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Original Research Article

Utility of triple tumor markers CA19-9, CA125 and CEA in predicting advanced stage of carcinoma gallbladder: a retrospective study

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ABSTRACT

Background: A combination of serum tumor markers are used in the evaluation and prognosis of carcinoma gallbladder (GBC). Aim of the study was to find the significance of combined use of CA19-9, CA125 and CEA in advanced stage of GBC and to find the cut-off value of each of these tumor markers in metastatic GBC.
Methods: This was a retrospective observational cohort study over 1 year, which was carried out in 42 cases of advanced GBC. The patients were grouped in to locally advanced and metastatic stage on the basis of CECT scan findings. CA19-9, CA125 and CEA were assayed in all patients. These tumor markers were analysed with these two groups of GBCs. Statistical analysis was performed using R statistical software v3.6.2.
Results: Out of 42 cases CA19-9 was elevated in 18 (78%), CA125 in 16 (70%) and CEA in 9 (39%) patients with metastatic disease. The cut-off value of CA19-9, CA125 and CEA was determined by ROC curve were >109 U/ml, 55.4 U/ml and 2.56 µg/l respectively. CA19-9 had the highest sensitivity 78.3% followed by CA125 69.6% and CEA has the highest specificity 68.4% for the diagnosis of metastatic stage of the disease. Specificity of these tumor markers were highest when used in combination.
Conclusions: Combined use of triple tumor markers increases its specificity in the diagnosis of advanced stage of GBC but their cut-off level is statistically not significant in predicting metastatic GBC.

Keywords: Tumor markers in carcinoma gallbladder, Cut-off value of CA19-9, CA125 and CEA in metastatic carcinoma gallbladder, Specific tumor markers in gallbladder cancer

INTRODUCTION

Carcinoma gallbladder (GBC) is a most common biliary malignancy.[1] Its incidence is very high in northern India. It is a very aggressive tumor.[2] Diagnosis of GBC is delayed because of non-specific presentations in early stage. Serum tumor markers carbohydrate antigen (CA) 19-9 and carcinoembryonic antigen (CEA) were widely used in GBC. These tumor markers were elevated in advanced GBC. One study from India showing use of CA125 as a tumor marker for GBC.[3] CA 125 is a high molecular weight glycoprotein and it is a differentiating antigen associated with coelomic epithelium. It is expressed from the epithelial cells of carcinoma ovary as well as malignancy of breast, pleura and peritoneal lining.[4,5] Half-life of CA125 is 4-5 days.[6] CA19-9 and CEA are generally used in combination. Tumor markers are used as a prognostic factor or a predictive factor. Prognostic factors are used to determine the risk of disease outcome in absence of treatment or to determine the residual risk after treatment whereas predictive factor is associated with the likelihood of sensitivity or resistance to a specific therapy.[7] In present study the Authors have used CA125 along with the CA19-9 and CEA as triple tumor markers. The aim of the study was to find the significance of combined use of triple tumor

markers CA19-9, CA125 and CEA in advanced stage of GBC and to find the cut-off value of each of these tumor markers for prediction of metastatic stage of GBC.

METHODS

This was a retrospective observational cohort study from January 2019 to December 2019 which was carried out on cases of advanced GBC admitted to a single unit of Department of General Surgery at All India Institute of Medical Sciences, Rishikesh. Ethical approval was not required because of retrospective nature of the study. However patient identity is not disclosed. Inclusion criteria was all the adult population of more than 18 years of age with final diagnosis of carcinoma gallbladder where contrast enhanced computed tomography (CECT) scan abdomen and chest and triple tumor markers CA19-9, CA125 and CEA were done. Cases with early GBC were excluded from the study. STROBE guidelines were used for this observational study. The data collected were the age, gender, serum CA19-9, and CA125, CEA and CECT scan findings. Total 42 cases fulfilled the inclusion criteria and were enrolled in the study. Cases were divided into locally advanced (LA) and metastatic (M) disease on the basis of CECT scan findings. All the patients with GBC were evaluated with triple tumor markers CA19-9, CA125 and CEA and were analysed with the LA and M stage of tumor. The upper normal reference values of these tumor markers were CEA >5 µg/l, CA125 ≤35 U/ml, and CA19-9 ≤39 U/ml.

Statistical analysis

Statistical analyses were performed using R statistical software v3.6.2. The non-parametric test used for comparison of data was the Wilcoxon-Mann-Whitney U Test. As the sample size was small so Fisher's exact test was employed to calculate the p value and t-test is used to compare the mean. The Chi-square test was applied to see the association of triple tumor markers with LA and M stage of GBC. The receiver operating characteristic curve (ROC) was constructed to see the cut-off value of the test and its diagnostic significance.

