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High Level of Serum Coiled-coil Domain Containing 25 (CCDC25) as a Diagnostic Marker for Cholangiocarcinoma But Not for Other Cancers

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Abstract. *Background/Aim: Recently, we reported that coiled-coil domain containing 25 (CCDC25) protein is elevated in the sera of patients with cholangiocarcinoma (CCA) and is suggested to be a diagnostic biomarker for CCA. This study aimed to examine whether serum CCDC25 level can be a unique biomarker for CCA. Bioinformatic analyses using Human Protein Atlas (HPA) database and Gene Expression Profiling Interactive Analysis 2 (GEPIA2) indicated that CCDC25 protein and mRNA are expressed not only in CCA but also in other cancers, such as colorectal cancer (CRC), breast cancer (BC), and hepatocellular carcinoma (HCC), all of which are the top 5 cancers highly prevalent in Thailand. Materials and Methods: Using a quantitative dot blot assay, serum CCDC25 levels were measured for 30 healthy controls (HC), 34 CRC, 42 BC, 43 HCC, and 83 CCA. Results: The serum CCDC25 levels of CCA patients (0.193±0.039 ng/μl) were significantly higher than those of CRC (0.019±0.006 ng/μl), BC (0.036±0.015 ng/μl), HCC (0.035±0.016 ng/μl), and higher than those of HC (0.012±0.003 ng/μl). The serum CCDC25 level can discriminate CCA from the HC, CRC, BC, and HCC with a sensitivity of 100, 99, 94, and 94%, respectively, and specificity of 100, 100, 98, and 95%, respectively. Conclusion: CCDC25 is a candidate diagnostic biomarker for CCA.*

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Key Words: CCDC25, CCA, highly prevalent cancers, diagnostic biomarker.

Cholangiocarcinoma (CCA) is a malignancy of cholangiocytes in the intrahepatic and extrahepatic bile ducts. CCA is highly endemic in the Greater Mekong Subregion, particularly Thailand (1). Currently, there is no screening or diagnostic test that can reliably detect CCA at the early stage. The clinicopathological examination is the most reliable method for the diagnosis of CCA. For routine CCA diagnosis, blood biomarkers including alkaline phosphatase (ALP), carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are currently considered as markers, but they have limited sensitivity and specificity, and CA19-9 is not considered a CCA specific biomarker (2, 3).

CCDC25 is a 25 kDa coiled protein present in various mammalian cells. Its encoding gene is located on human

