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Original Research Article

Evaluation of Tumor Marker as Better Prognosticator in Non-Metastatic Adenocarcinoma Gall Bladder

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Abstract:

Background: The diagnosis of gallbladder carcinoma mainly depends on non-invasive auxiliary imaging and invasive examination such as laparoscopy, cytology, and biopsy. No definitive tumor marker is yet available for diagnosis and prognosis. We aimed to investigate the cut-off value of tumor markers (CEA, CA19.9 and CA-125) for the diagnosis and prognosis of non-metastatic adenocarcinoma gall bladder.

Methods: Fifty histopathology/cytology positive with Contrast-Enhanced Computed Tomography (CECT) and Ultra Sonography (USG) confirmed non-metastatic adenocarcinoma of gall bladder patients aging between 50 and 60 years old and of both sexes were included in the study. These 50 individuals were subjected to 7 cycles of chemo and one cycle of radiation. Serum samples were collected in day 1 before each cycle. Thereafter, all samples were subjected to the estimation of serum CA 19.9, CA 125, and CEA.

Results: The result showed the mean of serum CA19.9 is significantly was decreased with progression of treatment cycle than CEA and CA125. Then to determine the prognostic value of the serum CA19.9, CEA and CA125, ROC analysis was performed and showed that for CA 19.9 the optimal threshold value being 32.15 IU/ml with a sensitivity of 92.5% and a specificity of 76%. 9.98 ng/ml was demonstrated as the optimal cut-off value for CEA as a predictor of non-metastatic CA gall bladder with a sensitivity of 88.2% and a specificity of 95.6% and serum CA 125 value of 35.37 IU/ml had got role in prognosis of the disease with a sensitivity of 91.4% and a specificity of 93.7%.

Conclusion: Among these tumor markers CA 19.9 was most sensitive, CEA was most specific prognosticator of non-metastatic adenocarcinoma gall bladder, however, in combination is most, specific and sensitive.

Keywords: Tumor markers, CA gall bladder, Prognostic marker, non-metastatic.

Introduction

Gall bladder cancer (GBC) is the one of the most common malignant neoplasm of the biliary tract and constitutes 80–95% of the biliary tract cancers.[1] Gallbladder carcinoma is usually has poor prognosis with 6 months mean survival rate and less than 5% five-years survival rate.[2] The reason for this is that more than 90% of the gallbladder carcinoma cases are diagnosed late in advance unresectable stage in which the tumor is already invading the adjacent organs with distant metastasis.[3] Complete surgical resection with wide free surgical margin is the only effective way to treat patients with gallbladder carcinoma.[2] Unfortunately because most of the cases diagnosed at advance unresectable stages, and it is very difficult to diagnose in the early stage as it lacks typical clinical early manifestations [4-6], this option is less likely available. So, it is critical to diagnose GBC as early as possible, as most patients present with advanced stage at the time of diagnosis, and thus loose the chance of radical cure and thus have a prolonged survival.

Currently, the diagnosis of gallbladder carcinoma mainly depends on non-invasive auxiliary imaging and invasive examination such as laparoscopy, cytology, and biopsy. No definitive better tumor marker for the diagnosis or prognosis is yet available.

However, certain tumor markers such as carbohydrate antigen (CA) 19.9, carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 125 are associated with hepatobiliary pathologies, including both benign and malignant ones.[7,8] These markers, when combined with clinical and imaging findings may guide further plan of the management. The mainstay of treatment of gall bladder cancer (GBC) remains radical resection. It is estimated that 75% of the cases of GBC are already unsuitable for resection in non-incidentally diagnosed patients. Preoperative imaging, available facilities and surgeon's expertise are the determining factors. However, not all patients have a resectable

disease at the time of diagnosis due to severe adhesion with surrounding structures. Moreover, there is no ideal single tumor marker for the diagnosis and prognosis of gallbladder carcinoma.[4] In earlier reports, CA19-9 and CA 125 have been studied and found to be raised in carcinoma gall bladder. But, no definite cutoff values have been calculated.[9,10] Therefore the present study was done to assess and find out any cutoff values for CA 19-9, CEA and CA 125 or in combination of them which can help to know the any prognostic role in cases of non-metastatic adenocarcinoma gall bladder to increase survival rate.

