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Evaluating msi2 Gene Expression as a Biomarker in Carcinoma Gallbladder

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ABSTRACT

Objectives: Gallbladder carcinoma (GBC) is an aggressive malignancy often diagnosed late, resulting in poor outcomes. Identifying reliable biomarkers is crucial for early diagnosis and prognostication. Musashi-2 (msi2), an RNA-binding protein implicated in oncogenesis, may play a role in GBC progression. To evaluate msi2 gene and protein expression in GBC and correlate it with clinicopathological parameters and tumor grade.
Methods: This prospective observational study included 50 histopathologically confirmed GBC patients and 50 controls with benign gallbladder disease. msi2 expression was analyzed using immunohistochemistry (IHC) and quantitative PCR (qPCR). Clinical data, tumor grade, and histopathology were correlated with msi2 expression. Statistical significance was set at $p < 0.05$.
Results: High msi2 protein expression (>50% IHC positivity) was observed in 70% of GBC cases. Moderate-to-high msi2 RNA expression (51–100%) was seen in 46% of cases. msi2 expression was significantly higher in GBC compared to cholelithiasis ($p < 0.0001$). Poorly differentiated tumors demonstrated the highest msi2 expression, suggesting a correlation with tumor aggressiveness.
Conclusion: msi2 is significantly overexpressed in GBC, correlating with histological grade and malignancy. It may serve as a diagnostic and prognostic biomarker, aiding differentiation from benign lesions..

Keywords: *Gallbladder carcinoma, msi2, Immunohistochemistry, Gene expression, Biomarker.*

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1. INTRODUCTION

Gallbladder carcinoma (GBC) is a highly aggressive and lethal malignancy with poor prognosis, particularly prevalent in northern India and parts of South America and East Asia. It is often diagnosed at an advanced stage due to vague and non-specific early symptoms, making the identification of reliable molecular biomarkers crucial for early detection and improved therapeutic targeting. One emerging molecular player in various cancers is the Musashi-2 (msi2) gene, an evolutionarily conserved RNA-binding protein involved in stem cell maintenance, differentiation, and oncogenic

transformation.

msi2, a member of the Musashi family alongside Musashi-1 (msi1), plays a pivotal role in post-transcriptional gene regulation, influencing mRNA translation, stability, and alternative splicing. Originally studied in neural stem cells, msi2 has been increasingly implicated in the pathogenesis of hematologic malignancies and several solid tumors [1]. Its dysregulation is associated with tumor progression, metastasis, and therapy resistance, highlighting its potential relevance in gallbladder cancer.

In hepatocellular carcinoma (HCC), for example, msi2expression is significantly upregulated in tumor tissues compared to adjacent non-tumorous samples. This overexpression correlates with tumor size, poor differentiation, recurrence, advanced TNM stage, and higher proliferative indices. msi2 expression was also found to independently predict poor survival outcomes, suggesting its role in cancer progression through mechanisms such as epithelial-mesenchymal transition (EMT) and activation of the Wnt/β-catenin signaling pathway [2,3].

Although no study has directly explored msi2 in gallbladder carcinoma, its consistent association with poor prognosis and tumor invasiveness across other gastrointestinal cancers suggests a possible involvement. In gastric cancer, for instance, msi2 expression did not vary significantly between tumor and non-tumor tissue; however, it showed notable differences between tumor grades, especially between Grade I and Grade II, suggesting a potential role in tumor differentiation and progression [4].

In breast cancer, particularly the aggressive subtype known as inflammatory breast cancer (IBC), msi2 is overexpressed and has been shown to confer resistance to chemotherapy and radiotherapy. Knockdown of msi2 in these cells results in decreased proliferation, reduced cancer stem cell markers, increased apoptosis, and diminished tumorigenic capacity. msi2 downregulation was associated with the reversal of stem cell-like properties and increased treatment sensitivity [5].

Similarly, in oral squamous cell carcinoma (OSCC), msi2 expression showed a positive correlation with Cyclin-D1, a key regulator of the cell cycle, further reinforcing its role in cellular proliferation and oncogenesis. While msi2 expression did not directly correlate with survival outcomes in OSCC, its link to cell cycle regulators suggests it could play an indirect role in tumor progression [6].

In the context of bladder carcinoma, msi2 appears to be broadly expressed across different cell lines, with msi1 showing more cancer-specific expression. However, the functional knockdown studies on msi1 highlight a significant increase in apoptosis and stress granule formation, suggesting that the Musashi family as a whole including msi2 plays a multifaceted role in tumor maintenance and survival [7].