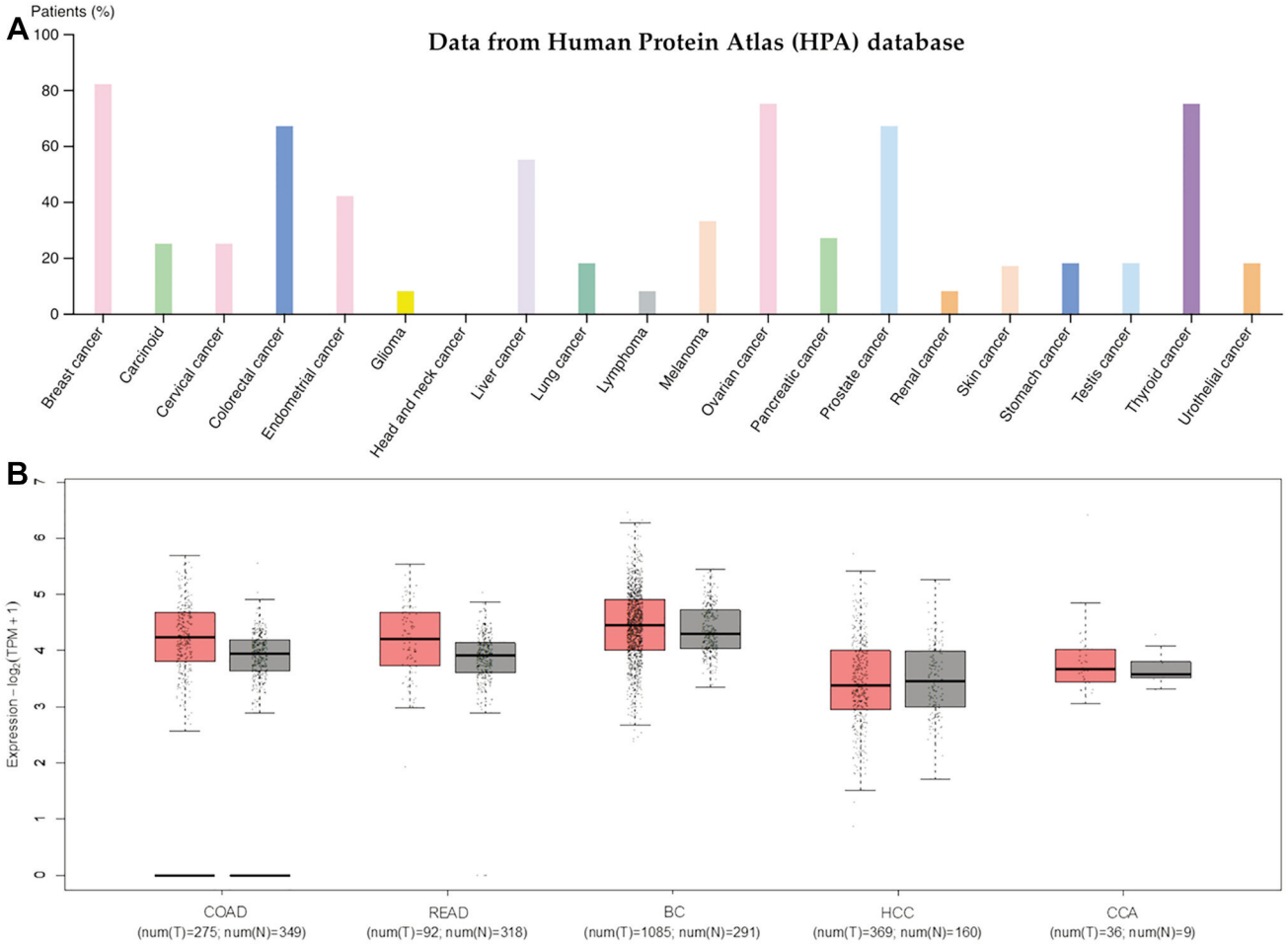

Figure 1. *Coiled-coil domain containing 25 (CCDC25) expression (A) in various cancer types in the Human Protein Atlas (HPA) database; (B) CCDC25 mRNA expression levels in various cancers and normal tissues were analyzed using Gene Expression Profiling Interactive Analysis 2 (GEPIA2) database. COAD: Colon adenocarcinoma; READ: rectum adenocarcinoma; BC: breast cancer; HCC: hepatocellular carcinoma; CCA: cholangiocarcinoma; T: tumor (red box); N: normal (grey box).*

chromosome 8p21.1 (4). CCDC25 is a membrane-bound protein identified as a receptor of neutrophil extracellular trap (NET) DNA. Thus, CCDC25 is considered as a precise navigator for NET-mediated metastasis of cancers (5). On the contrary, intracellular production, secretion, and biological roles of CCDC25 inside and outside of cancer cells remain unclear. In our previous study (6), we reported that CCDC25 was intracellularly overexpressed in CCA cells, especially after bile acid treatment, and was overexpressed in CCA tissues in comparison to the corresponding adjacent non-cancerous tissues. We also reported that the serum CCDC25 level of CCA patients was higher than that of healthy controls, suggesting that CCDC25 can be a diagnostic biomarker for CCA (7). In this study, therefore, we examined CCDC25 levels in the patients' sera from highly prevalence cancers in Thailand including CRC, BC, HCC, and CCA, to evaluate their diagnostic value. The results showed that CCDC25 can be a unique and specific biomarker for CCA.

Materials and Methods

Bioinformatic analysis. The Human Protein Atlas (HPA) Database (8) (accessed on 1 February 2022) was used to examine CCDC25 expression in various cancers. We also used the Gene Expression Profiling Interactive Analysis 2 (GEPIA2, accessed on 1 February 2022) to obtain the mRNA expression levels of CCDC25 in various cancers and normal tissues (9).

Serum sample size. To determine the sample size required for the evaluation of the diagnostic value of serum CCDC25 level as a biomarker for CCA patients, the serum CCDC25 levels of 18 colorectal cancers (CRCs), 20 breast cancers (BCs), 20 hepatocellular carcinomas (HCCs), 40 cholangiocarcinomas (CCAs) and 20 healthy controls (HCs) were individually determined using

Table I. *Demographic and clinical characteristics of healthy controls and cancer patients.*

Parameter (normal range)	HC (n=30)	CRC (n=34)	BC (n=42)	HCC (n=43)	CCA (n=83)	*p*-Value
Age	50±7 (25, 62)	58±9 (40, 75)	54±7 (25, 73)	60±5 (35, 72)	60±7 (31, 80)	<0.0001[1*,2*,3*,4*,5*, 6*]
Total protein (6.5-8.8 g/dl)	NA	NA	NA	7.90±3.6[c] (6.2, 9.6)	7.40±0.5 (4.6, 9.1)	0.0003[9*]
Total bilirubin (0.25-1.5 mg/dl)	NA	NA	NA	0.5±0.2[c] (0.2, 1)	0.6±1 (0.2, 16.8)	0.004[9*]
ALT (4-36 U/l)	16.5±5.1 (6, 30)	17±6.6[a] (6, 51)	NA	36±16.5[d] (10, 87)	38±20.8 (1, 795)	<0.0001[3*, 4*, 7*, 8*]
AST (12-32 U/l)	21±4.4 (13, 30)	26±4.9[a] (13, 82)	NA	48±15[d] (15, 238)	38±16.5 (4, 1,122)	<0.0001[3*, 4*, 7*, 8*]
ALP (42-121 U/l)	50.5±6.4 (31, 72)	87±18.1[a] (55, 199)	NA	103±21.5[d] (44, 244)	159±72.5 (46, 1,005)	<0.0001[1*, 3*, 4*, 8*, 9*]
CA19-9 (0-37 U/ml)	NA	NA	NA	NA	54.4±73.7[e] (0.6, 1,000)	NA
CEA (0-5 ng/ml)	NA	5±2.9[b] (0.6, 39.4)	NA	NA	5.6±5.7[f] (0.92, 125.5)	0.586
CA15-3 (<22 U/ml)	NA	NA	11.1±3.4 (3.4, 25.5)	NA	NA	NA
Survival days	NA	NA	NA	252±166 (24, 3,800)	377±109[g] (40, 1,407)	0.054

[a,b,c,d,e,f,g] represented the number of analyzed samples=22, 21, 40, 41, 75, 66, 73, respectively. NA: Not available; ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; CA19-9: carbohydrate antigen 19-9; CEA: carcinoembryonic antigen; CA15-3: cancer antigen. [1*]A significant difference in the value of each clinical parameter between healthy controls (HC) and colorectal cancer (CRC); [2*]A significant difference in the value of each clinical parameter between HC and breast cancer (BC); [3*]A significant difference in the value of each clinical parameter between HC and hepatocellular carcinoma (HCC); [4*]A significant difference in the value of each clinical parameter between HC and cholangiocarcinoma (CCA); 5*A significant difference in the value of each clinical parameter between BC and HCC; [6*]A significant difference in the value of each clinical parameter between BC and CCA; [7*]A significant difference in the value of each clinical parameter between CRC and HCC; [8*]A significant difference in the value of each clinical parameter between CRC and CCA; [9*]A significant difference in the value of each clinical parameter between HCC and CCA. *Statistically significant ($p<0.05$).

