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DIAGNOSTIC SIGNIFICANCE OF SERUM CA242, CA19.9 AND CEA IN DIAGNOSIS OF GALL BLADDER CANCER

Biochemistry

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ABSTRACT

Background: Tremendous progress of imaging technique does not result in early diagnosis of gall bladder cancer. In search of effective, inexpensive and non-invasive tool tumour markers shows promise. Serum CA242, CA19.9 and CEA are evaluated for this purpose. **Aim and objective:** To evaluate diagnostic significance of serum CA242, CA19.9 and CEA in gall bladder cancer either alone or in combination. **Material and methods:** It was an analytical cross sectional study done in Indira Gandhi Institute of medical Sciences. These markers were measured in 70 cases of gall bladder cancer and 70 healthycontrols. Serum CA242 was done by ELISA assay as per instruction given in pack insert (CD diagnostic). Serum CA19.9 and CEA were done by CLIA technique by Assess 2 analyser. **Results:** Mean value of these tumour marker were increased in gall bladder cancer group ($p<0.001$). CA19.9 was most sensitive tumourmarker (79.5%). Serum CA 242 was most specific test (83.3%) for diagnosis of gall bladder cancer among the three-tumour marker. On combination of tumour marker sensitivity (87.5%) and specificity (89.5%) were increased. In ROC curve analysis area under curve (AUC) for CA242 was highest. **Conclusion:** Combination of these tumour markers can be used as screening tool for gall bladder cancer.

KEYWORDS

Tumour marker, Gall bladder cancer, CA242, CA19.9, CEA.

INTRODUCTION

Gall Bladder cancer is one of the most aggressive tumour of biliary tract with very poor five year survival rates early manifestation of the disease is barely visible.**[1,2,3]**There is lack of serosal layer adjacent to liver which leads to early hepatic invasion, metastatic progression and so, measurable prognosis**[4].**World wise incidence of gall bladder cancer is 2/100000 individual but there is great variation in different geometrical location**[5].**Its incidence in resident of Indo-gangatic belt in Northern India is21.5/100000.It has been reported as highest affected region by gall bladder cancer**[5].**This cancer affects female gender two to three times more than male[**6].**Female hormone estrogens causes super saturation of cholesterol in bile that leads to formation of gall stone and hence involved in pathogenesis of gall bladder cancer**[7].**Porcelain gall bladder, Mirizzi syndrome and bile reflux are other predisposing factor of the disease **[8].**26% of the gall bladder carcinoma is familial in nature and there is significant risk in third degree relative [**9].** Clustering of gall bladder carcinoma in families is suggestive of role of genetics in its development **[10].**

Call bladder cancer has a very grave prognosis with median survival of 2-4 month in unresectable disease and 1 year survival in less than 5% cases**.[11]** Even with use of combination chemotherapy disease free survival is only 8 monthoverall median survival is 11.7 month**.[12]**More patients at the time of diagnosis is at advance stage thus radical cholecystectomy could not be performed.So, it is important to diagnosegall bladder cancer in earlier phase. **[13]**

Tumour markers are bioactive substances produced by tumour cell. They are non invasive, less expensive test with acceptable reliability. So, they are used for cancer screening and predicting prognosis of gall bladder cancer. Various tumour marker including CEA, CA125, CA19.9, CA242, are studied in connection with gall bladder cancer.CA 242 was obtained by immunization of mice with human cell line COLO205 fused with sp2/0 myeloma cell line**.[14]** It was used as tumour marker for pancreatic cancer**. [15-18]** Because of common embryonic origin is shared by both gall bladder and pancreases. This tumour marker is also increased in gallbladder cancer. **[19]** CA19.9 was isolated from Lewis Ag. **[20]** Level of CA 19.9 is elevated in patient of gall bladder cancer. But it is also increase in patients with gall stone especially with cholecystitis. **[21]** CEA is found in gastro intestinal tissue during foetal development and it is increased in gastro intestinal cancer such as gall bladder, stomach and colorectal. But it is also non-specifically increased in heavy smoker and in non specific colitis**. [20]**

The behaviour of tumour marker in biliary tract malignancy is not well known and has been scarcely studied. These markers could play important role in diagnosis of gall bladder cancer.

My study is aimed at to know significance ofCA242, CA19.9 and CEA in diagnosis of gall bladder cancer either individually or in combination.

MATERIALAND METHOD:

The study was an analytical cross-sectional study conducted in the department of biochemistry in Indira Gandhi Institute of Medical Sciences, Patna from march 2019 to February 2020. Cases of Gall bladder cancer were recruited from outpatient department of State cancer Institute.

