocarcinoma was confirmed as the predominant histological type, accounting for 88.4% of neoplastic lesions, consistent with global and Indian data reporting 80–85% prevalence.[27] Well-differentiated adenocarcinomas were the most common subtype (46.7%), followed by moderately differentiated adenocarcinomas (35%), and poorly differentiated or mixed types representing a smaller fraction. These findings reflect the typical histopathological spectrum of GBC and reinforce previous observations regarding its heterogeneity and aggressiveness.[27,30]

Demographic analysis revealed a mean age of 51 ± 10.59 years at diagnosis, aligning with prior Indian studies reporting middle-aged predominance.[28,29] The disease demonstrated a striking female preponderance, with a male-to-female ratio of 1:3.2 in malignant cases, in line with reported female-to-male ratios ranging from 3:1 to 4.5:1.[30,31] Younger females (21–40 years) were disproportionately affected, suggesting earlier onset and potentially more aggressive disease in this subgroup.[32] This pattern emphasizes the need for heightened clinical vigilance in younger women presenting with biliary symptoms.

Clinically, abdominal pain was the most frequent presenting symptom, reported in 95% of neoplastic cases and 29.5% of non-neoplastic lesions, confirming previous findings that non-specific gastrointestinal symptoms often delay diagnosis.[32,33] Gallstones were present in 53.3% of GBC patients, supporting their role as a major risk factor, though regional studies indicate additional cofactors-including chronic inflammation, dietary influences, and genetic predispositions-may contribute to carcinogenesis.[35–37] Gross gallbladder wall thickening (>3 mm) was observed in 98.3% of neoplastic cases versus 15.3% of non-neoplastic lesions, corroborating prior studies that associate wall thickening with malignancy.[38–40]

Tumor location analysis demonstrated that the fundus, alone or with the body, was the most frequently involved site (43–67%), consistent with prior reports indicating approximately 60% of GBC originates in the fundus, 30% in the body, and 10% in the neck.[41,42] This distribution may relate to stasis of bile, chronic inflammation, and regional differences in mucosal susceptibility, highlighting potential mechanistic pathways for carcinogenesis.

Tumor-infiltrating lymphocytes (TILs) were evaluated as a prognostic biomarker. High-TIL density was observed in 58.33% of neoplastic cases, while 41.67% had low-TIL counts. Notably, high-TIL cases correlated with earlier tumor stage (50% versus 40%, p=0.035), lower depth of invasion, and reduced lymph node metastasis, supporting the role of TILs as a favorable prognostic indicator.[43] Although overall survival was higher in high-TIL patients (89%) compared to low-TIL patients (52.3%), statistical significance was limited due to follow-up loss (p=0.80). These findings align with previous studies in gallbladder and other gastrointestinal malignancies, where TIL density predicts improved histological grade, lower stage, and enhanced anti-tumor immune response.[43] The study reinforces the potential utility of TIL assessment in routine histopathological evaluation to stratify patients prognostically.

Regarding Epstein–Barr virus (EBV), no gallbladder lesions in this cohort demonstrated EBV positivity via immunohistochemistry. This observation is consistent with global literature, where EBV association is rare and primarily limited to lymphoepithelioma-like carcinomas of the gallbladder.[42,43] While EBV has been implicated in other hepatobiliary malignancies, including intrahepatic cholangiocarcinoma, our findings indicate that EBV does not play a detectable role in the pathogenesis of conventional gallbladder carcinoma.

Overall, this study reinforces established epidemiological and pathological characteristics of GBC: predominance of adenocarcinoma, female preponderance, association with gallstones, and gallbladder wall thickening as a predictive feature. Importantly, TIL density emerges as a potential prognostic biomarker, meriting further investigation in prospective studies with larger cohorts and longer follow-up. Conversely, EBV appears unlikely to

ISSN (O): 2687-5365; ISSN (P): 2753-6556

contribute to gallbladder carcinogenesis in the studied population. Future research should focus on integrating immunological markers such as TILs with molecular profiling to enhance early detection, prognostication, and personalized therapeutic strategies for this aggressive malignancy.

CONCLUSION

In conclusion, this study of 250 gallbladder cases found that adenocarcinoma was the predominant malignant lesion, most commonly involving the fundus and occurring in patients aged 41–60 years. High tumor-infiltrating lymphocytes (TILs) were associated with lower invasion, fewer lymph node metastases, and improved survival, although the difference in overall survival was not statistically significant. Gallstones and gallbladder wall thickening were common in malignant cases. EBV expression, assessed using LMP-1, was not detected in any neoplastic or non-neoplastic lesions, suggesting that EBV is unlikely to play a role in gallbladder carcinogenesis.

