Original Article

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Biochemistry Section

Different Tumour Marker Kinetics as Prognosticators in Patients with Adenocarcinoma of Gall Bladder undergoing Chemotherapy: A Cohort Study

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ABSTRACT

Introduction: Gallbladder Cancer (GBC) is the most common malignancy of the biliary tract worldwide. Patients do not have specific symptoms, and therefore, GBC is usually diagnosed at an advanced stage. Carbohydrate Antigen (CA)-242, CA 19-9, and Cancer Antigen 125 (CA-125) have been found to be effective in the diagnosis and prognosis of GBC.

Aim: To determine the significance of tumour markers as prognosticators in adenocarcinoma of GBC with chemotherapy.

Materials and Methods: The present cohort study was conducted in the Department of Biochemistry, Murshidabad Medical College and Hospital, Baharampur, West Bengal, India, between December 2021 and November 2022. Thirty patients with pathologically confirmed adenocarcinoma of the gallbladder who were treated with gemcitabine-based chemotherapy were included in the study. Differences in serum CA-125 and CA 19-9 levels before and after chemotherapy (KINETICS) were measured along with haemoglobin, serum urea, creatinine, total bilirubin and Neutrophil-Lymphocyte Ratio (NLR). Receiver Operating Characteristic (ROC) curve analysis and Kaplan-Meier analyses of CA-125, CA 19-9, and combined changes were performed to assess survival rates and their statistical relevance as prognosticators in correlation with disease progression.

Results: The mean±Standard Deviation (SD) age was 52.4±5.275 years. A total of 25 (83.3%) patients were females. The associations of CA 19-9 change with other baseline variables were analysed, and there were significant associations with baseline Carbohydrate Antigen (CA19-9) levels (p-value=0.003) and metastatic changes (p-value <0.001). Patients with decreased tumour markers had significantly better Progression-Free Survival (PFS) than patients with increased tumour markers. The pre- and post-chemotherapy CA 19-9 ratio had the highest area-under-the-curve values (AUC 0.910) for predicting 6-month PFS, whereas for predicting metastatic changes, the combined pre- and post-chemotherapy marker ratio had the highest AUC 0.842. In the multivariate analysis, CA 19-9 ≥100 U/mL at diagnosis (p-value <0.001) and NLR_{change} ≥1.0 (p-value=0.003) were independent prognosticators of PFS, with hazard ratios of 1.007 (p-value <0.001) and 15.4 (p-value=0.003), respectively.

Conclusion: CA 19-9 kinetics proved to be a reliable prognosticator of metastatic changes in patients with adenocarcinoma of the gallbladder undergoing chemotherapy. The significant decline in tumour markers that were seen throughout the follow-up phase emphasises how important it is to use these markers as primary indicators of treatment response. To validate the present study findings, larger-scale studies involving a longer follow-up period and a greater number of patients are needed.

Keywords: Gallbladder cancer, Metastasis, Prognostic marker, Serum carbohydrate antigen

INTRODUCTION

Gallbladder cancer is the most common malignancy of the biliary tract (80.0-95.0% of biliary tract cancers) and the fifth most common gastrointestinal malignancy worldwide. Most gallbladder carcinomas are adenocarcinomas. About 5.0% have squamous cell carcinoma or adenosquamous differentiation [1]. The main factors that increase the chance of developing GBC are gallstone disease, gallbladder wall calcification, gallbladder polyps >10 mm, primary sclerosing cholangitis, pancreaticobiliary junction abnormality, smoking and obesity. Large differences in the incidence of GBC across geographic, ethnic, and cultural differences suggest a role for genetic and environmental factors, some of which are potentially modifiable [2].

Patients do not have specific symptoms, and therefore, GBC is usually diagnosed at an advanced stage. A poor prognosis corresponds to a large proportion of patients with advanced disease and ineffective treatment strategies. A more aggressive surgical approach is warranted in patients with early-stage disease. Only 20.0% of patients are candidates for curative surgery. Indeed, the global 5-year survival rate for GBC is less than 15.0% [3]. In contrast to South and West India, the most common areas of gallbladder disease are North, East, North East and Central India [4].

