

Validity of Dot ELISA using crude somatic antigen of cholangiocarcinoma tumor mass

ORIGINAL ARTICLE BY

Kanchana Tomanakan¹, Ph.D.
Jurairat Jongthawin², Ph.D.

¹Department of Medical Laboratory, Khon Kaen Hospital, Thailand; ²Department of Biochemistry, Faculty of Medicine, Mahasarakham University, Thailand

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Correspondence to: Kanchana Tomanakan;
tomanakan.k@gmail.com

ABSTRACT

OBJECTIVE

to investigate the validity of Dot ELISA using crude somatic antigen of cholangiocarcinoma (CCA) tumor mass.

METHODS

The cross-sectional diagnostic study was performed in patients who admitted at Khon Kaen Hospital, Thailand between October 2016 and March 2017. The overall patients enrolled to collect serum sample was 196 cases. The CHM1, a new prepared somatic antigen was collected and extracted from a liver tumor of one patient which clinically and histopathology confirmed as CCA. The enzyme-linked immunosorbent assay (ELISA) was performed to evaluate the diagnostic performance of reaction with that CHM1 crude somatic antigen.

RESULTS

There were 12 sera of proven CCA which nine sera gave positive results with Dot ELISA. For the 40 sera from healthy donors as the negative control group showed all negative results. We found that sensitivity, specificity, positive predictive value and negative predictive value were 75% (95% CI, 73.6 to 76.4%), 92.9% (95% CI, 91.5 to 94.4%), 40.9% (95% CI, 39.5 to 42.3%) and 98.3% (95% CI, 96.8 to 99.6%), respectively.

CONCLUSION

The results showed that Dot-ELISA using CHM1 antigen has a potential for rapid and simple performance to detect antibody in serum of cholangiocarcinoma patients.

INTRODUCTION

Cholangiocarcinoma (CCA) is one of the rare form of cancer with varied prevalence worldwide with the highest rates in the Northeastern Thailand; approximately 80 per 100,000 population.¹ The serum carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) are the most frequently used as serological markers with relatively low sensitivity and high specificity.² Other biomarkers such as MMP-9 and TuM2-Pk were also mentioned.³⁻⁵ However, no biomarkers have found to be useful for early diagnosis with no consensus to be used as standard diagnostic tools. Using somatic antigen have been used for serodiagnosis of leptospirosis⁶ and the crude somatic antigen prepared from *Fasciola gigantica* found to be a potential for diagnosis of fascioliasis.⁷ However, no study has examined the possibility of using somatic antigen from tumor mass for diagnosis of CCA and the use of liver biopsy as antigen is limited by its potential complications, including hemorrhage and tumor spread. In this study, we extracted the tumor mass for somatic antigen preparation by the method of Laemmli⁸ and we use Dot enzyme-linked immunosorbent assay (ELISA) using crude somatic antigen of tumor mass of CCA in various sera from various donors. Our aim of the study was to investigate the validity of Dot-ELISA using crude somatic antigen of tumor mass of CCA.

METHODS

STUDY DESIGN AND ETHICAL APPROVAL

The present study was a cross-sectional diagnostic study conducting at Khon Kaen Hospital, Thailand October 2016 through March 2017. Its protocol was approved by

Khon Kaen Hospital Institute Review Board (approval number: KE60008). The study complied with Declaration of Helsinki, October 2013.

CRUDE SOMATIC ANTIGEN PREPARATION

The 250 grams of fresh tumor mass which clinically and histologically diagnosed as cholangiocarcinoma was cut into small pieces and washed 3 times with phosphate buffered saline (PBS) pH 7.4 in a petri dish. Then added 50 ml. of PBS and 5 ml of phenylmethylsulfonyl fluoride (PMSF). Then the suspension was homogenized by a homogenizer, then centrifuge for 20 minutes at 10,000 rpm. Gently removed and aspirate the supernatant placed in a new tube, discard the pellet. The protein concentration was 3-5 mg/dl.⁹ This preparation was called human cholangiocarcinoma (CHM1) crude somatic antigen. Later the antigen was dotted with 3-5 mg/dl concentration of CHM1 protein coating on the nitrocellulose. The descriptive of this procedure can be found elsewhere.¹⁰