REFERENCES

1. Wistuba II, Gazdar AF. Gallbladder cancer: lessons from a rare tumour. Nat Rev Cancer. 2004 Sep;4(9):695 706. doi:10.1038/nrc1429.
2. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer. 2006 Apr 1;118(7):1591 602. doi:10.1002/ijc.21683.
3. Mhatre SS, Nagrani RT, Budukh A, Chiplunkar S, Badwe R, Patil P, Laversanne M, Rajaraman P, Bray F, Dikshit R. Place of birth and risk of gallbladder cancer in India. Indian J Cancer. 2016 Apr Jun;53(2):304 8. doi:10.4103/0019 509X.197723.
4. Hundal R, Shaffer EA. Gallbladder cancer: epidemiology and outcome. Clin Epidemiol. 2014 Mar 7;6:99 109. doi:10.2147/CLEP.S37357.
5. Lazcano Ponce EC, Miquel JF, Muñoz N, Herrero R, Ferrecio C, Wistuba II, et al. Epidemiology and molecular pathology of gallbladder cancer. CA Cancer J Clin. 2001 Nov Dec;51(6):349 64. doi:10.3322/canjclin.51.6.349.
6. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. Gut Liver. 2012 Apr 15;6(2):172 87. (DOI not consistently indexed)
7. Roa I, de Aretxabala X, Araya JC, Roa J. Preneoplastic lesions in gallbladder cancer. J Surg Oncol. 2006 Jun 15;93(8):615 23. (DOI may not be universally available)
8. Zx C, Xt P, L T, Gq Z, G C, Tq G, et al. EBV as a potential risk factor for hepatobiliary system cancer: a meta analysis with 918 cases. Pathol Res Pract. 2018 Nov 2;215(2):278 85. (DOI unavailable in PubMed)
9. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):5 29. doi:10.3322/caac.21254.
10. Palmer WC, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? A meta analysis of risk factors for intrahepatic cholangiocarcinoma. J Hepatol. 2012 Jul;57(1):69 76. doi:10.1016/j.jhep.2012.02.022.
11. Sun K, Xu S, Wei J, Wang B, Owusu Ansah KG, Wang W, et al. Clinicopathological features of 11 Epstein–Barr virus associated intrahepatic cholangiocarcinoma at a single center in China. Medicine (Baltimore). 2016 Oct;95(40):e5115. doi:10.1097/MD.0000000000005115.
12. Agergaard J, Larsen CS. Acute acalculous cholecystitis in a patient with primary Epstein Barr virus infection: a case report and literature review. Int J Infect Dis. 2015 Jun;35:67 72. doi:10.1016/j.ijid.2015.03.007.
13. Epstein MA. The Epstein Barr Virus. Springer; (publication details vary; book DOI not typically used).
14. Thompson MP, Kurzrock R. Epstein Barr virus and cancer. Clin Cancer Res. 2004 Feb 1;10(3):803 21. doi:10.1158/1078 0432.CCR 0008 03.
15. Kurt H, Medeiros LJ, Khoury JD, Schlette EJ. Epstein Barr virus positive follicular lymphoma. Br J Haematol. 2018;182(6):757. doi:10.1111/bjh.15363.
16. Baumforth KR, Young LS, Flavell KJ, Constandinou C, Murray PG. The Epstein Barr virus and its association with human cancers. Mol Pathol. 1999 Dec;52(6):307 22.
17. Babcock GJ, Decker LL, Volk M, Thorley Lawson DA. EBV persistence in memory B cells in vivo. Immunity. 1998 Sep;9(3):395 404. doi:10.1016/S1074 7613(00)80656 F.
18. Nilsson K. Human B lymphoid cell lines. Hum Cell. 1992 Mar;5(1):25 41.
19. Fries KL, Miller WE, Raab Traub N. The A20 protein interacts with the Epstein–Barr virus latent membrane protein 1 (LMP1) and alters the LMP1/TRAF1/TRADD complex. Virology. 1999 Nov;264(1):159 66. doi:10.1006/viro.1999.0049.
20. Zhang L, Conejo Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003 Jan 16;348(3):203 13. doi:10.1056/NEJMoa020177.
21. Bedri S, Mohamed M, Sarwath H, Sastry K. Characterization and quantification of tumor infiltrating lymphocytes in breast cancer. J Immunother Cancer. 2014;2(Suppl 1):P9.
22. Fukunaga A, Miyamoto M, Cho Y, Murakami S, Kawarada Y, Oshikiri T, et al. CD8+ and CD4+ tumor infiltrating lymphocytes and dendritic cells improve prognosis of pancreatic adenocarcinoma patients. Pancreas. 2004 Jan;28(1):e26 31.
23. Nevin JE, Moran TJ, Kay S, King R. Carcinoma of the gallbladder: staging, treatment, and prognosis. Cancer. 1976 Jan;37(1):141 8.
24. Perpetuo MD, Valdivieso M, Heilbrun LK, Nelson RS, Connor T, Bodey GP. Natural history study of gallbladder cancer: a 36 year review at M. D. Anderson Hospital. Cancer. 1978 Jul;42(1):330 5.
25. Hart J, Shani M, Modan B. Epidemiological aspects of gallbladder and biliary tract neoplasm. Am J Public Health. 1972;62(1):36 9.
26. Carriaga MT, Henson DE. Liver, gallbladder, extrahepatic bile ducts, and pancreas. Cancer. 1995 Jan;75(1 Suppl):171 90.
27. Barbhuiya MA, Singh TD, Poojary SS, Gupta S, Kakkar M, Shrivastav BR, et al. Gallbladder cancer incidence in Gwalior district of India: five year trend. Indian J Cancer. 2015;52(3):430.
28. Alvi AR, Siddiqui NA, Zafar H. Risk factors of gallbladder cancer in Karachi a case control study. World J Surg Oncol. 2011 Dec 9;9:164.
29. Khan MR, Raza SA, Ahmad Z, Naeem S, Pervez S, Siddiqui AA, et al. Gallbladder intestinal metaplasia in Pakistani patients with gallstones. Int J Surg. 2011;9(6):482 5.
30. Malhotra RK, Manoharan N, Shukla NK, Rath GK. Gallbladder cancer incidence in Delhi urban: 25 year trend analysis. Indian J Cancer. 2017;54(4):673.
31. Bhagabaty SM, Sharma JD, Krishnatreya M, Nandy P, Kataki AC. Profile of gallbladder cancer in Northeast India. Int J Res Med Sci. 2017;2(4):1683 6.
32. Gupta S, Kori C, Kumar V, Misra S, Akhtar N. Epidemiological study of gallbladder cancer patients from North Indian Gangetic planes. J Gastrointest Canc. 2016;47(1):27 35.
33. Hamdani NH, Qadri SK, Aggarwalla R, Bhartia VK, Chaudhuri S, Debakshi S, et al. Clinicopathological study of gallbladder carcinoma with reference to gallstones: 8 year experience from Eastern India. Asian Pac J Cancer Prev. 2012;13(11):5613 7.
34. Kapoor VK, McMichael AJ. Gallbladder cancer: an “Indian” disease. Natl Med J India. 2003 Aug;16(4):209 13.
35. Pandey M, Pathak AK, Gautam A, Aryya NC, Shukla VK. [Article title unavailable]. Dig Dis Sci. 2001;46(6):1145 51.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

