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RESEARCH ARTICLE

COMBINED ASSESSMENT OF LIPID PEROXIDATION, CEA AND CA19.9 BIO-MARKERS IN ADVANCED STAGE GALL BLADDER CANCER

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ABSTRACT

To clarify the value of combined use of bio-markers for the diagnosis of advanced stage gallbladder cancer and to observe the associated hepato-haematological abnormality. Serum level of MDA, CEA and CA19.9 were measured in 51 patients with gallbladder cancer (GBC), and 21 healthy controls using TBARs method for MDA and chemiluminescent microparticle immunoassay (CMIA) for CEA & CA19.9. In these patients LFT was estimated by the help of a commercial kit (APL, SGOT, SGPT, Total bilirubin, direct bilirubin, Total protein, Albumin & Globulin kit, Coral clinical systems, Goa, India) and haematological parameters were measured by standard procedure using Cell Counter (Medonic M-Series) which is used in Mahavir Cancer Institute, Patna, India. Serum MDA, CEA and CA19.9 levels in the GBC group were significantly higher when compared with those in healthy control groups ($P < 0.05$). With a single tumor marker for GBC diagnosis, the sensitivity of MDA was the highest (95.23%), with the highest specificity being in CEA (98.7%). Diagnostic accuracy was highest with a combination of MDA, CEA & CA19.9 (72.54%). Hepato-haematological parameters has been found to be significant differences of the mean ± SE of MDA, CEA, CA19.9, RBC, WBC, haemoglobin, ALP, SGOT, SGPT, Total bilirubin, Direct bilirubin & Globulin between the GBC and the normal groups ($P < 0.05$). There was no significant differences of the PLT, Total protein & Albumin between the GBC and normal groups ($P >0.05$). The combined detection of these markers may prove to be useful for diagnosis of GBC, assessing therapeutic effects, and predicting a prognosis. A majority of patients had an abnormal hepato-haematoparameters at the time of diagnosis requiring meaningful palliative treatment.

INTRODUCTION

Carcinoma of gallbladder is the fifth most common gastrointestinal cancer and most common and aggressive malignant neoplasm of the biliary tract in the USA (Jemal *et al.* 2008). The incidence rate of gallbladder cancer is observed very high in northern Indian cities and low in southern India (Dhir *et al*. 1999). Gallbladder cancer (GBC) is a disease in which cancer cells are found in the tissues of the gallbladder. Majority of the gallbladder tumors are found in the glandular tissue within the gallbladder [Adenocarcinoma]. Others originate in the connective tissue [sarcoma] or other tissues [squamous cell carcinoma] (De *et al*. 1999). The prognosis of GBCs is extremely poor with high mortality. Over 90% of GBC patients are diagnosed at inoperable stage with serious invasion and metastasis to other organs (Hawkins *et al*. 2004). Metastasis is an indicative of poor prognosis for various cancers (Iiizumi *et al*. 2008). Early diagnosis is generally impossible because of the lack of specific sign, symptoms and makers (Reid *et al*. 2007). In modern clinical practice, the diagnosis of GBC's is made on non-invasive auxiliary imaging and invasive examination for example laparoscopy and biopsy. However, there is no specific single tumor marker for the diagnosis and prognosis of GBC (Okada *et al*. 2012, Eil *et al*. 2013 & Zhai *et al*. 2012).

Malondialdehyde (MDA), which is an end product of the oxidation of polyunsaturated fatty acids (PUFA) and can determine the degree of lipid peroxidation (LPO), has been used as a marker for oxidative stress (Bae *et al*. 2010). The super oxide ion generation is responsible for DNA, protein and lipids damage in our body (Yang *et al*. 2007). Such as MDA is an intermediate which forms DNA-MDA adduct leading to

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cause mutation by forming M_1A, M_1G and M_1C with dA, dG and dC respectively (Ward *et al*. 1988 & Esterbauer *et al*. 1990). However, MDA has been established to promote the development of cardiovascular diseases, atherosclerosis and various types of cancers in human (Bast *et al*. 1993).