a dot blot assay. Then, the serum sample size was calculated using G*Power program version 3.1.9.6 (10). The results showed that the minimum sample sizes required for discrimination between CCA from HC, CRC, BC, and HCC were 6, 9, 10 and 30, respectively. The 34 CRCs patients (median age±quartile deviation, 58±9) and 42 BCs (median age±quartile deviation, 54±7) were collected from the Clinical Laboratory of Udonthani Cancer Hospital, Udon Thani, Thailand. The serum samples of 43 HCCs patients (median age±quartile deviation, 60±5) and 83 CCAs (median age±quartile deviation, 60±7) were supplied by the Cholangiocarcinoma Research Institute, Faculty of Medicine, Khon Kaen University, Thailand. All cancer cases were diagnosed by clinicopathological examination. The 30 HCs samples (median age±quartile deviation, 50±7) were the leftover sera from the annual health check-up at the Faculty of Associated Medical Sciences (AMS-KKU Excellence Laboratory), Khon Kaen University, Thailand. The HC group had normal levels in laboratory tests. All serum samples were kept at –20˚C until use. The use of human samples was approved by the Human Ethics Committee of Khon Kaen University (approval no. HE641285).

Western blot assay. The detection of CCDC25 in the sera was validated using western blotting performed as previously described (11) with some modifications. In brief, protein concentration of the samples was determined using the Bradford assay, and 30 μg of protein from each serum sample were dissolved in buffer [10% sodium dodecyl sulfate (SDS), 1M Tris-HCl, pH 6.8], and boiled for 5 min. The gel was activated at 70 V for 20 min at room temperature. Samples were loaded and run in parallel with molecular weight markers at 150 V, 20 mA for 90 min at room temperature. The separated protein bands were transferred onto the PVDF membrane. The membrane was incubated with 1:1,000 dilution of rabbit polyclonal primary antibody against human CCDC25 (Cat#orb2517, Biorbyt, Cambridge, UK), overnight at 4˚C, and then incubated with 1:2,000 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Cat#orb7083, Biorbyt) for 1 hour (h) at room temperature. The chemiluminescence was detected and visualized using an Amersham Imager 600 apparatus (GE Healthcare, Buckinghamshire, UK).

Dot blot assay and acquisition of data. To determine serum CCDC25 levels, dot blot assay was performed as previously described with minor modifications (7). In brief, for each membrane, the pooled CCA sera were used as a positive control for the normalization of the intensity of CRC, BC, HCC, CCA, and HC serum samples. Based on the preliminary results, serum CCDC25 levels in the sera of CCA patients were much higher than those of other cancer patients. Thus, CRC, BC, HCC, and HC serum samples were used

as neat sera, while CCA serum samples were diluted to 1:3 with normal saline solution (NSS). Then, 2 μl of each serum sample was spotted onto the membrane. The membrane was incubated with 1:1,000 dilution of rabbit polyclonal primary antibody against human CCDC25 (Cat#orb2517, Biorbyt) overnight at 4˚C, and then incubated with 1:2,000 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Cat#orb7083, Biorbyt) for 1 h at room temperature. The chemiluminescent signal was detected and quantified on an Amersham Imager 600 (GE Healthcare, Chicago, IL, USA). The experiment was performed in triplicate. The relative intensity of each spot was obtained from normalizing each intensity with the intensity of pooled CCA serum samples by using ImageJ software (version 1.53a; National Institutes of Health, Bethesda, MD, USA). The CCDC25 concentration was calculated based on the standard curve prepared using serially diluted (0.5, 0.25, 0.125, 0.0625, 0.0313, and 0.0156 ng/μl) of recombinant CCDC25 protein (Cat#orb424527, Biorbyt).

Statistical analysis. The mean±standard deviation or median±quartile deviation with the range (minimum-maximum) were used for the description of normally and non-normally distributed data, respectively. The comparisons of two or three sample groups were performed using the following criteria and statistics; normal distribution was analyzed using T-test, or ANOVA; non-normal distribution was analyzed using the Mann Whitney U test, or Kruskal-Wallis test. The correlation between serum CCDC25 levels and commonly used serum biomarkers was analyzed using Spearman's correlation test. A receiver operating characteristic (ROC) curve was used to obtain the sensitivity and specificity values, and respective areas under the curves (AUCs) with 95%CI. All analyses were conducted using GraphPad Prism software (version 7; GraphPad Software Inc., La Jolla, CA, USA) and SPSS software (version 28; SPSS, Inc., IBM, Armonk, NY, USA). $p<0.05$ was considered to indicate a statistically significant.

Results

Bioinformatic analysis of CCDC25 expression. Initially, CCDC25 expression in various cancer tissues were examined using the HPA database. The frequency of CCDC25 positive cases was high in several cancers, such as breast, colorectal, liver, ovarian, prostate, and thyroid cancers (Figure 1A). Then, we accessed the GEPIA2 database to assess the CCDC25 mRNA expression levels in colon adenocarcinoma (COAD), rectum adenocarcinoma (READ), BC, HCC, and CCA tissues. The results showed that CCDC25 mRNA in the COAD, READ, BC, HCC, and CCA cancer types was highly expressed compared with the normal tissues (Figure 1B).