Studies also support that high msi2 expression is correlated with poor clinical outcomes in hematologic malignancies such as B-cell acute lymphoblastic leukemia (B-ALL). Here, msi2 overexpression was associated with increased NOTCH1 expression and significantly worse overall and event-free survival, identifying it as an independent prognostic biomarker [8].

Given the stem cell-associated functions of msi2, it is also of interest that studies in testicular tissues have demonstrated its expression during spermatogenesis. Although its expression was not significantly altered in pathological conditions like varicocele, its presence confirms a physiological role in tissue development and cellular renewal [9].

These findings collectively suggest that msi2 may act as a molecular driver in carcinogenesis through multiple mechanisms including stemness maintenance, EMT induction, Notch and Wnt pathway modulation, and cell cycle regulation. Despite the lack of direct studies on msi2 in gallbladder carcinoma, its consistent oncogenic role in closely related cancers such as hepatocellular, gastric, and oral carcinomas provides a strong rationale for investigating its expression and functional significance in GBC. Such studies could yield valuable insights into gallbladder cancer pathogenesis and help develop msi2 as a diagnostic, prognostic, or therapeutic target in Indian clinical settings where the disease burden is high [10].

This study aimed to assess msi2 gene expression in gallbladder carcinoma and its correlation with tumor grade, stage, and clinical outcomes, to evaluate its potential as a diagnostic and prognostic biomarker.

2. MATERIALS AND METHODS

2.1 Study Population

The records and pathological reports of 100 patients newly diagnosed with gallbladder carcinoma (GBC) by histopathology and radiology at Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh, India, were prospectively analyzed from July 2023 to July 2025. Among these, 50 histologically confirmed resected GBC cases were selected for the study based on inclusion criteria. A structured pro forma was used to collect demographic data, disease characteristics, and clinicopathological profiles. Follow-up information was obtained during outpatient visits or telephonic communication with patients or their relatives. The median follow-up duration was 6 months after surgery or biopsy.

50 patients with benign gallbladder disease (cholelithiasis/chronic cholecystitis) undergoing elective cholecystectomy were included as controls after obtaining written informed consent. The study protocol was approved by the Institutional Ethics

Committee.

Inclusion Criteria:

Patients ≥18 years with histologically or radiologically confirmed GBC undergoing surgical resection or biopsy.

Willingness to provide tissue samples and informed consent.

Exclusion Criteria:

Patients with distant metastasis, unresectable disease, or prior history of other malignancies.

Patients refusing consent or with inadequate tissue samples.

Patients with incomplete clinicopathological data.

2.2 Tissue Specimen

Mass tissue specimens from resectable GBC patients and control cholecystectomy specimens were collected intraoperatively. Samples were divided into two parts: one fixed in 10% neutral buffered formalin for histopathology and immunohistochemistry (IHC), and the other immediately snap-frozen in liquid nitrogen and stored at –80 °C for RNA extraction and gene expression analysis.

2.3 Histopathology

Formalin-fixed, paraffin-embedded (FFPE) tissues were sectioned at 4 µm thickness and stained with hematoxylin and eosin (H&E). Histopathological diagnosis and tumor grading were done as per WHO classification into well, moderately, and poorly differentiated adenocarcinomas. Two independent pathologists blinded to clinical data reviewed the slides.

2.4 Immunohistochemistry

FFPE sections were mounted on poly-L-lysine coated slides, deparaffinized with xylene, and rehydrated through graded alcohol. Antigen retrieval was done by heat-induced epitope retrieval using citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 minutes. Slides were incubated with non-immune goat serum to block non-specific binding sites.

Sections were then incubated overnight at 4 °C with primary antibody against msi2 (Rabbit monoclonal antibody, Clone EPR22862-134, Abcam; dilution 1:200). After washing with PBS, slides were treated with HRP-polymer secondary antibody and developed with 3,3′-diaminobenzidine (DAB) chromogen. Counterstaining was done with hematoxylin. Positive and negative controls were included in every batch.

Scoring of IHC:

Five random high-power fields per slide were analyzed. Staining was semi-quantitatively scored based on staining intensity (0 = none, 1 = weak, 2 = moderate, 3 = strong) and percentage positivity. Final immunoreactive score (IRS) was obtained by multiplying intensity and percentage scores, yielding a range of 0–12. Expression was categorized as low (<10%), moderate (10–50%), and high (>50%).

2.5 RNA Extraction and Gene Expression Analysis

Total RNA was extracted from frozen tissue using TRIzol reagent (Thermo Fisher Scientific). RNA purity and integrity were assessed by spectrophotometry (A260/A280 ratio) and agarose gel electrophoresis. Complementary DNA (cDNA) synthesis was performed using a high-capacity cDNA reverse transcription kit (Takara Bio).