Some group of researchers have been widely used tumor markers such as carcinoembryonic antigen (CEA) and cancer antigens (CA) CA19.9 for the diagnosis of different types of cancer [*e.g*., liver, gastric, colorectal, and pancreatic] (Ghosh *et al*. 2013, Zhang *et al*. 2013, He *et al*. 2013 & Zur *et al*. 2012). Together with CEA, elevated CA19.9 is suggestive of gallbladder neoplasm in the setting of inflammatory gallbladder disease (Strom *et al*. 1990). This tumor associated antigen may also be elevated in some non-malignant conditions (Steinberg *et al*. 1990). CEA is a substance normally found in fetus, but the same on elevated level in adult blood has been shown to be involved with colorectal cancer and other types of cancer, also referred to as an "oncofetal antigen" because of its similarity to fetal tissue (Ohuchi *et al*. 1989).

In gallbladder cancer, liver is the primary organ for metastasis because the gallbladder lies under the liver. The liver has a complex functions, out of which some important functions are to synthesis of plasma proteins such as albumin, fibrinogen etc. and metabolism of bilirubin, proteins, fats, carbohydrates etc. They are also responsible for detoxification of some drugs and alcohol (Mohan *et al*. 2000). The liver damage can occur following viral infection or exposure to drugs and toxins. Drugs and toxins can injure cell through direct damage to cell membranes or through the release of reactive intermediary metabolites with resultant release of oxygen free radicals and lipid peroxidation. Viruses can damage the liver directly, as in hepatitis A, d agent (hepatitis D virus), or hepatitis C infection or through immunologically mediated mechanisms, as in hepatitis B infection (Henry *et al*. 2001). If liver is damaged then all functions of liver may be affected.

Therefore, the present study was undertaken to investigate that upto what extent the lipid peroxidation and bio-markers are elevated in gallbladder cancer patients. This may be evaluated by the assessment of lipid peroxidation and bio-markers in serum of gallbladder cancer patients and by observing the associated hepato-haematotoxicity parameters in gallbladder cancer patients.

MATERIAL AND METHODS

Blood samples were collected from 51 gallbladder cancer patients as well as 21 normal volunteers who came for their treatment at Mahavir Cancer Institute, Patna, India, from the department of pathology, after prior consent of the patients. All 51 patients of advance stage of GBC as per clinical and radiological staging are selected for sample collection. The study has been approved from Human Ethical Committee (HEC) of Mahavir Cancer Institute, Patna, India.

Determination of MDA

Serum of 51 gallbladder cancer patients as well as 21 normal volunteer were assayed for lipid peroxidation by determining their malondialdehyde (MDA) levels. MDA level in each subjects was determined by standard procedure of TBRA method with slight modifications (Ohkawa *et al*. 1979). The blood samples were centrifuged at 3000 rpm for 10 min and serum was collected and stored at -80˚ C. 2.5 ml of 10 % of trichloroacetic acid (TCA) was added to 100 µl of test serum, incubated at 95˚ C for 15 min and the solution was centrifuged at 3000 rpm for 10 min. The supernatant was collected and 0.675% of tributaric acid (TBA) was added to the same and incubated again at 95˚ C for 15 min. The colour reaction was obtained to measure optical density using spectrophotometer and concentration was determined from standard prepared formula.

Haematological parameter

RBC count, WBC count, platelet count and haemoglobin levels were estimated by standard procedure using Cell Counter (Medonic M-Series) in the department of haematology, Mahavir Cancer Institute, Patna, India.

Detection of serum tumor markers

Serum CEA and CA19.9 levels were detected by chemiluminescent microparticle immunoassay (CMIA) at the Department of pathology of the Mahavir Cancer Institute, Patna, India. The normal reference values were as follows: CEA < 3.0 ng/ml and CA19.9 < 40.0 U/ml.

Estimation of liver function test

Alkaline phosphatase (ALP), serum glutamates oxalo-acetate transferase (SGOT), serum glutamates pyruvate transferase (SGPT), Total bilirubin, Direct bilirubin, Total protein, albumin and globulin levels were estimated by the help of a commercial kit (APL, SGOT, SGPT, Total bilirubin, Direct bilirubin, Total protein, Albumin & Globulin kit, Coral clinical systems, Goa, India).

Statistical analysis

The data were expressed as mean ± SE. The obtained data between groups were compared with the *t* test. Statistical analysis was performed using SPSS version 20.0 statistical software. All tests were two-tailed and $P < 0.05$ was considered statistically significant.

