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Efficacy of Strongyloides Stercoralis Peptides Antigen for Serology Diagnosis

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Abstract

Humans can become infected with *Strongyloides stercoralis* from soil-transmitted helminthiasis as a neglected disease. The human strongyloidiasis can involve vital organs leading to serious consequences. The gold standard method for diagnosis is detecting *S. stercoralis* larvae in feces under microscopy but the method needs more personnel expertise. The method itself also has low sensitivity and specificity. Serological methods are another important method for the diagnosis of strongyloidiasis by detecting antibody against *S. stercoralis*. Our aim was to evaluate the short chain antigen peptide for detection antibody in *S. stercoralis* infected patient. A total of 88 serum samples were tested by ELISA. The sensitivity by using designed peptide number 1, 2 and *S. ratti* as antigen were 66.67%, 77.78% and 88.83%, respectively. The specificity of designed peptide number 1, 2 and *S. ratti* were 69.62%, 94.94% and 64.56%, respectively. Even the designed peptides 2 have lower sensitivity than *S. ratti* but the higher specificity also detected. More proportions of sample needed to evaluate the potential of peptide for using as strongyloidiasis diagnosis tool.

Keywords: *Strongyloides stercoralis*, Strongyloidiasis, peptides, ELISA

1. Introduction

Human strongyloidiasis is a soil-transmitted helminthiasis caused by *Strongyloides stercoralis* (*S. stercoralis*) infection. The transmission occurr in tropical areas, including Thailand (Laoraksawong *et al*., 2018; Olsen *et al*., 2009). The infection can range from being clinically asymptomatic to showing signs of serious health, especially in severe condition in immunocompromised people, malnutrition or those who have been taking corticosteroids for a long time. The patient may not have been aware that he had a parasite infection because the symptoms may not have been obvious. This can cause hyperinfection and lead to more dangerous illnesses (Keiser & Nutman, 2004; Mejia & Nutman, 2012). *S. stercoralis* can live in the human body for up to 10 years without showing any symptoms. If the body has low immunity or is given immunosuppressive drugs, the number of worms may increase. The infectious parasite stage is a filariform larva (L3), which can be found in areas where soil is moistened and contaminated with infected people's feces. It can grow into an adult worm and survive in the soil without a host. The parasite spreads through unwashed food and vegetables, as well as penetrable during barefoot walking. It can penetrate the skin, enter the bloodstream, heart, lungs, and respiratory tract, then swallow through the gastrointestinal

tract to the small intestine and live until an adult and can re-infect the host (Schär *et al*., 2013). However, it is still a neglected disease, which must be recognized in order to avoid becoming infected with the parasite. To save the patient's life, an accurate diagnosis and treatment are required.

Diagnostic tests to reveal strongyloidiasis infection are very important for screening patients. The majority of adult parasitic worm detection methods involve a fecal examination by using the following techniques, such as light microscopy, the Kato-Katz technique, but the sensitivity remains low (Steinmann *et al*., 2007). The Baermann method and Koga agar plate cultures (APC), in which larva are cultured on medium for two days, are more sensitive than regular microscopy, they still miss a large proportion of infection, however (Buonfrate *et al*., 2020). There is a highly specific polymerase chain reaction (PCR) technique for detection. But it is required high-cost tools and often practiced at a specified organization (Buonfrate *et al*., 2015). More importantly, high sensitivity and specificity antigens are needed for those using serological methods to identify acute instances, severe and fatal hyperinfection strongyloidiasis (Arifin, Hanafiah, Ahmad, & Noordin, 2019). The enzyme-linked immunosorbent assays (ELISA) technique is one of the popular techniques for detecting specific antibodies in people who infected with *S. stercoralis*. Most ELISA tests rely on different crude extracts of *S. stercoralis* worm antigen, other Strongyloides species or recombinant *S. stercoralis* antigens (Eamudomkarn *et al*., 2018). *Strongyloides ratti* (*S. ratti*) is commonly used as a compared antigen for antibodies detection in patients with strongyloidiasis.

