Original Article
The value of serum HE4 in pancreatic adenocarcinoma diagnosis

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Abstract: Early diagnosis of Pancreatic adenocarcinoma is a world wide challenge due to the lack of early detection methods. Currently, carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) markers play a key role in the Pancreatic adenocarcinoma diagnosis. However, they are not sensitive for early diagnosis. Here, we characterized a new promising biomakers for early diagnosis and prediction of longterm outcome of pancreatic adenocarcinoma. Human epididymis protein 4 (HE4) is recently identified serum tumor marker, here we identified its function in pancreatic adenocarcinoma. HE4, CEA, CA19-9 and CA242 were detected by chemiluminescence in control and tumor patients, sensitivity and specificity of HE4, CEA, CA19-9 and CA242 for diagnosis of pancreatic adenocarcinoma were checked. The AUC value of ROC curve for HE4 was higher than CEA, CA242 (0.866 vs 0.577 vs 0.864. Surprisingly, combination of HE4 and CA199 revealed the best sensitivity (0.953). Furthermore, HE4, CEA, CA199 and CA242 of pancreatic adenocarcinoma patients before and after surgery or chemotherapy were also been analysed, but no significant difference were observed, Moreover, HE4 can predict pancreatic adenocarcinoma recurrence and metastasis ($P<0.05$). Together with these finding, HE4 is a promising biomakers for early diagnosis and prediction of longterm outcome of pancreatic adenocarcinoma.

Keywords: Human epididymis protein 4, pancreatic adenocarcinoma, carbohydrate antigen 19-9, carbohydrate antigen 242, carcinoembryonic antigen

Introduction

Pancreatic cancer (PC) is one of the most aggressive and lethal human cancer, and the incidence of PC increased almost three times in the past 10 years [1]. PC is the fourth leading cause of death around the world, which also ranks the seventh in China [2]. Pancreatic adenocarcinoma is the most common and deadly form of pancreatic cancer [3]. Despite of the availability of several treatment madalities, surgical resection remains the only chance for curing pancreatic adenocarcinoma, and the five-year survival rate remains to be lower than 5%. There are no early detection tests and the majority of patients with localized disease have no recognizable symptoms or signs. As a result, pancreatic adenocarcinoma has metastasized to other organs [4]. Multiple serum tumor markers such as carbohydrate antigen 19-9 (CA199), carbohydrate antigen 242 (CA242), carcinoembryonic antigen (CEA) has been widely used in clinical application [5-7]. However, so far, none of these biomarkers has reached the sensiti-

vity and specificity required for standard clinical practice.

Human epididymis protein 4 (HE4) is recently identified serum tumor marker [8], specifically expressed in ovarian cancer [9], endometrial cancer [10], lung cancer [11, 12] and gastric cancer [13]. HE4 encoding genes first to be separated from epididymis epithelium, a member of a broader family of secreted putative protease inhibitors, all containing 2-4 whey acidic protein (WAP) domains. The exact function of these proteins is poorly understood, but their status as likely extracellular protease inhibitors suggests that they may be involved in the regulation of extracellular matrix, cell migration and cell invasion [14]. This type of role could also explain different tissue-specific patterns of expression in metaplasias. In contrast, metaplasias in the pancreas are organized into simpler ductular structures, which are lined by mucinous metaplastic lineages. Thus, in the case of pancreatic cancers, up-regulation of HE4 may be more indicative of changes towards
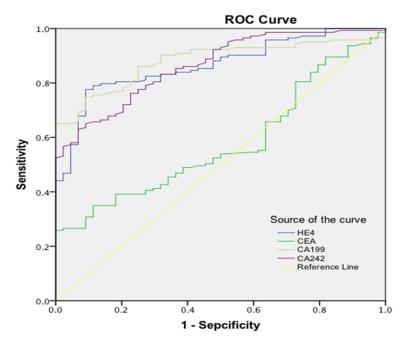
Figure 1. ROC curves of serum markers HE4, CEA, CA19-9 and CA242 for the diagnosis of pancreatic adenocarcinoma. Training set ROC Curves for pancreatic adenocarcinoma patients' serum vs normal controls.

