RESEARCH COMMUNICATION

Improved Diagnostic Accuracy of Pancreatic Diseases with a Combination of Various Novel Serum Biomarkers - Case Control Study from Manipal Teaching Hospital, Pokhara, Nepal

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Abstract

Background: Pancreatic cancer is a distressing disease with a miserable prospects and early recognition remains a challenge due to ubiquitous symptomatic presentation, deep anatomical location, and aggressive etiology. False positives and problems in distinguishing pancreatitis from adenocarcinoma limit the use of CA 19-9 as both disorders can present with similar symptoms and share radiographic physiognomies. This study aimed to assess the relative increase in accuracy of diagnosing the patients with chronic pancreatitis, benign neoplasm of pancreas and adenocarcinomas with CA 19-9, haptoglobin, and serum amyloid A in comparison to CA 19-9 alone. Materials and Methods: This hospital based case control study was carried out in the Departments of Medicine and Biochemistry of Manipal Teaching Hospital, Pokhara, Nepal, between 1st January 2010 and 31st December 2011. The variables assessed were age, gender, serum CA19-9, serum haptoglobulin, serum Amyloid A. The data were analyzed using Excel 2003, R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows Version. Results: Out of 197 cases of pancreatic disease, maximum number of assumed cases were of adenocarcinoma of pancreas (95). Number of males (59) were more than females (36) in assumed cases of adenocarcinoma of pancreas. The mean values of CA19-9 raised considerably in cases of chronic pancreatitis, benign neoplasm and adenocarcinoma of pancreas when compared to controls. The highest augmention in CA19-9 values were in cases of adenocarcinoma of pancreas. The p-value indicates that in cases of chronic pancreatitis, there was not significant increase in precision of diagnosis. Conclusions: These statistics established that haptoglobin and SAA are useful in discriminating cancer from benign conditions as well as healthy controls.

Keywords: Pancreatic diseases - serum biomarkers - Nepal

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Introduction

Pancreatic cancer is a distressing disease with a miserable prospects and early recognition remains a challenge due to ubiquitous symptomatic presentation, deep anatomical location, and aggressive etiology. Pancreatic cancer is currently fourth leading cause of cancer-related deaths in the United States and on the whole 5-year survival has been accounted to be less than 5% (Greenlee et al., 2000). In 2010, an estimated newly diagnosed cases of pancreatic cancer were 43,140 in USA and amazingly 36,800 cases perishes from the disease(Randall et al., 2011). The process of pancreatic carcinogenesis is a multifactorial phenomenon (Shrikhande et al., 2009). The most widely used and best validated tumor marker for adenocarcinoma of pancreas is CA19-9. The false positives results in distinguishing pancreatitis from adenocarcinoma of pancreas limit the use of CA 19-9 as both disorders can present with similar symptoms and share radiographic physiognomies. No single test could effectively discriminate PA from benign conditions. The current absence of reliable biomarker testing for pancreatic cancer mandates the development of novel strategies for identifying and characterizing additional biomarkers. One of novel biomarker is serum amyloid A (SAA). Serum amyloid A (SAA) which shares antigenicity with amyloid AA, has been recognized as acute-phase reactant (Kushner et al., 1994). The infection, trauma, or presence of malignant disease, may results in the elevation of plasma SAA (Biran et al., 1999). Another biomarker is haptoglobulin, which is glycoprotein produced in liver. The appearance of fucosylated

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haptoglobin has been reported in various diseases such as pancreatic cancer, hepatocellular carcinoma, liver cirrhosis, gastric cancer and colon cancer (Okuyamaet al., 2006). The enhanced diagnostic precision over CA 19-9 alone and the ability to discriminate between PA and pancreatitis have become the minimal standard for validation of novel serum biomarkers. This study aims to assess the relative increase in accuracy of diagnosing the patients with chronic pancreatitis, benign neoplasm of pancreas and adenocarcinomas with CA 19-9, haptoglobin, and serum amyloid A in comparison to CA 19-9 alone.

Materials and Methods

It was a hospital based case control study carried out in the Department of Medicine and Biochemistry of Manipal Teaching Hospital, Pokhara, Nepal between 1st January 2010 and 31st December 2011. The variables collected were age, gender, serum CA19-9, serum haptoglobulin, Serum Amyloid A. Approval for the study was obtained from the institutional research ethical committee. Quantitative Analysis of Human Serum Amyloid A and CA19-9 was performed by ELISA reader for all cases. The standard procedure was followed as per manufacturer's instructions for ELISA with minor modifications (Sell., 1990). Estimation of serum haptoglobins was done by colorimetric method that was based on the peroxidase activity of haptoglobin-methaemoglobin complexes (Owen et al., 1960). All these laboratory parameters were analyzed using Human reagent kits and with the help of ELISA and semi autoanalyser (Humalyser 3500, Germany). Analysis was done using descriptive statistics and Confidence Interval (CI). The data was analyzed using Excel 2003, R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows Version.