Quantitative real-time PCR (qRT-PCR) was carried out using SYBR Green Master Mix (Applied Biosystems) on the ABI 7500 system. GAPDH served as endogenous control. Relative quantification of msi2 expression was performed using the 2^-ΔΔCt method, and results were expressed as fold change relative to control tissues.

2.6 Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY). Continuous variables were expressed as mean ± SD, and categorical variables as percentages. Group comparisons were done using Student's t-test or Chi-square test as appropriate. Correlation between msi2 expression and clinicopathological parameters was assessed using Pearson's correlation coefficient. Survival analysis was performed using Kaplan-Meier method, with log-rank test applied to compare survival curves. $p < 0.05$ was considered statistically significant.

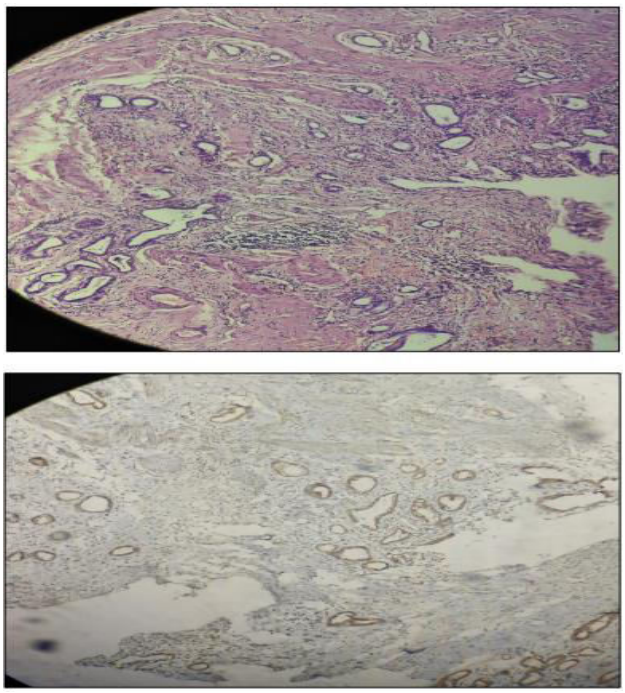


Figure 1: Low msi2 Gene Expression (IHC) in Gall Bladder Carcinoma (less than 10%)

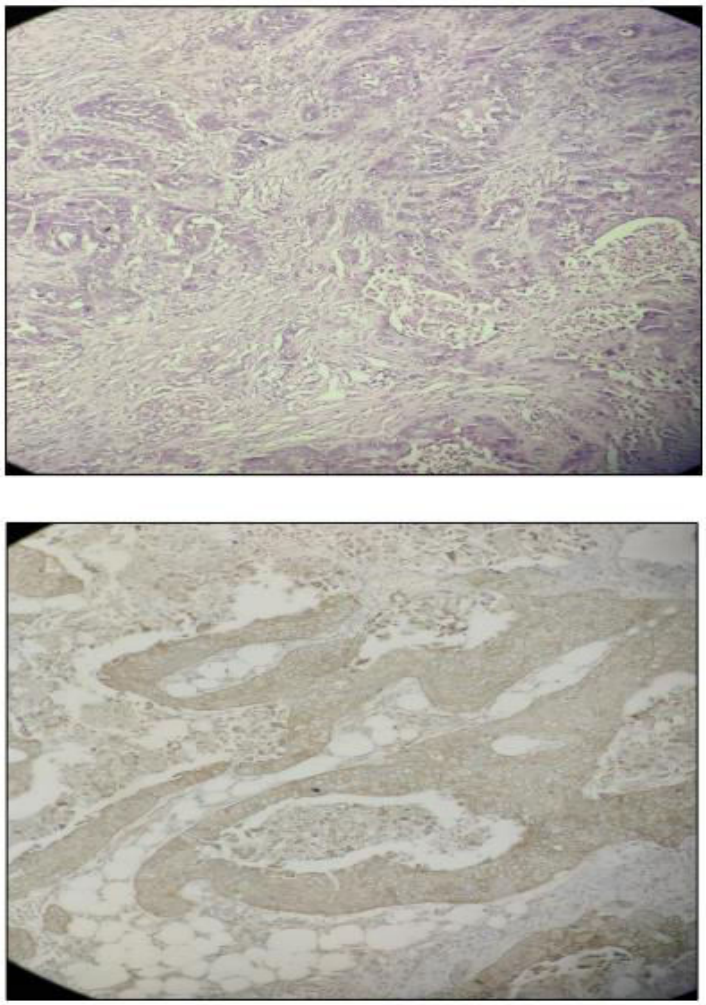


Figure 2: Moderate msi2 Gene Expression (IHC) in Gall Bladder Carcinoma (10%-50%)

