LIM and SH3 Protein 1 Stands for a Bio-Marker for Cholangiocarcinoma Detection

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Abstract

Background: *LIM and SH3 protein 1* (*LASP-1*) has been demonstrated to be overexpression in several types of cancers. The aim of this study was to verify the serum level of *LASP-1* and investigate its diagnostic value in cholangiocarcinoma (CCA) patients.

Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to detect the expression level of *LASP-1* in CCA patients and healthy controls. The correlation of *LASP-1* expression with clinicopathological characteristic of CCA patients was analyzed via Chi-square test. Receiver operating characteristic (ROC) curve was built to evaluate the diagnostic value of serum *LASP-1* in CCA.

Results: Serum levels of *LASP-1* were upregulated in CCA compared with healthy controls (P<0.01). And the serum level and tissue level of *LASP-1* mRNA exhibited significant correlation (R=0.454, P=0.000). Serum expression of *LASP-1* was closely associated with lymph node metastasis (P=0.018) and TNM stage (P=0.021). ROC curve analysis revealed that serum *LASP-1* was of great value in differentiating CCA patients from healthy individuals. The area under the ROC curve (AUC) value was 0.879 corresponding with a sensitivity of 81.9% and a specificity of 79.6%.

Conclusions: Serum *LASP-1* might be an useful diagnostic biomarker for CCA.

Background

Cholangiocarcinoma (CCA) is the bile duct cancer, one of the highly aggressive malignant cancer, arising from ductular epithelium of biliary tree [1]. This cancer contains two major types including extrahepatic CCA (eCCA) and intrahepatic CCA (iCCA) according to anatomical location [2]. Previous studies have found several risk factors for CCA, such as primary sclerosing cholangitis (PSC), intrahepatic biliary stones, cirrhosis, viral hepatitis C and B, as well as CEA or CA19-9 [3-7]. But the diagnosis of CCA is still difficult due to its location, size and demoplastic characteristics [8]. Surgery is perhaps the main treatment method for cholangiocarcinoma, and despite improvements in surgical techniques, the survival of patients with this neoplasm was still poor. At present, there is no effective tool or specific biomarker that can detect the early stage or monitor status of CCA. Hence, there is a crucial need to explore novel reliable CCA diagnostic biomarker for improving the prediction and therapy efficacy.

The *LIM and SH3 protein 1* (*LASP-1*) gene, also called metastatic lymph node gene 50 protein (*MLN50*), was initially identified from a cDNA library of metastatic axillary lymph nodes (MLN) from human breast cancer and the gene was mapped to human chromosome 17q21 [9-11]. *LASP-1* has been demonstrated to play an important role in cancer development and progression and might function as a tumor oncogene in several types of cancers [12-14], such as ovarian cancer [12], breast cancer [13] and colorectal cancer [14]. Furthermore, Zhang et al. have analyzed the effect of *LASP-1* on biology and function of human CCA cell lines HCCC-9810 and RBE. The results showed that *LASP-1* was overexpressed in CCA tissues and was involved in the metastasis and growth of CCA [15]. However, the clinical significance of *LASP-1* in the diagnosis of CCA was few reported.

Quantitative real-time polymerase chain reaction (QRT-PCR) is a sensitive, faster and affordable detection method for gene expression in different biological systems [16]. Currently, qRT-PCR technique is widely used to explore cancer biomarkers in human tissue and fluid samples. For example, Koshkin et al. reported that the expression patterns of 10 mature microRNA (7, 10a, 17, 20a, 21, 23a, 26a, 137, 222) exhibited significant different between glioma specimens and non-cancerous tissues through qRT-PCR analysis, suggesting their potential as diagnostic biomarker for brain tumors [17]. The qRT-PCR analysis carried out by Sun et al. demonstrated that serum levels of *miR-770* levels showed significant difference between lung cancer patients and healthy controls, which could be employed as an indicator for early diagnosis and prognosis evaluation of the cancer [18]. QRT-PCR is an easy and reliable method to explore tumor-related biomarkers.

In the present study, we investigated the expression level of *LASP-1* in CCA serum samples and tissue specimens using qRT-PCR. In addition, we also estimated the diagnostic value of serum *LASP-1* in CCA patients.