Material and Methods

Study Area

This hospital based observational descriptive longitudinal study was conducted in the Department of Radiotherapy with the collaboration of Department of Biochemistry of Medical College, Kolkata, West Bengal, India.

Ethics Statement

The study was approved and permitted by the institutional ethics committee for care and use of laboratory and started after obtaining the written consent from the concerned ethics committee.

Study population

The present study was conducted between February 2022 and July 2023. Sample size was calculated at 95% confidence interval, with a power of 80% [11] using the formula

$N = [Z(1-\alpha/2)]^2XpX(1-p)/d^2$

As shown in Figure 1, fifty histopathology/cytology along with Contrast-Enhanced Computed Tomography (CECT) and Ultra Sonography (USG) proven non-metastatic adenocarcinoma of Gall bladder patients aging between 50 and 60 years old and of both sexes were included in the study. Cases were selected by a systematic random sampling method. Every patient was informed about the details of the study through individual interviews and all the provided written informed consent. Patients with M1 (Metastatic) adenocarcinoma of Gallbladder, patients having CA gall bladder with pathological staging T1A and T2N0 are not included in this study. Patients with pregnancy, lactation were excluded from the study. Patients showing Functional status based on Eastern Cooperative Oncology group (ECOG) grade ≥3 also removed from the present study.

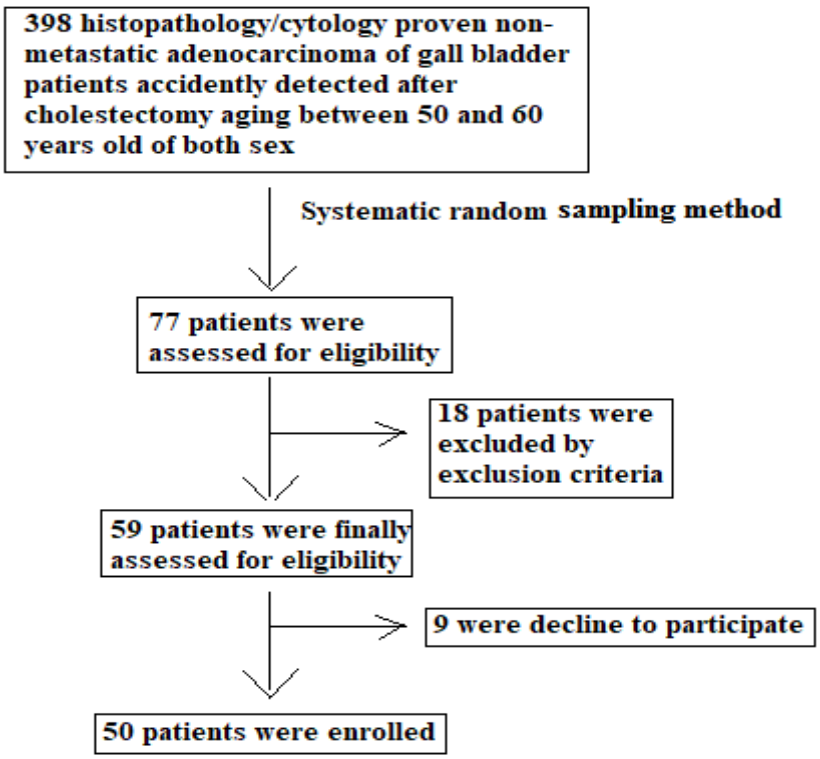

Figure 1: Study design of present study

Then these 50 subjects were subjected to 7 cycles of chemo-radiation and serum samples were collected as follows -

- Sample 1: Day 1 before 1st cycle of chemo-

radiotherapy (baseline)

- Sample 2: Day 1 before 2nd cycle of chemotherapy (after 2 weeks of 1st cycle)
- Sample 3: Day 1 before 3rd cycle of chemotherapy (after 2 weeks of 2nd cycle)
- Sample 4: Day 1 before 4th cycle of chemotherapy (after 2 weeks of 3rd cycle)
- Sample 5: Day 1 before 5th cycle of chemotherapy (after 2 weeks of 4th cycle)
- Sample 6: Day 1 before 6th cycle of chemotherapy (after 2 weeks of 5th cycle)
- Sample 7: At the end of chemotherapy (after 2 weeks of 6th cycle)

Chemotherapy

Chemotherapy Patients were treated by multimodality approach with Capecitabine-based chemotherapy in both the adjuvant and palliative setting.