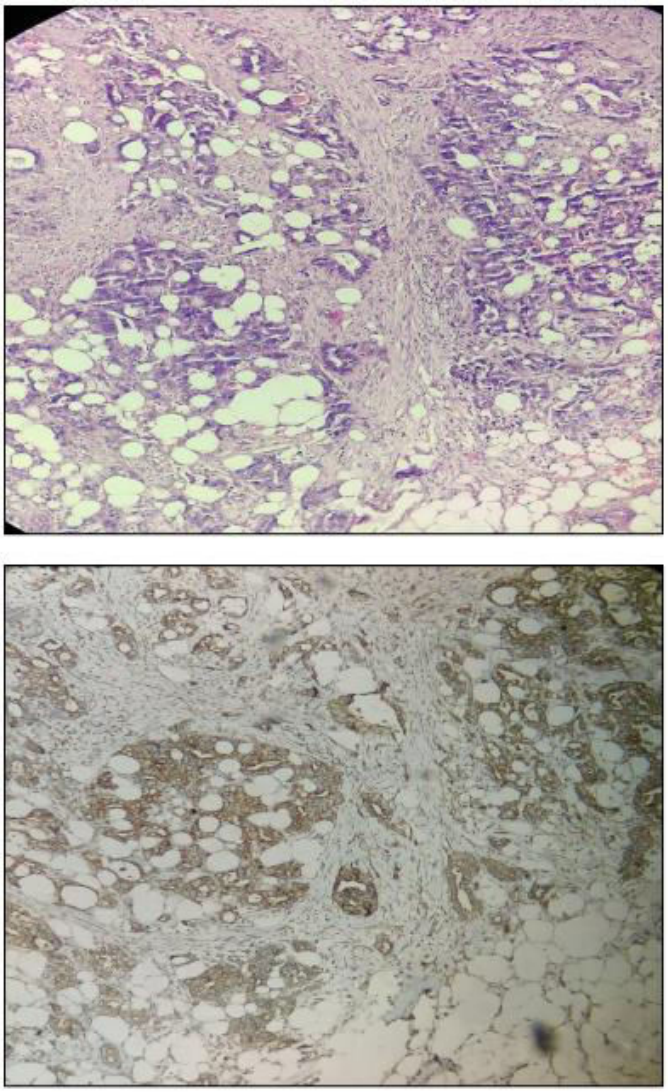


Figure 3: High msi2 Gene Expression (IHC) in Gall Bladder Carcinoma (more than 50%)

3. RESULTS

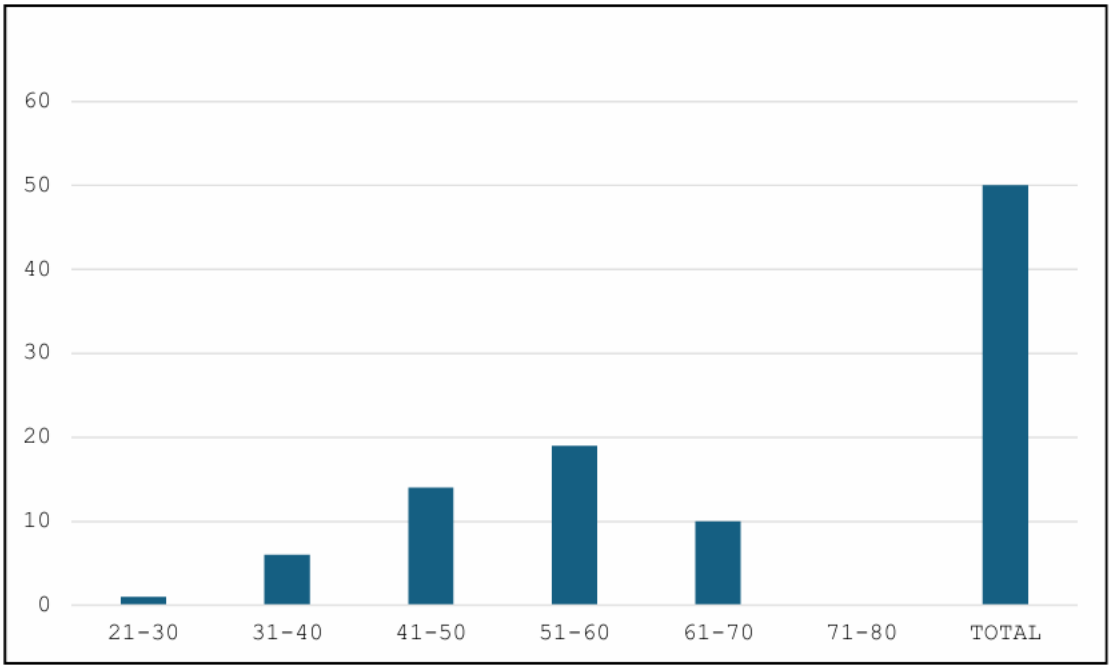
1. Age-wise distribution of gall bladder carcinoma cases

The majority of GBC cases occurred in patients aged 51–70 years, with a mean age of 45.95 years. This suggests a higher prevalence in middle-aged to elderly individuals (Table 1).