The standard curve preparation for CCDC25 quantification using dot blot assay. As the first step, we examined to find out whether CCDC25 protein could be detected in the sera of various cancer patients using an anti-CCDC25 antibody. For this purpose, pooled sera of HC, CRC, BC, HCC, and CCA were examined using a western blot assay. The results showed that antibody used in this study was able to detect a clear single band of about 25 kDa, corresponding to the predicted molecular weight of CCDC25 (Figure 2). Then, CCDC25 levels of the individual serum sample of HCs and patients with various cancers were measured quantitatively using dot blot assay based on the standard curve created by serial dilutions of recombinant CCDC25 (Figure 3A and B).

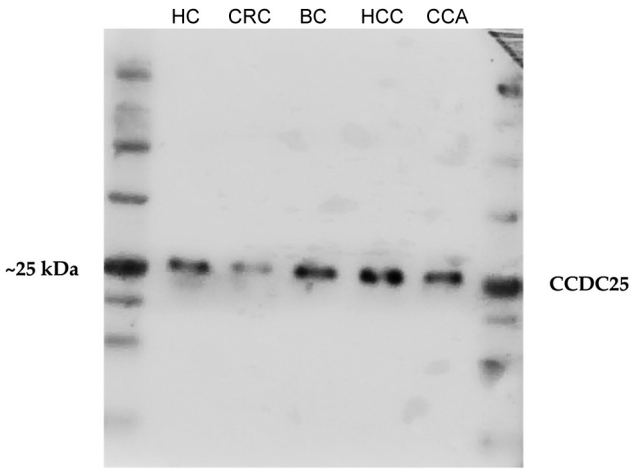

Figure 2. *Western blot assay was applied to examine the expression of coiled-coil domain containing 25 (CCDC25) in the sera using an anti-CCDC25 antibody.*

Serum CCDC25 levels of CRC, BC, HCC, CCA patients, and healthy controls. The serum CCDC25 levels of the patients with CRC (n=34), BC (n=42), HCC (n=43), CCA (n=83), and HC (n=30) were measured quantitatively using a dot blot assay. The characteristics and clinical data of these patients are shown in Table I. As presented in Figure 4, the median of CCDC25 levels in the sera of CCA patients was significantly higher than those of other cancers ($p<0.0001$). Moreover, no correlation was observed in between serum CCDC25 levels and age in HC (Figure 5).

Evaluation of the diagnostic value of CCDC25 levels for various cancer patients, based on the ROC analysis and Youden's index (YI). To examine whether serum CCDC25 level can be used as the diagnostic biomarker for patients with cancer, ROC curve analysis was performed for CCA, CRC, BC, and HCC against HC groups. We established the best cut-off value derived from YI to distinguish patients with each cancer from HC. The ROC analysis revealed that CCDC25 can effectively distinguish CCA patients from the HC with 100% sensitivity and specificity ($p<0.0001$, AUC=1.000, 95%CI=1.000-1.000, cut-off: 0.017 ng/μl) (Figure 6A). The serum CCDC25 levels had a sensitivity and specificity of 68% and 100%, respectively, in the CRC group ($p<0.0001$, AUC=0.850, 95%CI=0.754-0.946, cut-off: 0.017 ng/μl) (Figure 6B). The serum CCDC25 levels' sensitivity and specificity were 88% and 100%, respectively, in the BC

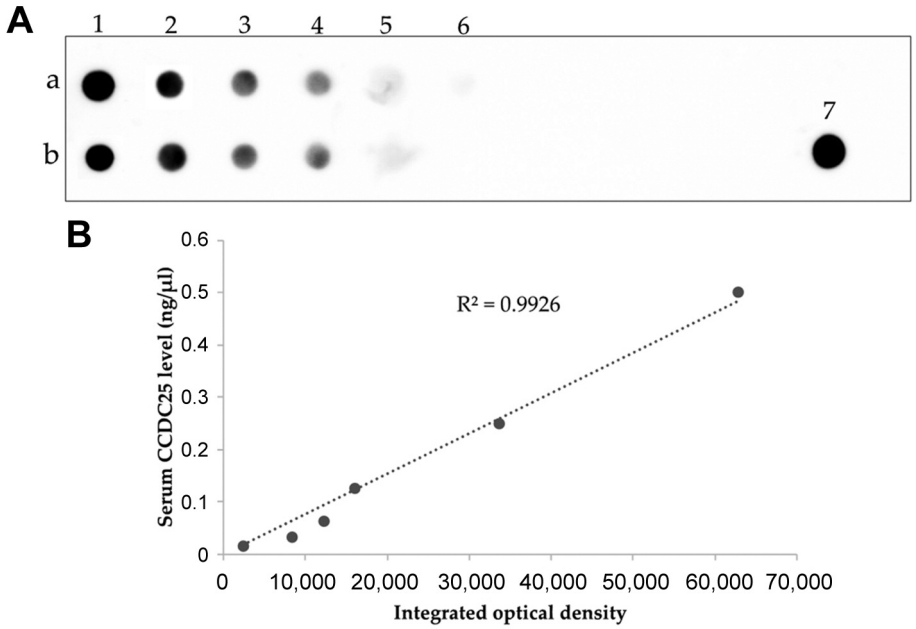

Figure 3. *The standard curve for the quantitative measurement of coiled-coil domain containing 25 (CCDC25) in the sera using dot blot assay. (A) Representative dot blot assay to create the standard curve for CCDC25 quantification. Samples in line a and b are duplicates of two-fold serial dilutions of standard recombinant CCDC25 protein. Sample 7 is a positive control using pooled CCA sera; (B) The standard curve for CCDC25 measurement.*

Table II. *The performance of serum coiled-coil domain containing 25 (CCDC25) and alkaline phosphatase (ALP) biomarker for differential diagnosis of cholangiocarcinoma (CCA) from other cancers.*