Methods

Patients and specimens

In total, 127 CCA patients and 113 healthy controls were contained during their hospitalization and physical examination in the PLA Rocket Force Characteristic Medical Center. The patients were newly confirmed with CCA through pathological examinations. None of them had received surgical treatments, chemotherapy, or radiotherapy before sample collection. The healthy individuals exhibited normal range of all laboratory examinations, and had no history of malignant diseases. We collected 5mL blood from each participator for serum isolation. Blood was collected during physical examination and the serum was separated from whole blood by centrifugation for 10 min at 3000 rpm within 2 h after obtained, and then stored at -80℃ until use. In addition, 96 CCA patients in case group accepted surgical treatment in our study. The CCA tissues and corresponding normal tissues were collected from these patients.

The study protocol was approved by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center, and all patients provided written informed consents prior to sampling. All specimens were made anonymous according to the ethical and legal standards.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA from serum and tissue samples was extracted using TRIzol reagent (Invitrogen) and reverse transcribed to first-strand cDNA using the SuperScript™ II Reverse Transcriptase Kit (Invitrogen) according to the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was performed with an Applied Biosystems 7900HT system (Applied Biosystems) using SYBR Premix Ex Taq (Takara). *GAPDH* was used as endogenous control. The sequences of the primers used for *LASP-1* amplification were 5'-TGTCTCCTGACTGGTTGCGT-3', and 5'-TGATCTGGTCCTGGGTCTTC-3'. Primers for *GAPDH* were: 5'-

ATCAAGAAGGTGGTGAAGCAG-3’ and 5’-TACTCCTTGGAGGCCATGTG-3’. The $2^{-\Delta\Delta Ct}$ method was used to determine relative quantitation of *LASP-1* expression. Each sample was performed in triplicate.

Statistical analysis

The SPSS version 21.0 and GraphPad Prism 5 softwares were used to conduct all statistical analyses. The Student’s t-test was used to determine the statistical significance between tumor and healthy control groups. The association between *LASP-1* expression and clinicopathological parameters was evaluated using χ^2 test. The receiver operating characteristic (ROC) curves was generated to distinguish CCA patients from healthy individuals and the diagnostic potential of serum *LASP-1* evaluated by calculating the area under ROC curve (AUC). *P* value less than 0.05 was considered statistically significant.

Results

Baseline characteristics of the study population

In our study, 127 CCA patients including 73 males and 54 females were employed as case group. The control group included 66 men and 47 women. The mean age of case group was 53.78±18.75 years, while the average age of control group was 55.16±17.12 years. The case and control groups did not show significant differences in age and gender distributions (*P*=0.552 and 0.105, respectively). The body mass index (BMI) value of CCA patients was 22.56±2.15 kg/m^2, and the data in control group was 21.78±1.56 kg/m^2, without obvious difference (*P*=0.754) (**Table 1**).

Expression level of LASP-1 was increased in CCA

The relative mRNA expression level of *LASP-1* in serum specimens collected from 127 CCA patients and 113 healthy controls were analyzed by qRT-PCR. The results showed that expression levels of *LASP-1* in CCA patients were significantly higher than in healthy controls (*P*<0.01) (**Figure 1A**). Furthermore, we collected CCA tissues from 96 patients. The relative expression of *LASP-1* mRNA was also detected in CCA tissues and adjacent normal tissues. QRT-PCR assay suggested that compared to non-cancerous tissues, CCA tissues exhibited significant up-regulation of *LASP-1* mRNA (**Figure 1B**).

In addition, the relationship between serum and tissue levels of *LASP-1* was also estimated in present study. Pearson’s correlation analysis indicated that the serum level and tissue level of *LASP-1* mRNA exhibited significant correlation (R=0.454, *P*=0.000) (**Figure 2**).

Relationship between LASP-1 expression and clinicopathological features of CCA patients

To investigate whether *LASP-1* was involved in the development of CCA, the correlation between *LASP-1* expression and clinicopathological characteristics was analyzed. The included CCA patients were divided into high expression group (n=76) and low expression group (n=51), according to their median serum levels of *LASP-1* mRNA (the cut-off was 1.99). As shown in **Table 2**, the expression of *LASP-1* was significantly correlated with lymph node metastasis (*P*=0.018) and TNM stage (*P*=0.021). However, there

were no significant correlations between serum *LASP-1* expression and age, gender, tumor size, or differentiation (all, $P>0.05$).