Radiotherapy

Patients receive radiation at 45 Gy in 28 fractions IMRT boost up to 50.4 Gy during 1st cycle of chemo-radiation.

Collection of samples

Peripheral venous blood was drawn under aseptic precautions and collected from all participants in clotted vial. Then blood samples was collected and allowed to clot for 30 min at room temperature and then centrifuged at 2400×g for 10 min to separate serum. This serum is used for the determination of serum concentration of CA19.9, CEA and CA125. All serum samples were stored at (-70^{0}C) and kept under these conditions until chemical analysis was performed. All parameter assays should be done as soon as possible.

Parameters assay

Serum CA19-9, CEA, CA-125 levels were estimated by chemiluminescence (CLIA) on ADVIA Centaur® XP system (Siemens Healthineers; Germany). The samples were run only after satisfactory level of performance by two levels of internal quality controls (low and high) as per manufacturer's instructions. The ADVIA Centaur CA 19-9 assay is a two-step sandwich immunoassay using direct CLIA technology which uses a single monoclonal antibody, 1116-NS-19-9, for both the solid phase and lite reagent. The antibody is covalently coupled to the paramagnetic particles in the solid phase and the same clone of antibody is labeled with acridinium ester in the lite reagent. The sample and solid phase were incubated at 37°C for 7.5 minutes followed by a wash step to remove excess unbound antigens. The lite reagent was then reacted with solid phase-bound CA 19-9 antigens for an additional 20 min incubation, whereas CEA and CA 125 are a two-site sandwich immunoassay using direct CLIA technology, which uses constant amounts of two antibodies. In case of CEA, first antibody, in the lite reagent, is a purified polyclonal rabbit anti-CEA and rabbit antibody labeled with acridinium ester. The second antibody, in the solid phase, is a monoclonal mouse anti-CEA antibody covalently coupled to the paramagnetic particles. In case of CA 125, the first antibody is directed toward the M11 antigenic domain, and is labeled with acridinium ester. The second antibody is directed toward the OC 125 antigenic domain and was labeled with fluorescein. The immune-complex formed with CA 125 was captured with monoclonal mouse anti-fluorescein antibody coupled to paramagnetic particles in the solid phase. The normal reference values were as follows: CEA ≤ 10 ng/ml, CA125 ≤ 35 IU/ml, CA242 ≤ 15 IU/ml, and CA19.9 ≤ 39 IU/ml

Follow up

All patients were followed up for a period of minimum 6 months after complete chemo-radiation.

Statistical analysis

Data were entered using Microsoft Excel 2007. Then the data for biochemical analysis was subjected to standard statistical analysis such as Student's t test using the Statistical Package for Social Science (SPSS) 20 software. For all tests 'p' value was considered to be significant if it was less than 0.05 at a confidence level of 95 %.

Result

The characteristics and their comparison among different groups of study population – Chi-square test.

Baseline personal profile and clinical details of the study population shown in Table 1.

Table 1: Biochemical and anthropometric variables and their comparison between the groups of the study population

Characteristics	
Number of participants (n)	50
Age (years)	55.26 ± 5.32
Sex Male Female	 22 (44) 28 (56)
Demographic data Urban background	 24 (48)

Rural background	26 (52)
BMI (Kg/m^2)	23.8 ±2.8
Hemoglobin (g/dl)	11.8 ± 2.1
Bilirubin (mg/dl) Total Direct Indirect	 0.9 (0.6-1.2) 0.3 (0.2-0.5) 0.6 (0.4-0.7)
ALP in IU/L at 37^{0}C	119.5 ± 32.68
SGOT in U/L at 37^{0}C	34.0 ± 7.33
SGPT in U/L at 37^{0}C	27.0 ± 9.52
INR	1.1 (1.0-1.1)
Serum creatinine (mg/dl)	0.8 (0.7-0.9)
BUN (mg/dl)	12.3 (9.0-14.4)
Comorbidities Diabetic Hypertensive Both	 2(4) 1(2) 1(1.4)
Smoking Yes No	 9 (18) 41 (82)
Alcohol Yes No	 3 (6) 47 (94)

Data are expressed as numbers (group percentages in parentheses) for categorical variables and mean values ± SD for continuous variables

Comparison of serum CA19.9, CEA and CA125 between pre-treatment and after different cycles of chemo-radiation therapy in non-metastatic CA gall bladder patients – Unpaired t test

The mean of serum CA19.9, CEA and CA125 were decreased with progression of treatment cycle (Table 2).

