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The importance of CA 19-9 in diagnosis and monitoring of Hepatobiliary and Gastrointestinal malignancies

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Abstract
Introduction: *CA 19-9 (carbohydrate antigen), also called Cancer Antigen 19-9. or sialylatedlewisa antigen is a tumor marker that is used primarily in the management of pancreatic cancer. Carbohydrate antigen sialyllewisa (CA 19-9) is the most frequently applied serum tumor marker for diagnosis of cancers in the Hepatobiliary & Gastrointestinal tract.*
Aims and Objectives: *To evaluate the significance of CA 19-9 levels in Hepatobiliary and Gastrointestinal malignancies.*
Material and Methods: *The present study was conducted on 50 diagnosed cases of Hepatobiliary and GIT cancers constituted the study group and 30 age and gender-matched individuals were comprised the control group. The study was conducted in the Department of Biochemistry, Government Medical College Patiala. Venous blood sample was collected in plain vial and serum CA 19-9levels were evaluated by ELISA technique and the results were statistically analysed.*
Results: *The mean CA 19-9 levels were significantly higher in the study group as compared to control group (p value<0.001). And the CA 19-9 levels were<35 U/ml, in the cases those were on treatment of cancers.*
Conclusion: *From our study, it was concluded that for diagnosis of Hepatobiliary and GIT malignancies, serum CA 19-9 is simple, non invasive& cost effective biomarker which can be used along with other investigations such as imaging and cellular pathology, it increases the efficacy of diagnosis. For pancreatic carcinoma, it is a single & best diagnostic biomarker.*
Keywords: *CA 19-9, Tumor marker, Hepatobiliary Ca., Pancreatic tumor, stomach cancer, colon cancer.*

Introduction

A Neoplasm refers to any abnormal new growth of tissue. It may be benign or malignant in nature. The term cancer is usually associated with malignant tumors. Tumors can arise in any organ in the body and result in different clinical features, depending on the location of the growth. Gastrointestinal cancer refers to malignant condition of the gastrointestional tract (GIT) and accessory organs of digestion, including the esophagus, stomach, biliary system, pancreas, small intestine, large intestine, rectum and anus.(1)
Tumor marker is a substance produced by a tumor, or by the host in response to a tumor, that is used to differentiate a tumor from normal tissue or to determine the presence of a tumor based on

measurements in the blood or secretions. Such substances are found in cells, tissues, or body fluids and are measured quantitatively or qualitatively by chemical, immunological, or molecular diagnostic methods.[2]

CA 19-9 (carbohydrate antigen), also called cancer antigen 19-9.[3]And also known as sialylatedlewisa antigen is tumor marker that is used primarily in the management of pancreatic cancer.[4] CA 19-9 was discovered in the serum of patients with colon cancer and pancreatic cancer in 1981.[5] Carbohydrate antigen sialyllewis a (CA 19-9) is the most frequently applied serum tumor marker for diagnosis of cancers in the digestive organs.[6]

Tumour markers are most useful in evaluating the progression of disease status after the initial therapy and monitoring subsequent treatment modalities. The clinical staging of cancer is aided by quantitation of the marker. The marker value at the time of diagnosis may be used as a prognostic indicator for disease progression and patient survival.[2]

Aims and Objectives

1) To analyse the significance of CA 19-9 levels in Hepatobiliary and Gastrointestinal malignancies.
2) To estimate CA 19-9 levels in Hepatobiliary and GIT cancers for treatment and monitoring of above patients.

Material and Methods

The present hospital based, analytical, case control study was conducted in the Department of Biochemistry on 50 diagnosed cases of Hepatobiliary and Gastrointestinal Malignancies, referred by Department of Surgery, Rajindra Hospital Patiala, constituting the study group. 30 age and gender-matched individuals comprised the control group. Special investigation measurement i.e. Serum CA 19-9 levels was done using standard commercially available immunoassay - ELISA sandwich Assays based on streptavidin-biotin technology.

Inclusion criteria for cases: Diagnosed cases of following malignancies:

1. Pancreatic carcinoma
2. Gall Bladder carcinoma
3. Cholangiocarcinoma
4. Hepatocellular carcinoma
5. Gastric carcinoma
6. Colorectal carcinoma.

Exclusion criteria for cases: Patients having history of-

1) More than one malignancy
2) Other inflammatory pathologies (such as Cholangitis, Pancreatitis etc).