The diagnostic value of LASP-1 in CCA

To assess the feasibility of serum *LASP-1* as a diagnostic tool for the detection of CCA, ROC curve analysis was performed. As shown in **Figure 3**, *LASP-1* was proved to have a relatively high accuracy in differentiating CCA patients from healthy individuals yielded AUC of 0.897 (95% CI: 0.837-0.921) with a sensitivity of 81.9% and a specificity of 79.6% at the optimal cutoff point 1.77.

Discussion

CCA is a type of malignant tumor and patients with CCA mostly appear in late clinical presentation that resulted in high mortality with poor prognosis [19]. Owing to highly chemo-resistant characteristic of CCA, the only curative treatment is surgical resection, however, which may not be suitable for all cases. The incidence and prevalence of CCA vary markedly worldwide and it is still a refractory disease with a low 5-year survival rate [20, 21]. The current major problems are lack of effective biological indicators for monitoring tumor occurrence. Thus, it is critical to find effective indicator markers for early detection of CCA.

Nowadays, more and more specific biological markers were studied which play important roles in the detection and treatment of patients with different cancer types, including CCA [22-28]. Kawin Leelawat et al. measured the level of *CEA*, *CA19-9*, *MMP-7* and *MMP-9* in the serum of cholangiocarcinoma and benign biliary tract diseases patients and found only the serum *MMP-7* appears to be a valuable diagnostic marker in the discrimination of CCA from benign biliary tract disease [25]. Zhang et al. pointed that the biology of calretinin and CK5/6 expression in CCA is unclear but calretinin and CK5/6 immunohistochemical stains may be useful for diagnosing a CCA [26]. Yu et al. determined the expression of *PKM2* in and its impact on biology and clinical outcome of human hilar cholangiocarcinoma (HC), and pointed that *PKM2* is an independent prognostic factor and potential therapeutic target for human HC [27]. In recent years, serum tumor markers have been the objects of extensive investigation to assist CCA diagnosis because of the easy obtaining samples and relatively low cost. However, until now no marker was adequate specificity for CCA diagnosis.

LASP-1 is a specific focal adhesion protein that has been demonstrated to play an important role in cancer development and progression [13, 29-31]. For instance, Frietsch et al. analyzed the prognostic significance of *LASP-1* in breast cancer patients and provided evidence that nuclear *LASP-1*-positivity may serve as a negative prognostic indicator for long-term survival of breast cancer patients [13]. The *LASP-1* was found overexpressed in gastric cancer tissues and cell lines, and associated with poor prognosis, which plays an important role in growth and metastasis of gastric cancer [29]. Wang et al. determined the expression of *LASP-1* in primary HCC and the results showed that the high cytosolic *LASP-1* expression was associated with poor overall survival in HCC patients [30]. Yang et al. examined *LASP-1* expression in ccRCC tissues and in two cell lines and the results indicated that *LASP-1* might serve as a

prognostic biomarker for ccRCC patients [31]. Based on the previous reports, we studied the clinical diagnostic value of *LASP-1* in CCA.

In this present study, we examined the expression level of *LASP-1* in serum and tissues of the CCA patients. The results revealed that serum level of *LASP-1* in CCA patients was increased compared with healthy control. The high expression of *LASP-1* was tightly correlated with positive lymph node metastasis and advanced TNM stage, which indicated that *LASP-1* as an oncogene participated in the development and progression of CCA. The study carried out by Zhang et al. reported that the knockdown of *LASP-1* could obviously promote cell apoptosis, inhibit cell proliferation, migration and invasion in vitro and tumorigenesis in vivo. The up-regulation of *LASP-1* in CCA showed close association with malignant clinical characteristics, and poor prognosis [15]. However, the molecular mechanism of *LASP-1* in development of CCA was poorly known. Further investigations are still required.

ROC curve analysis demonstrated that serum *LASP-1* be a valuable indicator for CCA detection. *LASP-1* is a specific focal adhesion protein at cell surface, and its over-production in tumor cells or tissues may lead to alterations in peripheral blood concentration. In our study, we also found that the expression patterns of *LASP-1* in serum and tissue samples exhibited high consistency. Compared to tissue specimens, the blood was easily obtained via non-invasive methods, having repeatability. Thus, serum *LASP-1* might be a potential non-invasive biomarker for early diagnosis of CCA.