Table 1: Age-wise distribution of gall bladder carcinoma cases

Variable	Minimum	Maximum	Mean	Std. Deviation
Age	30	75	45.95	12.91
Age Group (years)	**Number of Patients**		**Percentage (%)**	
21–30	1		2.00%	
31–40	6		12.00%	
41–50	14		28.00%	

51–60	19	38.00%
61–70	10	20.00%
71–80	0	0.00%
Total	50	**100.00%**



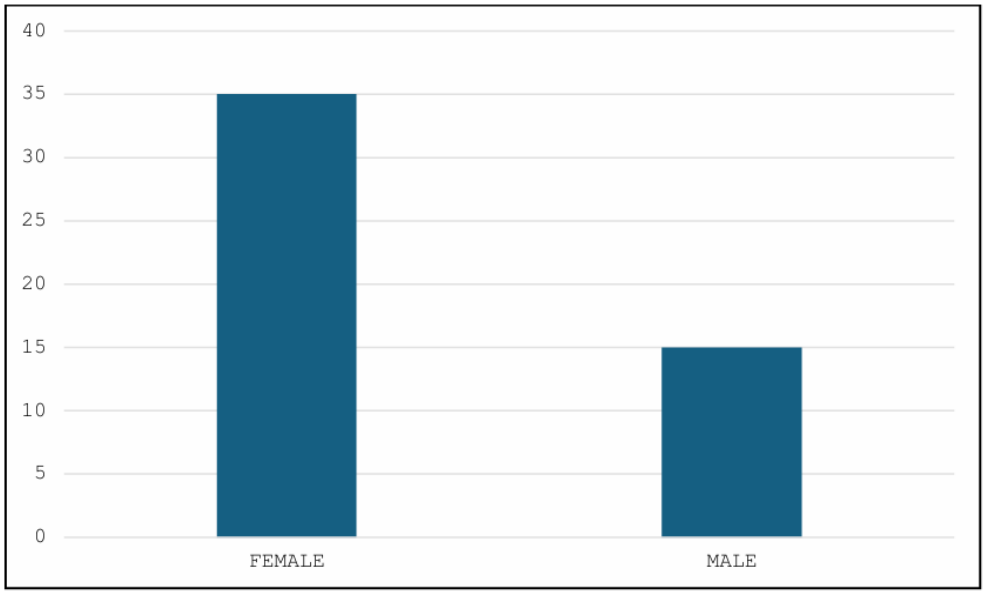
Graph 1: Age-wise distribution of gall bladder carcinoma cases

2. Sex distribution among patients with gall bladder carcinoma

Females accounted for 70% of the cases, indicating a significant female predominance in gall bladder carcinoma (Table 2).

Table 2: Sex distribution among patients with gall bladder carcinoma

Sex	**Number of Patients**	**Percentage (%)**
Female	35	70.0%
Male	15	30.0%
Total	**50**	**100.0%**



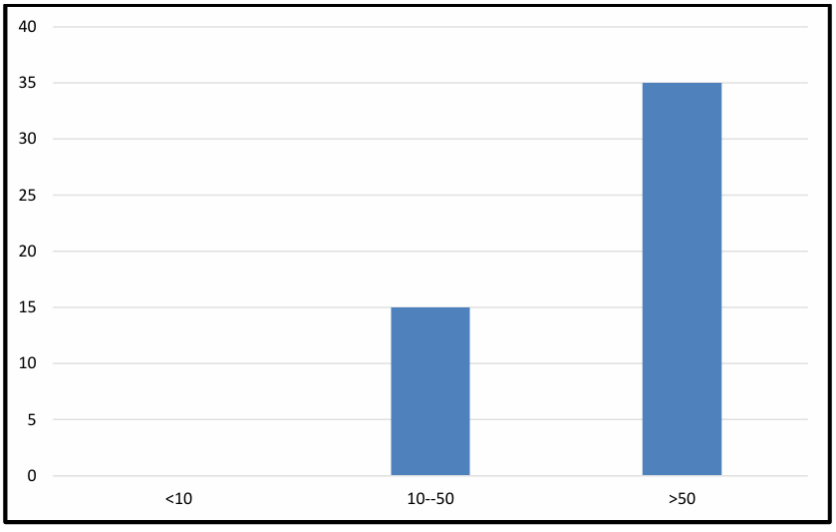
Graph 2: Sex distribution among patients with gall bladder carcinoma

3. msi2 protein expression (IHC) in gall bladder carcinoma

High msi2 expression (>50%) was observed in 70% of carcinoma cases, suggesting a potential role in tumor biology (Table 3).

