International Journal of Research in Medical Sciences
Misra M et al. Int J Res Med Sci. 2023 Dec;11(12):4360-4368
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20233700

Original Research Article

Inclusion of carbohydrate antigen 242 in addition to carbohydrate antigen 19.9 in serological workup of carcinoma gall bladder: a case series analysis

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Received: 03 September 2023
Revised: 22 November 2023
Accepted: 23 November 2023

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ABSTRACT

Background: Common antigenic pool is seen because of shared embryonic origins of gall bladder cancer (GBC) and pancreas. Hence, we analyzed the role of serum carbohydrate antigen 242 (CA242) which has been studied in pancreatic cancer, in GBC. The objectives were to identify whether serum CA242 provides added advantage in diagnosis of GBC when compared to controls and to determine its cut-off value.
Methods: Serum CA 19-9 level was determined by chemiluminescent micro particle assay and CA242 by enzyme linked immunosorbent assay (ELISA) of age matched cases and controls.
Results: Total enrolled patients were 83 including 10 (11.7%) healthy volunteers, 22 (25.9%) chronic cholecystitis cases, and 53 (62.4%) patients with histological evidence of carcinoma. Mean age of presentation of GBC was 51.64 SD10.88 years with F: M ratio of 5.6:1. Pain (90.6%, 48/53) accompanied with jaundice was significantly associated with GBC well reflected by significantly raised serum total bilirubin (p=0.011), direct bilirubin (p=0.008) along with alkaline phosphatase levels (p=0.001). Significantly higher median value of CA 19-9 and CA242 was observed in GBC when compared to CC and healthy volunteers (p<0.001) with a significant correlation between tumor size (>2.5 cm) and serum levels of CA242. The best cut-off limit for CA242 was 45.25 IU/ml. The specificity for carcinoma diagnosis increased to 100% when CA242 was included along with CA 19.9 in serological estimation.
Conclusions: We recommend that CA antigen 19-9 may be complimented with CA242 for serological identification of malignancy in the gall bladder.

Keywords: CA 242, Gall bladder cancer, Tumor antigens, CA19.9, ELISA

INTRODUCTION

Gall bladder cancer (GBC), a notoriously lethal malignancy having marked ethnic and geographical variations, was first described in 1717 by Stoll.[1,2] It is a common cancer of the biliary tract representing 80-95% biliary tract cancers worldwide.[3] GBC is also found to be quite frequent with respect to digestive tract neoplasm; moreover, that it has been ranked fifth amongst malignant neoplasms of the digestive tract by few authors. The prognosis is found to be poor with a high fatality rate. Gender and geographic variations have been seen markedly in GBC; studies have found it to be more frequent in central and South America, central Europe,

Japan, and Northern India.[4] In India, although the incidence rate is 2.5/100,000 compared to other countries; however, the overall disease burden is high, this is due to a large population base. The residents of Indo-Gangetic belt particularly the females of Northern India (21.5/100,00) and South Karachi, Pakistan (13.5/100,00) were reported as one of the most affected regions.[5,6] As India is a vast country with multiple geographic, cultural and lifestyle variations, the incidence of GBC is also variable here. GBC is more prevalent in northern and north-eastern states (e.g., Uttar Pradesh, Bihar, Orissa, West Bengal, and Assam) compared to other parts of our country.[7]

Radical cholecystectomy is the preferred modality of treatment; however, more than 70% of cases are non-resectable at the time of diagnosis due to local invasion and metastasis.[7] Chemo-radiation has shown limited promise for this cancer.[8] For incidental GBC, revision surgery with resection of the tumor bed and lymph node dissection is a standard approach, good results are seen in T1 disease only.[9] In most of the cases, the prognosis is dismal with limited results.[10] The best resort left is if we can detect the disease early by any means. Serum tumor marker carbohydrate antigen 19-9 (CA 19-9) is an extensively studied marker used in pancreatic carcinoma and GBC. Its role is seen in diagnosis, post-operative assessment of complete resection, and as a marker for early recurrence in pancreatic adenocarcinoma. However, it's detectable; defined cut-off levels have been seen in benign hepatobiliary diseases (gallstones, cholestasis), pulmonary disease, and renal failure in various studies.[11-13] Combinations of CA 19-9 with other serum tumor markers like Carcinoembryonic antigen (CEA) have been tested by multiple studies in literature. One such combination is CA 19-9 and carbohydrate antigen 242 (CA 242). There are reports of their combination having greater diagnostic value in pancreatic cancer. In cases of suspected gall bladder mass (GB mass) or GBC patients, serum CA 19-9 and Carcinoembryonic antigen (CEA) have been used in management.[14]