RESULTS

Table1: shows the characteristic profiles of subjects enrolled in the study. In this study 21 normal volunteer (7 male & 14 female) & 51 gallbladder cancer patients (15 male & 36 female) were enrolled. Out of 51 patient's gallbladder cancer is more prevalent in women (70.59%) than men (29.41%). **In table2** different symptoms pattern of GBC patients are mentioned, recorded at clinical presentation. Clinical presentation were as follows: abdominal pain 72.54%, loss of appetite 33.33%, nausea & vomiting 17.64%, fever 13.72%, anorexia11.76%, jaundice 5.88%, weakness 5.88% and others symptoms 29.41%. In the **text fig.1** shows that the liver function test (ALP, SGOT, SGPT, Total bilirubin, Direct

bilirubin, Total protein, Albumin & Globulin), in the **text fig.2** shows that the complete blood cell count (RBC, PLT, WBC & haemoglobin) & in the **text fig.3** shows that the MDA level and bio-marker level (CEA & CA19.9) of normal and gallbladder cancer patients.

Table1 Characteristics of controls and gallbladder cancer (GBC) patients.

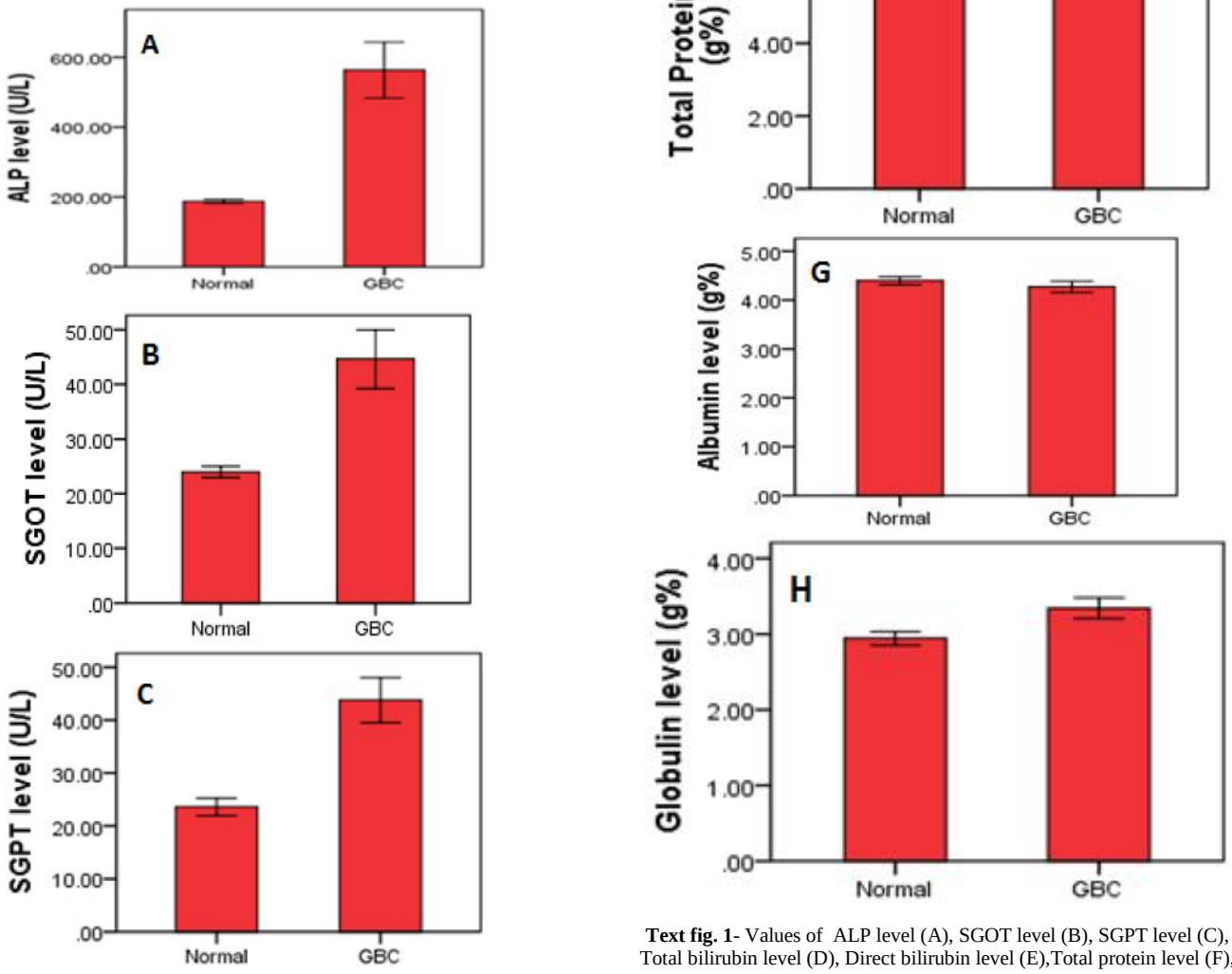
Characteristic profile	Normal,)	GBC,)
Total	21	51
Male	7(33.33)	15(29.41)
Female	14(66.67)	36(70.59)
Mean age (years) ± SE.	45.57±2.12	50.45±1.58
Type of carcinoma		
Adenocarcinoma	N/A	49(96.08)
Sq. cell carcinoma	N/A	2(3.92)

