

## Original Research Article

# Evaluation of serum carbohydrate antigen 19-9 as a diagnostic marker for pancreatic malignancy

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## ABSTRACT

**Background:** Carbohydrate antigen (CA) 19-9 is considered as a tumor marker in biliary-pancreatic malignancy. Though a high level may indicate the presence of a malignant disorder, it may rise even in benign condition. Similarly, the value may be normal even in malignant condition.

**Methods:** An observational comparative study was conducted in the Department of Surgery of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from 01 June 2016 to 31 May 2017 to find out the sensitivity and specificity of CA 19-9 as a tumor marker in pancreatic malignancy in our perspective and to find out a cut-off value of CA 19-9 which might prove as a definitive indication of pancreatic malignancy.

**Results:** The study shows when the cut off value of CA 19-9 is 37 U/ml. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) were 77.8%, for all four characteristics respectively. But if the serum CA 19-9 threshold used to diagnose pancreatic cancer was raised to 100 and 120, sensitivity decreased to 72.2% and 66.7% and NPV decreased to 76.2% and 73.9% respectively. However, specificity increased to 88.9% and 94.4% and PPV increased to 86.7% and 92.3% respectively.

**Conclusions:** Serum CA 19-9 level may be considered as an important determinant in the diagnosis of malignant pancreatic diseases and to assess the resectability of the lesions preoperatively, but other adjuncts are necessary in the overall management of pancreatic diseases.

**Keywords:** Carbohydrate antigen 19-9, Tumor marker, Pancreatic cancer

## INTRODUCTION

Pancreatic cancer is the sixth leading cause of cancer death in the United Kingdom and the incidence is ten cases per 100000 population per year. Worldwide, it constitutes 2-3 per cent of all cancers and in the United States, is the fourth highest cause of cancer death. The incidence has declined slightly over the last 25 years. There is no simple screening

test; however, patients with an increased inherited risk of pancreatic cancer should be referred to specialized units for screening and counseling.<sup>1</sup> More than 85 per cent of pancreatic cancers are ductal adenocarcinomas. The remaining tumors constitute a variety of pathologies with individual characteristics. Endocrine tumors of the pancreas are rare. Jaundice secondary to obstruction of the distal bile duct is the most common symptom that draws

attention to ampullary and pancreatic head tumors. It is characteristically painless jaundice but may be associated with nausea and epigastric discomfort. Pruritus, dark urine and pale stools with steatorrhea are common accompaniments of jaundice. In the absence of jaundice, symptoms are often non-specific, namely vague discomfort, anorexia and weight loss, and are frequently dismissed by both patient and doctor. Upper abdominal symptoms in a recently diabetic, especially in one above 50 years of age, with no family history or obesity, should raise suspicion. Occasionally, a patient will present with an unexplained attack of pancreas. Tumors of the body and tail of the gland often grow silently, and present at an advanced unresectable stage. Back pain is a worrying symptom, raising the possibility of retroperitoneal infiltration.<sup>1</sup>

Among recently diagnosed pancreatic cancer patients, 65-70% will have advanced disease (stage III-IV) at initial presentation. Advanced pancreatic cancer has a very poor prognosis, with a median survival of 2-6 months for stage IV disease and 6-11 months for stage III disease. Overall, the 5-year survival among these patients is only 5-7% and the majority of patients survive less than 1-2 years. Even among patients who undergo surgery with curative intent, >90% develop disease progression within 12-18 months. This poor prognosis is attributable to late stage of presentation, lack of effective treatments, early recurrence and absence of clinically useful biomarker(s) which can detect pancreatic cancer in its precursor form(s) or earliest stages.<sup>2-4</sup>

A wide variety of tumor markers derived from serum, pancreatic tissue, pancreatic juice, saliva and/or stool has been proposed for early diagnosis as well as to predict prognosis in pancreatic cancer patients. Nevertheless, utility of those markers is often significantly limited by poor sensitivity, high false positive rates and lack of large-scale validation.<sup>5</sup> Despite the vast number of potential pancreatic cancer biomarkers, very few have been thoroughly evaluated and none to the extent of carbohydrate antigen 19-9 (CA 19-9). Koprowski and his team first described CA 19-9 in colorectal cancer cell line (SW 1116) using a monoclonal antibody (1116-NS-19-9) i.e. hybridoma technology in 1979.<sup>6</sup> CA 19-9 is also identified in the tissue and sera of patients with other gastrointestinal tumors including esophageal, gastric, biliary and pancreatic cancer. CA 19-9 also termed as sialyl Lewis-a (sLea), is expressed on the surface of cancer cells as a glycolipid and as an o-linked glycoprotein. CA 19-9 is derived from an aberrant pathway during production its normal counterpart disialyl Lewis-a that has one extra sialic acid residue attached through a 2 to >6 linkage. Epigenetic silencing of the gene for 2 to >6 sialyl transferase during early stages of carcinogenesis leads to abnormal synthesis and accumulation of sialyl lewis-a (CA 19-9).<sup>7</sup> Elevation of CA 19-9 level correlate with the degree of tumor differentiation as well as the extent of tumor mass.<sup>8</sup> Steinberg in 1990 analyzed diagnostic value of CA 19-9 serum levels (37-40 U/ml) in 1040 patients (24