Inclusion criteria: Suspected cases of chronic pancreatitis were enrolled as evidenced by 2 out of the following 4 criteria: (a) calculi on abdominal X-ray, (b) dilated pancreatic duct on ultrasound, (c) changes of pancreatitis on ERCP and/or MRCP and (d) EUS appearances of pancreatitis. Mass lesions on ultrasound or CT scan for suspected cases of benign and malignant neoplasm of pancreas. Confirmation of pancreatic cancer was done by histological confirmation of malignancy by biopsy (radiology/endoscopy or surgery) and establishment of benign nature of disease by follow-up of more than 3 years without any evidence of recurrence of mass or metastatic disease.

Exclusion criteria: The patients suffering from any other disease except chronic pancreatitis, benign neoplasm and adenocarcionma of pancreas were excluded from our study.

Results

Table 1 illustrates that out of 197 cases of pancreatic disease, maximum number of assumed cases were of adenocarcinoma of pancreas(95). Number of males(59) were more than females(36) in assumed cases of adenocarcinoma of pancreas. Number of patients suspected of Stage IIB (27) and Stage IV(26) were almost equal. The total number of suspected cases of benign tumor of pancreas were 57 and females were more prone to benign tumor of pancreas. Maximum number of suspected cases were of Intraductal Papillary Mucinous Tumor(19). Furthermore ,the total number of chronic pancreatitis were 45 and median age was 47 years. For comparison with cases, healthy controls (170) were also taken with average age of 65 yrs.

Table 1. Distribution of Cases and Controls According to their Gender, Types and Stages

Genderwise variation	No.	Age (Range)
Controls		
Total	170	65 (34-86)
Female	68	63 (34-81)
Male	102	67 (37-86)
Suspected Cases		
Chronic inflammation of Pancreas		
Total	45	47 (28-70)
Female	21	48 (28-63)
Male	24	46 (29-70)
Benign tumor of pancreas		
Total	57	73 (45-85)
Male	24	75 (45-82)
Female	33	71 (48-85)
Distribution of cases according to its type		
Intraductal Papillary Mucinous Tumor	19	
Mucinous Cystic Neoplasm	13	
Neuroendocrine Tumor	13	
Serous Cystadenoma	6	
Cystic Neuroendocrine Neoplasm	6	
Solid-Cystic Papillary Neoplasm	2	
Maligmant carcinoma of pancreas (Adenocarcinomas)		
Total	95	72 (54-90)
Male	59	69 (57-90)
Female	36	75 (54-87)
Distribution of cases according to stages		
Stage IA	4	
Stage IB	8	
Stage IIA	9	
Stage IIB	27	
Stage III	21	
Stage IV	26	

Table 2 illustrates that mean values of CA19-9 raised considerably in cases of chronic pancreatitis, benign neoplasm and adenocarcinoma of pancreas when compared to controls. The highest augmention in CA19-9 values were in cases of adenocarcinoma of pancreas. There was inconsequential increase in cases of chronic pancreatitis in comparison to controls for haptoglobulin. There was noteworthy enhancement in haptoglobulin levels in cases of benign neoplasm and adenocarcinoma of pancreas. Similarly, there was significant increase in mean values of serum Amyloid A in cases of chronic pancreatitis, benign neoplasm and adenocarcinoma of pancreas when compared to controls.

Table 3 depicts that there was significant difference in cases of pancreatic adenocarcinomas and benign neoplasm of pancreas when percentage of accuracy was calculated with the help of other serum markers i.e. haptoglobulin

Table 2. Mean Values of CA19-9, Haptoglobulin, Serum Amyloid A in Controls and Cases

Variables	Controls	Chronic pancreatitis	Benign neoplasm of pancreas	Adenocarcinomas of pancreas	p Value
CA19-9	24.74±06.38	114.11±87.76†	204.53±097.73†	561.21±315.63†	0.0001**
(<40 U/ml)	(23.77,25.70)	(87.74,140.48)	(178.59,230.46)	(496.91,625.51)	
Haptoglobulin	118.53±059.78	134.22±69.83	142.58±078.56†	178.48±069.28†	0.0001**
(33 to 213 mg/dl)	(109.48,127.58)	(113.24,155.20)	(121.73,163.43)	(164.37,192.60)	
Serum Amyloid A	4.41±1.35	6.86±1.50†	9.49±03.50†	15.35±03.59†	0.0001**
(<8ug/ml)	(4.21,4.62)	(6.41,7.31)	(8.56,10.42)	(14.61,16.08)	