Table 1. Area-under-the-receiver operating characteristic (ROC) curves of four serum markers (HE4, CEA, CA19-9, CA242) were shown in the table respectively

Markers	Area	Standard error	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
HE4	0.866	0.027	0.000	0.813	0.919
CEA	0.577	0.044	0.123	0.490	0.664
CA199	0.880	0.025	0.000	0.831	0.928
CA242	0.864	0.027	0.000	0.810	0.918

more invasive phenotypes [15]. However, serum HE4 which associated with occurrence, development, and prognosis of pancreatic carcinoma is still unknown [16]. Therefore, this study further analyzed the value of serum HE4 compared to conventional serum markers for diagnosis of pancreatic carcinoma.

Methods

Study population

The case group included 143 patients with pancreatic carcinoma admitted into Anhui Provincial Cancer Hospital, all patients was confirmed by pathological examination. In addition, 45 age-matched healthy persons who admitt-

ed to the same hospital for physical examination and had no medical history of malignant disease were recruited as controls. 42 patients were underwent surgery and 42 patients were underwent chemotherapy, and 40 pancreatic adenocarcinoma patients were followed-up survey for recurrence and metastasis. This study had protocols approved by the Ethics Committee of Anhui Provincial Cancer Hospital.

Serum measurements

Morning fasting peripheral blood samples were collected from study subjects of the three groups. For patients in case and benign control groups, collection time was before the patients received relevant treatment. Samples were retained for 1 hour at room temperature, then serum was isolated by centrifugation at 4000 rpm, isolated serum was stored at -20°C.

The level of HE4, CEA, and CA199 were detected by electrochemiluminescence, and the matched reagents purchased from Roche (Elecsys and cobas e601 analyzers, Germany). CA242 were detected by enzyme-linked immunosorbent assay (CanAg Diagnostics, Sweden). All detection was conducted according to protocols.

Statistical methods

SPSS 19.0 statistical package was used to establish a database and perform statistical analyses. Measurement data were expressed as mean standard deviation. Independent sample T test, single factor analysis of variance (ANOVA) was adopted for intergroup comparison, and LSD method was utilized for pairwise comparison. Area-under-the-receiver operating characteristic (ROC) curve was used to compare serum marker diagnostic efficiency. $P < 0.05$ was considered statistically significant.
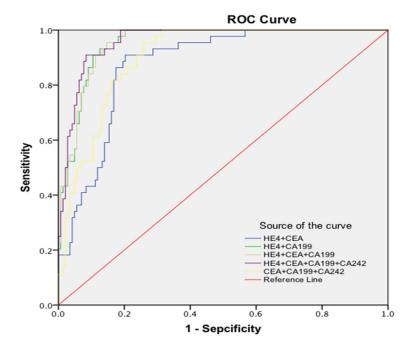
Figure 2. The ROC curves of serum markers for the diagnosis of pancreatic adenocarcinoma, the multiple tumor marker combined detection of pancreatic adenocarcinoma vs normal control.

Table 2. Area-under-the-receiver operating characteristic (ROC) curves of HE4 combination with other serum markers for pancreatic adenocarcinoma

Markers	Area	Standard error	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
HE4+CEA	0.875	0.026	0.000	0.824	0.926
HE4+CA199	0.953	0.014	0.000	0.926	0.980
HE4+CEA+CA199	0.953	0.014	0.000	0.926	0.980
HE4+CEA+CA199+CA242	0.960	0.013	0.000	0.935	0.985
CEA+CA199+CA242	0.903	0.021	0.000	0.861	0.945

Results

Comparison of serum marker levels

The values of HE4 in ovarian cancer, endometrial cancer and lung cancer have been recognized. To assess the diagnostic value of serum HE4 in pancreatic adenocarcinoma, we calculated sensitivity and specificity of HE4 in tumors and controls groups. Area-under-the-receiver operating characteristic (ROC) curve was used to compare serum marker diagnostic efficiency. Serum markers HE4 (AUC=0.866),

CA199 (AUC=0.880) and CA-242 (AUC=0.864) shown the most promise as diagnostic markers of pancreatic adenocarcinoma (**Figure 1** and **Table 1**).