Tumour markers are a key tool in the field of oncology because they act as biochemical indicators that help identify tumours. These markers can be present in abnormal concentrations in the body when a tumour is present. They can be produced either by neoplastic cells themselves or by normal tissues in response to the presence of a tumour [5]. What makes tumour markers particularly valuable is that they offer a less invasive and less harmful way to detect and monitor cancer than other diagnostic methods such as radiological imaging or endoscopic procedures. This non invasive nature makes them very useful for patients and doctors. Several tumour markers are used not only in diagnosis but also in prognosis [6].

It is important to note that although tumour markers play an essential role in the diagnosis and treatment of cancer, they are not exclusive to cancer and can be elevated in non cancerous conditions, as well. Therefore, the interpretation of tumour marker results requires careful consideration and correlation with clinical findings and

other diagnostic methods. Serial assessment of tumour marker levels during the follow-up period and the resulting pattern can be correlated with the response to chemotherapy [7].

Gemcitabine plus Cisplatin (GEMCIS), the combination chemotherapy widely used as first-line treatment for unresectable GBC based on a recent clinical trial showing favourable outcomes of the combination chemotherapy. Gemcitabine-based adjuvant chemotherapy has also been found to improve the prognosis in patients who underwent radical resection of GBC [8,9]. Some tumour markers, such as Carcinoembryonic Antigen (CEA), CA-242, CA 19-9, and CA-125, have been found effective in the diagnosis and prognosis of GBC [10]. The authors worked with only two parameters due to availability and funding constraints.

The antigen CA 19-9, widely used as a serum marker for pancreatic ductal adenocarcinoma, is a mucin-type glycoprotein expressed on the surface of pancreatic cancer cells. The CA 19-9 epitope is normally present within the biliary tree. CA-125, also known as MUC16, is a carbohydrate epitope on a glycoprotein carcinoma antigen and is most commonly used as a tumour marker for ovarian carcinoma [11].

Previous reports in the literature have discussed the significance of either CA-19-9 or CA-125 levels in the prognosis of GBC. Confusion is also caused by differences in the prognostic cut-off values found in various research studies [6,12]. Furthermore, no research has been done to determine the prognosis by comparing the levels of serum tumour markers with the results of a Computed Tomography (CT) scan. Therefore, these markers will be assessed as prognosticators in this tertiary care setup in West Bengal. The objective of the present investigation was to ascertain the predictive significance of CA 19-9 and CA-125 in GBC during the course of chemotherapy.

MATERIALS AND METHODS

The present cohort study with a prospective study design was conducted in the Department of Biochemistry, Murshidabad Medical College and Hospital, Baharampur, West Bengal, India, between December 2021 and November 2022. Patients with pathologically confirmed GBC, who were treated at the study Institution were included in the study. All patients were followed-up until November 30, 2022. Before commencing data collection, approval for the research project was obtained from the Institutional Ethics Committee (Registration No. ECR/1620/Inst/WB/2021 under CDSCO). Informed consent was obtained from every participant before their participation.

Incluision criteria: Patients aged 40-70 years with histopathologically/cytology-proven carcinoma of the gallbladder, receiving gemcitabine-based chemotherapy, and who underwent at least three cycles of chemotherapy with baseline and post-chemotherapy tumour marker records were included in the study [13].

Exclusion criteria: Patients who were pregnant or had other diagnosed malignant tumours, Eastern Cooperative Oncology Group (ECOG) performance status [14] grade 4 and 5, a history of systemic chemotherapy or immunosuppressive disorders, and patients requiring Endoscopic Retrograde Cholangio-pancreatography (ERCP) were excluded from the study [13].