Table 3: msi2 protein expression (IHC) in gall bladder carcinoma

msi2 IHC Expression Level	Number of Patients	Percentage (%)
<10	0	0.0%
10–50	15	30.0%
>50	35	70.0%
Total	**50**	**100.0%**



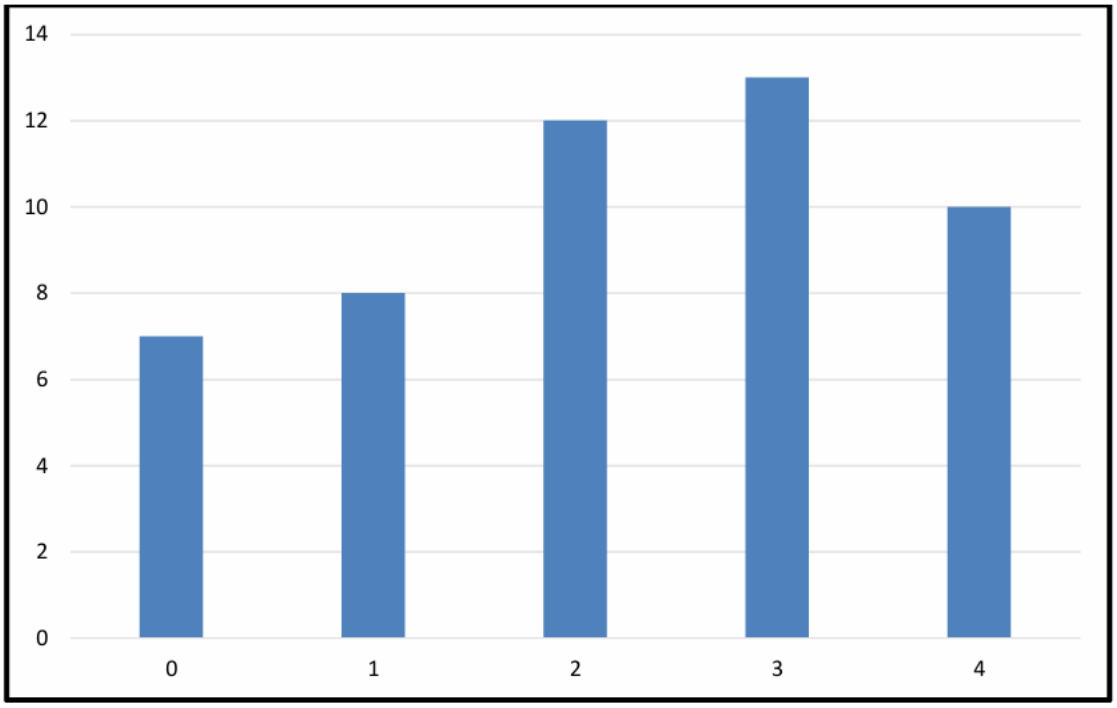
Graph 3: msi2 protein expression (IHC) in gall bladder carcinoma

4. msi2 gene expression (RNA level) among gall bladder carcinoma cases

Over 50% of GBC patients had moderate-to-high msi2 RNA expression, supporting its upregulation in malignancy (Table 4).

Table 4: msi2 gene expression (RNA level) among gall bladder carcinoma cases

msi2 RNA Expression Level	Number of Patients	Percentage (%)
0	7	14.0%
1	8	16.0%
2	12	24.0%
3	13	26.0%
4	10	20.0%
Total	**50**	**100.0%**