Shared embryonic origin may lead to a common tumor antigen pool for both gallbladder and pancreas; hence serum carbohydrate antigen 242 (CA 242), which has been studied in pancreatic cancer, may be explored in GBC as well. The present study intends to measure serum levels of CA 242 and CA 19-9 in patients with GBC and compare these levels with gall stone disease (GS) and in healthy volunteers.

METHODS

A case series analysis was conducted to assess the role of serum tumor markers CA19-9 and CA 242 for the diagnosis and prognosis of gall bladder cancer and to evaluate the variability of these markers among healthy individuals, patients with gall stone disease and suspected gall bladder cancer patients. The study was carried out after approval from Institutional ethical committee approval via letter number 650/Ethics/R. cell-2019 at King George's Medical University, Lucknow, Uttar Pradesh, India from December 2019 to June 2021. Study population comprised of 85 subjects and was divided into three groups. Group I, the control group of the study (10 subjects, 11.7%), comprised of normal healthy volunteers with no radiological evidence of gall stone disease or no clinical suspicion of any malignancy, group II (22 patients, 25.9%) constituted patients having preoperative radiological evidence of gall stone disease and no clinical suspicion of malignancy and group III (53 patients, 62.4%) comprised of carcinoma gall bladder patients diagnosed by histological examination of the gall bladder specimens received in department of pathology. This included both incidental GBC diagnosed on simple cholecystectomy or patients with clinical and radiological diagnosis of GBC undergoing radical cholecystectomy. The cases/ volunteers who were not willing to participate from the study were excluded. Any epithelial abnormality seen in the gall bladder mucosa like dysplasia, anaplasia were excluded from group II. Additionally patients with prior history of chemotherapy were excluded from group III. Computed tomography (CT) abdomen and magnetic resonance imaging (MRI) abdomen were the radiological investigations used to support clinical diagnosis of malignancy. Accrual patient records were analyzed for preoperative serological values [serum total bilirubin, direct bilirubin, serum glutamic oxaloacetic transaminase (SGOT/AST), serum glutamic pyruvate transaminase (SGPT/ALT), alkaline phosphatase, total protein and serum albumin] in all cases and controls. Final histology and cytology as applicable for patients after admission in the institute was also followed (Figure 1).

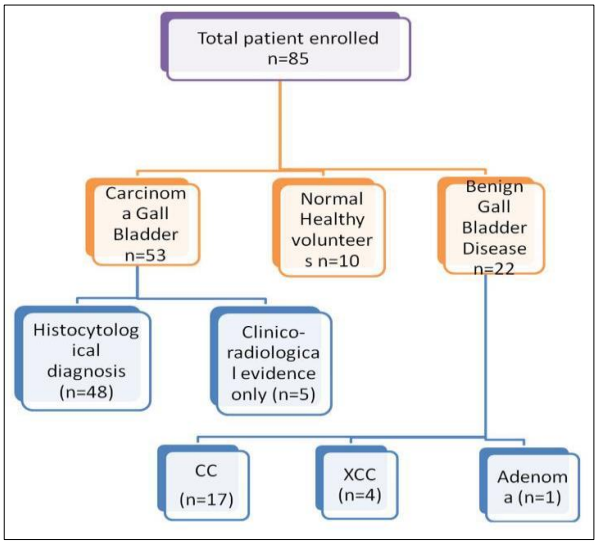

Figure 1: Flowchart of the diagnostic breakup of the study.