Study Procedure

In total, 30 patients were enrolled and analysed. Patient characteristics such as age, sex and ECOG performance status were obtained. Variables in tumour characteristics, including cancer stage {according to the standard Tumour Node Metastasis (TNM) staging system} and distant metastasis, were collected [3]. With strict adherence to aseptic precautions, a total of 4 mL of fasting venous blood was meticulously collected using a syringe. It was equally aliquoted into red and lavender vacutainers. Samples were refrigerated at 2-8°C if not assayed within eight hours or frozen at -20°C if not assayed within 48 hours. Laboratory variables included haemoglobin, serum urea, creatinine, total bilirubin; CA-125 (reference range <35 U/mL), CA 19-9 (reference range <37 U/mL), and Neutrophil Lymphocyte Ratio (NLR), which were measured at baseline. NLR values were defined as the number of absolute neutrophils divided by the absolute lymphocyte count from samples of peripheral blood.

Each cycle of combination chemotherapy consisted of cisplatin and gemcitabine administered intravenously on days 1 and 8 every three weeks. Serum CA-125, CA 19-9 levels, and NLR values at baseline and post-chemotherapy were evaluated. Baseline levels were measured before chemotherapy initiation ($CA\text{-}125_{pre}$ and $CA\ 19\text{-}9_{pre}$). Post-chemotherapy levels were measured 21 days after the end of the third cycle of chemotherapy ($CA\text{-}125_{post}$ and $CA\ 19\text{-}9_{post}$). Serum CA-125 and CA 19-9 were measured using a commercially available chemiluminescence immunoassays (Advia Centaur CP, Siemens).

Data on PFS were collected. PFS is defined as the time from initial treatment to the confirmation of disease progression or death. The best overall tumour response was assessed by contrast-enhanced CT scan based on the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria. The minimum duration for the definition of stable disease was one month, and tumour response was evaluated at intervals of three cycles. Tumour marker kinetics were defined as individual tumour marker kinetics and combined tumour marker kinetics derived as following [15]:

$$CA\ 19\text{-}9_{change}=\frac{CA\ 19\text{-}9_{post}}{CA\ 19\text{-}9_{pre}} \text{ and } CA\text{-}125_{change}=\frac{CA\text{-}125_{post}}{CA\text{-}125_{pre}}$$

$$COMB_{change}=CA19\text{-}9_{change}\times CA\text{-}125_{change}$$

STATISTICAL ANALYSIS

The data were entered into Microsoft Excel (version 2019) and then analysed using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) software version 29.0.0 (IBM Corp., Armonk, NY, USA) and MedCalc version 20.116. The categorical variables were described as number and percentage, and the Pearson's Chi-square test was applied to determine differences between them. Continuous variables were reported as mean and SD, if normally distributed, or median and Interquartile Range (IQR), if non normally distributed. The median, first and third quartile values of the kinetic parameters were calculated. ROC curve analysis of $CA\text{-}125_{change}$, $CA\ 19\text{-}9_{change}$ and $COMB_{change}$ was performed to assess metastatic change and disease progression. The cut-off value for the highest sum of sensitivity and specificity was used for further analyses. Kaplan-Meier analyses were performed for survival evaluation. The log-rank test was used to assess the relationships between tumour marker kinetic parameters and PFS. Trend analysis of variables CA 19-9, CA-125, and NLR was done for each patient individually in Minitab, and a trend equation was derived for each patient for each parameter. Spearman's Rank correlation coefficients have been calculated between Response Evaluation Criteria in Solid Tumours (RECIST) scores and the slopes of trend equations in Minitab and R software version 4.2.1. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Patient demographics: Baseline patient characteristics of a total of 30 cases are listed in [Table/Fig-1]. The mean±SD age was 52.4±5.275 years (range: 44-67 years). A total of 12 (40%) patients had metastasis at diagnosis, whereas 15 (50%) patients had unresectable disease due to extensive liver invasion or regional lymph node metastasis. The median serum CA-125 and CA 19-9 levels were 11 U/mL (range: 4.5-426.2 U/mL) and 102.7 U/mL (range: 8.26-2458 U/mL), respectively. The mean NLR (mean±SD) was 2.5±0.441. The median PFS was six months (range 2-12 months). Following three months of chemotherapy, three patients had a complete response, whereas 11 patients had progressive disease.