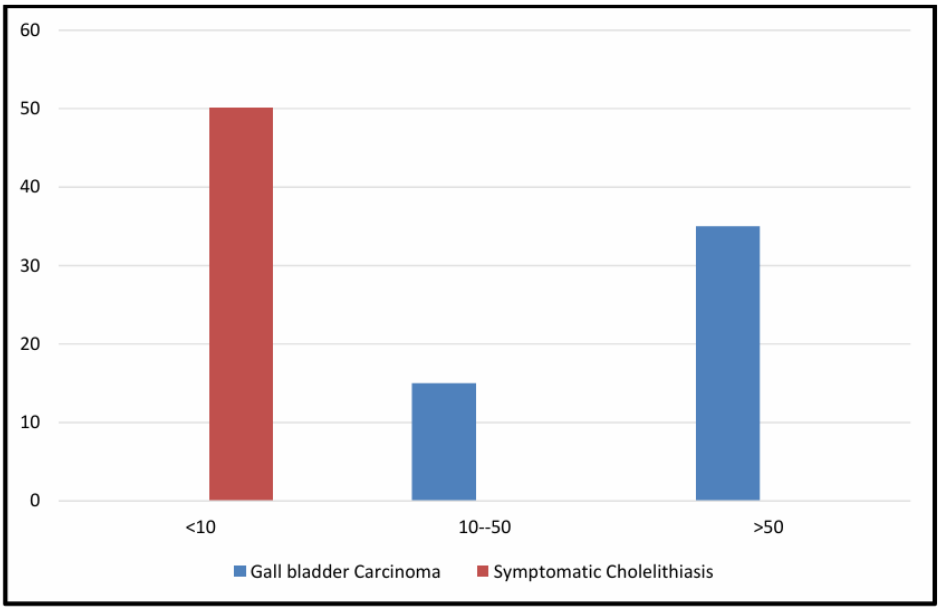
Graph 4: msi2 gene expression (RNA level) among gall bladder carcinoma cases

5. Correlation between msi2 IHC expression and diagnosis

msi2 IHC expression was significantly higher in GBC than in cholelithiasis ($p < 0.0001$), indicating diagnostic relevance (Table 5).

Table 5: Correlation between msi2 IHC expression and diagnosis

msi2 IHC Expression	Gall Bladder Carcinoma	Symptomatic Cholelithiasis	Total	p-value
<10	0	50	50	
10–50	15	0	15	
>50	35	0	35	<0.0001
Total	**50**	**50**	**100**	



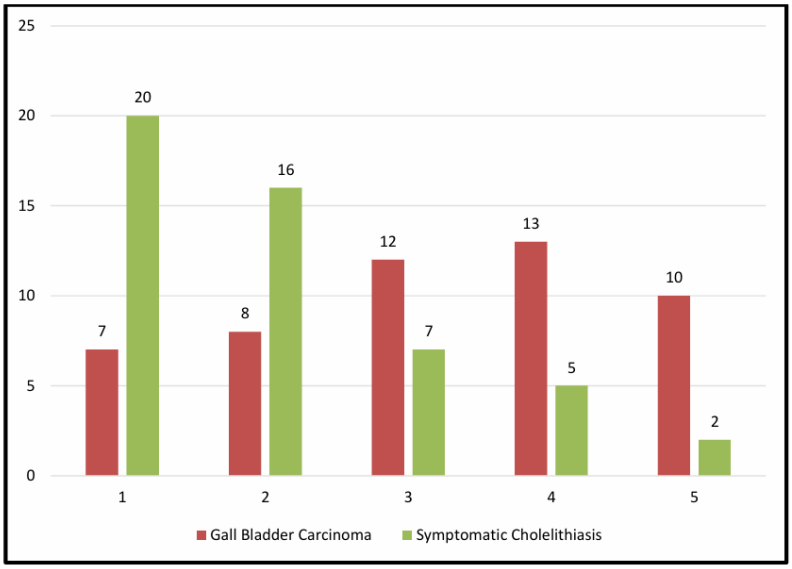
Graph 5: Correlation between msi2 IHC expression and diagnosis

6. msi2 RNA expression by clinical diagnosis

High msi2 RNA expression was significantly more common in GBC compared to cholelithiasis ($p < 0.0001$), reinforcing its tumor specificity (Table 6).

Table 6: msi2 RNA expression by clinical diagnosis

msi2 RNA Expression	Gall Bladder Carcinoma	Symptomatic Cholelithiasis	Total	p-value
0	7	20	27	
1	8	16	24	
2	12	7	19	
3	13	5	18	
4	10	2	12	<0.0001
Total	**50**	**50**	**100**	