Biomarker	Group comparison	Cut-off	AUC (95%)	YI	SN (%)	SP (%)
CCDC25 (ng/µl)	CCA *vs*. HC	0.017	1.000 (1.000-1.000)	1.00	100	100
	CCA *vs*. CRC	0.054	0.999 (0.997-1.000)	0.99	99	100
	CCA *vs*. BC	0.081	0.991 (0.981-1.000)	0.92	94	98
	CCA *vs*. HCC	0.084	0.981 (0.964-1.000)	0.89	94	95
ALP (U/l)	CCA *vs*. HC	73	0.990 (0.974-1.006)	0.95	95	100
	CCA *vs*. CRC	109	0.813 (0.721-0.905)	0.51	73	77
	CCA *vs*. HCC	141	0.770 (0.688-0.852)	0.51	58	93

SN: Sensitivity; SP: specificity; AUC: area under the ROC curve; YI: Youden's index. Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were not available.

group (p<0.0001, AUC=0.961, 95%CI=0.922-1.000, cut-off: 0.017 ng/µl) (Figure 6C). The serum CCDC25 levels had a sensitivity and specificity of 98% and 100%, respectively, in the HCC group (p<0.0001, AUC=0.992, 95%CI=0.976-1.000, cut-off: 0.017 ng/µl) (Figure 6D).

Evaluation of the diagnostic value of CCDC25 levels for differential diagnosis of patients with CCA from patients with other cancer based on the ROC analysis and Youden's Index (YI). To examine whether serum CCDC25 level can be used as the diagnostic biomarker for CCA patients, ROC curve analysis was performed for CRC, BC, HCC, and CCA group. ROC analysis revealed that CCDC25 can effectively distinguish patients with CCA from patients with CRC, BC, HCC with sensitivity of 99%, 94% and 94%, respectively, and specificity of 100%, 98% and 95%, respectively (Figure 7). Moreover, the performance of CCDC25 levels was higher than that of ALP levels for CCA diagnostic as shown in Table II.

The correlation between serum ALP, CEA, CA19-9 and CCDC25 levels in patients with CCA. Currently, serum biomarkers for CCA include CA19-9, CEA, and ALP (12). Thus, correlation analysis was performed between commonly

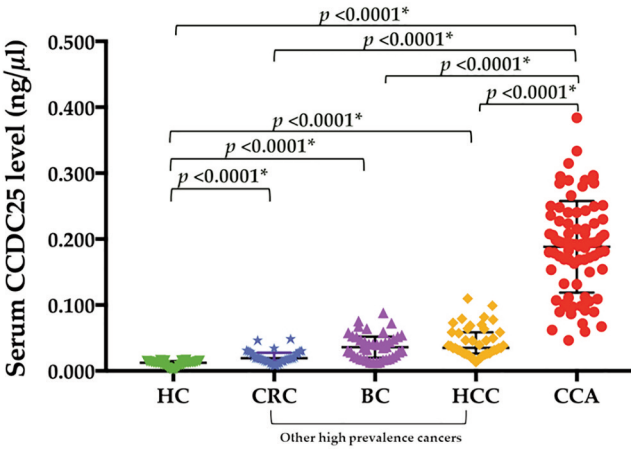

Figure 4. *Comparison of serum coiled-coil domain containing 25 (CCDC25) levels among cholangiocarcinoma (CCA), other high prevalence cancers and healthy controls. Scatter plot represented median level±quartile deviation of the CCDC25 level was 0.012±0.003 ng/µl in the healthy control (HC) group, 0.019±0.006 ng/µl in the colorectal cancer (CRC) group, 0.036±0.015 ng/µl in the breast cancer (BC) group, 0.035±0.016 ng/µl in the hepatocellular carcinoma (HCC) group, and 0.193±0.039 ng/µl in the CCA group. *Statistically significant (p<0.05).*

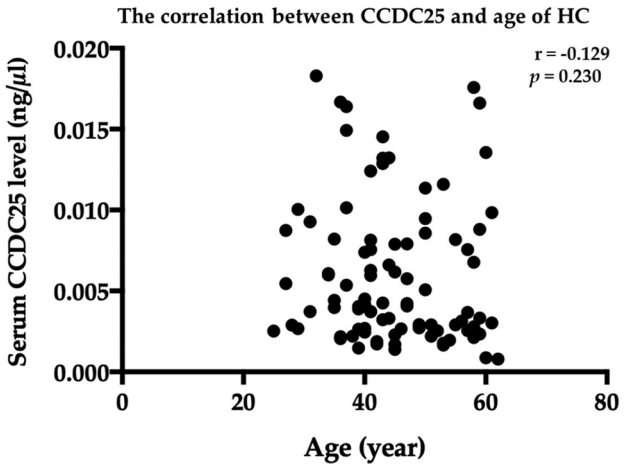

Figure 5. *The correlation between coiled-coil domain containing 25 (CCDC25) levels and age in healthy controls (HC).*

used serum biomarkers in patients with CCA and serum CCDC25 levels. The results showed no correlation between serum ALP, CEA, CA19-9 levels, and serum CCDC25 levels in patients with CCA (Figure 8). Thus, CCDC25 was identified as an independent biomarker for CCA patients.

Since serum levels of ALP, but not those of CEA and CA19-9, were available for all patients with various cancers in this study, correlation between CCDC25 and ALP levels of them were analyzed on a scattered graph (Figure 9). The results showed that only patients with CCA showed wide distribution in both directions, whereas patients with other cancers are clustered near the crossing of the X and Y axes. Thus, the scattered graph showed a clear discrimination pattern between CCA and other cancer groups.