RESULTS

Out of 42 cases 19 (45%) had LA disease and 23 (55%) had M disease. Nine cases were male and 33 females. Mean age at presentation was 53 years. CA19-9 was normal in 11 (26%) and elevated in 31 (74%), whereas CA125 was normal in 14 (33%) and elevated in 28 (67%) and CEA was normal in 27 (64%) and elevated in 15 (36%) cases of GBC (Table 1). When comparing the triple tumor marker with stage of the GBC, CA19-9 was elevated in 78% (18), CA125 in 70% (16) and CEA in 39% (9) of patients with metastatic disease. (Table-2) Although none of the tumor markers were statistically significantly associated ($p<0.05$) with stage of the GBC (Table 2).

Table 1: Description of all parameters of GBC (n=42).

All parameters	Mean ±SD; Median (IQR); (Min-Max)
Age in years	52.71±12.79; 52.50 (15.00); (22.00-84.00)
Gender N (%)	
Male	9 (21.4)
Female	33 (78.6)
CA-19-9 (U/ml)	1789.56±8824.66; 220.25 (696.00); (2.00-5750.00)
CA-19-9 N (%)	
WNL	11 (26.2)
Raised	31 (73.8)
CA125 (U/ml)	422.28±1186.27; 59.40 (221.50); (9.70-6900.00)
CA125 N (%)	
WNL	14 (33.3)
Raised	28 (66.7)
CEA (µg/l)	72.95±250.66; 7.64 (12.82); (0.00-1500.00)
CEA N (%)	
WNL	27 (64.3)
Raised	15 (35.7)
Tumor stage N (%)	
Locally advanced	19 (45.2)
Metastatic	23 (54.8)

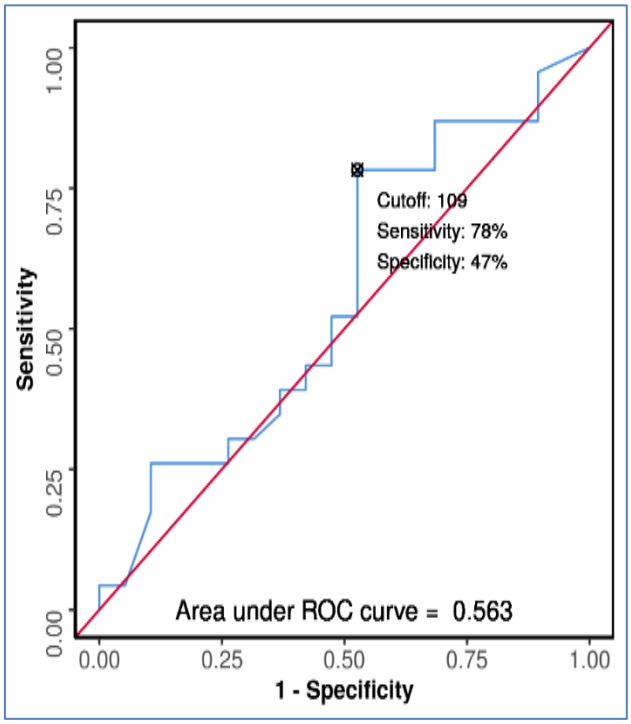

Figure 1: ROC curve analysis showing diagnostic performance of CA-19-9 (U/ml) in predicting metastatic GBC vs locally advanced (n=42).

Table 2: Association of tumor markers with stage of GBC.

All parameters	Tumor stage		P value
	Locally advanced (n=19)	Metastatic (n=23)	
CA-19-9 (U/ml)	461.67±707.82	2886.51±1191.83	0.495[3]
CA-19-9 N (%)			0.504[2]
WNL	6 (31.6)	5 (21.7)	
Raised	13 (68.4)	18 (78.3)	
CA125 (U/ml)	134.09±155.63	660.34±1572.18	0.419[3]
CA125 N (%)			0.661[4]
WNL	7 (36.8)	7 (30.4)	
Raised	12 (63.2)	16 (69.6)	
CEA (µg/l)	45.49±134.34	95.64±318.02	0.980[3]
CEA N (%)			0.611[4]
WNL	13 (68.4)	14 (60.9)	
Raised	6 (31.6)	9 (39.1)	

*1: t-test, 2: Fisher's exact test, 3: Wilcoxon-Mann-Whitney U test, 4: Chi-squared test.

Table 3: Performance of study parameters for predicting metastatic GBC.