case series) with symptomatic pancreatic cancer and reported a median sensitivity and specificity of 81% and 90% respectively. The positive predictive value (PPV) and negative predictive value (NPV) of an elevated serum CA 19-9 level was 72.3% and 95.8% respectively.<sup>9</sup> If the serum CA 19-9 threshold used to diagnose pancreatic cancer was raised to 100 U/ml or 1000 U/ml, the specificity increased to 98% and 99.8%, however the sensitivity decreased to 68% and 41% respectively.<sup>7</sup> High CA 19-9 levels have been associated with unresectable lesions and a poor prognosis for patients presenting with pancreatic carcinoma.

Owing to the reported high degree of false negative and false positive cases in the context of CA 19-9 as a diagnostic marker for cancers, the American Society of clinical oncology guidelines implicitly discourage the use of CA 19-9 as a screening test, particularly in pancreatic cancer. Current practice dictates the main use of CA 19-9 in establishing confirmation on first, whether a pancreatic tumor is secreting it at all. If so, the levels should visibly fall when the tumor is treated, and they may increase in levels on recurrence of the lesion. CA 19-9, thus, serves well as a prognostic indicator in patients with pancreatic tumors.<sup>10</sup>

Several studies have been conducted over the past years trying to establish the diagnostic and prognostic value of the marker (CA 19-9) both singly and in combination with other known tumor markers. Some of these studies have been focused on establishing the predictive values and sensitivity for detection of different cancers of the gastrointestinal tract. Other studies have been conducted to evaluate the use of this marker in the context of inflammatory processes of the hepatobiliary system, like cholangitis and pancreatitis, for example. Serum CA 19-9 levels have a sensitivity and specificity of 79-81% and 80-90% respectively for the diagnosis of pancreatic cancer in symptomatic patients.<sup>7</sup> There was a higher positivity of CA 19-9 in cancers than in benign masses (23/34; 68%, 15/50; 30%,  $p < 0.01$ ) with cut off values 37 U/ml.<sup>11</sup> CA 19-9 serum levels are often significantly elevated in the setting of obstructive jaundice resulting in further increase false positives in benign conditions thereby reducing the overall accuracy and specificity of it as a diagnostic marker. On the other hand, sialyl Lewis negative phenomenon seen in 5-10 % population is associated with false negative results for CA 19-9 levels even in presence of advanced pancreatic cancer. Further studies from these aspects may be necessary. Elevated CA 19-9 levels are not pathognomonic of cancer of pancreas; it may be elevated in other malignancies as well as benign conditions. It is known that there are some CA 19-9 positive patients with benign diseases such as pancreatitis, hepatitis, and cholelithiasis.<sup>12</sup> The purpose of this study was to observe the serum levels of CA 19-9 in patients suffering from different clinical conditions (both benign, like infections, inflammations and malignant) related to the pancreatic diseases and compare between benign and malignant conditions.

## METHODS

This observational comparative study was conducted in the Department of Surgery of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from 01 June 2016 to 31 May 2017. Serum CA 19-9 assay was done in Department of Clinical Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.

### Inclusion criteria

Patient having pancreatic diseases of both sexes and patients aged 18 or more than 18 years.

### Exclusion criteria

Patients not willing to enroll in the study and patient having severe co-morbidities.

By applying purposive sampling technique 18 cases were included in the malignant group and 18 cases were included in the benign group and a structured questionnaire was developed which contained background information including clinical presentation, investigation parameter during the disease process of the patients. Prior to data collection both verbal and written consent was taken from the respondents. Baseline information was collected from the patient. All information was recorded in a data collection sheet. All the data were checked and edited after collection and statistical analysis of the results were obtained by Statistical Packages for Social Sciences (SPSS). The results have been presented in tables and figures as necessary. Numerical data were presented as mean and standard deviation and categorical data were presented as frequency and percentage. Statistical test was done by chi-square test in case of categorical data and by Mean Whitey U test and t-test in case of numerical data.