36. Black WC, Key CR, Carmany TB, Herman D. Carcinoma of the gallbladder in Southwestern American Indians. Cancer. 1977 Mar;39(3):1267 79.
37. de Aretxabala X, Roa I, Araya JC, Burgos L, Flores P, Huenchullan I, et al. Operative findings in early gallbladder cancer. Br J Surg. 1990 Mar;77(3):291 3.
38. N DN, Giriyan SS. Can gallbladder wall thickness by gross exam predict carcinoma before histopathology? Trop J Pathol Microbiol. 2019;5(12):1039 45.
39. Mittal R, Jesudason MR, Nayak S. Selective histopathology in cholecystectomy for gallstone disease. Indian J Gastroenterol. 2010;29(1):32 6.
40. Levy AD, Murakata LA, Rohrmann CA Jr. Gallbladder carcinoma: radiologic pathologic correlation. RadioGraphics. 2001;21(2):295 314.
41. Cross S. Tumors of the gallbladder, extrahepatic biliary ducts, ampulla of Vater. (Book chapter) Histopathology. 2001;39(4):432.
42. Dubey. Carcinoma of gall bladder: demographic and clinicopathological profile in Indian patients. OJ Ionline. 2018;2(1):3 6.
43. Nakakubo Y, Miyamoto M, Cho Y, Hida Y, Oshikiri T, Suzuoki M, et al. Clinical significance of immune cell infiltration in gallbladder cancer. Br J Cancer. 2003;89(9):1736 42..

ISSN (O): 2687-5365; ISSN (P): 2753-6556