Variables	Category (s) suggesting outcome present	Category (s) suggesting outcome absent	Total positive N (%)	True positive N (%)	True negative N (%)	False positive N (%)	False negative N (%)
Tumor stage	Metastatic	Locally advanced	23 (54.8)	-	-	-	-
CA-19-9 (U/ml) (cut off: 109 by ROC)	≥109	<109	28 (66.7)	18 (43)	9 (21)	10 (24)	5 (12)
CA-19-9	Raised	WNL	31 (73.8)	18 (43)	6 (14)	13 (31)	5 (12)
CA125 (U/ml) (cut off: 55.4 by ROC)	≥55.4	<55.4	23 (54.8)	15 (36)	11 (26)	8 (19)	8 (19)
CA125	Raised	WNL	28 (66.7)	16 (38)	7 (17)	12 (29)	7 (17)
CEA (µg/l) (cut off: 2.56 by ROC)	≤2.56	>2.56	12 (28.6)	8 (19)	15 (36)	4 (10)	15 (36)
CEA	Raised	WNL	15 (35.7)	9 (21)	13 (31)	6 (14)	14 (33)

Table 4: Primary diagnostic parameters of metastatic GBC.

Variable	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy
CA-19-9 (U/ml) (cut off: 109 by ROC)	78.3 (56-93)	47.4 (24-71)	64.3 (44-81)	64.3 (35-87)	64.3 (48-78)
CA-19-9	78.3 (56-93)	31.6 (13-57)	58.1 (39-75)	54.5 (23-83)	57.1 (41-72)
CA125 (U/ml) (cut off: 55.4 by ROC)	65.2 (43-84)	57.9 (33-80)	65.2 (43-84)	57.9 (33-80)	61.9 (46-76)
CA125	69.6 (47-87)	36.8 (16-62)	57.1 (37-76)	50.0 (23-77)	54.8 (39-70)
CEA (µg/l) (cut off: 2.56 by ROC)	34.8 (16-57)	78.9 (54-94)	66.7 (35-90)	50.0 (31-69)	54.8 (39-70)
CEA	39.1 (20-61)	68.4 (43-87)	60.0 (32-84)	48.1 (29-68)	52.4 (36-68)

The receiver operating characteristic curve (ROC) was constructed to see the cut off value of the triple tumor markers in predicting the M stage of the disease and its diagnostic significance. At a cut-off value of CA19-9 >109 U/ml it predicts metastatic disease with a sensitivity of 78% and specificity of 47.4% (Figure 1, Table 3 and 4) The area under ROC curve (AUROC) for CA 19-9 (U/ml) in predicting M vs LA was 0.563 (95% CI: 0.382-

0.744), which is demonstrating poor diagnostic performance. It was statistically not significant (p=0.495). The odds ratio (95% CI) for metastatic tumor when CA19-9 (U/ml) is ≥109 was 2.55 (0.7-9.31). The relative risk (95% CI) for Metastatic tumor when CA19-9 (U/ml) is ≥109 was 1.57 (0.86-3.32).

The AUROC for CA125 (U/ml) in predicting M vs LA tumor was 0.574 (95% CI: 0.397-0.752), thus again demonstrating poor diagnostic performance. It was statistically not significant (p=0.419). At a cut-off of CA125 (U/ml) ≥55.4, it predicts Metastatic tumor with a sensitivity of 65%, and a specificity of 58% (Figure 2 and Table 3 and 4) The odds ratio (95% CI) for metastatic disease when CA125 (U/ml) is ≥55.4 was 2.14 (0.62-7.37). The relative risk (95% CI) for metastatic disease when CA125 (U/ml) is ≥55.4 was 1.41 (0.81-2.62).

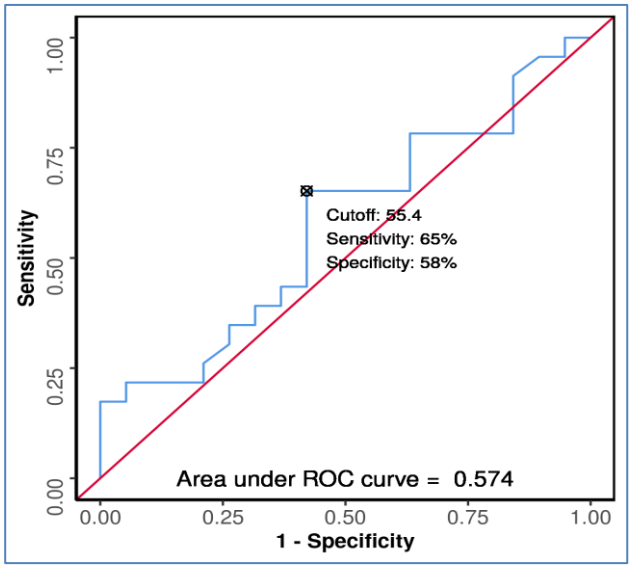

Figure 2: ROC curve analysis showing diagnostic performance of CA125 (U/ml) in predicting metastatic GBC vs locally advanced (n=42).