Conclusions

In conclusion, *LASP-1* expression is increased in CCA patients, and its elevated expression is positively correlated with lymph node metastasis and TNM stage. Serum *LASP-1* may be a promising diagnostic marker for CCA patients.

List Of Abbreviations

LIM and SH3 protein 1 (*LASP-1*)

cholangiocarcinoma (CCA)

Quantitative real-time polymerase chain reaction (qRT-PCR)

Receiver operating characteristic (ROC)

area under the ROC curve (AUC)

extrahepatic CCA (eCCA)

intrahepatic CCA (iCCA)

primary sclerosing cholangitis (PSC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of the PLA Rocket Force Characteristic Medical Center and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

N.W. design of the work; Y.L. the acquisition, analysis, Y.Z. interpretation of data; H.C. the creation of new software used in the work; X.W., Z.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Tables

Table 1. Baseline characteristics of the CCA patients and healthy controls

Parameters	CCA group (n=127, %)	Healthy controls (n=113, %)	*P* values
Age (years)			0.304
<50	68 (53.54)	53 (46.90)	
≥50	59 (46.46)	60 (53.10)	
Average age (years)	53.78±18.75	55.16±17.12	0.552
Gender			0.105
male	73 (57.48)	66 (58.41)	
female	54 (42.52)	47 (41.59)	
BMI values (kg/m^2)	22.56±2.15	21.78±1.56	0.754

Notes: CCA: Cholangiocarcinoma; BMI: Body mass index.

Table 2. Relationship between *LASP-1* expression level and clinicopathologic parameters of CCA patients

Parameters	Cases (n=127)	*LASP-1 expression*		χ^2	*P*
		Low	High		
Age				1.796	0.180
<50	68	31	37		
≥50	59	20	39		
Gender				0.967	0.326
Male	73	32	41		
Female	54	19	35		
Tumor size				0.381	0.537
<5cm	73	31	42		
≥5cm	54	20	34		
Lymph node metastasis				5.595	0.018
negative	71	35	36		
positive	56	16	40		
Differentiation				3.697	0.055
poor	69	33	36		
moderate+well	58	18	40		
TNM stage				5.320	0.021
I-II	74	36	38		
III-IV	53	15	38		

Figures

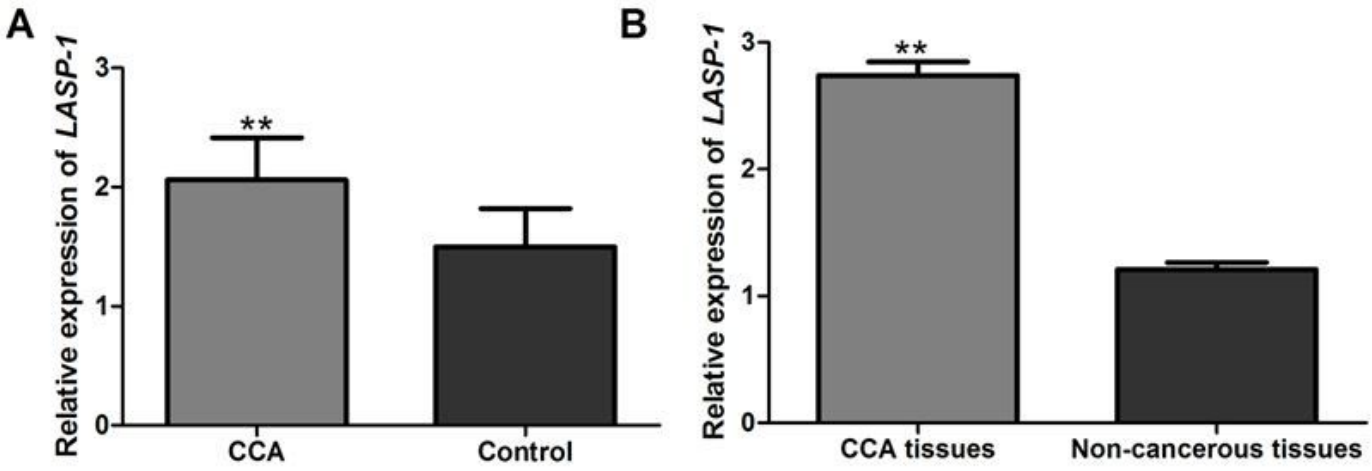

Figure 1

The relative LASP-1 expression levels in CCA patients. A: The expression of LASP-1 in CCA serum was significantly higher than that in healthy control individuals. B; Compared to adjacent non-cancerous tissues, CCA tissues showed obviously increased expression of LASP-1 (**, P<0.01).