Sample collection and processing

After informed consent serum was collected and stored in two labeled aliquots for each patient sample, and stored at

-20 degrees Celsius in a deep freezer for serum CA 19-9 and CA 242 estimations. Each aliquot was labelled with unique identification number and the diagnosis and the subgroup to which the sample belonged was not shared with the team performing the biochemical tests. Serum CA 19-9 level was determined by ARCHITECTTM CA 19-9 XR Assay, which is a chemiluminescent micro-particle assay for the quantitative determination of CA 19-9 (1116-NS-19-9) reactive determinants in human serum and plasma using the ARCHITECT i1000 system. Assay of CA 242 was carried using E lab sciences human CA 242 pancreatic carcinoma marker ELISA KIT [Production No: E-EL- H2240, Lot No. KPHI7W3CA7]. Serum levels of CA 19-9 and CA 242 were analyzed in relation to histological cytological diagnosis.

Follow-up

All cases and controls were followed using hospital records as well as telephonically at regular intervals until the end of the study and survival data was collected. All controls were also checked for ultrasound evidence of gall bladder disease. Confidentiality of data was maintained in all cases, i.e., patients and controls.

Statistical analysis

The data were expressed in number (%), mean and SD. Measurement data between groups were compared with the t-test, while enumerative data were compared with the χ^2 test. The prediction value was calculated by receiver operating characteristic (ROC) curve analysis. Statistical analysis was performed using statistical package for social sciences (SPSS) Version 21.0 statistical analysis software.

RESULTS

Demographic profile of the subjects and clinical parameters

The study population comprised of 85 subjects with mean age and gender distribution of group I, group II, and group III as 48.70SD14.83, 46.91 SD 14.36, 51.64 SD 10.88 years, and 1:1.5, 1:1.44 and 1:5.63 respectively. The mean ages of the three groups were comparable. On comparing the duration of symptoms, the majority of group II patients had symptoms for >6 months (54.5%), whereas majority of group III patients had symptoms for 3 to 12 months (62.3%), however, the difference was not significant statistically. Among the symptoms higher proportion of group III subjects (28.3%) had jaundice as compared to group II and I in which none of the subject had it (p=0.004); whereas significantly higher proportion of group II (90.9%) and group III (90.6%) patients had pain as compared to group I (p<0.001) (Table 1). Majority of subjects in the three groups did not provide history of tobacco and/ or alcohol intake. Statistically, there was no significant difference among the groups with respect to their use (p>0.05).

Bio-chemical parameters

No significant intergroup difference was observed for SGOT, SGPT, total protein, and albumin levels (p>0.05), however a significant intergroup difference was observed for serum bilirubin (total and direct) and serum alkaline phosphatase levels (p<0.05). Mean serum bilirubin (total and direct) was found to be significantly higher in group III cases compared to that in group II, whereas mean serum alkaline phosphatase levels were significant higher in group III cases compared to group I and group II (p<0.05) (Table 2).

Clinico-pathological profile of cases

Maximum number of cases, i.e., group II and group III (47.2%) were Eastern Cooperative Oncology Group (ECOG) performance status 2 followed by ECOG 1 (22.6%), ECOG 0 (17%) and ECOG 3 (13.2%) respectively. Multiple stones were seen in 21 patients from group III cases (39.6%) while 12/53 (22.6%) had a single stone. There were 20 (37.7%) patients who did not have gall stones. Thickened growth was seen in 19/53 (35.8%) cases. The growth was >2.5 cm in 20/53 (37.7%) cases. Liver infiltration was seen in 25/53 (47.2%) while lymph node enlargement was seen in 28 (52.8%) cases. Common bile duct was largely unremarkable in the majority (86.8%) of the cases. A total of 4/53 (7.5%) had dilated ducts while 1/53 (1.9%) ducts had calculi. IHBR dilation was seen in 12/53 (22.6%) cases. A total of 12/53 (22.6%) had involvement of other organs. There were 8 (15.1%) cases with distant metastasis. Incidental GBC was seen in 10 (18.9%) cases. Neck alone or in combination with others was the most commonly involved (n=26; 49.1%), followed by fundus (n=24; 45.3%) and body (n=18; 34.0%) cases respectively. Histologically/cytologically (n=48) all GBCS except one were diagnosed as Adenocarcinoma. The single remaining case was diagnosed as squamous cell carcinoma. Pathological lymph node metastasis could be assessed in 48 cases. It was absent in 29 (63%), whereas in 12 (26.1%) it was N1 and in 5 (10.9%) it was N2. Necrosis was seen in 12/32 (22.6%) cases while lymph vascular/perineural invasion was seen in 6/32 (15.8%) cases (Table 3).