Characteristics	Value (N=30)*
Age (years)	
<55	20 (66.7)
≥55	10 (33.3)
Gender	
Male	5 (16.7)
Female	25 (83.3)
CA19-9 (U/mL)	
<100	14 (46.7)
≥100	16 (53.3)
CA-125 (U/mL)	
<11	13 (43.3)
≥11	17 (56.7)
NLR	
<2.5	15 (50.0)
≥2.5	15 (50.0)
Postoperative	
Yes	15 (50.0)
No	15 (50.0)
Metastasis	
Yes	12 (40)
No	18 (60)
Metastatic change	
Yes	8 (26.7)
No	22 (73.3)
Best response to chemotherapy	
Complete Response (CR)	3 (10)
Partial Response (PR)	8 (26.7)
Static Disease (SD)	8 (26.7)
Progressive Disease (PD)	11 (36.7)
PFS, months	6.13±3.391
CA 19-9^{change}	0.34 (0.23-4.58)
CA-125^{change}	0.55 (0.30-1.80)
COMB^{change}	0.14 (0.08-6.94)
NLR^{change}	0.97±0.173

[Table/Fig-1]: Baseline characteristics of eligible patients.
*Data presented as n (%) or median (range) or mean±SD; PFS: Progression free survival; COMB^{change}: Combined tumour marker kinetics

Prognostic value of CA 19-9$_{change}$, CA-125$_{change}$ and COMB$_{change}$: In the ROC analysis, the areas under the curve of the CA 19-9$_{change}$, CA-125$_{change}$ and COMB$_{change}$ for predicting 6-month PFS were 0.910, 0.871 and 0.873, respectively, whereas those for metastatic changes were 0.805, 0.819 and 0.842, respectively [Table/Fig-2a,b].

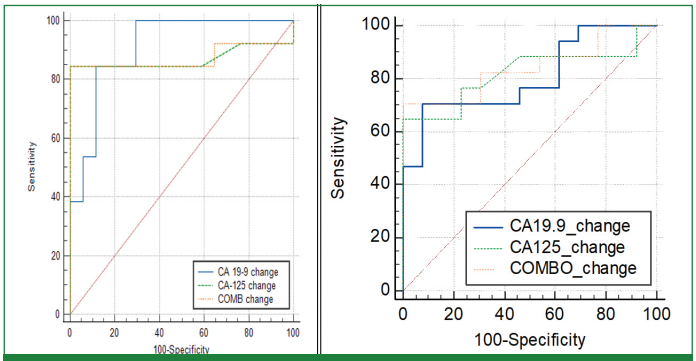
The relationships between tumour marker kinetics and survival are shown in [Table/Fig-2a]. Although all three parameters are significantly associated with PFS, the AUC of CA19-9$_{change}$ was maximum (0.910) among them. However, in predicting metastatic change, the AUC of COMB$_{change}$ was maximum and significantly associated. Survival curves according to tumour kinetic parameters using a cut-off value of 1.0 showed that the mean PFS was 8.41 and 3.15 months in patients with a CA 19-9$_{change}$ of <1 and ≥1, respectively (p-value <0.001). The mean PFS was 8.21 and 2.55 months in those with a CA-125$_{change}$ of <1 and ≥1, respectively (p-value <0.001). The mean PFS was 8.41 and 3.15 months in patients with a COMB$_{change}$ of <1 and ≥1, respectively (p-value <0.001). The mean PFS was 7.29 and 3.44 months in patients with NLR$_{change}$ of <1 and ≥1, respectively (p-value <0.001). CA 19-9 change was the most valuable prognostic marker. Kaplan-Meier analyses according to CA 19-9$_{change}$, CA-125$_{change}$, and COMB$_{change}$, NLR change cut-off value of 1.0 are shown in [Table/Fig-3,4]. Although patients with CA 19-9$_{change}$ <1.0, CA-125$_{change}$ <1.0, and COMB$_{change}$ <1.0 had significantly better survival, patients with a CA-125$_{change}$ ≥1.0 had significantly worse survival compared to other changes.