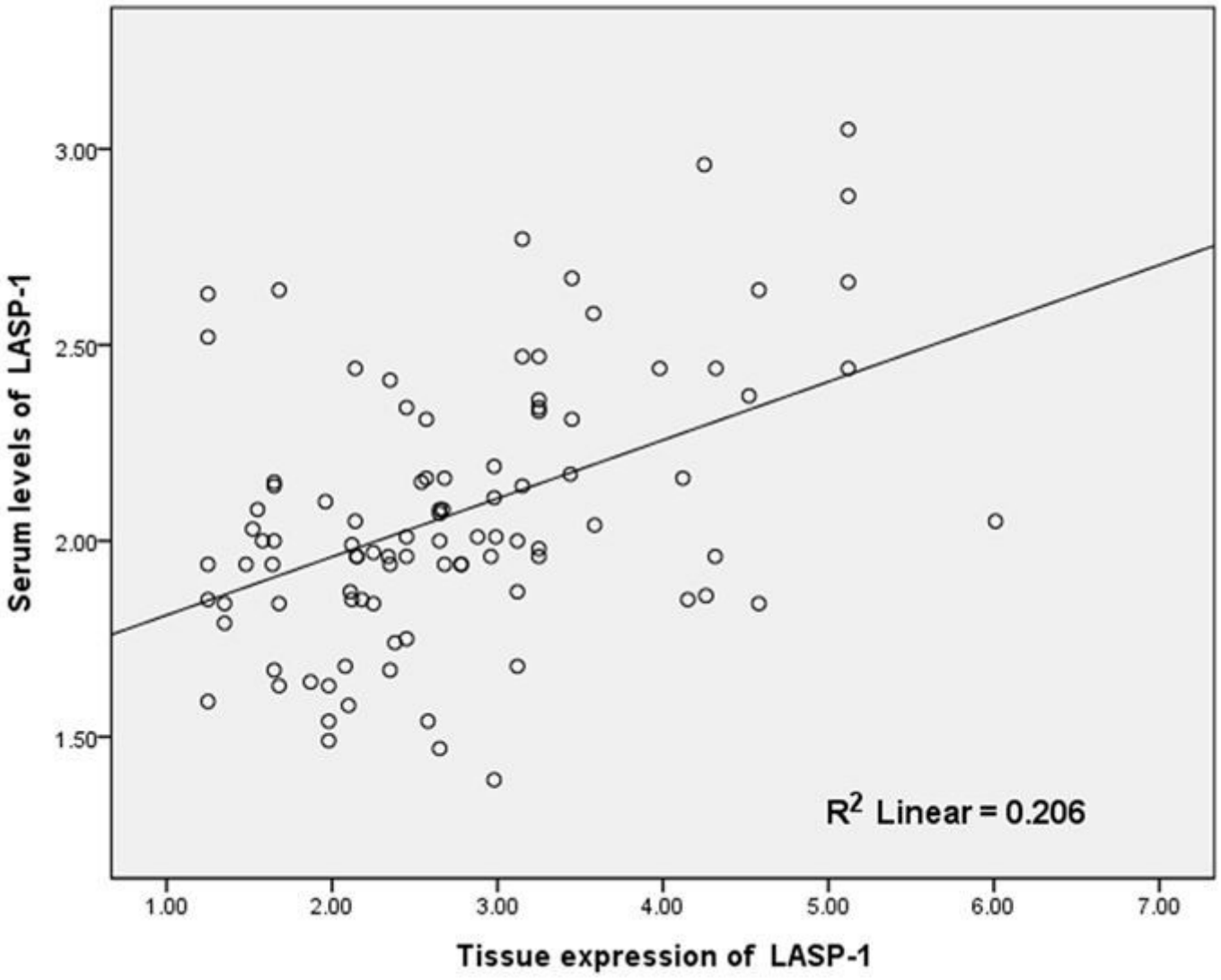

Figure 2

The interaction between serum and tissue expression of LASP-1 in CCA patients. The serum levels of LASP-1 was significantly correlated with its expression in CCA tissues (R=0.454, P=0.000).