Evaluation of tumor markers

CA19-9 levels ranged from 1.8 to 85950 IU/ml in the study population. Mean CA19-9 values were 6.41 SD 2.24, 9.74 SD 8.33 and 3796.14 SD 13148.01 IU/ml in groups I, II and III respectively. On comparing the mean values of group I and group III as well as group II and group III, statistically significant difference was observed (p<0.001). However, there was no significant difference between group I and group II. Median [IQR] values of CA19-9 in groups I, II and III were 6.71 [4.29-8.81], 7.74 [2.41-13.23] and 78.40 [15.28-482.75] respectively (Table 4). Statistically, there was a significant difference among groups (p<0.001). It was observed that the median CA19-9 values in GBC group (III) were significantly higher

compared to CC(II) and control(I), however, there was no significant difference between group I and group II (p=0.626).

CA-242 levels ranged from 5.87 to 183.60 IU/ml. Mean CA-242 levels were 11.04 SD 3.19, 21.50 SD 19.29 and 70.95 SD 24.80 IU/ml in groups I, II and III respectively. Statistically, this difference was significant (p<0.001). Median [IQR] CA-242 levels were 10.84 [8.31-14.35], 13.20 [11.57-17.41] and 63.50 [56.20-77.95] IU/ml in group I, II and III respectively. On evaluating the differences between the groups, only GCB group was found to have a significant difference from both control and CC cases (p<0.001), however, there was no significant difference between control and CC cases (p=0.064). Mean serum CA-242 levels were significantly higher in patients having radiological growth size >2.5 cm compared to those having radiological growth size <2.5 cm (p=0.001) (Table 3).

Except for a significant relationship of CA-242 with radiological growth size, there was no significant association of CA19-9 and CA-242 with the clinical, pathological, or survival profile of group III cases. Using both positive criteria, the sensitivity of the test was 58.5% and specificity was 100%. The positive and negative predictive values were 100% and 59.3%, respectively. Using either positive criterion was 94.3% positive and 87.5% specific. It had positive and negative predictive values of 92.6% and 90.3% and an accuracy of 91.8% (Table 5).

The patients were followed up till the end of the study, a total of 34 (64.2%) were alive, 16 (30.2%) succumbed to disease, while 3 (5.7%) were lost to follow-up. We tried to define the cutoff for CA242 in our study and found that the best results were found for the cutoff of 45.25 IU/ml (Table 6).

Table 1: Comparison of presenting complaints their duration and personal habits among different study groups namely healthy volunteers (controls), chronic cholecystitis (CC) and gall bladder cancer (GBC).

S. no.	Variable	Controls (n=10)	CC (n=22)	GBC (n=53)	Statistical significance	
		No. (%)	No. (%)	No. (%)	χ^2	P
1	Jaundice	0	0	15 (28.3)	11.0	0.004
2	Pain	0	20 (90.9)	48 (90.6)	45.33	<0.001
3	Lump	0	0	7 (13.2)	4.61	0.100
4	Duration of symptoms					
	No symptoms	-	0	1 (1.9)	7.79	0.100
	0-3 months	-	8 (36.4)	10 (18.9)		
	3-6 months	-	2 (9.1)	18 (34)		
	6-12 months	-	5 (22.7)	15 (28)		
	>12 months	-	7 (31.8)	9 (17)		
5	Tobacco addiction	1 (10%)	7 (31.8)	13 (24.5)	1.762	0.414
6	Alcohol use	0	4 (18.2)	2 (3.8)	5.78	0.056

Table 2: Group comparison of S. bilirubin (total and direct) and S. alkaline phosphatase (Tukey HSD test) among different study groups namely healthy volunteers (controls), chronic cholecystitis (CC) and gall bladder cancer (Ca GB).

Parameter	Control versus CC			Control versus Ca GB			CC versus Ca GB		
	Mean diff.	SE	'p'	Mean diff.	SE	'p'	Mean diff.	SE	'p'
Serum bilirubin (total)	-0.14	1.24	0.993	-2.35	1.12	0.098	-2.21	0.83	0.024
Serum bilirubin (direct)	-0.05	1.12	0.098	-2.14	1.01	0.091	-2.09	0.74	0.017
Serum alkaline phosphatase	-62.97	137.42	0.891	-370.26	124.23	0.010	-307.29	91.38	0.003

Table 3: Association of CA19-9 and CA-242 with diagnostic and clinical profile of carcinoma gall bladder cases (n=53).