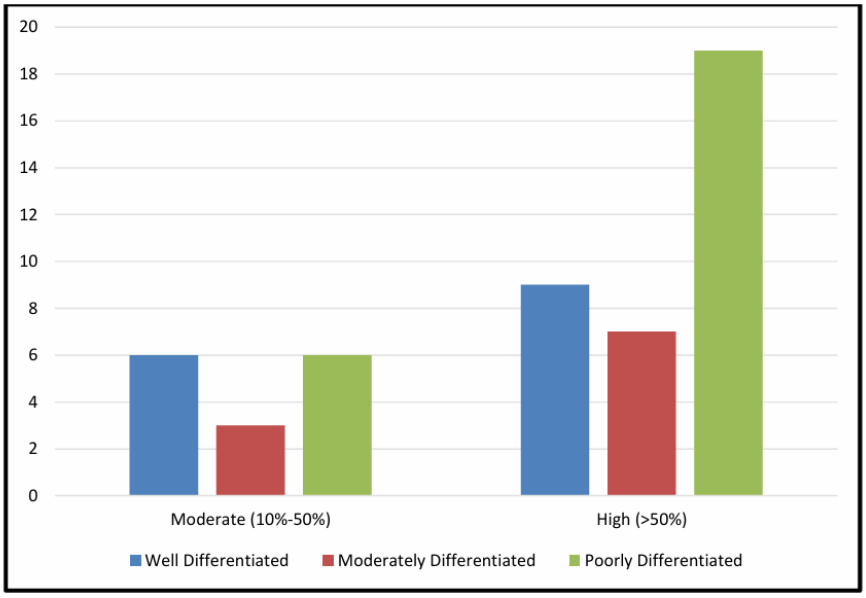
Graph 6: msi2 RNA expression by clinical diagnosis

7. msi2 IHC expression and grading

High msi2 IHC expression was predominantly seen in moderately differentiated tumors, suggesting correlation with tumor grade (Table 7).

Table 7: msi2 IHC expression and grading

Grading	Low (<10%)	Moderate (10%–50%)	High (>50%)
Well Differentiated	0	6	9
Moderately Differentiated	0	3	7
Poorly Differentiated	0	6	19



Graph 7: msi2 IHC expression and grading

4. DISCUSSION

In the present study, msi2 (Musashi-2) gene and protein expression were significantly elevated in gallbladder carcinoma

(GBC) cases compared to controls with symptomatic cholelithiasis. High msi2 expression (>50%) was detected in 70% of patients at the protein level (IHC), and moderate to high RNA expression (51–100%) was observed in over 46% of cases. These findings highlight the probable oncogenic role of msi2 in GBC progression and diagnosis.

Our results align with findings by Liu et al. (2010), who studied the Musashi-1 (msi1) protein and demonstrated significantly higher expression in GBC tissues versus benign gallbladder lesions. Though msi1 and msi2 are homologous RNA-binding proteins, their combined overexpression was associated with poor prognosis and tumor aggressiveness [11]. Our study adds further insight by establishing msi2 specifically as a marker of tumor burden and grade.

Furthermore, msi2 has been well studied in hematological malignancies and solid tumors. For instance, Jiang et al. (2023) demonstrated msi2's ability to promote tumorigenesis in neuroblastoma via MYC-mediated metabolic regulation [12]. Similarly, Siddique et al. (2017) found that msi2 supports oncogenesis through MYC IRES activation and microRNA suppression in liver cancer models [13]. Although not focused on GBC, these studies support the biological plausibility of msi2 acting as an oncogene across malignancies.

No prior study has directly assessed msi2 expression in gallbladder adenocarcinoma. Thus, our research fills a critical gap by correlating msi2 expression with histological grading and clinical diagnosis. A significant correlation was found between msi2 IHC expression and tumor grade, with poorly differentiated tumors showing the highest expression, suggesting its role in tumor aggressiveness. Importantly, msi2 was not expressed in cholelithiasis samples, making it a potential diagnostic marker to differentiate malignant from benign gallbladder conditions. This finding parallels earlier observations that certain stem cell markers like ALDH1 and MSI1 are absent in chronic cholecystitis and benign polyps.

Our study supports msi2 as a novel molecular marker with diagnostic and possibly prognostic implications in GBC. Further studies involving survival correlation, therapeutic targeting, and molecular pathways are warranted to validate msi2 as a candidate for clinical application.

msi2 gene and protein expression were significantly upregulated in gallbladder carcinoma compared to benign controls, with higher levels correlating with poor differentiation and tumor aggressiveness. Its absence in cholelithiasis suggests diagnostic specificity, making msi2 a potential biomarker for distinguishing malignant from benign lesions. These findings highlight msi2 as a promising candidate for prognostication and a potential therapeutic target. Larger multicentric studies and survival analyses are warranted to validate its clinical utility and explore its mechanistic role in gallbladder carcinogenesis.

5. LIMITATIONS OF THE STUDY

The study was limited by its single-center design, relatively small sample size, and short follow-up period, which may restrict generalizability. Molecular correlation was limited to msi2 expression without functional assays, warranting larger multicentric studies with extended survival analysis.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

he datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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