## RESULTS

This was an observational comparative study conducted in the Department of Surgery of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from 01 June 2016 to 31 May 2017. We found in malignant group, there is no patient in less than 30 years but 9 patients in 51-60 years age group, whereas in benign group 6 patients in less than 30 years and no patients in 51-60 years age group. From this table it can be said that malignant pancreatic diseases occur more in elderly age and benign pancreatic diseases more occur in younger age ( $p < 0.05$ ) (Table 1). Result shows male was more affected than female by both malignant and benign diseases of pancreas. The result shows that sex is not significant issue (Table 2). Result shows that the value of CA 19-9 was classified into four groups and showed that CA 19-9 value of 10 malignant patients was  $>200$  and 4 patients was  $\leq 37$ . In case of benign disease 15 patients had CA 19-9 value  $\leq 37$  and one patient had  $>200$ . About 78% of malignant patients had the CA 19-9 value above the upper limit of normal range (0-37 U/ml) (Table 3). Result shows if the value of CA 19-9

is 37 U/ml, the sensitivity, specificity, PPV and NPV were 77.8%, 77.8%, 77.8%, 77.8% respectively. If the serum CA 19-9 threshold used to diagnose pancreatic cancer was raised to 100 and 120, sensitivity decreased to 72.2% and 66.7% and NPV decreased to 76.2% and 73.9% respectively, however specificity increased to 88.9% and 94.4% and PPV increased to 86.7% and 92.3% respectively (Table 4). Figure shows that a receiver operator characteristics (ROC) curve analysis was conducted to evaluate the cutoff values for CA 19-9 in order to be able to detect malignant conditions of the pancreas. With a cutoff value of 38 U/ml, the study showed that one can achieve 77.8% sensitivity and 77.8% specificity for detection of malignant pancreatic neoplasms (AUC 0.858) (Figure 1). Table shows that CA 19-9 of malignant group ( $1279 \pm 1602$ ) was higher than benign group ( $66.46 \pm 186$ ), which was statistically significant ( $p < 0.05$ ) (Table 5). Table shows serum CA 19-9 level of resectable pancreatic cancer ( $406 \pm 520$ ) was much lower than non-resectable pancreatic cancer ( $1615 \pm 1763$ ) (Table 6). Table shows that serum bilirubin, serum alkaline phosphatase, prothrombin time and INR tests are significant markers for the differentiating between benign and malignant groups with p values of  $< 0.001$ , 0.003, 0.020 and 0.049 (Table 7).

**Table 1: Distribution of study subjects according to age.**

Age (years)	Group (n=36)		P value
	Malignant (n=18)	Benign (n=18)	
21-30	0 (0.0)	6 (33.3)	0.004
31-40	4 (22.2)	6 (33.3)	
41-50	3 (16.7)	4 (22.2)	
51-60	9 (50.0)	0 (0.0)	
>60	2 (11.1)	2 (11.1)	
Mean±SD	52.27±11.03	37.27±14.39	

Chi-square test was done to measure the level of significance.

**Table 2: Distribution of study subjects according to gender.**

Age (years)	Group (n=36)		P value
	Malignant (n=18)	Benign (n=18)	
Male	12 (66.7)	11 (61.1)	0.729
Female	6 (33.3)	7 (38.9)	

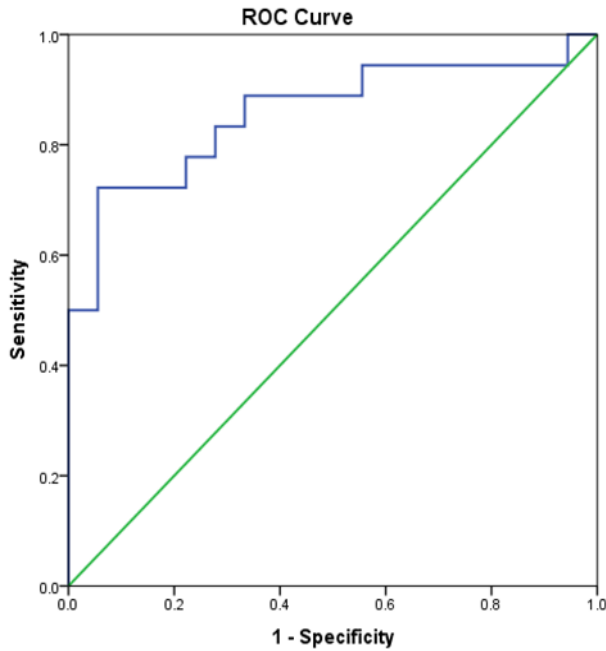
Chi-square test was done to measure the level of significance.