S. no.	Characteristic	No. of patients	CA 19-9		CA 242	
			Mean	SD	Mean	SD
1	Eastern cooperative oncology group (ECOG) performance status					
	0	9	12.95	9.91	61.39	11.91
	1/2/3	44	5525.61	15678.85	72.91	26.35

Continued.

S. no.	Characteristic	No. of patients	CA 19-9		CA 242	
			Mean	SD	Mean	SD
	Statistical significance		't'=1.047; p=0.300		't'=1.277; p=0.207	
2	Radiological growth size					
	≤2.5 cm	33	1496.96	4063.25	62.66	11.67
	>2.5 cm	20	7589.78	20517.84	84.64	33.72
	Statistical significance		't'=1.663; p=0.102		't'=3.346; p=0.001	
3	Liver infiltration					
	Yes	25	6490.95	18464.12	76.48	31.41
	No	28	1390.05	4142.97	66.02	15.94
	Statistical significance		't'=1.424; p=0.161		't'=1.554; p=0.126	
4	Involvement of other organs					
	Yes	12	5826.36	11356.55	68.78	12.14
	No	40	3281.79	13863.97	71.80	27.83
	Statistical significance		't'=0.579; p=0.565		't'=0.363; p=0.718	
5	Incidental GBC					
	Yes	10	207.46	465.60	65.33	14.26
	No	43	4630.71	14498.41	72.26	26.62
	Statistical significance		t=0.957; p=0.343		t=0.793; p=0.431	
6	Histological/cytological diagnosis (n=48)					
	Adenocarcinoma	47	4099.33	14012.59	71.37	25.86
	Squamous cell Ca	1	7.48		63.20	
	Statistical significance		-		-	
7	Histological grade					
	Poorly differentiated	1	9179		61.25	
	Mod. differentiated	12	295.92	460.37	66.09	20.14
	Well differentiated	19	5821.45	19875.44	73.01	24.85
	Statistical significance		F=0.517; p=0.601		F=0.402; p=0.673	
8	Tumor site					
	Neck	20	4486.2	9767.3	67.32	14.28
	Neck + Body	4	1503.4	2373.01	86.93	34.73
	Neck + Body + FUUS	2	4985.12	7041.20	65.35	17.47
	Body	5	372.13	640.44	63.28	8.23
	Body + FUUS	9	683.94	1509.23	66.67	16.76
	FUUS	13	6728.58	23803.65	78.41	39.94
	Statistical significance		F=0.314; p=0.902		F=0.807; p=0.550	
9	Tumor thickness					
	≤1 cm	16	388.34	1161.14	64.69	17.20
	>1 cm	35	4441.18	14830.21	73.85	27.85
	Statistical significance		't'=1.086; p=0.283		't'=1.211; p=0.232	
10	Necrosis					
	Yes	12	1746.05	5315.70	74.68	23.08
	No	27	5286.70	17864.33	72.72	29.84
	Statistical significance		't'=0.669; p=0.508		't'=0.201; p=0.842	
11	LVI/PNI					
	Absent	32	5739.94	18101.72	71.40	22.50
	Present	6	5016.54	7493.10	59.54	7.51
	Statistical significance		't'=0.095; p=0.924		't'=1.265; p=0.214	
13	Pathological N status					
	No	29	4226.52	15988.94	73.77	29.49
	Yes	17	3866.48	10242.19	68.15	18.14
	Statistical significance		't'=0.083; p=0.934		't'=0.709; p=0.482	
14	No. of gallstones					
	No	20	3764.95	9534.97	68.26	16.93
	Single	12	8280.69	24697.11	75.97	25.89

Continued.