Table 2: Serum CA19.9, CEA and CA125 between pre-treatment and after different cycles of chemo-radiation therapy in non-metastatic CA gall bladder patients

Parameters	Pre-treatment	After 1st cycle of Chemo-radiation	2nd cycle of chemo-therapy	3rd cycle of chemo-therapy	4th cycle of chemo-therapy	5th cycle of chemo-therapy	6th cycle of chemo-therapy
Serum CA19.9 (IU/ml)	188.8 ± 45.62	178.47 ± 63.28	148.28 ± 37.29	139.37 ± 52.92	90.28 ± 28.48	48.84 ± 17.71	22.29 ± 6.23
Serum CEA (ng/ml)	16.9 ± 5.21	12.4 ± 2.69	9.39 ± 2.97	7.72 ± 2.24	8.83 ± 2.18	7.18 ± 1.39	7.23 ± 0.92
Serum CA125 (IU/ml)	56.82 ± 11.98	51.03 ± 17.93	46.38 ± 14.92	35.97 ± 9.35	32.43 ± 3.96	24.97 ± 4.99	28.47 ± 1.58
Data are expressed in Mean values ± SD							

Comparison of tumor markers before and after individual cycle of chemo-radiation management of non-metastatic CA gall bladder patients (Pairwise multiple comparison of different tumor markers within the case group) - Post hoc ANOVA analysis with Bonferroni correction: Pairwise multiple comparisons in the post hoc ANOVA analysis with Bonferroni correction within the group was performed and it became evident that CA 19.9 showed a statistically significant reduction from baseline at after almost all the cycles of chemo-radiation and were also significantly reduced as cycle of chemo-radiation was progress. Regarding CA 125 and CEA markers, the serum concentration were also significantly reduced from baseline as chemo-radiation was progressed but not significantly reduced in between the all cycles of the treatment. (Table 3).

Table 3: ANOVA with Bonferroni correction showing multiple comparisons of different tumor markers within the case group with significance of difference

Dependent variable	Factor (I)	Factor (J)	Mean difference (I-J)	Significance at 95% CI
Serum CA19.9 (IU/ml)	1	2	10.33	0.071
		3	40.52	0.038*
		4	49.43	0.029*
		5	98.52	<0.001*
		6	139.96	<0.001*
		7	166.51	<0.001*
	2	1	- 10.33	0.071
		3	30.19	0.047*
		4	39.1	0.040*
		5	88.19	<0.001*
		6	129.63	<0.001*
		7	156.18	<0.001*
	3	1	-40.52	0.038*
		2	- 30.19	0.047*
		4	8.91	0.168
		5	58	0.049*
		6	99.44	<0.001*
		7	125.99	<0.001*
	4	1	- 49.43	0.029*
		2	- 39.1	0.040*
		3	- 8.91	0.168
		5	49.09	0.044*
		6	90.53	<0.001*
		7	117.08	<0.001*
	5	1	-98.52	<0.001*
		2	-88.19	<0.001*
		3	-58	0.049*
		4	-49.09	0.044*
		6	41.44	0.039*
		7	67.99	<0.001*
	6	1	-139.96	<0.001*
		2	-129.63	<0.001*
		3	-99.44	<0.001*
		4	-90.53	<0.001*
		5	-41.44	0.039*
		7	26.55	0.056
	7	1	-166.51	<0.001*
		2	-156.18	<0.001*
		3	-125.99	<0.001*
		4	-117.08	<0.001*
		5	-67.99	<0.001*
		6	-26.55	0.056
Serum CEA (ng/ml)	1	2	4.5	0.072
		3	7.51	0.033*
		4	9.18	<0.001*
		5	8.07	<0.001*
		6	9.72	<0.001*
		7	9.67	<0.001*
	2	1	-4.5	0.072
		3	3.01	0.097
		4	4.68	0.061
		5	3.57	0.083
		6	5.52	<0.001*
		7	5.17	<0.001*
	3	1	-7.51	0.033*
		2	-3.01	0.097