**Table 3: Distribution of number of subjects in different CA 19-9 values (n=36).**

CA 19-9	Malignant (n=18)	Benign (n=18)
$\leq 37$	4	15
38-100	1	1
101-200	3	1
$>200$	10	1

**Table 4: Serum CA 19-9 in diagnosis of pancreatic malignancy at different cutoff value (n=36).**

Cut off value	Sensitivity	Specificity	PPV	NPV	Accuracy
37	77.8	77.8	77.8	77.8	77.8
100	72.2	88.9	86.7	76.2	80.6
120	66.7	94.4	92.3	73.9	80.6



**Figure 1: Receiver operator characteristics (ROC) curve of CA 19-9 in diagnosis of pancreatic malignancy area under curve=0.858.**

**Table 5: Comparison of serum CA 19-9 between malignant and benign groups.**

	Group (n=36)		P value
	Malignant (n=18)	Benign (n=18)	
Mean serum CA 19-9 (U/L)	1279±1602	66.46±186	0.001

Mann-Whitney U test was done to measure the level of significance.

**Table 6: Serum CA 19-9 level in resectable and non-resectable malignant cases.**

	Group (n=18)		P value
	Resectable (n=5)	Non-resectable(n=13)	
Mean serum CA 19-9 (U/L)	406±520	1615±1763	0.143

Mann-Whitney U test was done to measure the level of significance.

**DISCUSSION**

CA 19-9 is defined by the monoclonal antibody obtained from the human colorectal cancer line, but the usefulness of serum CA 19-9 as a tumor marker is higher in pancreatic cancer than in colorectal cancer, as can be seen in previous reports.<sup>13-15</sup> CA 19-9 is related to the Lewis blood group antigens and only patients belonging to the Le (α-β+) or Le (α+β-) blood groups will express the CA 19-9 antigen. Le (α-β-) phenotypes occur in population which lack the enzyme 1,4-fucosyl transferase required for antigen epitope production.<sup>7</sup>

**Table 7: Comparison of serum liver function test between malignant and benign groups.**

Investigation findings	Group (n=36)		P value
	Malignant (n=18)	Benign (n=18)	
Serum bilirubin (mg/dl)	7.18±5.70	0.71±0.70	<0.001
Serum glutamic-pyruvic transaminase (SGPT) (U/L)	98.06±115.05	56.89±56.75	0.153
Alkaline phosphatase (U/L)	355.56±184.28	220.43±259.75	0.003
Prothrombin time (PT) (sec)	13.31±1.12	12.94±2.48	0.020
International normalized ratio (INR)	1.11±0.11	1.09±0.21	0.049
Serum albumin (gm/L)	31.00±7.84	34.88±6.26	0.089

Mann-Whitney U test was done to measure the level of significance.

CA 19-9, one of the most monosialogangliosides has been identified as a sialylated derivative of the normal Lewis blood group-active glycolinkage. This antigen is presumed to appear subsequent to sialylation that is caused by mechanism related to carcinogenesis. The Lewis antigen-negative blood group, Le (α-β-) is said to be found in about 10% of the total population.<sup>12</sup> In such persons who lack Lewis antigen CA 19-9 is not expected to appear even after carcinogenesis. This population may be associated with false negative results of CA 19-9 serum levels even in

presence of advance pancreatic cancer. In the present study it was observed, if the value of CA 19-9 is 37 U/ml, the sensitivity, specificity, PPV and NPV were 77.8% for all respectively. If the serum CA 19-9 threshold used to diagnose pancreatic cancer was raised to 100 and 120, sensitivity decreased to 72.2% and 66.7% and NPV decreased to 76.2% and 73.9% respectively, however specificity increased to 88.9% and 94.4% and PPV increased to 86.7% and 92.3% respectively (Table 4).