SERUM TESTING

A total of 196 individual serums using quota sampling were collected from leftover specimens after finish for laboratory testing in Khon Kaen Hospital (Table 1). Of these, 12 cases were histopathologically diagnosed as CCA (positive control), 40 samples from blood donors were used as negative control. We tested all 196 patient's serum with dot-ELISA based on the CHM1 which adsorbed on the nitrocellulose sheet. The nitrocellulose strip was blocked by placing into 5% skim milk in PBS pH 7.4 at room temperature for 10 minutes. Then washed 3 times with PBS. The nitrocellulose strip was incubated with serum sample 0.5 ml in PBS 10 ml. in small box 2 hours on a rotator. After the incubation, the nitrocellulose was washed with

Table 1. Demographic data and clinical characteristics of the specimen donors

Source of specimen	Gender Male:Female	Age	Reaction +/-
Cholangiocarcinoma (positive control)	7:5	52-71	9/3
Surgery Department	38:40	17-57	9/69
Medicine Department	30:26	22-78	0/0
From annual check-up	5:5	35-60	4/6
Blood donors (negative control)	24:16	26-39	0/0

PBS 3 times as described above and then incubated for 2 hours with alkaline phosphatase conjugated streptavidin (1:2,000; Dakopatts). Later, the strip was rinsed three times with PBS and immersed in a substrate solution for color development 10 minutes, washed with distilled water and air dried. The positive reaction appeared as a blue or purplish-blue dot on the test strip distinguishable from the negative as clear spot. The positive and negative control were tested in all run.

DATA ANALYSIS

The validity of the test was calculated regarding sensitivity, specificity, positive predictive value and negative predictive value were analyzed by calculation from a 2x2 table as described by Galens presenting together with 95% confidence interval (CI).¹¹

RESULTS

A total of 196 samples were examined using Dot ELISA. The patient's characteristics were shown in Table 1. The positive reaction with 22 serum out of 196 cases. Only 9 cases of positive control from total 12 proven cases demonstrated visualized dot.

The same as 40 cases of healthy donor serum showed negative results. Interestingly, 9 cases from patients who admit in Surgery Department showed the positive results, and only 4 cases from check-up individual represented as positive with Dot ELISA.

For validity of the tools in the current study The sensitivity, specificity, positive predictive value and negative predictive value were 75% (95% CI, 73.6 to 76.4%), 92.9% (95% CI, 91.5 to 94.4%), 40.9% (95% CI, 39.5 to 42.3%) and 98.3% (95% CI, 96.8 to 99.6%), respectively.

DISCUSSION

Although our current study indicates that CHM1 may be a potential tool for detection CCA. The need for better tests for detection early stage of CCA is an important issue. However, several studies have reported that many biomarkers served as the tools to diagnose CCA but there is still no specific serum tumor marker as the most potential diagnostic markers for CCA.²⁻⁵ Based on our results in this study, the sensitivity and specificity of anti CHM1 are 75% and 92.93% which demonstrated quite high when compared with CA19-9 (sensitivity

33.0-58.5%, specificity 62.5-84.0%) and CEA (sensitivity 38.0-70.4%, specificity 43.8-98.0%) and for the MMP7 (sensitivity 76.3%, specificity 46.9%). In addition, CA 19-9 levels can rise in other pancreatic and gastric cancers.¹³ We suggested that the differences of the patients included in the study should be concerned. In our study, the four patients with no clinical sign of tumor mass in surgery unit gave positive results due to acute cholangitis. So that, it was not clear whether these patients were in the early stage of CCA because it should take a long time to progress as CCA. The subjects in our control group were healthy donors and the annual checkup individuals instead of patients with benign bile

duct diseases because of we need to discriminate CCA from a normal population. The utility of molecular techniques on bile samples or brushing material for diagnosis of CCA remains unknown.¹² Interestingly, the study of molecular evidence of CCA should be emphasized because of mitochondrial DNA mutation, whose levels changes during treatment could be potential biomarkers for diagnosis CCA. However, our study provides the CHM1 antigen which is, to our knowledge, the first local antigen preparation to use in Dot ELISA method. This method is one of the non-invasive serological screening tests with relatively high diagnostic value.

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