		4	1.67	0.124
		5	0.56	0.169
		6	2.21	0.115
		7	2.16	0.117
	4	1	-9.18	<0.001*
		2	-4.68	0.061
		3	-1.67	0.124
		5	-1.11	0.136
		6	0.54	0.172
		7	0.49	0.177
	5	1	-8.07	<0.001*
		2	-3.57	0.083
		3	0.56	0.169
		4	1.11	0.136
		6	1.65	0.126
		7	1.6	0.130
	6	1	-9.72	<0.001*
		2	-5.52	<0.001*
		3	-2.21	0.115
		4	-0.54	0.172
		5	-1.65	0.126
		7	0.05	0.346
	7	1	-9.67	<0.001*
		2	-5.17	<0.001*
		3	-2.16	0.117
		4	-0.49	0.177
		5	-1.6	0.130
		6	0.05	0.346
Serum CA125 (IU/ml)	1	2	5.79	0.126
		3	10.44	0.085
		4	20.85	<0.001*
		5	24.39	<0.001*
		6	31.85	<0.001*
		7	28.35	<0.001*
	2	1	-5.79	0.126
		3	4.65	0.189
		4	15.06	0.073
		5	18.60	0.061
		6	26.06	<0.001*
		7	22.56	<0.001*
	3	1	-10.44	0.085
		2	-4.65	0.189
		4	10.41	0.085
		5	13.95	0.078
		6	21.41	<0.001*
		7	17.91	0.067
	4	1	-20.85	<0.001*
		2	-15.06	0.073
		3	-10.41	0.085
		5	3.54	0.284
		6	11.00	0.081
		7	7.50	0.124
	5	1	-24.39	<0.001*
		2	-18.60	0.061
		3	-13.95	0.078
		4	-3.54	0.284
		6	7.43	0.126
		7	3.96	0.233
	6	1	-31.85	<0.001*
		2	-26.06	<0.001*

		3	-21.41	<0.001*
		4	-11.00	0.081
		5	-7.43	0.126
		7	-3.50	0.248
	7	1	-28.35	<0.001*
		2	-22.56	<0.001*
		3	-17.91	0.067
		4	-7.50	0.124
		5	-3.96	0.233
		6	-3.50	0.248

*p value significant ($p < 0.05$) at 95% Confidence interval (CI); 1 = Baseline activity, 2 = after 1st cycle of Chemo-radiation, 3 = 2nd cycle of chemo-therapy, 4 = 3rd cycle of chemo-therapy, 5 = 4th cycle of chemo-therapy, 6 = 5th cycle of chemo-therapy, 7 = 6th cycle of chemo-therapy

Determination of prognostic value of the serum CA19.9, CEA, CA125 and in combination of tumor markers in non-metastatic CA gall bladder patients - Receiver Operating Characteristic (ROC) Curve Analysis -

The ROC curves were analyzed and were plotted as shown in Figure 2 for the prognostic value of the serum CA19.9, CEA and CA125 in non-metastatic CA gall bladder patients as shown in Table 3. The analysis of the ROC curve illustrated a 0.86 area under the curve (AUC) for CA19.9 levels as a prognostic indicator in non-metastatic CA gall bladder patients (95% CI, 0.82–0.89, $P < 0.05$). The AUC of this biomarker indicated a high predictive value for the outcome, with the optimal threshold value being 32.15 with a sensitivity of 92.5% and a specificity of 76%. The corresponding positive and negative predictive values were 76.5% and 92%, respectively (Table 4) Regarding the ROC curve for the detection of prognosis, a CEA of 9.98 was demonstrated as the optimal cut-off value as a predictor of non-metastatic CA gall bladder with a sensitivity of 88.2% and a specificity of 95.6%, AUC 0.94, (95% CI 0.91–0.96; $p = 0.0001$) (Figure 2). But ROC curve of CA125 was shown that AUC was 0.74 and CA 125 of 35.37 had got role in prognosis of the disease (95% CI, 0.72–0.78, $P < 0.05$) with a sensitivity of 91.4% and a specificity of 93.7%.