Case comprises of 70 newly diagnosed cases of Gall bladder carcinoma .The diagnosis of gall bladder carcinoma was based on clinical, radiological, and histopathological finding. An ultrasound and CT scan were used to stage cancer using Henson staging criteria.**[22]** Proper written consent was taken.

Inclusion criteria include a) Newly diagnosed case of gall bladder cancerand b) Age between 20 – 72 years

Exclusion criteria includes a) Patient with benign tumor in gastrointestinal tract such as pancreas and colon or stomach and b) Patients with other coexisting malignancy.

70 healthy volunteer were taken as control controls were selected after matching age and gender of cases. There was no difference in baseline data.

Detection of tumour marker

5 ml of venous blood was collected and after clotting centrifuged at 3000 rpm/min at room temperature for 10 minute to separate serum.Separated serum was stored at -20^0 C until use.

The concentration of CA 242 was measured by direct sandwich ELISA assay (CD diagnostic). The procedure was implemented using manufacturer protocol.The inter-assay and Intra assay variation was 4.1% and 3.8% respectively. Normal reference range of serum CA242 was <20 U/ml. **[23]**

Serum CEA and serum CA 19–9 levels were also done. CEA levels were assessed using chemiluminensense immunoassay (Assess2 Beckman Coulter) and cut-off of 3 U/ml as considered to be elevated as per manufacturer's recommendations. CA 19–9 levels were assessed

using chemiluminensense immunoassay (Assess2 Beckman Coulter) and cut off value was <37 U/ml.

STATISTICALANALYSIS

Student's t test was used to compare quantitative variables across the two groups i.e. case and control**.** The ROC curve was displayed using SPSS 16.0. Area under curve (AUC), 95% CI was calculated for each tumour marker. Sensitivity, specificity, positive predictive value (PPV) and Negative predictive value were also determined. All the above mentioned statistical calculations were done by SPSS 16.0 software (SPSS Inc. Chicago IL, USA).p<0.05 was considered statistically significant**.**

RESULT

The Demographic and clinical Data is presented in table 1, The mean of patients with Gall bladder carcinoma were 49±12 years and that of control was 47±10 years. Prevalence of cases in female is high 46(65.7%).Maximum cases are present in IIIB and IVB stage in my study**. (Table 1)**

Table 1 Showing Clinical and demographic finding of cases and control in Gall Bladder Carcinoma

		case	control
Age(years)		49±12	47±10
sex	Female	46(65.7%)	45(64.2%)
	Male	24(34.3%)	25(35.8%)
Gall Stone	Present	51 (72.8%)	--
	Absent	19 (27.2%)	
Stage	II	4	------
	IIIA	9	
	IIIB	21	
	IVA	8	
	IVB	28	

Mean level of serum 242 was significantly higher in patients of gall bladder cancers compared to control p<0.001). There was also significant difference among the mean level of CEA and CA 19.9 in case and control (p<0.001). **(Table 2)**

Table 2: showing mean value of tumour markers (CEA, CA19.9, CA242) in gall bladder carcinoma and healthy control.

	CA242(U/ml) (Mean±SD)	CA19.9(U/ml) (Mean±SD)	CEA(ng/ml) (Mean±SD)
case	44.12±26.2	270.6±192.6	22.6±21.9
control	14.57±7.5	23.75±20.22	2.96±3.01
P*	0.001	0.001	0.001

P* student´s t test

Serum CA 242 was most specific test (83.3%) for diagnosis of gall bladder cancer among the three tumour marker. Ca 19.9 was most sensitive diagnostic tumour marker (79.5%) among these three. CEA has highest PPV and CA 19.9 has highest NPV for Gall bladder cancer diagnosis. Use as single tumour marker CA242 has very good specificity but low sensitivity than other two markers. Combination of all the tumour markers gave highest sensitivity and specificity. **(Table3)**

Table 3: showing Sensitivity, Specificity, PPV, and NPV of CEA, CA19.9 and CA242 in gall bladder cancer individually and in combination.

Parameter	Sensitivity	Specificity	PPV	NPV
CA242	77.3	83.3	85	75.1
CA19.9	79.5	78.6	77.5	80.2
CEA	64.7	60.9	55	70
Combination of Tumour Markers	85.7	89.5	87.5	89.5

The ROC curve analysis for CA242, CA19.9 and CEA in Gall bladder cancer are lotted in figure 1 The area under curve(AUC) of ROC analysis for CA242, CA19.9 and CEA in gall bladder carcinoma were 0.912, 0.893 and 0.749.AUC of CA242 was highest. This shows very good diagnostic performance. Diagonal line represents reference line. **(Figure 1)**

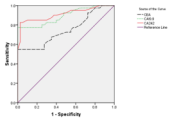

Figure 1 showing ROC curve of different tumour markers (CEA, CA19.9, and CA242) in gall bladder cancer.