S. no.	Characteristic	No. of patients	CA 19-9		CA 242	
			Mean	SD	Mean	SD
	Multiple	21	1263.23	2969.46	70.65	30.59
	Statistical significance		F=1.092; p=0.344		F=0.356; p=0.702	
15	Gallstones					
	Yes	33	3815.04	15064.3	72.58	28.67
	No	20	3764.95	9534.97	68.26	16.93
	Statistical significance		't'=0.013; p=0.989		't'=0.612; p=0.543	
16	Survival					
	Alive	34	4055.01	14976.30	72.18	26.66
	Expired	16	3908.88	10157.47	71.50	22.56
	Statistical significance		't'=0.035; p=0.972		't'=0.087; p=0.931	

Table 4: Comparison of CA 19-9 and CA 242 expression among different study groups (IU/ml).

S. no.	Group	No. of subjects	Mean and SD	Median [IQR]	Mean and SD	Median [IQR]
			CA 19-9		CA242	
1	Group I: healthy volunteers (controls)	10	6.41 SD 2.24	6.71 [4.29-8.81]	11.04 SD 3.19	10.84 [8.31-14.35]
2	Group II: chronic cholecystitis (CC)	22	9.74 SD 8.33	7.74 [2.41-13.23]	21.5 SD 19.29	13.20 [11.57-17.41]
3	Group III: gall bladder cancer (GBC)	53	3796.14 SD 13148.01	78.40 [15.28-482.75]	70.95 SD 24.80	63.50 [56.20-77.95]
Statistical significance			c^2=30.71; p<0.001 (Kruskal Wallis test)		c^2=49.95; p<0.001 (Kruskal Wallis test)	

Table 5: Comparison of diagnostic efficacy of CA 19-9 (>43 IU/ml) and CA-242 (>45.25 IU/ml) combination for diagnosis of GBC.

Criteria	Sens	Spec	PPV	NPV	Accuracy	PLR	NLR
Both positive	58.5	100	100	59.3	74.1	¥	0.42
Either positive	94.3	87.5	92.6	90.3	91.8	7.55	0.06

Table 6: CA-242 diagnostic efficacy at different cut-off levels (GBC).

Cut-off	Sens	Spec	PPV	NPV	Accuracy	PLR	NLR
≥28.0	100	87.5	93.0	100	95.3	8	0
≥45.25	96.2	87.5	92.7	93.3	92.9	7.7	0.04
≥70.0	37.7	100	100	49.2	61.2	∞	0.62
≥57.75	79.2	93.7	97.7	73.8	85.9	12.57	0.22

DISCUSSION

The burden of GBC has increased in recent years worldwide. The question as how to treat and assess the therapeutic effect, evaluate the prognosis, and predict post-operative recurrence in GBC cases has prompted increased attention of both clinicians as well as surgeons worldwide, to look for ways of detecting carcinoma at early stage. In the present observational study, we evaluated serum levels of tumor marker (TM) CA 242 and TM CA 19-9 in cases with benign gall bladder disease like chronic cholecystitis (CC), histologically diagnosed GBC (cases) and normal healthy controls (HC). We studied 53 patients with GBC (62.3%), 22 patients with CC (25.9%), and 10 healthy voluntary controls (11.8%). The mean age of presentation of GBC was comparable to published Indian literature that is between 50 to 55 years.[15,16] However, the peak incidence of GBC in western countries is almost two decades higher (i.e. 70-79 years) compared to India.[17-20]

On comparing the symptoms, i.e. the association of pain and jaundice with serological values of CA 19-9 and CA242, it was found that serological values correlate with the clinical symptoms discussed above i.e., obstructive jaundice which was seen in a significantly higher number in GBC compared to CC.[6,16,21] Majority of gallbladder cancer cases present with nonspecific symptoms similar to

benign gallbladder disease and thus are usually diagnosed late leading to high mortality and morbidity. As reported in literature in our study we found that the bulk of patients (42/53; 79.2%) presented with either T2/T3 i.e., late-stage disease incidental post-cholecystectomy carcinoma seen in 11 cases (11/53 cases; 20.8%).[20,21] Therefore, there is a need for a means to diagnose the disease early in the disease course, when a cure may be possible. Tumor markers may help in this case scenario to reliably differentiate between benign causes and malignancy in patients presenting with symptoms related to gall bladder disease.