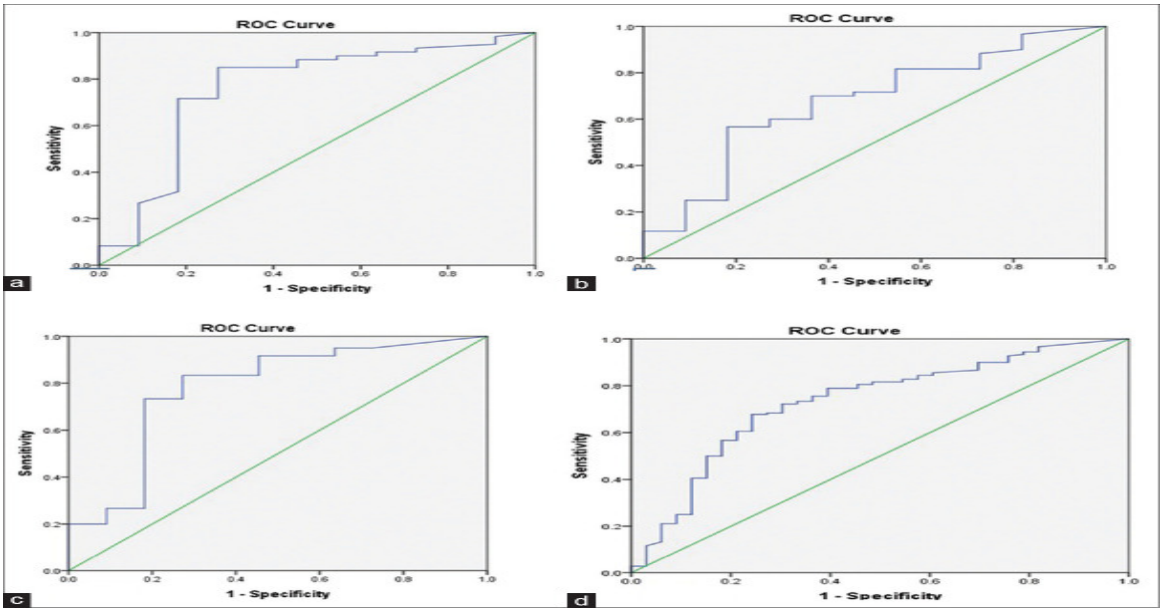

Figure 2: Receiver operating characteristic plots demonstrating discriminatory ability of tumor markers regarding prognostic value of non—metastatic gall bladder cancer cases (a) carbohydrate antigen 19.9 AUC: 0.86; (b) carcinoembryonic antigen AUC: 0.94; (c) Carbohydrate antigen CA125 AUC: 0.74; (d) Combination of three markers AUC: 0.88

Table 4: ROC Value for serum CA19.9, CEA and CA125 in prognosis of non-metastatic CA gall bladder patients

Variables	AUC	Cutoff	Sensitivity	Specificity	PPV	NPV
Serum CA19.9 (IU/ml)	0.86	32.15	92.5%	76%	76.5%	92%
Serum CEA (ng/ml)	0.94	9.98	88.2%	95.6%	62.2%	95.9%

Serum CA125 (IU/ml)	0.74	35.37	91.4%	85.1%	67.9%	93.7%

NPV: Negative predictive value; PPV: Positive predictive value.

Table 5: Performance of combination of tumor markers for prognosis in non-metastatic CA gall bladder patients

Variables	Sensitivity	Specificity	PPV	NPV
Combination of CA 19-9 and CEA	63.8%	96%	86.3%	85.2%
Combination of markers CA 19-9 and CA125	76%	91.7%	95.2%	84.5%
Combination of 3 markers	42%	100%	100.0%	70.8%

NPV: Negative predictive value; PPV: Positive predictive value. But when combination tumor markers were used there were increase in sensitivity, specificity and as well as PPV and NPV for the prognosis of non-metastatic GBC (Table 5).

Discussion

The incidence of GBC has increased in recent years worldwide. On the contrary, regarding modality of treatment, therapeutic effect assessment, prognostic evaluation, and predict post-operative recurrence in the early stage have been aroused increasing attention in both clinical studies and practice [12,13]. This study was conducted in patients with non-metastatic adeno-carcinoma gall bladder to assess prognostic accuracy of different tumor markers separatelyand in combination.

Among these tumor markers, CA 19.9 showed a statistically significant reduction from baseline and after almost all the cycles of chemo-radiation. Regarding CA 125 and CEA markers, the serum concentration were also significantly reduced from baseline as chemo-radiation was progressed but not significantly reduced in between the all cycles of the treatment.