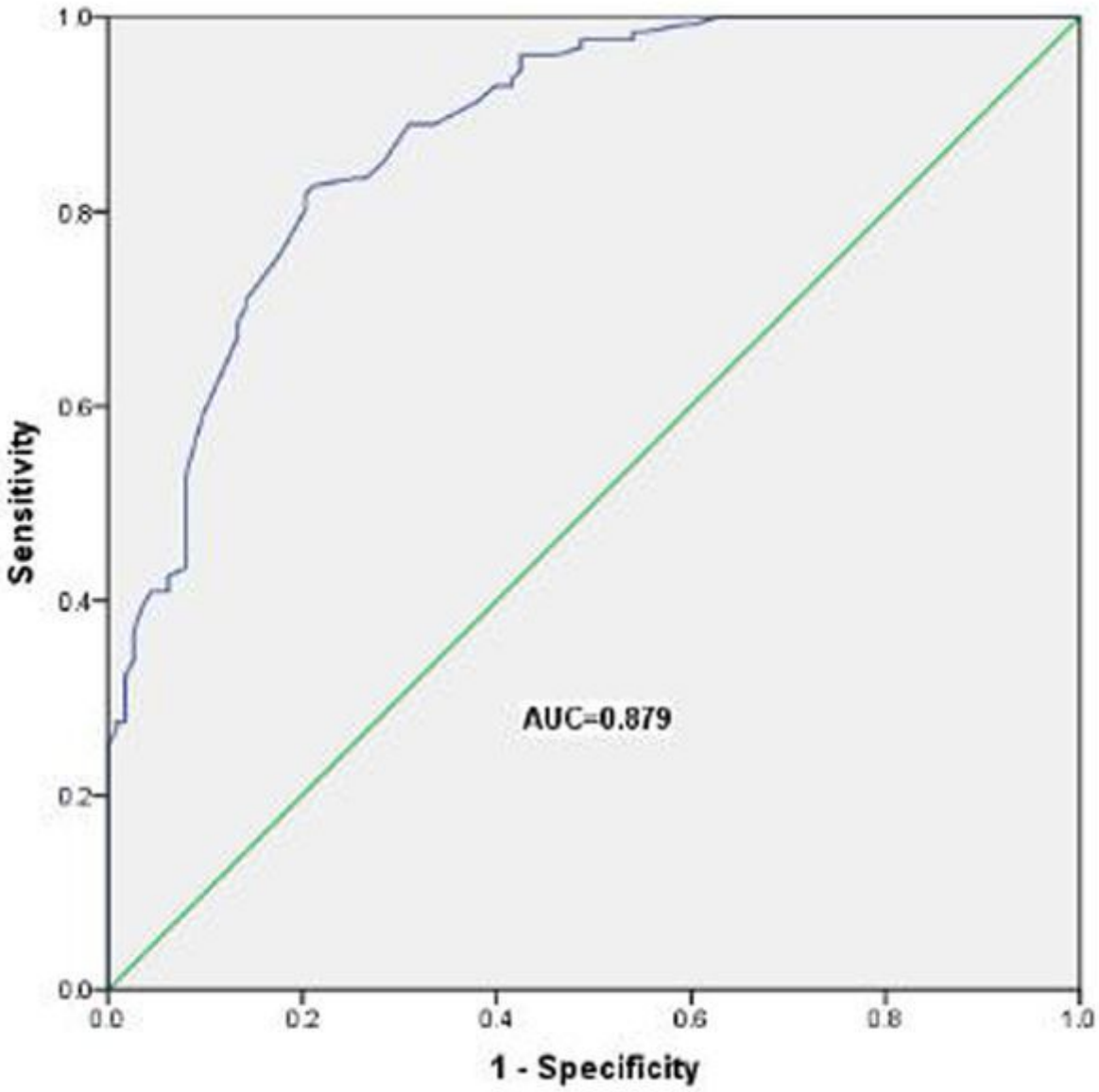

Figure 3

The diagnostic significance of LASP-1 was analyzed via establishing ROC curve. ROC curve analysis results showed serum LASP-1 expression to discriminate CCA patients from healthy controls.