The AUROC for CEA (µg/l) predicting M vs LA disease was 0.503 (95% CI: 0.324-0.683), thus demonstrating poor diagnostic performance. It was not statistically significant (p=0.980). At a cut off of CEA (µg/l) ≤2.56, it predicts metastatic disease with a sensitivity of 35%, and a specificity of 79% (Figure 3 and Table 3 and 4). The odds ratio (95% CI) for metastatic disease when CEA (µg/l) is ≤2.56 was 1.64 (0.4-6.76) and the relative risk (95% CI) for metastatic disease when CEA (µg/l) is ≤2.56 was 1.23 (0.64-2.07).

All these cut-off and diagnostic parameters are not reliable as the test is not statistically significant. Result shows that CA19-9 and CA125 are more consistently elevated in metastatic GBC than CEA. We further analysed the PPV and NPV of triple tumor markers. The sensitivity and specificity of CEA was nearly 39% and 68% respectively whereas it was nearly 78% and 32% for CA19-9 and 70% and 37% for CA125 (Table 4).

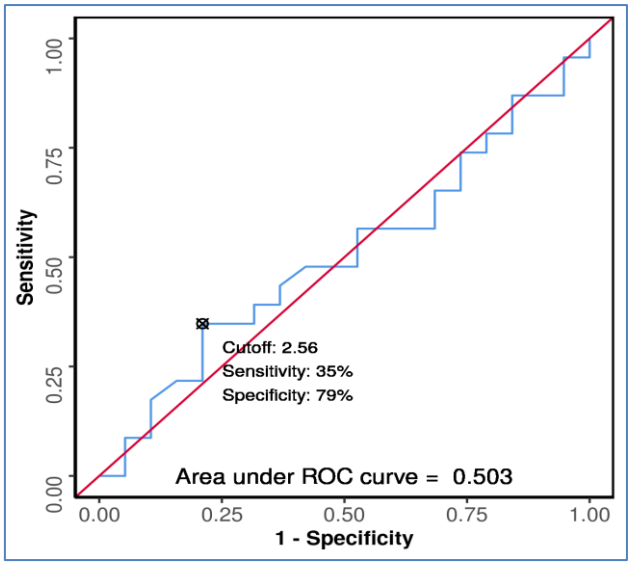

Figure 3: ROC curve analysis showing diagnostic performance of CEA (µg/l) in predicting metastatic GBC vs locally advanced (n=42).

DISCUSSION

Tumor markers are a molecular or tissue-based process that gives information about the future behaviour of a malignancy. These markers are the result of changes in malignant tissue itself or the type of malignancy which distinguishes it from other malignancy. Some of the tumor markers are detected from the tissue of origin of malignancy or regional lymph nodes or the distant metastatic organs and some are detected in the circulation. Tumor markers are commonly non-specific to the tissue of origin so it is uncommonly used to identify the tissue of origin of malignancy.[7] CA19-9 and CEA are most commonly used serum tumor markers for the diagnosis and prognosis of the GBC. In combination they are superior to either one alone in predicting the prognosis.[8,9] CA19-9 is a mucinous protein whereas CEA is a protein polysaccharide complex. CA19-9 is elevated in neoplasm of pancreas, stomach and bile duct whereas CEA is elevated in gastrointestinal malignancy, pancreas and biliary tract and embryonic gut.[10,11] Serum CEA was first used in the patient with colorectal cancer and CA125 was used in ovarian cancer.[7] Although CA19-9 and CEA is used in combination as prognostic markers of malignancy of pancreas and stomach but the sensitivity and specificity of CEA for biliary tract malignancy is poor.[11] Sensitivity of CA19-9 and CA125 gradually increased with progression of the stage of the disease.[12] The result of present study was consistent with the previous study which is showing increased sensitivity of CA19-9 and CA125 in metastatic disease. The sensitivity and specificity of CEA in GBC was poor which was nearly 39% and 68% respectively, which is consistent with the previous study.[13] CA19-9 had the highest sensitivity 78.3% followed by CA125 69.6% and the CEA has the highest specificity 68.4% for the diagnosis and prediction of advanced GBC which is consistent with

the previous study.[12] In the present study, the cut-off value of CA19-9, CA125 and CEA as determined by ROC curve were >109 U/ml, 55.4 U/ml and 2.56 µg/l respectively. In a study by Shukla, the cut value of CA19-9 and CA125 in GBC was 211.27 U/ml and 253.6 U/ml which was in contrast to the present study.[14] In the present study when the value of CA19-9, CA125 and CEA was more than the cut-off level then the specificity and diagnostic accuracy for metastatic disease were 47.4%, 57.9% ,78.9% and 64.3%, 61.9% and 54.8% respectively, which suggest that combined use of these tumor markers increases its specificity in the diagnosis of advanced GBC.

CONCLUSION

This study identified the role of combined use of triple tumor markers CA19-9, CA125, and CEA as independent predictor of advanced stage of GBC. However, cut-off value of none of these tumor markers are statistically significant in diagnosing metastatic GBC. Advanced stage of GBC was more commonly predicted by CA19-9 and CA125 than CEA.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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