Then the prognostic value of the serum CA19.9, CEA, CA125 and in combination of tumor markers in non-metastatic CA gall bladder patients performed and found that CA19.9 levels as a better prognostic indicator in non-metastatic CA gall bladder patients as this biomarker indicated a high predictive value for the outcome, with the optimal threshold value being 32.15 with a sensitivity of 92.5% and a specificity of 76%. The corresponding positive and negative predictive values were 76.5% and 92%, respectively. It was also clear that a CEA of 9.98 was demonstrated as the optimal cut-off value as a predictor of these patients with a sensitivity of 88.2% and a specificity of 95.6% But CA125 was shown that CA 125 of 35.37 had got role in prognosis of the disease with a sensitivity of 91.4% and a specificity of 93.7%.

A recent study of 71 patients diagnosed with GBC showed that CA 19-9 had the highest sensitivity of 85% and CA 125 had the highest specificity of 81.8%.[14] A prospective study reported that CA 19-9 had better sensitivity and specificity (52% and 80%, respectively) than CEA (51% and 72%, respectively) for the prediction of tumor burden in patients with GBC.[15] Another study reported that CA 19-9 and CA 242 had the highest sensitivity and specificity of 71.7% and 98.7%, respectively. [16]

GBC can be detected using serum CA 19-9, which had moderate sensitivity and good specificity.[17] In a meta-analysis, it was noted that GBC can be detected using serum CA 19-9, which had moderate sensitivity and good specificity. [17] These findings suggest that the sensitivity and specificity of tumor markers were inconsistent when used individually for the diagnosis of GBC; however, better sensitivity was observed when the markers were used in combination.[18-20] In the current study, sensitivity was 3.8% when all four markers exceeded the critical values. This is in accordance with a previous study with a sensitivity of 8.9% and a diagnostic accuracy that was better when CA 19-9, CA 125, and CA 242 were used in combination. These results suggest that the diagnosis of GBC based on combined detection of the tumor markers could increase the sensitivity and specificity of the diagnosis.

It is the interesting finding of our study is that when combination tumor markers were used as prognostic marker for non-metastatic GBC there were increase in sensitivity, specificity and as well as PPV and NPV. Most researchers believe that CA19.9 is a better marker of malignant tumors as serum CA19.9 is elevated most obviously in tumors of the gastro-intestinal, pancreas, and biliary tract.[21] CA19.9 is not only a diagnostic indicator, but also a predictor of the therapeutic effect and prognosis of GBC. However, CA19.9 is not specific for GBC. Therefore, CA19.9 should be combined with other tumor markers to diagnose GBC. CA125 is a good marker for the diagnosis of cholangiocarcinoma. Previous studies had shown that CA125 has a relatively high specificity because it is rarely affected by serological levels of inflammation and liver stones.[19,22,23] Our research was not exactly consistent with that of Shukla et al. But combined use of CEA and CA19.9 or CA125 can improve the diagnosis of cholangiocarcinoma, [24-30] which is consistent with our study. The expression of CEA is high in most gastrointestinal tumors.[29,30] It was found

that CEA expression was significantly increased in GBC.[31] However, It was suggested that CEA and AFP had little value for the diagnosis and prognosis of GBC.[32] Our study also showed that CEA had limited value for the diagnosis and prognosis of GBC. In terms of a single marker for the diagnosis of GBC, CA19.9 has the highest sensitivity with relatively low specificity, but cannot be used alone as an effective tumor marker to identify GBC.

Our study has a few limitations. It was a randomized observational study but a relatively small sample size and a short follow-up duration of only 6 month. The study included only operable and suspicious cases of GBC to determine early indications of malignancy by assessing different tumor markers in resource-constrained situations. Further studies with a large number of patients with longer duration of follow-up are required to validate our results. Post-operative tumor recurrence and metastasis are major causes of death in GBC patients.

To search a comprehensive and accurate understanding about the probability of post-operative recurrence and metastasis of GBC, efforts have been made to explore more effective prognostic predictors. The tumor-related indicators in all individuals cannot be detected systemically and comprehensively due to storage of fund and newer techniques.

Conclusion

Among three tumor markers, CA 19.9 was most sensitive and CEA was most specific as prognosticator of non-metastatic adenocacinoma GB and in combination of all three shows most specificity and sensitivity.

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