However, new technological models make it easier to develop peptide antigens in present time. To monitor the patient's serum, we created a *S. stercoralis*-specific peptides. We performed ELISA using peptide 1 and peptide 2 compared with conventional ELISA using *S. ratti* crude extract. The aim of this study is to determine the efficacy of antigenic peptides that may be useful for the diagnosis of Strongyloidiasis.

2. Purposes

To evaluate the short chain antigen peptide for detection antibody in *S. stercoralis* infected patient.

3. Research Methodology

1) Ethic approval

This study was approved by Suranaree University of Technology International Standard Human Ethics Committee, with project code EC-64-57. Patient samples were identified by an anonymous code.

2) Peptides designing

The amino acid sequence of fatty acid and retinol-binding protein (Masoori et al., 2019) was retrieved from GENBANK database accession number BBB03675.1. The linear B-cell epitope region was analyzed using the IEDB Analysis Resource program (http://tools.iedb.org/bcell/). The designed peptides were analyzed for *S. stercoralis* specificity by using BLAST program. The 2 peptides which have the highest similarity were selected. The peptides were synthesized by GenScript USA, Inc. in the United States.

3) *S. ratti* crude extracted antigens

The filariform larva (L3) of *S. ratti* were generously provided from Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. The *S. ratti* crude extracted antigens were prepared according to the protocol previously described (Eamudomkarn *et al*., 2018).

Briefly, the dead worms of *S. ratti* were washed in normal saline and stored at -20 °C. The larvae were dispersed in 2 ml of phosphate-buffered saline (PBS) pH 7.4, frozen them at -76 °C for 30 minutes and thawed, repeat again 4-5 times. Then, created a subsequent disruption of L3 by Ultrasonic Processors (Cole Parmer - CPX 750, Inc. USA) and homogenized, stored overnight at 4°C, then centrifuged at 15,000×g for 30 minutes at 4°C. The supernatant was taken to measure with NanoDrop (Thermo scientific, Inc. USA) and kept at -20°C until use.

4) Serum samples collection

A total of 88 human serum samples were collected and divided into 2 groups. The positive group for strongyloidiasis (n = 9), while the negative group (n = 78) were evaluated by developed ELISA.

5) The enzyme-linked immunosorbent assay (ELISA)

Human serum samples were analyzed using enzyme-linked immunosorbent assay (ELISA) for the detection of *S. stercoralis* antibody as reported by Eamudomkarn C, *et al*. and Varatharajalu R, *et al*., respectively

(Eamudomkarn *et al*., 2018; Varatharajalu, Parandaman, Ndao, Andersen, & Neva, 2011). Briefly, 96-well microplates (Greiner Bio-ones, Inc. USA) were coated with 10 µg/ml of peptide 1, peptide 2 and/or crude antigen of *S. ratti* in 0.05 M carbonate buffer at pH 9.6 then plates were kept at 4 °C overnight. The plates were then washed three times with PBS plus Tween 20 (PBS-T) 0.05%. Inc. After washing and added serum samples diluted 1:3,000 in 3% BSA and incubated 2 hours at room temperature. After three washing cycles, peroxidase-conjugated goat anti-human IgG diluted 1:6,000 in 3% BSA were added and incubated 1 hour at room temperature. And after another washing cycle, the reaction was developed by a ABTS substrate solution (ABTS Single Reagent, Millipore, Inc. USA) and incubated for 15 minutes at room temperature in the dark. The reaction was stopped by adding 1% Sodium Dodecyl Sulfate (SDS, Affymetrix, USA) The optical density (OD) value was determined for each well in a microplate reader (PerkinElmer, Inc. USA) using wavelength 405 nm.

6) Data analysis

The receiver operating characteristic curve (ROC) curves were used to evaluate the diagnostic performance of peptide antigens. The area under the curve (AUC), the best cut-off point, sensitivity, specificity was calculated using MedCalc software version 20.218 (MedCalc Software, Ostend, Belgium).