Discussion

Currently, the prognosis of CCA patients is mostly poor because of the difficulties in early diagnosis owing to the lack of accurate diagnostic tools. In the clinical process, there is no reliable tumor marker for CCA diagnosis and/or prognosis. Moreover, although serum CA19-9 level is recommended as a diagnostic marker for CCA, it still is insufficient to diagnose CCA (2, 3). Other tumor biomarkers such as CEA, mucin 5AC (MU5AC) or matrix metallopeptidase 7 (MMP-7) also have limited value for detection owing to their inaccuracy (13, 14). We previously reported that CCDC25 was highly expressed in cancer tissues and sera of CCA patients compared with those of the HC group (6, 7), suggesting that CCDC25 can be a potential biomarker for CCA. Since the bioinformatic analysis revealed that CCDC25 is a ubiquitous protein, it might be used as a potential biomarker not only for CCA but at least for some other cancers. In this study, to evaluate whether CCDC25 is a unique biomarker for CCA patients, we determined the serum CCDC25 levels in healthy controls and compared them to those of patients with CCA and other highly prevalent cancer types in Thailand. The results showed that serum CCDC25 levels of CCA patients were significantly higher than those of the other cancer patients. High serum CCDC25 levels in patients with CCA are consistent with those of our previous study, which demonstrated that CCDC25 was overexpressed in CCA tissues and CCA serum (6, 7). Importantly, the present results showed that the elevation in serum CCDC25 levels was remarkable in patients with CCA, but it was slight or marginal in the patients with other cancers. Interestingly, as shown in Figure 1, the HPA database showed over-expression of CCDC25 protein in various cancers and the GEPIA2 database showed over-expression of CCDC25 mRNA in various cancers. Since CCDC25 is a membrane-bound protein (5), overproduction and oversecretion of this molecule from CCA cells might be responsible for the elevation in serum CCDC25 levels. Further study is necessary to elucidate the possible mechanisms of the elevation of serum CCDC25 levels in CCA.

In terms of diagnostic value, the present study showed that CCDC25 could distinguish patients with CCA from the HC group with 100% sensitivity and specificity and also from patients with other cancers with 94-99% sensitivity and 95-100% specificity. In addition, our previous study revealed that the serum CCDC25 level could distinguish

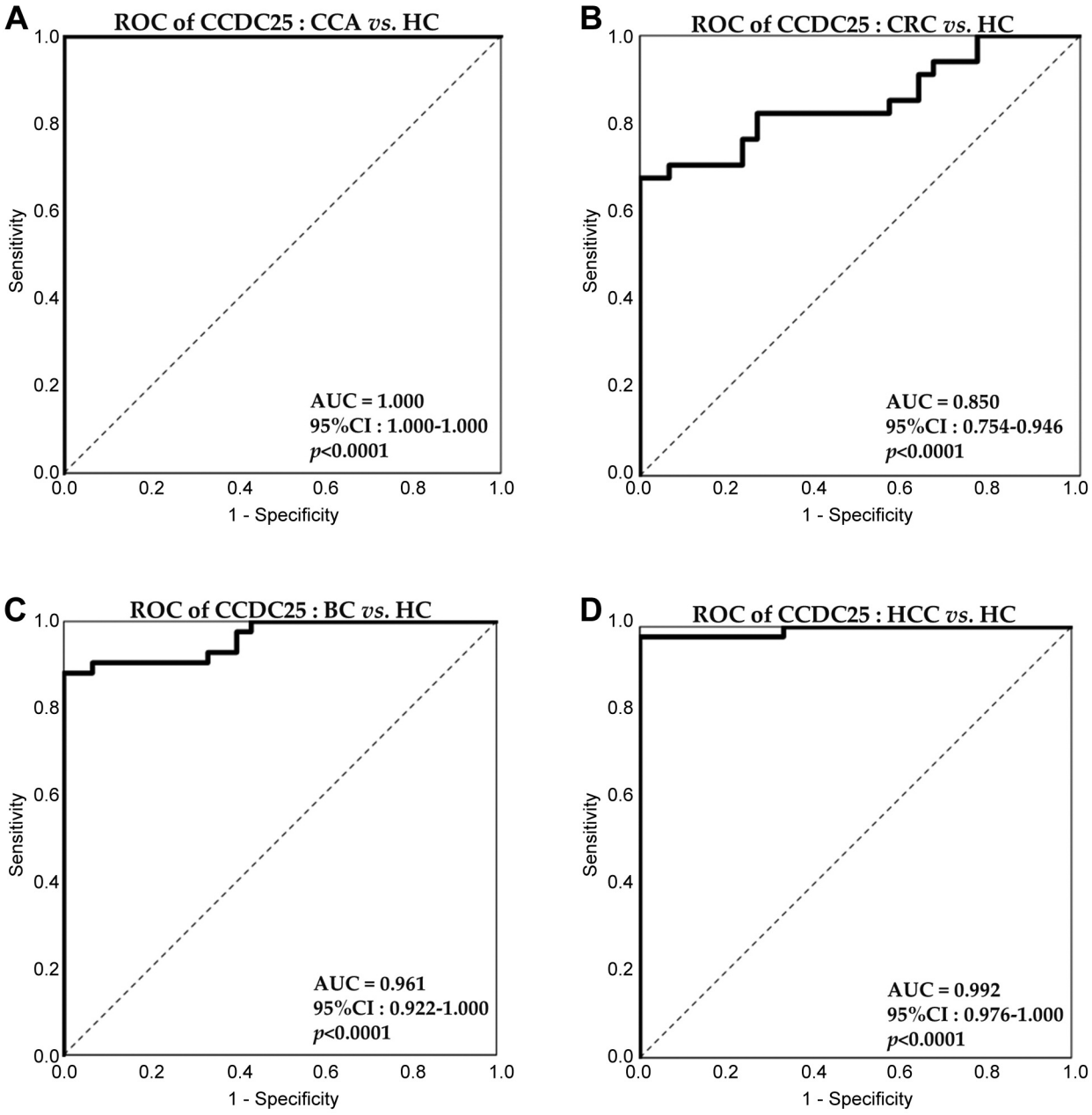

Figure 6. *Receiver operating characteristic (ROC) curve evaluation of serum coiled-coil domain containing 25 (CCDC25) levels as a potential biomarker for the diagnosis of cancers. (A) Comparison of ROC curve of CCDC25 levels between the cholangiocarcinoma (CCA) and healthy control (HC) groups; (B) Comparison of ROC curve of CCDC25 levels between the colorectal cancer (CRC) and HC groups; (C) Comparison of ROC curve of the CCDC25 levels between the breast cancer (BC) and HC groups; (D) Comparison of ROC curve of CCDC25 levels between the hepatocellular carcinoma (HCC) and HC groups. p<0.05 was considered statistically significant.*