The study was conducted after obtaining the ethical clearance from institutional Ethics Committee. A written informed consent was obtained from each participant before commencement of study.

Venous blood sample was collected in plain vial and serum CA 19-9 levels were evaluated by enzyme linked immunosorbent assay (ELISA) method. The data was analysed statistically. Chi Square test, student's t- test were applied. The p-value less than 0.05 was considered statistically significant. Sensitivity, specificity, NPV, PPV, accuracy were calculated. All analysis was done using SPSS (statistical package for social sciences) 17.0 Version.

Results

In present study the gender-wise distribution, were 27 females (54.0%) & 23 males (46.0%) in study group. Comprising 16 females 53.3% & 14 males (46.7%) in control group. There was no statistically significant difference in gender distribution among the study and control groups

The comparison of age in cases and controls group, Mean±SD of cases group was 53.18 ± 12.02and in controls was 48.20 ± 16.24. The p value was 0.121. So there was no significant difference between cases and control group. The maximum number of patients were in the age group of 51-60 years.

Table 1: Mean CA 19-9 levels in study and control groups

Groups	Mean ± SD U/ml	P value	Significance
Pre-treatment cases (n=39)	164.10 ± 217.35	<0.001	HS
Post-treatment cases (n=11)	17.54 ± 22.31	-	-
Total cases (n=50)	131.8 ± 201.2	0.002	S
Controls (n=30)	14.63 ± 12.1	-	-

(Normal level is upto 35 U/ml)

Table 1 shows, the Mean±SD CA 19-9 levels were131.8 ± 201.2U/mlin total cases, 164.10 ± 217.35 U/ml in Pre-treatment. And in controls Mean±SD were 14.63±12.1 U/ml. Mean CA 19-9 levels were significantly higher in cases than controls (p<0.001) i.e. significant.

11 cases were on treatment of cancer, the Mean ± SD CA 19-9 levels were 17.54 ± 22.31 U/ml. Our study demonstrated the mean CA19-9 levels were <35 U/ml, in the cases those were on treatment of cancers, i.e. lower than the cases those were without cancer treatment.

Table 2: Distribution of study and control groups according to CA 19-9 levels

CA 19-9 (U/ml)	Controls	Cases	Total
<35	28	17	45
>35	2	22	24
Total	30	39	69

Table 2 shows CA 19-9 levels in out of 30 controls, in 28 controls were <35 U/ml, in 2 controls >35 U/ml, in 2 controls >35 U/ml (but mildly elevated). And in our 50 cases, in 17 cases CA 19-9 levels were <35 U/ml in 22 cases >35 U/ml.