Serum HE4 in combination with classical pancreatic adenocarcinoma biomarker

Traditionally, clinicians tended to use the combined detection of multiple tumor markers for diagnosis of pancreatic adenocarcinoma. However, in our study, we showed that the sensitivities of 4 kinds of routine combined detection modes (HE4+CEA, HE4+CA-19-9, HE4+CEA+CA19-9, HE-4+CEA+CA19-9+CA242, CEA+CA199+CA242) with HE4 alone (AUC=0.866). Combination analysis revealed that AUC for HE4 and CA199 was the most effective and convenient (0.953 for HE4 and CA199), is equal to HE4+CEA+CA199, slightly lower than HE4+CEA+CA199+CA-242 (0.960) and higher than CEA+CA199+CA242. The data is shown in the **Figure 2** and **Table 2**.

Serum concentration levels of HE4, CEA, CA199 and CA242 before and after surgery for pancreatic adenocarcinoma

Serum HE4, CEA, CA199 and CA242 in 42 pancreatic adenocarcinoma patients underwent surgery and chemotherapy were detected by electrochemiluminescence assay, and results showed no significant decrease of HE4, CEA, CA199 and CA242 after surgery and chemotherapy, on the contrary, there are HE4 and CA199 little higher than pre-operation (pre-operation, HE4, 92.68±12.44 pmol/L, CA199, 197.50±85.61 U/ml; Post-operation, HE4, 112.54±16.71 pmol/L, CA199, 219.32±90.54 U/ml; P>0.05) (**Table 3** and **Figure 3**).

Serum HE4, CEA, CA19-9, CA242 in pre-operation and post-operation of pancreatic adenocarcinoma

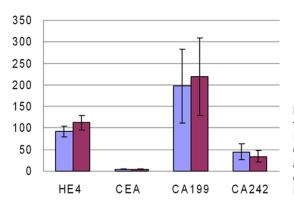| Group	HE4 (pmol/L)	CEA (ng/ml)	CA199 (U/ml)	CA242 (U/ml)
Pre-operation	92.68±12.44	4.19±0.98	197.50±85.61	44.83±18.78
Post-operation	112.54±16.71	3.77±0.96	219.32±90.54	34.35±13.94

Figure 3. The histogram of serum HE4, CEA, CA19-9, CA242 in pre-operation and post-operation of pancreatic adenocarcinoma.

Serum concentration levels of HE4, CEA, CA199 and CA242 before and after chemotherapy for pancreatic adenocarcinoma

Pre-chemotherapy and post-chemotherapy HE4, CEA, CA199 and CA242 concentration difference also had no statistically significant (P>0.05), CA242 also little higher than pre-chemotherapy (pre-chemotherapy, 95.44±26.41 U/ml; post-chemotherapy, 133.37±27.03 U/ml; P>0.05) (**Table 4** and **Figure 4**).

Serum HE4 in pancreatic adenocarcinoma recurrence and metastasis

The case group included 40 pancreatic adenocarcinoma patients, and no- recurrence and metastasis HE4 concentration positive rate was 14.29%, recurrence and metastasis positive rate was 80.77%. Follow-up survey found that 26 patients had arisen recurrence and metastasis (HE4, 236.98±53.96 pmol/L) and 21 patients HE4 had shown positive. Compared with no recurrence and metastasis, the difference have statistically significant (*P*=0.033) (**Table 5**). The results were shown that serum HE4 can well predict pancreatic adenocarcinoma recurrence and metastasis.