The observed mean of serum CA 19-9 and CA242 values were found to be significantly raised in GBC patients compared to CC and HC. Similarly, higher median values were noted for both serum markers in GBC patients compared to CC and HC. On contrary, similar value of mean serum CA 19-9 were seen in both CC and HC. Thus, we found that serum CA 19-9 was able to differentiate between benign and malignant groups, i.e., CC/HC versus GBC, but was not able to differentiate between healthy people and patients with chronic cholecystitis. Serum CA242 values showed significantly higher mean and median values in GBC cases compared to controls. Our results were concordant to the reported literature where it has been found that CA19-9 and CA242 are significantly raised in GBC as compared to CC and HC.[18-28] The results displayed in table 5 clearly show that complimenting it with CA242 will not only add to specificity by which both may predict presence or absence of malignant change in gall bladder but also sensitivity while screening if we consider elevated levels of either of them inclusive.

The difference between levels of both CA 19-9 and CA 242 with the incidental diagnosis of GBC cases (10/53) versus non-incidental category, were not statistically significant (p=0.431); ascertaining that both the markers were able to pick up incidental GBC almost as much as radiologically and clinically evident GBC. This is a very important finding as it suggests strongly that inclusion of CA242 along with CA19.9 in preoperative workup of cholecystectomy patients may provide information underlying pathologic process being malignant or not. Preoperative information like this rendered by the above marker can be of irreplaceable value in patient care. The clinician can hence counsel the patient accordingly and add intraoperative frozen section evaluation in these cases. The surgery may be modified based on intraoperative tissue diagnosis of these cases.

There are reports in literature where higher mean levels of CA 19-9 and CA242 were seen in patients with nodal metastasis.[26] However, our results were contrary and no significant results were obtained in this regard. However; we observed a significant correlation between tumor size more than 2.5 cm and a raised value of CA242 (p=0.001) in our study, the above parameter could be a reflection of tumor burden and hence once positive it may be used as a predictor of tumor load and may serve to be a reflection of response to treatment.[25] To the best of our knowledge, no study in the published English literature has tried to correlate histological parameters like tumor thickness, tumor necrosis, LVI, and PNI with serum levels of tumor markers CA19-9 and CA 242. We tried to explore these parameters with respect to CA19-9 and CA242, however no significant differences were observed for these in our patient population.

Cutoff of 45.25 IU/ml was found to have an acceptable sensitivity and specificity with positive likelihood ratio of 7.7 and negatively likelihood ratio of 0.04. This was contrary to the cutoff provided by the product insert [E lab sciences human CA 242 pancreatic carcinoma marker ELISA KIT Production No: E-EL- H2240, Lot No. KPHI7W3CA7]. Authors have also supported cut-off between 42-45 IU/ml for best results. [16, 26-27] As shown in table 6 if we use only CA242 for diagnosis 100% specificity may be achieved with compromised sensitivity of 37.7% that too when the cutoff is made at 70 IU/ml. This will make this test unsuitable for screening purposes owing to low sensitivity. Studies have shown that combination of multiple markers is always provides with more reliable results rather than single marker evaluation for disease prediction.[29] Our results when interpreted for assessing the dual marker (CA242 with CA19-9) with respect to prediction of malignancy in GBC. We found that when, both CA19-9 and CA242 were elevated (both positive), the specificity of the dual test was 100% for carcinoma prediction and if we considered Either Positive then the sensitivity is 94.3% with a diagnostic accuracy of 91.8%. This supports that combination of CA242 and CA19-9 may be more informative rather than applying single test. CA19-9 however cannot be totally replaced as still larger multicentric studies to establish the role of CA242 in follow up are deficient.

The limitations of the present study were that gender ratio could not be made comparable in GBC and CC groups, which might have influenced the results. Secondly, serial measurements of TM on treatment could have provided a better correlation. Moreover, multicentric and international collaboration may also provide with robust data in this regard.

CONCLUSION

Hence, our results clearly show that, if we compliment CA 19-9 with CA 242, the probability to pick-up the affected population would be increased using either positive criterion and when both markers are raised, the probability of the patient having an underlying malignancy in the gallbladder would be high.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Misra M, Mishra M, Agarwal P, Gupta S, Anand A, Ali W, et al. Inclusion of carbohydrate antigen 242 in addition to carbohydrate antigen 19.9 in serological workup of carcinoma gall bladder: a case series analysis. Int J Res Med Sci 2023;11:4360-8.