N/A: not applicable.

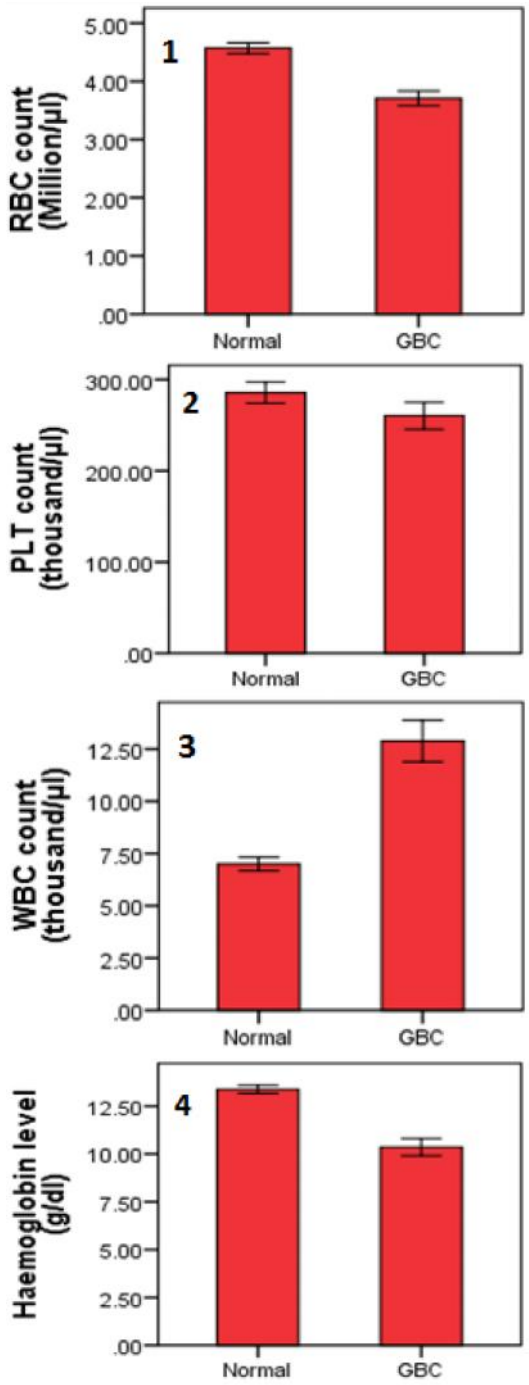
Table2 Different recorded symptoms Pattern at clinical presentation in gall bladder cancer patients (n=51).