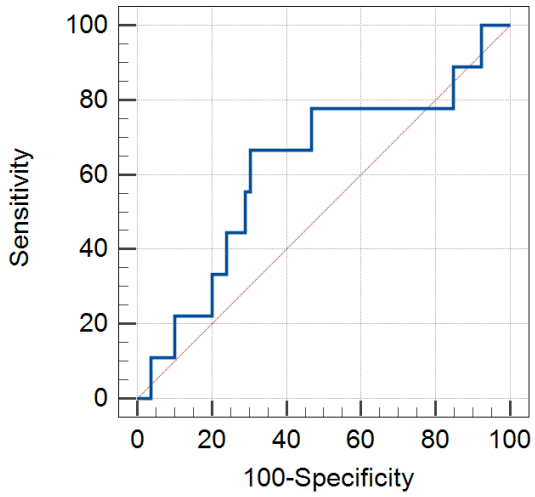
4. Results

The amino acid sequences at positions 21-50 and 61-90 were analyzed for the linear epitope of *Strongyloides*, which were identified as linear epitopes corresponding to that of *S. stercoralis* with 100% similarity were selected and use as peptides antigen in ELISA.

The threshold to obtain the diagnostic cut-off for positivity and negativity using serum ELISA as determined by the receiver operating characteristic (ROC) curve (Figure 1). The ROC curve was constructed using the model including negative controls (n = 78) and *S. stercoralis*-infected patients (n = 9). The separated cut-off OD value was 0.3655 for peptide 1, 0.3625 for peptide 2 and 0.506 for *S. ratti* antigen by serum antibody detection ELISA. These were used to determine negative and positive tests. The area under the ROC curve (AUC) describes the probability of correctly identifying a positive individual and a negative individual. An AUC of 1 would describe a diagnostic test that would correctly identify all positive and all negative 100% of the time. The Figure 1 is labeled with the AUC values of each ROC curve. The peptide 2 showed the greatest AUC value (0.840) following by *S. ratti* (0.764) and peptide 1 (0.620), respectively, as shown in Fig 2. The efficiency of peptide antigens for diagnosis of strongyloidiasis according to sensitivity and specificity is shown in Figure 1. The crude *S. ratti* antigen revealed the highest sensitivity (88.89%). Whereas, the highest specificity was found in peptide 2 (94.94%). Although, peptide 2 has lower sensitivity than *S. ratti* but the higher specificity was detected.

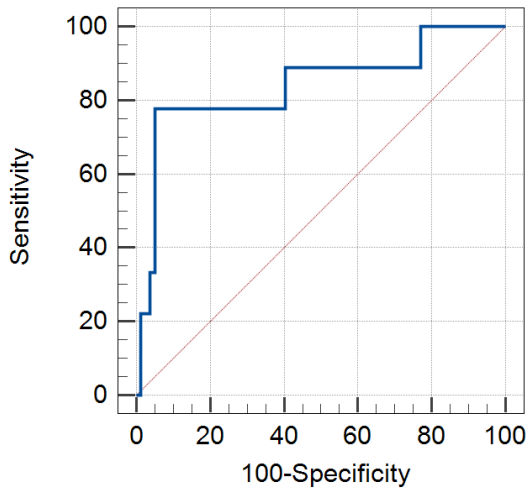
However, it was less specific than peptide 2, possibly due to the fact that it is not a purified protein for diagnosis of Strongyloidosis.

(A)



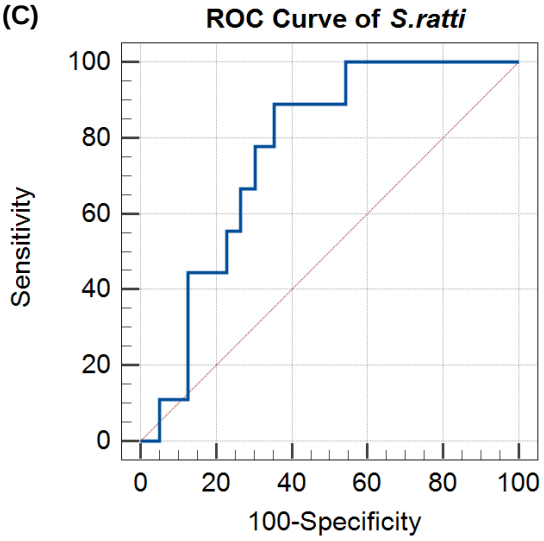
AUC = 0.620
Cut-off = 0.3655
Sensitivity = 66.67
Specificity = 69.62

(B)



AUC = 0.840
Cut-off = 0.3625
Sensitivity = 77.78
Specificity = 94.94

(C)



AUC = 0.764
Cut-off = 0.506
Sensitivity = 88.89
Specificity = 64.56

Figure 1: The ROC curve was constructed by plotting the sensitivity against 100-specificity for the entire range of the OD values obtained in serum ELISA of the study participants (*S. stercoralis*-infected patients, n = 9 and negative controls, n = 78). The ROC indicating area under the curve (AUC), cut-off, sensitivity and specificity (A) ROC curve of peptide 1 (B) ROC curve of peptide 2 (C) ROC curve of *S. ratti* antigen.