CCA from benign biliary disease (BBD) (7). Furthermore, serum CCDC25 levels could discriminate CCA patients from HC more effectively compared with commonly used biomarkers including CEA and CA19-9 (15-19), and liver function tests including AST, ALT, and ALP. In addition, the results showed no correlation between serum ALP, CEA, CA19-9 levels and CCDC25 levels in CCA which are consistent with our previous study and indicated that CA19-9 and CEA were not correlated with serum CCDC25 levels in CCA patients (7). Thus, CCDC25 was identified as an independent biomarker for CCA patients. The correlation between serum CCDC25 and ALP levels can discriminate

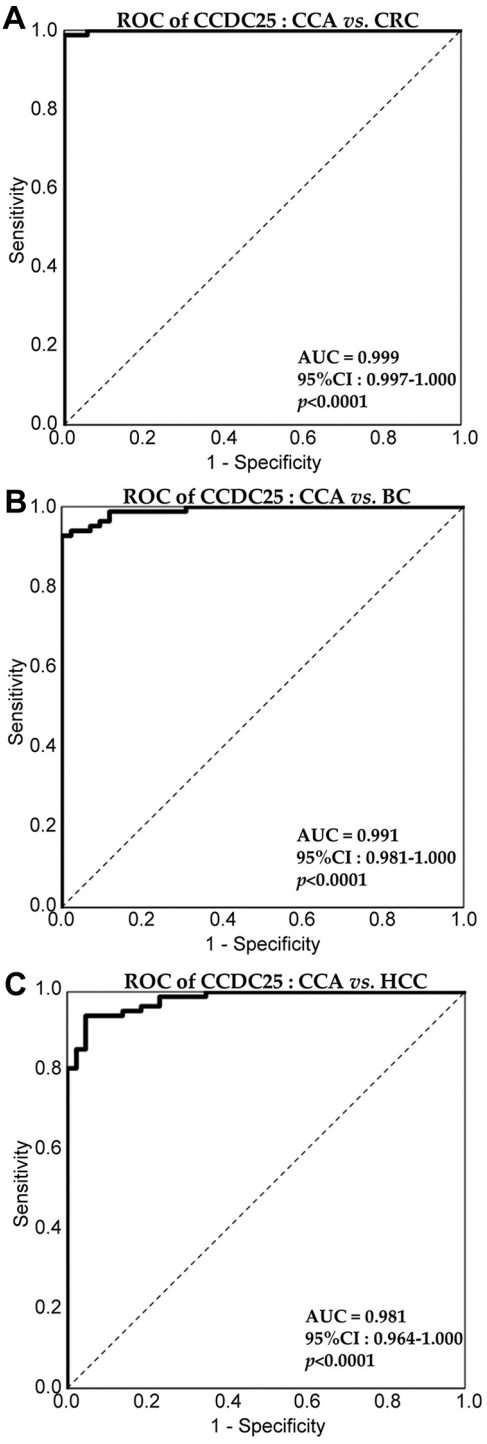

Figure 7. *Receiver operating characteristic (ROC) curve evaluation of serum coiled-coil domain containing 25 (CCDC25) levels for the differential diagnosis of cholangiocarcinoma (CCA) from other cancers. (A) Comparison of ROC curve of CCDC25 levels between the CCA and colorectal cancer (CRC) groups; (B) Comparison of ROC curve of CCDC25 levels between the CCA and breast cancer (BC) groups; (C) Comparison of ROC curve of CCDC25 levels between the CCA and hepatocellular carcinoma (HCC) groups. p<0.05 was considered statistically significant.*