CA 19-9$_{change}$ as a predictor of response to chemotherapy: Responses after three cycles (rather than 3 months due to the variety of chemotherapy regimens) of chemotherapy were assessed according to the modified Response Evaluation Criteria in Solid Tumours (version 1.1). Three (10.0%) patients had a complete response, eight (26.7%) patients had a partial response, 8 (26.7%) had stable disease, and 11 (36.7%) showed Progressive Disease (PD). A high CA 19-9$_{change}$ and CA-125$_{change}$ were associated with PD (p-value <0.001), but a low CA 19-9$_{change}$ or CA-125$_{change}$ was not associated with a complete response (p-value=0.110, p-value=0.165, respectively). Also, a low CA-125$_{change}$ was associated with stable disease (p-value=0.012). Three patients with a CA 19-9$_{change}$ >10.0 showed PD after three cycles of chemotherapy. In the current study, Spearman's and Kendall's rank correlation coefficients were derived, establishing the presence of a positive correlation [Table/Fig-5].

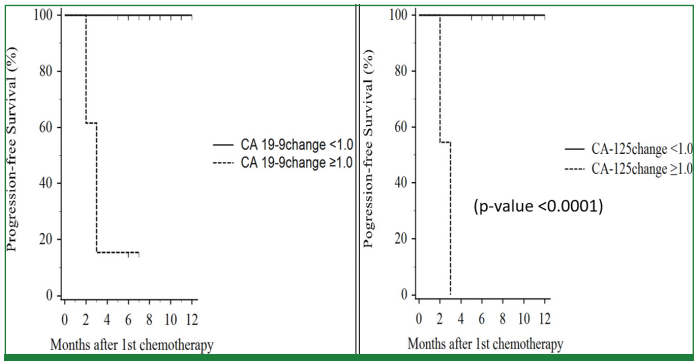
Prognostic value of CA 19-9$_{change}$ associated with PFS: The associations of CA 19-9 changes and CA-125 changes with other

Variables	PFS			Metastatic changes		
	CA 19-9$_{change}$	CA-125$_{change}$	COMB$_{change}$	CA 19-9$_{change}$	CA-125$_{change}$	COMB$_{change}$
AUC	0.910	0.871	0.873	0.805	0.819	0.842
Youden index	0.7285	0.8462	0.8462	0.6290	0.6471	0.7059
95% CI	0.747-0.983	0.698-0.965	0.701-0.966	0.621-0.926	0.636-0.935	0.663-0.948
p-value	<0.0001	<0.0001	<0.0001	0.0002	0.0001	<0.0001

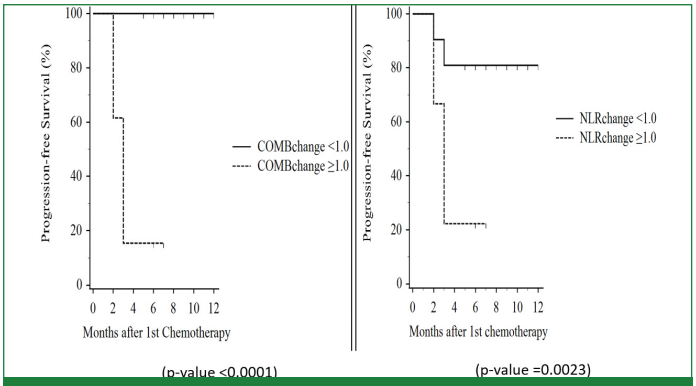
[Table/Fig-2a]: Area Under Curve (AUC) of tumour marker kinetics.
*DeLong ER et al., 1988 [16]; Binomial exact; The p-value <0.05 was considered statistically significant



[Table/Fig-2b]: Receiver Operating Characteristic (ROC) curve analysis of tumour marker kinetics as predictor of survival and predictors of metastatic change.