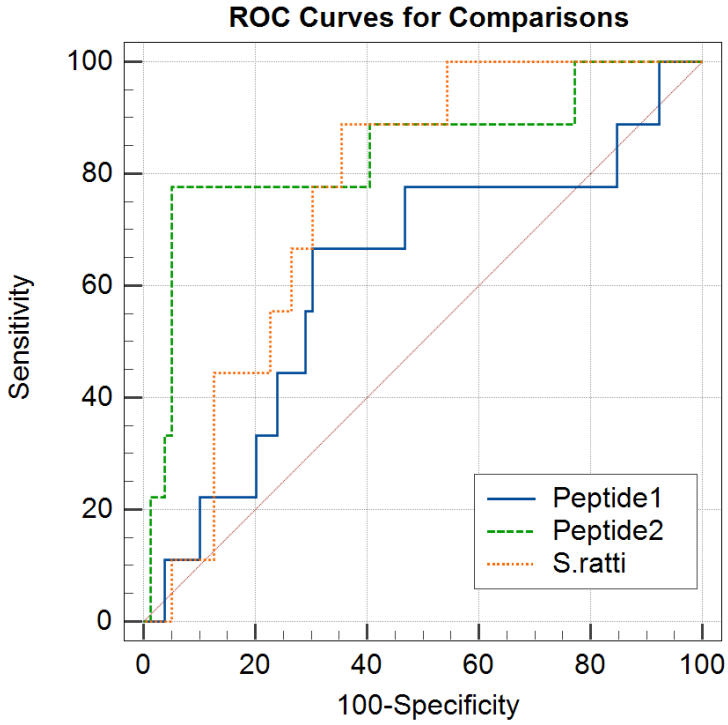


Figure 2: Comparison the AUC of ROC curves of peptide 1, peptide 2, and *S. ratti* antigen. The ROC curve illustrates the comparison between the diagnostic performance of antibody detection using 3 different peptides antigen.

5. Discussion

This research was to determine the efficiency of *S. stercoralis* peptide antigens for detection of antibody in patient infected with *S. stercoralis.* The sensitivity of peptide 1, 2 and crude antigen of *S. ratti* were 66.67%, 77.78% and 88.89% respectively. Whereas, the specificity of peptide 1, 2 and crude antigen of *S. ratti* were 69.62%, 94.94 % and 64.56% respectively. The lower sensitivity of peptides 1 and 2 when compared with crude *S. ratti* may cause by non-specific antigen in crude *S. ratti* antigen. The result confirmed by the higher specificity presented by using peptide 2 (Masoori *et al.*, 2019). The advance of biotechnology allows us to easy to manipulate which is the benefit using peptide as antigen. The popular using *S. ratti* crude antigen to detect *S. stercoralis* infection may come from several reasons; higher amount of obtained antigen from culture in rat, safety for operated person (*S. ratti* non infected in man) (Viney & Kikuchi, 2017). However, the lower specificity for *S. stercoralis* was also addressed. To increase the sensitivity of ELISA by using peptide antigen may need to use combined more peptides of *S. stercoralis*. Moreover, to evaluate potency of designed peptides also need more amount of sample from several parasite infected patient.

6. Conclusions

Serological methods, which have a higher sensitivity than parasitological approaches, are helpful tools for the diagnosis *S. stercoralis* infection. Synthetic peptides are useful because they are easy to produce in large quantities with high purity. Despite this, peptide-1 may not be suitable as it is less distinguishable between two groups. The peptide 2 are still needs further testing and should increase the number of samples to confirm its accuracy and reliability.

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