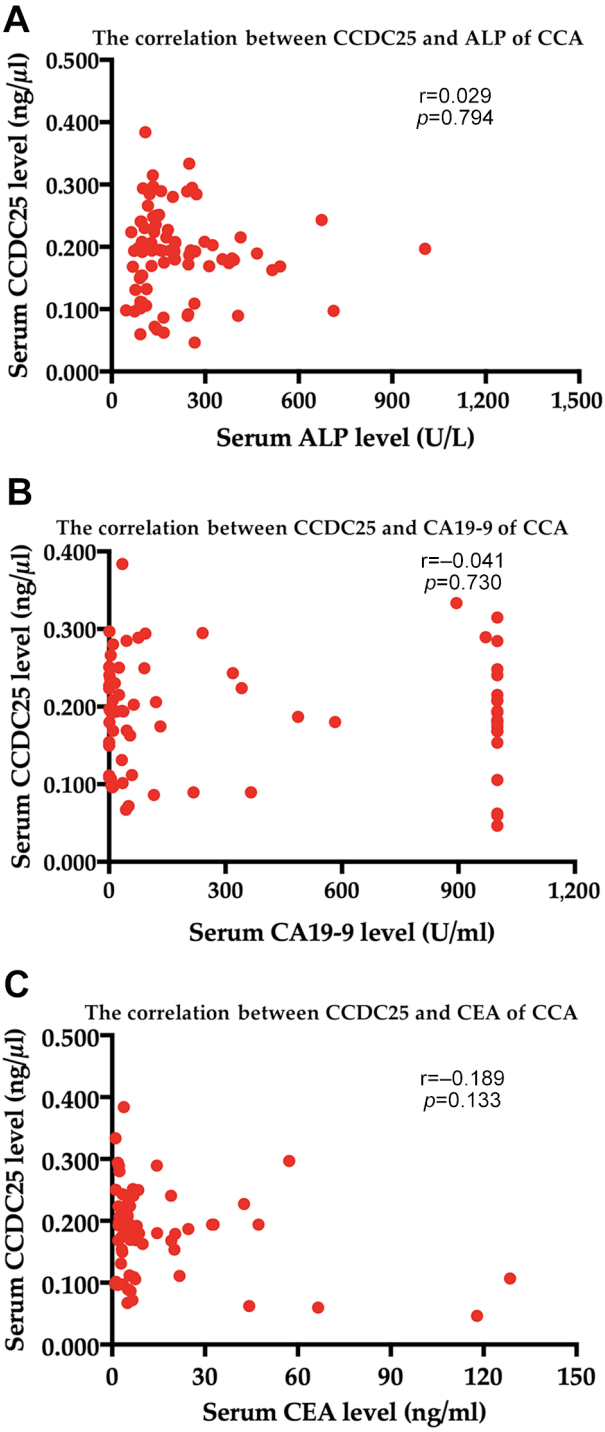

Figure 8. *The Spearman's correlation tests were performed between commonly used serum biomarkers and coiled-coil domain containing 25 (CCDC25) serum levels. (A) Correlation between CCDC25 and alkaline phosphatase (ALP) serum levels in cholangiocarcinoma (CCA) patients (r=0.029, p=0.794); (B) Correlation between CCDC25 and carbohydrate 19-9 antigen (CA19-9) serum levels in CCA patients (r=-0.041, p=0.730); (C) Correlation between CCDC25 and carcinoembryonic antigen (CEA) serum levels in CCA patients (r=-0.189, p=0.133). Using the Spearman's correlation test. p<0.05 was considered statistically significant.*

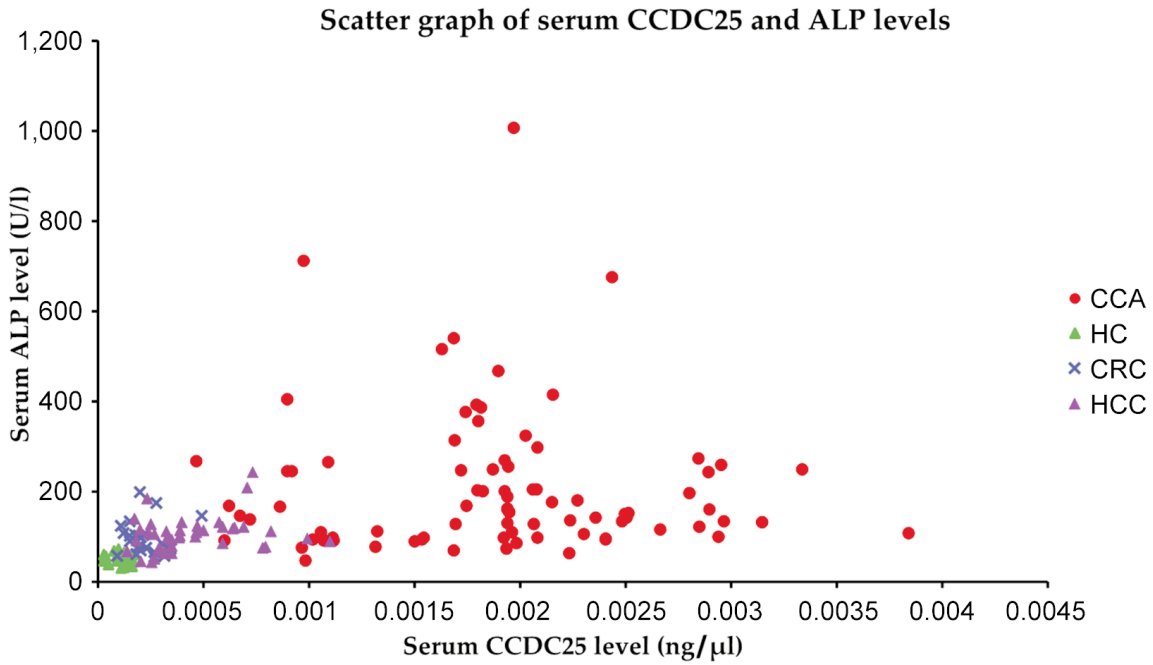

Figure 9. *Correlation between serum coiled-coil domain containing 25 (CCDC25) and alkaline phosphatase (ALP) levels in various cancer patients. CCA: cholangiocarcinoma; HC: healthy control; CRC: colorectal cancer; HCC: hepatocellular carcinoma.*

CCA from other cancers. Moreover, only serum CCDC25 levels can distinguish CCA from other cancers with high efficiency compared with ALP levels. In addition, a previous study revealed that higher serum CCDC25 levels of patients with CCA was correlated to a longer survival time (7). These suggest that serum CCDC25 level is a good diagnostic biomarker and is a unique biomarker for patients with CCA.

Conclusion

In conclusion, the results of this study demonstrated that CCDC25 can be a unique clinical biomarker for patients with CCA. We also found that the levels of serum CCDC25 were highest in patients with CCA and the serum CCDC25 levels could distinguish patients with CCA from those with other cancers with high efficiency.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Conceptualization of study design; A.S., W.S., T.P., M.W., S.R., I.C., and S.P. Methodology; A.J., A.S., W.S., T.P., M.W., and S.P. Data analysis; A.J. Investigation; A.J., T.A., and S.P. Original draft preparation; A.J. Final manuscript; T.P., and S.P.

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