Graph 1: CA 19-9 levels in study & control group

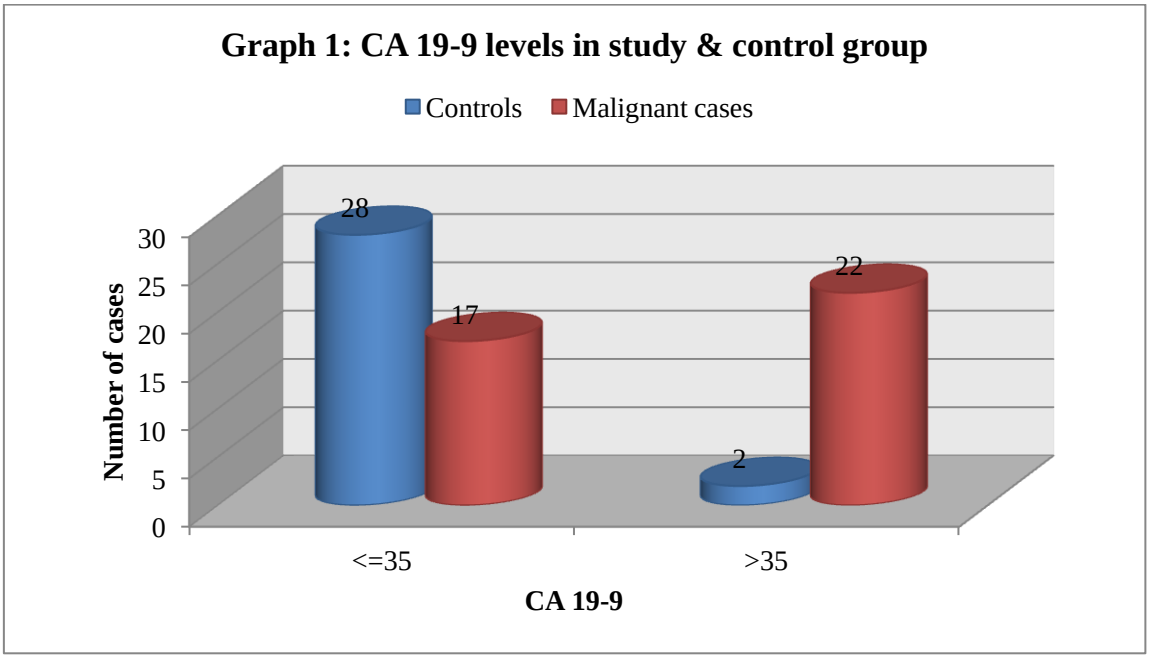

Table 3: Distribution of patients according to diagnoses

Malignancy	CA-19-9 <=35 U/ml		CA-19-9 >35 U/ml		Total
	N	%	N	%	
1. Pancreatic	-	-	2	100.0	2
2. Gall bladder	1	20.0	4	80.0	5
3. CCC	-	-	1	100.0	1
4. HCC	-	-	1	100.0	1
5. Gastric	4	57.1	3	42.9	7
6. Colorectal	12	52.2	**11**	47.8	23
Total	17	43.6	22	56.4	39

Table 3 shows out of 50 cases of Hepatobiliary & GIT malignancies, there were 1(20%) case of Gallbladder, 4(57.1%) cases of Gastric, 12 (52.2%) cases of Colorectal the CA19-9 levels were <35 U/ml and there were 2(100%) cases of Pancreatic, 4(80%) cases of Gallbladder, 1(100%) Cholangiocarcinoma (CCC), 1(100%) case of Hepatocellular carcinoma, 3(42.9%) cases of

Gastric, 11 (47.8%) cases of Colorectal the CA-19-9 levels were >35 U/ml. Remaining 11 cases were on treatment of cancer & the CA 19-9 levels were<35 U/ml.