Discussion

Pancreatic adenocarcinoma is one of the most aggressive human cancer of which 5-year survival rate is 5%. Most pancreatic adenocarci-noma patients are diagnosed at advanced stages. Early diagnosis of Pancreatic adenocarcinoma is a challenge for sensitivity and specificity of tumor biomarkers. Numerous serum classical biomarkers, including CA19-9, CEA, CA242, CA724 and CA125 have been investigated, but none of them have reached the standard clinical practice [17].
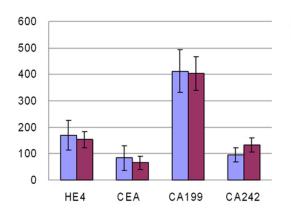
The potential use of HE4 as a tumor marker have been reported in an increasing number of studies, such as ovarian cancer, endometrial cancer, lung cancer and breast cancer [18]. The serological detection of HE4 have increased specificity and sensitivity compared with CA125, a classical marker of ovarian cancer [19, 20]. However, the diagnostic and predictive value of serum HE4 in pancreatic adenocarcinoma is still unknown. In our study, Area-under-the-curves operating (AUC) of serum HE4 was higher than CEA, CA242 (0.866 for HE4, 0.577 for CEA and 0.864 for CA242). The results demonstrated the potential value of HE4 serum levels as a promising biomarker for the diagnosis of pancreatic cancer patients. Moreover, increased diagnostic value of HE4 has been achieved through combination with other biomarkers. In this study, the serum levels of HE4 and previously investigated markers including CA19-9, CA242 all elevated in pancreatic adenocarcinoma. Indeed, combination of HE4 and CA199 revealed the best sensitivity.

However, HE4, CEA, CA199 and CA242 of pancreatic adenocarcinoma patients before and after surgery or chemotherapy were also been analysed, but no significant difference were observed, which maybe due to the patients that we included were already in later stage. Recent studies had indicated that CA199 and CEA increased in metastatic adenocarcinoma [21, 22]. CEA and CA199 levels of 189 patients with advanced gastric cancer who received first-line chemotherapy were measured, changes of CEA and CA199 levels can accurately predict the efficacy of first-line chemotherapy in advanced

Table 4. Serum HE4, CEA, CA19-9, CA242 in pre-chemotherapy and post-chemotherapy of pancreatic adenocarcinoma

Group	HE4 (pmol/L)	CEA (ng/ml)	CA199 (U/ml)	CA242 (U/ml)
Pre-chemotherapy	170.56±55.11	83.81±46.47	412.55±81.84	95.44±26.41
Post-chemotherapy	153.07±30.07	65.35±26.11	403.08±63.07	133.37±27.03

Figure 4. The histogram of serum HE4, CEA, CA19-9, CA242 in pre-chemotherapy and post-chemotherapy of pancreatic adenocarcinoma.

Table 5. Serum HE4 in pancreatic adenocarcinoma recurrence and metastasis

Group	n	HE4 (x±sd, pmol/L)	Positive rate	P
No-Recurrence and metastasis	14	73.10±6.34	14.29% (2)	0.033
Recurrence and metastasis	26	236.98±53.96	80.77% (21)	

adenocarcinoma and do not promote the use of any individual biomarker considered herein for diagnostic purposes. Moreover, while the nature of our investigation does not permit the identification of specific mechanistic links between any particular biomarker and the development of pancreatic cancer, our results provided a sound basis for subsequent targeted analyses of pancreatic cancer biomarkers.

Acknowledgements

gastric cancer [23]. The controversy may be due to the difference in sample selection. We would like to include more samples to evaluate the combination value of HE4 and CA199 in screening the pancreatic adenocarcinoma in our future study. In addition, we will detect the change of tumor markers in patients with carcinoma in situ and distant metastases of pancreatic cancer before and after treatment in next studies. Although HE4 had no significant difference before and after surgery and chemotherapy, high level of HE4 related to recurrence and metastasis of pancreatic adenocarcinoma. HE4 can be an early monitoring biomarker of the pancreatic adenocarcinoma.

In conclusion, our study confirmed increased expression of serum HE4 in pancreatic adenocarcinoma. Considering the increased diagnostic value by combination of HE4 with other biomarkers, particularly CA19-9 and CA242, HE4 act as a promising biomarker have great potential and clinical value for pancreatic adenocarcinoma. These findings represent an evaluation of biomarkers potentially related to pancreatic

Disclosure of conflict of interest

None.

Abbreviations

HE4, Human epididymis protein 4; CEA, Carcinoembryonic antigen; CA19-9, Carbohydrate antigen 19-9; CA242, Carbohydrate antigen 242.

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