[Table/Fig-3]: Kaplan-Meier plot of Progression-Free Survival (PFS) according to serum CA 19-9 and serum CA-125 using a cut-off value of 1.0.



[Table/Fig-4]: Kaplan-Meier plot of Progression-Free Survival (PFS) according to combination change NLR changes using a cut-off value of 1.0.

Statistical analysis	CA 19-9	CA-125	NLR
Spearman's rank correlation coefficient	0.7597357	0.5846986	0.8200553
Kendall's rank correlation coefficient	0.61807	0.42289	0.6973018

[Table/Fig-5]: Spearman's and Kendall's Rank correlation coefficient of prognostic indicators with RECIST score.

baseline variables were analysed [Table/Fig-6]. A CA 19-9 level ≥100 U/mL before starting chemotherapy (p-value=0.003) and metastatic change (p-value <0.001) were significantly associated with increased CA 19-9 changes. Also, a CA-125 level <11 U/mL before starting chemotherapy was significantly associated with decreased CA-125 changes (p-value <0.001). Univariate analysis revealed that age ≥55 years (p-value=0.023), CA 19-9 ≥100 U/mL at diagnosis (p-value 0.003), CA-125 ≥12 U/mL at diagnosis (p-value <0.001), metastatic change after chemotherapy (p-value <0.001), CA 19-9_{change} ≥1.0 (p-value <0.001), CA-125_{change} ≥1.0 (p-value <0.001), $COMB_{change}$ ≥1.0 (p-value <0.001), and NLR_{change} ≥1.0 (p-value <0.001) had a p-value of <0.05 for PFS. However, CA 19-9 ≥100 U/mL at diagnosis (p-value <0.001) and NLR_{change} ≥1.0 (p-value=0.003) were independent prognosticators of PFS [Table/Fig-7].

Variables	CA 19-9 changes		p-value	CA-125 changes		p-value
	Decrease	Increase		Decrease	Increase	
Age (years)						
<55	11	9	0.794	11	9	0.180
≥55	6	4		8	2	
Gender						
Male	4	1	0.249	4	1	0.397
Female	13	12		15	10	
CA 19-9 (U/mL)						
<100	12	2	0.003	14	0	<0.001
≥100	5	11		5	11	
CA-125 (U/mL)						
<11	9	4	0.225	11	2	0.034
≥11	8	9		8	9	
NLR						
<2.5	6	9	0.065	8	7	0.256
≥2.5	11	4		11	4	
Postoperative						
Yes	6	9	0.0701	11	4	0.256
No	11	4		8	7	
Metastasis						
Yes	9	3	0.098	10	8	0.279
No	8	10		9	3	
Metastatic change						
Yes	0	8	<0.001	19	3	<0.001
No	17	5		0	8	
Best response						
CR	3	0	<0.001	3	0	<0.001
PR	8	0		8	0	
SD	6	2		8	0	
PD	0	11		0	11	

[Table/Fig-6]: Associations between changes in CA 19-9, CA-125 and other variables. The p-value <0.05 was considered statistically significant