†p-Value <0.001, statistically significant between controls vs cases groups, **p-Value <0.05, statistically significant

Table 3. Diagnostic Precision of the Haptoglobin/SAA/ CA19-9 in Comparison to CA19-9 Alone

Cases	CA19-9*	Haptoglobulin/SAA/CA19-9*	p-Value
Chronic inflammation of Pancreas			
	91.1% (41/45)	88.8% (40/45)	0.153
Benign tumor of pancreas			
	73.7% (42/57)	77.19% (44/57)	0.0001**
Pancreatic Adenocarcinomas			
	80.0 %(76/95)	83.2% (79/95)	0.0001**

* Percentage (Confirmed/suspected)

and serum amyloid along with CA19-9. The p-value indicates that in cases of chronic pancreatitis, there was not significant increase in precision of diagnosis.

Discussion

Chronic pancreatitis, benign neoplasm of pancreas and pancreatic cancer are accountable for most of the burden of exocrine pancreatic disease (Raimondi et al., 2010). Even though improvements in imaging techniques, it may be problematic to segregate inflammatory head masses, benign grazes from malignant masses. Discrepancy between benign (inflammatory) and malignant masses has important therapeutic repercussions – evade unnecessary resection in inflammatory masses (Boll et al., 2003). various complement of proteins and antigens synthesized by tumor cells indcate that no single common marker would be effective in diagnosing of disease. Numerous prospective serum and tissue markers for pancreatic cancer are presently enduring evaluation, none are sufficiently validated for routine clinical use (Benson et al., 2007). Thus, CA 19-9 which remains the serum pancreatic cancer marker, new markers in combination for this malignancy should be judged (Katz et al., 2008). In our present study, mean values of CA 19-9 in cases of chronic pancreatitis 114.11 ± 87.76 (87.74,140.48) and benign neoplasm 204.53±97.73(178.59,230.46) were below 300U/ml. The mean levels of CA19-9 in cases of adenocarcinoma of pancreas was 561.21±315.63 (496.91, 625.51). Our findings concurred with the findings of Rocha et al which revealed that an elevated CA 19-9 greater than 300 U/ mL in the setting of head mass with chronic pancreatitis stalwartly advocates malignancy (Rocha et al., 2007). In our present study, the mean levels of both haptoglobulin and serum amyloid had found to be increased linearly with the progression of severity of disease. The recognition of true positive cases of chronic pancreatitis remains almost same with CA19-9 alone (91.1%) and with the combination other biomarkers(88.8%). In contrast to that the recognition of true positive cases in cases of benign neoplasm(77.19%) and pancreatic adenocaercinoma (83.2%) had been increased in comparison of suspected cases of benign neoplasm (73.7%) and adenocarcinoma of pancreas(80.0%)(Okuyamaet al.,2006). The synthesis of acute-phase protein is mainly takes place in cancer cell lines that reflect the growth of malignancy and largely regulated by inflammation- associated cytokines such as IL-1, IL-6, and tumor necrosis factor. Serum amyloid A synthesis is induced in various tissues by the stimulation of inflammation-related cytokines. Haptoglobin is a glycoprotein produced in the liver. Other studies also illustrates that interleukin-6 (IL-6) increases the secretion of fucosylated haptoglobin from pancreatic cancer tissue Both haptoglobin and serum amyloid A are acute-phase proteins play role in angiogenesis, antioxidant, cell migration, lipid metabolism, induction of extracellular matrix degrading enzymes , conscription of inflammatory cells to sites of inflammation, tumor growth, metastasis, and neovascularization (Narisada M et al., 2008). Although synthesized mainly in the liver, local differential expression of haptoglobin and serum amyloid A has been demonstrated in cancer tissues. Expression in cancer cells as well as potential roles in angiogenesis, cell migration and extracellular matrix remodeling suggests that haptoglobin and SAA may directly contribute to tumorigenesis. Additionally, it is likely that levels of proteins secreted or released by the tumor will correlate together and therefore mitigate the advantage of their use in combination. Accurate diagnostic and prognostic biomarkers for PA could improve outcomes through early detection, selection of appropriate treatment strategies, monitoring intervention efficacy, and surveillance of groups at high-risk for developing PA. Thus, the evaluation of serum biomarker levels not only capable of discriminating and diagnosing pancreatic cancer from benign conditions and chronic pancreatitis with high sensitivity and specificity, but also offer improved insight into the complex network of factors involved in pancreatic tumorigenesis.

In conclusions, these statistics established that haptoglobin and SAA are useful in discriminating PA from benign conditions as well as healthy controls. This study supports the use of combined biomarkers for improved accuracy in the diagnosis of pancreatic adenocarcinomas.

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