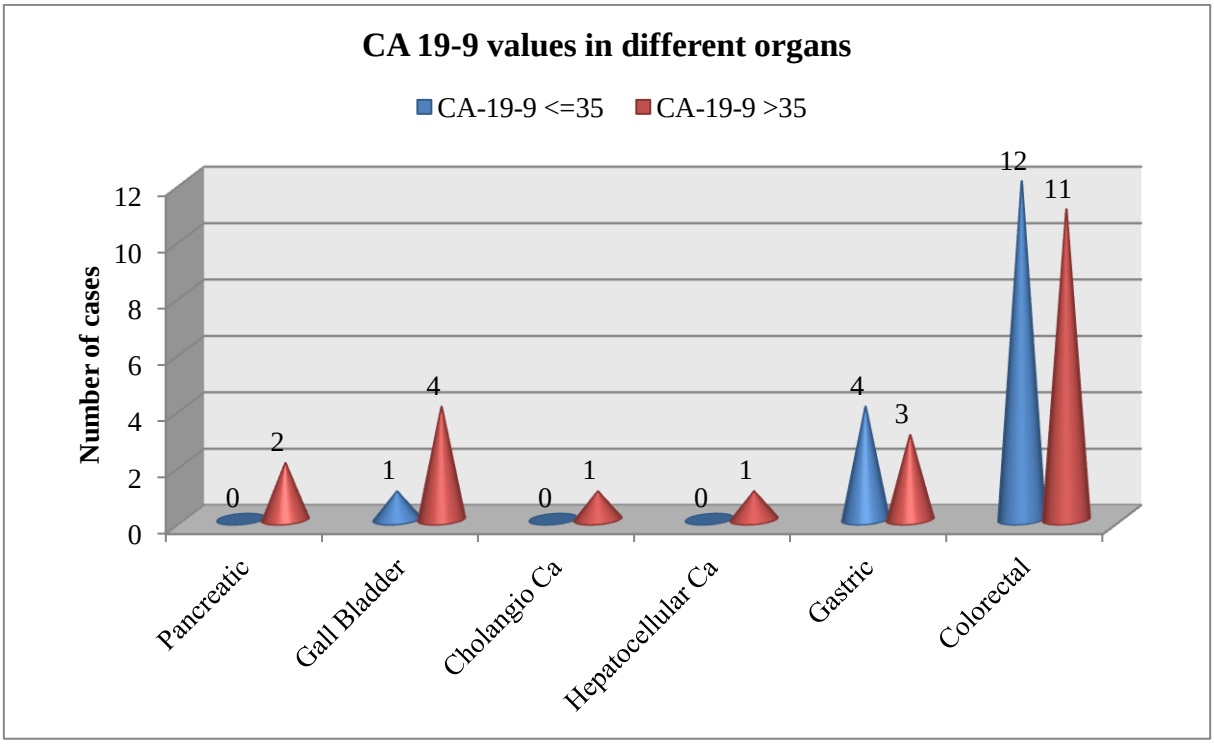

Table 4: Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of CA 19-9

Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Accuracy (%)
56.41	93.33	91.67	62.22	72.46

As shown in table 4, Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value & Accuracy of CA 19-9 were 56.41%, 93.33%, 91.67, 62.22 & 72.46% respectively.

Table 5: Mean CA 19-9 levels in different malignancies

Malignancies	**n**	**Minimum** (U/ml)	**Maximum** (U/ml)	**Mean** (U/ml)	**± SD** (U/ml)	**p value**	**Significance**
1. Pancreatic	2	600.00	600.00	600.000	0.000	<0.001	HS
2. Gall Bladder	5	20.37	600.00	430.694	238.908		
3. Cholangiocarcinoma	1	600.00	600.00	600.000	0.00		
4. HCC	1	260.40	260.40	260.400	0.00		
5. Gastric	7	8.36	258.30	78.820	95.424		
6. Colorectal	23	11.12	600.00	71.054	122.024		

As shown in table 5, the mean CA 19-9 levels in Pancreatic carcinoma were 600.00 U/ml, in Gall bladder carcinoma 430.6 U/ml, in Cholangiocarcinoma 600.00 U/ml, in Hepatocellular carcinoma 260.4 U/ml, in Gastric carcinoma 78.8 U/ml, in Colorectal carcinoma 71.0 U/ml & p value was <0.001 i.e. highly significant. So CA 19-9 levels were highest in pancreatic as well as in Cholangiocarcinoma.

Discussion

Carbohydrate antigens such as CA19-9 contain oligosaccharide structures present on heavily glycosylated high molecular weight mucins.[7] The CA 19-9 is a tumour associated, but not a tumour specific antigen. It is synthesized by normal human pancreatic and biliary ductular cells, as well as by gastric, colonic and salivary epithelia. This explains the elevated levels of CA 19-9 in

many malignancies.[8]CA 19-9 is derived from an aberrant pathway during production of its normal counterpart disialyl Lewis-a that has one extra sialic acid residue attached through α 2→6 linkage. Normally, disialyl Lewis-a is expressed on the epithelial surface of digestive organs, acts as a ligand for monocytes and macrophages and helps in immuno surveillance. Epigenetic silencing of the gene for α 2→6 sialyltransferase during early stages of carcinogenesis leads to abnormal synthesis and accumulation of sialyl Lewis-a (CA 19-9) because decreases disialyl Lewis-a which is synthesized normally.[9-13]

As shown in table 1 there was significance difference between cases and controls andthe mean CA 19-9 levels in controls was 14.6 ± 12.1, range was 0- 59 U/ml and in cases 164.10 ± 217.35 U/ml and the p value was <0.001. Out of 30 controls the CA 19-9 levels, in 28 controls were <35 U/ml, in 2 controls >35 U/ml (but mildly elevated). In these 2 controls probably there was a unnoticed benign gastrointestinal disease, because CA 19-9 also increases in benign conditions in Hepatobiliary as well as Gastrointestinal tract (acute and chronic pancreatitis, cholecystitis, cirrhosis, alcoholic hepatitis, acute hepatic necrosis, gallstones and cholestasis of any cause, etc). Similar results showing significant relation have been concluded by Pavai S et al in their study in two individuals in whom the tumour marker had been requested as a screening procedure, CA 19-9 was found to be elevated, albeit only mildly.[14]Amar H et al in their studythe mean CA 19-9 levels were7.7 ± 4.9 U/ml and range was 1-19 U/ml & p value was <0.005.[15]