Symptoms	Proportion of patients (%)
Abdominal pain	37(72.54%)
Loss of appetite	17(33.33%)
Nausea & vomiting	9(17.64%)
Fever	7(13.72%)
Anorexia	6(11.76%)
Jaundice	3(5.88%)
weakness	3(5.88%)
Others symptoms	15(29.41%)

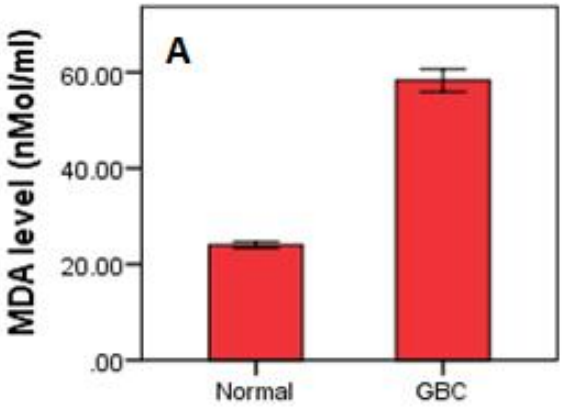
*Abdominal pain was present in most of the patients.

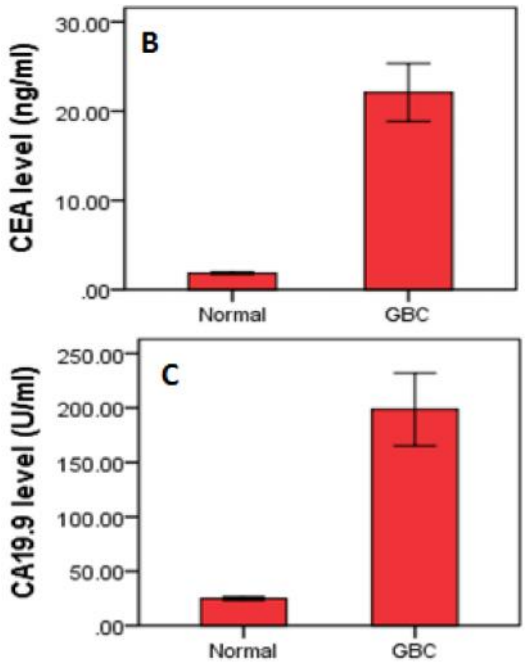


Text fig. 1- Values of ALP level (A), SGOT level (B), SGPT level (C), Total bilirubin level (D), Direct bilirubin level (E),Total protein level (F), Albumin level (G) and Globulin level (H) of normal and GBC patients.



Text fig. 2- Values of RBC count (1), PLT count (2), WBC count (3) and haemoglobin level (4) of normal and GBC patients.





Text fig. 3 Values of MDA level (A), CEA level (B) and CA19.9 level (C) of normal and GBC patients.

In table3: it is mentioned that were significant differences of the mean ± SE of MDA, CEA, CA19.9, RBC, WBC, haemoglobin, ALP, SGOT, SGPT, Total bilirubin, Direct bilirubin & Globulin between the GBC and normal groups ($P < 0.05$). There was no significant differences of the PLT, Total protein & Albumin between the GBC and normal groups ($P >0.05$). The positive rates of MDA, CEA & CA19.9 in normal volunteer & GBC patients is given in the **table 4.**

Table3 Mean ± SE of different pathological parameters of normal persons and GBC patients.

Pathological parameter	Normal group (Mean ± SE.)	GBC Patients (Mean ± SE.)	p-value
MDA	24.26±0.73	59.17±1.48	0.000
CEA	1.85±0.14	22.08±3.23	0.000
CA19.9	24.98±1.80	198.71±33.41	0.000
RBC	4.57±0.09	3.86±0.09	0.000
PLT	285.57±11.55	260.29±14.68	0.180
WBC	7.00±0.32	12.87±0.98	0.000
Haemoglobin	13.37±0.21	10.75±0.29	0.000
ALP	187.61±6.03	581.01±65.77	0.000
SGOT	24.00±1.00	44.60±5.38	0.000
SGPT	23.61±1.65	48.39±6.00	0.000
Total bilirubin	0.47±0.04	1.39±0.36	0.015
Direct bilirubin	0.24±0.01	0.75±0.23	0.032
Total protein	7.40±0.11	7.66±0.09	0.073
Albumin	4.39±0.08	4.20±0.08	0.097
Globulin	2.94±0.09	3.44±0.08	0.000

Table4 Positive rates of lipid peroxidation, carcinoembryonic antigen and cancer antigen 19.9 n (%)

	GBC (n=51)	Normal group (n=21)
MDA pc	50(98.03)	3(14.28)
CEA pc	42(82.35)	1(4.76)
CA19.9 pc	37(72.54)	2(9.52)
Combination pc	30(58.82)	0(0.0)

Combination: MDA + CEA + CA19.9; Pc: Positive cases; GBC: Gallbladder cancer.