Variables	Univariate			Multivariate		
	n	PFS	p-value	HR	95% CI	p-value
Age (years)						
<55	20	5.05±2.819	-	-	-	-
≥55	10	8.30±3.529	0.023	0.632	0.317-1.260	0.193
Gender						
Male	5	8.60±3.975	-	-	-	-
Female	25	5.64±3.121	0.177	-	-	-
CA 19-9 (U/mL)						
<100	14	8.00±2.512	0.003	1.007	1.000-1.013	<0.001
≥100	16	4.50±3.266				
CA-125 (U/mL)						
<11	13	8.46±3.126	<0.001	1.035	0.988-1.085	0.152
≥11	17	4.35±2.396				
NLR						
<2.5	15	6.07±3.788	0.185	-	-	-
≥2.5	15	6.20±3.075		-	-	-
Postoperative						
Yes	15	5.87±3.815	0.277	-	-	-
No	15	6.40±3.019		-	-	-
Metastasis						
Yes	12	5.92±2.999	0.771	-	-	-
No	18	6.28±3.707		-	-	-
Metastatic change						
Yes	8	2.63±0.51	<0.001	38668.8	0.276-5.42+9	0.081
No	22	7.41±3.06				
Best response						
CR+PR+SD	19	8.21±2.44	<0.001	3.84E+009	3.91-151-37.77+168	0.9067
PD	11	2.55±0.52				
CA 19-9_{change}						
<1.0	17	8.41±2.50	<0.001	0.149	0.012-1.893	0.142
≥1.0	13	3.15±1.57				
CA-125_{change}						
<1.0	19	8.21±2.440	<0.001	0.242	0.38-1.542	0.133
≥1.0	11	2.55±0.522				
$COMB_{change}$						
<1.0	17	8.41±2.501	<0.001	1.181	0.962-1.448	0.111
≥1.0	13	3.15±1.573				
NLR_{change}						
<1.0	21	7.29±3.273	<0.001	15.4E+009	0.47-497.22+18	0.003
≥1.0	9	3.44±1.810				

[Table/Fig-7]: Prognosticators of PFS in patients with adenocarcinoma of gallbladder. The p-value <0.05 was considered statistically significant

DISCUSSION

Tumour markers are tissue or molecular-based processes that provide insight into how cancer may behave in the future. The most widely used serum tumour markers for GBC diagnosis and prognosis are CA 19-9 and CA-125. When combined, they outperform each other in terms of prognostic prediction [1,17]. The sensitivity of CA 19-9 and CA-125 gradually increases with the progression of the disease stage [10]. The current study's findings were in line with those of the earlier investigation, which demonstrated that metastatic

illness increases the sensitivity of CA 19-9 and CA-125. For the diagnosis and prediction of advanced GBC, CA 19-9 showed the best sensitivity, followed by CA-125, which is in line with the findings of the prior study [10,11].

The present primary objective was to evaluate the prognostic significance of serum tumour marker kinetics following chemotherapy in patients diagnosed with unresectable GBC. The study's findings have provided crucial insights, indicating that changes in tumour markers after the initial two cycles of chemotherapy independently predict patient survival. Serum CEA, serum CA 19-9, or combinations of the two were valuable prognosticators. The gallbladder's perimuscular connective tissue is in direct contact with the liver, and there is no serosal layer covering it in the vicinity of the liver. As a result, GBC invasion of the liver occurs frequently, and hepatic involvement is recognised to be linked to a harmful prognosis [1,18]. Yu T et al., examined the changes in serum CA 19-9 and CEA following GBC resection and found that these changes were independent predictors of poor survival [19].

By combining serum tumour marker kinetics, postoperative pathology results, and other pertinent information, the authors were able to effectively stratify postsurgical patients with incurable GBC into discrete prognostic groups, which facilitated subsequent decision-making. Patients with unresectable GBC receive palliative treatment, and unlike those with resectable GBC, they may experience a wide range of tumour responses. Hence, accurate and valid assessments of these responses become paramount in guiding subsequent treatment decisions.

The goal of the current study was to determine criteria for early imaging follow-up or chemotherapy withdrawal based on changes in tumour markers and the association between the serum markers and chemotherapy response.

In their article, Wang YF et al., found that individuals with lymph node metastases had serum CA-125 levels that were noticeably higher than those without the metastasis. They concluded that CA-125 was helpful in prognostic prediction, monitoring recurrence and evaluating LN metastasis [10].