As shown in table 3, out of 50 cases of Hepatobiliary & GIT malignancies, there were2 (100%) cases of Pancreatic ca. CA-19-9 levels were >35 U/ml and mean levels of these malignancies are shown in table 4. Normal adult pancreas has been observed to express CA19-9 in about 80% of cases, usually in the apical border of ductal cells and often more strongly in large ducts, whereas acinar structures and Langerhans islets are negative.[16] This probably reflects the propensity of pancreatic cancer to cause back-secretion of mucin into the blood rather than differential expression of the epitope.[17] Although CA19-9 is not accurate enough to be used in screening asymptomatic subjects for pancreatic cancer, it is currently the single most useful blood test in differentiating pancreatic cancer from chronic or recurring pancreatitis with a sensitivity ranging from 70–90% and a specificity from 68–91%.[18-21] Similar result were also obtained by Aseem B et al the mean CA 19-9 levels were94.4 ± 29.2 U/ml & p value <0.001. Currently, the best known and examined pancreatic cancer marker is CA 19-9.[22]

4(80%) cases of Gallbladder ca. CA-19-9 levels were >35 U/ml and 1(20%) case was <35 U/ml. Production and secretion of CA 19-9 from malignant cells is considered to be responsible for the elevated serum CA 19-9 levels found in malignancy. The reason for the CA 19-9 elevation in acute cholangitis is not clear. Several mechanisms have been postulated, including: 1) Leakage of condensed CA 19-9 due to biliary tract obstruction from the bile into blood circulation.[23] 2) Enhanced CA 19-9 production by irritated bile duct cells exposed to increased biliary.[24] 3) Enhanced production of CA 19-9 in the bile duct epithelium and the mucosa of the gallbladder induced by the inflammatory process.[23] 4) The inflammatory cytokines produced in sepsis due to cholangitis may be a contributing factor.[24]

1(100%) Cholangiocarcinoma (CCC) case levels was >35 U/ml. CCC typically refers to mucin – producing adenocarcinomas (different from HCC) that arise from the bile ducts. Similar result were also obtained by Aseem B et althe mean CA 19-9 levels were35 ± 10.6 U/ml & p value was <0.001.[22]And also by Xing LQ et althe mean CA 19-9 levels were310.1 ±7.1 U/ml and p value <0.001.[25]Chen YL et al studied and found that in the 106 hepatocellular carcinoma patients with preoperative CA19-9 levels were >27 U/mL.[26]

4(57.1%) cases of Gastric ca. CA 19-9 levels <35 U/ml and 3(42.9%) cases of Gastric ca. CA 19-9

levels >35 U/ml. Similar result were also obtained by Aseem B et al The mean CA 19-9 levels were37.7 ± 16.2 U/ml & p value was <0.001.[22] And also by Amar H et al the mean CA 19-9 levels were42.6 ± 3 U/ml and range was 10-76 U/ml & p value <0.05.[15]

12(52.2%) cases of Colorectal ca. CA 19-9 levels <35 U/ml and 11 (47.8%) cases of Colorectal ca. CA-19-9 levels were >35 U/ml. Similar result were also obtained by Aseem B et al The mean CA 19-9 levels were54.8 ± 22.3 U/ml & p value was <0.001.[22] And also by Amar H et al the mean CA 19-9 levels were47 ± 0.3 U/ml and range was 46.5-47.5 U/ml & p value <0.05.[15] Remaining 11 cases were on treatment of cancer & the CA 19-9 levels were<35 U/ml.

In the present study as shown in table 4, the Specificity & Sensitivity of CA 19-9 was 93.33%, 56.41% respectively. Specificity was more than Sensitivity, so this test can be used to confirmation of Hepatobiliary & GI malignancies. Moreover, the mean of CA 19-9 levels in the study group was 164.09 and in the controls group was 14.62. The p value was (p <0.001) which was statistically highly significant as shown in Table 1.

Conclusion

From our study, it was concluded that the mean CA 19-9 levels was significantly higher in the study group as compared to control group (p value <0.001). CA 19-9 represents the best prognostic biomarker for predicting disease recurrence following curative resection. CA 19-9 is a simple, non invasive and cost effective assay recommended in diagnosing and monitoring in individuals known to have or suspected of having Hepatobiliary and Gastrointestinal malignancies. Which can be used along with other investigations such as imaging and cellular pathology, it increases the efficacy of diagnosis. For pancreatic carcinoma, it remains the single & best diagnostic biomarker.

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