Steinberg in 1990, analyzed diagnostic value of CA 19-9 serum levels (37-40 U/ml) in 1040 patients (24 case series) with symptomatic pancreatic cancer and reported a median sensitivity and specificity of 81% and 90% respectively.<sup>9</sup> The positive predictive value (PPV) and negative predictive value (NPV) of an elevated serum CA 19-9 level was 72.3% and 95.8% respectively. If the serum CA 19-9 threshold used to diagnose pancreatic cancer was raised to 100 U/ml or 1000 U/ml, the specificity increased to 98% and 99.8%, however the sensitivity decreased to 68% and 41% respectively. A receiver operator characteristics (ROC) curve analysis was conducted to evaluate the cutoff values for CA 19-9 in order to be able to detect malignant conditions of the pancreas. To establish a cut off value for achieving maximal sensitivity and specificity to diagnose malignant conditions of pancreas was set at 38 U/ml achieving 77.8% sensitivity and 77.8% specificity whereas Bhattarai and his team set the cut off value at 92 U/ml.<sup>10</sup> In this study it was found that the mean level of CA 19-9 of malignant group (1279±1602) was significantly ( $p=0.001$ ) higher than of benign group (66.46±186) (Table 5). Bhattarai and his team showed that, patients with malignant conditions of the pancreas had a higher mean value of serum CA 19-9 compared to patients with non-malignant and inflammatory conditions of the pancreas (121.0±16.7 U/ml compared to 74.5±17.86 U/ml,  $p<0.001$ ).<sup>10</sup> Steinberg showed in his research, the mean serum values of CA 19-9 were significantly higher in patients with malignant pancreatic lesions compared to benign lesions.<sup>9</sup> The present study also showed significant result like above mentioned works which is similar to results obtained by other researchers (Table 5).<sup>16-18</sup>

In the present research it was observed that about 78% malignant patients had above the normal limit of CA 19-9 level which was approximately similar to the study done by Jalanko and his team in where they showed CA 19-9 concentration were above the upper limit of normal range were 76% in pancreatic carcinoma.<sup>16</sup> Bhattarai and his team showed 100% of patients had elevated CA 19-9 level.<sup>10</sup> Kau and his team showed that mean levels of CA 19-9 were lower in patients with surgically resectable stages of cancers of the pancreas in comparison to patients who had a surgically unresectable mass of the pancreas (524±70 U/ml compared to 3114±1643 U/ml,  $p=0.002$ ).<sup>19</sup> Steinberg also mentioned in his study the higher mean values of CA 19-9 were found to be associated with higher surgical stages of the tumors of the pancreas, as shown by the present study 406±520 U/ml in resectable cases of pancreatic cancer compared to 1615±1763 U/ml in surgically inoperable cases (Table 6).<sup>9</sup> Other similar results were found in Paganuzzi and his team (94±59 U/ml compared to 563±768 U/ml,  $p>0.05$ ) and Bhattarai and his team (65.91±30.11 U/ml, compared to 99.36±33.51 U/ml,  $p=0.002$ ).<sup>10,20</sup>

Though the mean values of CA 19-9 were higher in malignant patients in this study, 4 out of 18 malignant patients, the values were below 37 (Table 3). The cause of this may include Lewis antigen negative blood group patients and the patients that might come in the earlier

stage of the disease.<sup>7,12</sup> On the other hand in benign condition some patients have higher CA 19-9 values (Table 3). This fallacy limits the universal applicability of serum CA 19-9.

In different study CA 19-9 level has been suggested as a prognostic indicator of patient survival. However, we are unable to verify this in the present study, as there was no further follow up of the study subjects in most instances.<sup>8</sup> On socio demographic view the present study showed that, in case of age, malignancy occur more in 50-60 years age group and the mean value was 52.27±11.03 years (Table 1). On the other hand, benign disease occurred more in younger age (mean value 37.27±14.39 years). A research in California showed that the mean age of the malignant disease was 68.1±12.<sup>21</sup> In case of sex, male was more affected by both malignant (12 out of 18) and benign (11 out of 18) diseases (Table II). Jason et al showed that male (50.7%) was more affected than female (49.3%) in malignant pancreatic disease.<sup>21</sup>

### Limitations of the study

This was a single centre study with small sample size. So, the study results may not reflect the scenarios of the whole country.

### CONCLUSION

The serum levels of CA 19-9 may rise both in benign and malignant pancreatic diseases, but in malignant conditions it was significantly higher than benign conditions. Higher cutoff value can potentially serve as a more specific marker for the diagnosis of pancreatic malignancy. Cutoff value was set at 38 U/ml. In lower value the sensitivity increases but the specificity decreases much. Advanced malignancy and resectability also may be determined by CA 19-9 level as the mean value of CA 19-9 level was much higher in non-resectable than resectable conditions. Serum CA 19-9 level may be considered as an important determinant in the diagnosis of malignant pancreatic diseases and to assess the resectability of the lesions preoperatively, but other adjuncts are necessary in the overall management of pancreatic diseases.

### Recommendations

Large scale study may be done including diseases of other organs like hepatobiliary, pulmonary, renal and stomach as CA 19-9 also increases in those diseases. To avoid error, the study can be done with a large sample size in a long period of time. As there is no screening test for pancreas, more study can perform about the utility of this CA 19-9 as a screening test.

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