DISCUSSION

In this study out of seventy cases of gall bladder cancer age ranges from 25 years to 84 years. 28 cases are in stage IV B i.e., in advance stage. As the incidence of Gall bladder cancer is increasing and prognosis is very poor in in advance stage. Finding of cases in early stage is very important to save life. Tumor markers are molecular process or tissue-based process that gives information about behavior of malignancy. **[24]** They are used for diagnosis and predicting prognosis of malignant tumor.

In this study we analyze diagnostic value of three tumor marker CEA, CA19.9 and CA242 in gall bladder carcinoma. Mean value of Serum CA19.9 is increased in case of gall bladder cancer. Kankonkar et al. also found the similar value (847.6 U/mL) in his study. **[25]** Shukla et al. also reported the raised value of serum CA19-9 in malignant lesions as compared to the benign lesions of gallbladder (211.27 U/mL vs. 86.06 U/mL). **[26]** Other studies also identified serum CA 19-9 as a sensitive marker for carcinoma gallbladder**.[27-32]** Serum CA242 was also significantly increased in case of gall bladder cancers compared to control. Rana et al in (2012)found that the median levels of CA 242 were higher in the gall bladder cancer group (59 [199]) compared to the stomach cancer group (10 [13]; p<0.001) and the control group (3 [14.5]; p<0.001).**[33]** Wang et al found significant differences in serum CA125, CA199, and CA242 levels between GBC cases at different stages, tumor size, and differentiation **.[34]** Serum CEA was also significantly increased in case of gall bladder cancer compared to control.Rana et al also found increasedmedian CEA level was 9.5 (IQR 28) U/ml in patients with GBC in contrast to 6 (IQR 11.5) U/ml in patient with GS (p=0.791).**[33]**

Specificity of CA 242 is highest among three tumor marker. Wang et al in also found highest specificity of serum 242 (98.5%) in gall bladder cancer cases among CEA, CA19.9 and CA242**.[34]** Rana et al reported that CA242 was better than CEA and CA199 as a tumor marker for the diagnosis of GBC. He found sensitivity, specificity, PPV, and NPV of CA 242 were 64%, 84%, 88%, and 53%, respectively. **[33]** CA19.9 was most sensitive test among three tumor marker in our study. Kumar et al found that sensitivity and specificity of CA19.9 is 78.3% in case of gall bladder cancer**[35].**Wang et al found sensitivity and specificity of CA19.9 was71.7% with 96.1%. He also reported CA19.9 was most sensitive tumor marker than CEA and CA242 for diagnosis of gall bladder cancer. **[34]** Our study also showed that CEA had limited value for the diagnosis of GBC. This study is in similar to other studies of Vij et al who suggested that CEA and AFP had little value for the diagnosis and prognosis of GBC**.[36]** Kumar et al**[35]** also reported poor sensitivity and specificity of CEA in GBC which was nearly 39% and 68% respectively. Grunnet M et al also reported low sensitivity and specificity of CEA in diagnosis of Gall bladder cancer. **[37]**

In terms of a single marker for the diagnosis of GBC, CA19.9 has the highest sensitivity with relatively low specificity. It cannot be used alone as an effective tumor marker to identify GBC. CA242 has the highest specificity, However, the expression of CA242 is high in pancreatic cells , and therefore cannot be used to differentiate between GBC and pancreas cancer**.[38]** The Joint detection of CA242, CA125, and CA199 may prove to be useful for the diagnosis of GBC, assessing therapeutic effects, and predicting a prognosis. When we combine all the three tumor marker sensitivity and specificity was increased. Yawen Deng et al (2017) also found increased sensitivity and specificity when he combine serum CEA, CA125, CA19-9, and CA724 for diagnosis of GBC**.[39]** Other studies also reported increase in sensitivity and specificity when a combination approach of tumor marker was used**.[32,34,40].**

CONCLUSION

When we use a single tumour marker as screening test for diagnosis of gall bladder cancer results are inconsistent . Combined detection of CA242, CA19.9 and CEA was very effective than single detection of tumour marker. So, a combination approach this triple tumour marker can be considered as important tool for diagnosis of gall bladder cancer.

Conflict of interest: There was no conflict of interest

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