The MDA alone had the highest sensitivity of 98.03%, and CEA alone had the highest specificity of 95.23% for the diagnosis of GBC. MDA and CEA had the most accurate

validity (**Table 5**). The sensitivity, specificity, and positive predictive values are shown in **table 6**.

Table5 Evaluation of diagnostic value of a single MDA, CEA and CA19.9 in 51 gallbladder cancer cases

Diagnostic value	n	Sensitivity	Specificity	Positive likelihood ratio	Negative likelihood ratio
MDA	50	98.03%	85.71%	6.86	0.02
CEA	42	82.35%	95.23%	17.26	0.18
CA19.9	37	72.54%	90.47%	7.61	0.30

Sensitivity = true positive/patients × 100%; Specificity = true negative/normal × 100%; Positive likelihood ratio = sensitivity/(1 - specificity); Negative likelihood ratio = (1 - sensitivity)/specificity. MDA: Malondialdehyde; CEA: Carcinoembryonic antigen; CA: Cancer antigen.

Table6 Analyses of different combinations of MDA, CEA and CA19.9 in normal persons & gallbladder cancer patients.

Group	n	1 item (+)	2 item (+)	3 item (+)
Normal	21	3(14.28)	1(4.76)	0(0.0)
GBC	51	50(98.03)	42(96.07)	30(72.54)
Positive likelihood rate		6.86%	20.18%	100%

1 item (+): MDA; 2 item (+): MDA+CEA and 3 item (+): MDA+CEA+CA19.9.

DISCUSSION

The incidence of gall bladder cancer is two to six times more in women than men and is increasing steadily with age (Nakayama *et al*. 1991 & Scott *et al*. 1999). In our study also, women patients overcomes the male patients. In this study we analysed the different symptoms patterns, lipid peroxidation and bio-markers (CEA & CA19.9) and also observing the associated hepato-haematotoxicity in advanced stage gallbladder cancer patients in comparison with normal group. Abdominal pain was the most common symptoms (72.54%) in this study followed by loss of appetite (33.33%) but these are not specific symptoms for GBC. These clinical features of the patients are matches to other published reports (Malik *et al*. 2003)**.** Most of the associated hepato-haematotoxicity parameters (ALP, SGOT, SGPT, Total bilirubin, Direct bilirubin, Globulin, RBC, WBC and Haemoglobin) were significant in comparison to normal volunteers ($p<0.05$).

The associated hepato-haematoparameters are severely affected in our study in advanced stage GBC. Pandey *et al*. reported comparable finding in other study (Pandey *et al*. 2001). Serum levels of MDA, CEA, and CA19.9 were significantly higher in the GBC than normal volunteers. The sensitivity of MDA was the highest, reaching 98.03%, with the highest specificity being that of CEA at 95.23%. When all three parameters (MDA, CEA, and CA19.9) exceeded the critical values, the sensitivity was 72.54%, the specificity was 100%, and the positive predictive rate was also 100%. All these findings suggest that the combined use of these parameters for the diagnosis of advanced stage GBC could increase the specificity of diagnosis, but not the sensitivity.

Malondialdehyde (MDA) has been described previously used as a marker for oxidative stress (Bae *et al*. 2010). The expression of CEA is found to be high not only in GBC but also in most of the gastrointestinal tumors (Stefanović *et al*. 1993 & Zhou *et al*. 2012). Many group of researchers reported CA19.9 as a better maker of malignant tumors in digestive system, pancreas, and biliary tract (Zhou *et al*., 2012).

A group of researchers reported that 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 (Yun *et al*., 2014).

CONCLUSION

This study has highlighted the importance of combined assessment of all three biomarker (MDA, CEA and CA19.9) for diagnosis of advanced stage gallbladder cancer, although there are no specific tumour markers for the diagnosis of GBC. Joint detection of these markers may prove to be useful for diagnosis of GBC, assessing therapeutic effects, and predicting a prognosis. A majority of patients had an abnormal hepato-haematoparameters at the time of diagnosis requiring meaningful palliative treatment.

Conflicts of interests The authors hereby declare that there is no conflict of interests.

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