According to Sachan A et al., patients with metastatic cancer had a substantially higher median score for CA19-9 than patients with either resectable or unresectable illness (79 vs 53.9 vs 21.35 IU/mL; p-value <0.001) and a cut-off value of 72 IU/mL for CA19-9 in predicting metastatic disease [12].

In the ROC analysis, the areas under the curve of the CA 19-9_{change}, CA-125_{change} and $COMB_{change}$ for predicting 6-month PFS were 0.910, 0.871, and 0.873, respectively. Though all three parameters are significantly correlated with PFS, the AUC of CA19-9_{change} was the maximum (0.910) among them. However, in predicting metastatic change, the AUC of $COMB_{change}$ was the maximum and significantly correlated.

Survival curves according to tumour kinetic parameters using a cut-off value of 1.0 are shown in [Table/Fig-2,3]. The mean PFS was 8.41 and 3.15 months in patients with a CA 19-9_{change} of <1 and ≥1, respectively (p-value <0.001). The mean PFS was 8.21 and 2.55 months in those with a CA-125_{change} of <1 and ≥1, respectively (p-value <0.001). The mean PFS was 8.41 and 3.15 months in patients with a $COMB_{change}$ of <1 and ≥1, respectively (p-value <0.001). The mean PFS was 7.29 and 3.44 months in patients with NLR_{change} of <1 and ≥1, respectively (p-value <0.001).

The antigen CA 19-9_{change} was the most valuable prognostic marker. Kaplan-Meier analyses according to CA 19-9_{change}, CA-125_{change}, $COMB_{change}$ and NLR_{change} with a cut-off value of 1.0 are shown in [Table/Fig-3,4].

Although patients with CA 19-9_{change} <1.0, CA-125_{change} <1.0 and $COMB_{change}$ <1.0 had significantly better survival, patients with a CA-125_{change} ≥1.0 had significantly worse survival compared to other changes. These equivocal results led us to conduct univariate and multivariate analyses to detect the most valuable prognosticator.

The authors also derived Spearman's rank correlation coefficient between CA 19-9 with RECIST score, CA-125 with RECIST score, and NLR with RECIST score, with their values being 0.759, 0.585 and 0.820, respectively. The authors related the prognosis of the patients to the trend of changing indicators and established the presence of a positive correlation.

Furthermore, in one study, the neutrophil-to-lymphocyte ratio and CEA were associated with poor OS of GBC patients [20]. Lee JW et al., assessed CEA and CA 19-9 levels post-chemotherapy and found CA19-9 to be the independent and most valuable prognosticator [15]. Yu T et al., who investigated CA 19-9 and CEA as independent prognostic markers in resectable GBC, obtained similar results [18]. On the other hand, Agarwal A et al.,'s research revealed inconsistent findings, suggesting that elevated blood levels of CA 19-9 and CA 242 were associated with a worse median survival rate, though not to the point of statistical significance [21].

Limitation(s)

There were certain restrictions in the present study investigation. The study used an observational, non randomised design with a small sample size and a brief follow-up period of just 12 months. In order to identify early signs of malignancy and metastatic alterations by evaluating various tumour indicators in nations with limited resources, the study covered both treatable and incurable cases. To validate the present findings, larger-scale studies involving a longer follow-up period and a greater number of patients are needed.

CONCLUSION(S)

The combined use of the tumour markers CA 19-9 and CA-125 was found to be an independent predictor of the advanced stage of GBC in this investigation. However, none of these tumour markers' cut-off values were statistically significant for identifying metastatic GBC. CA19-9 and CA-125 were more frequently used to predict advanced stages of GBC. The significant decline in tumour markers that was observed throughout the follow-up phase emphasises the importance of using these markers as primary indicators of treatment response. It is expected that this strategy will help in prompt detection and raise the survival rates of those affected.

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