

Detection of Circulating Antibody of *Parastrongylus cantonensis* in Sera with Eosinophilic Meningitis by Dot-Blot ELISA

Kanchana Tomanakan *, Nattiya Srisurach **,
Surin Sae-tung ***, Churat Pengpinich ****

* Division of Immunology, Department of Clinical Pathology, Khon Kaen Hospital, Khon Kaen

** Division of Bacteriology, Department of Clinical Pathology, Khon Kaen Hospital, Khon Kaen

*** Division of Neuro-Medicine, Department of Medicine, Khon Kaen Hospital, Khon Kaen

**** Division of Laboratory, Wang-Yai Hospital, Khon Kaen

Background: Eosinophilic meningitis caused by *Parastrongylus cantonensis*, the rat lung worm is a major public health problem in Thailand. Humans acquire this parasite by eating raw food containing infective larvae. A specific diagnosis of Parastrongyliasis is difficult to make because identification of parasite materials by biopsy or chance finding is rarely possible.

Objective: Develop alternative approaches of *Parastrongylus cantonensis* infection employing crude antigen by dot-blot ELISA.

Material and Method: The investigation was carried out between October 2003 and July 2004 in Khon Kaen, which is an endemic area. One hundred thirty two serum samples from several villagers of the present study area were divided into five groups. Group 1 consisted of 30 patients with Cryptococcal meningitis, group 2 were 22 cases of Bacterial meningitis, group 3 were 32 cases of eosinophilic meningitis, group 4 were other parasitic infections (4 from Cysticercosis, 2 from Fascioliasis, 12 from Malaria), and group 5 were 30 negative healthy control.

Results: The result demonstrated that 26 cases of eosinophilic meningitis, were positive with Dot-blot ELISA.(81.3%). None of the other groups of sample reacted with this antigen.

Conclusion: The data obtained showed that Dot-blot ELISA has a potential for diagnosis of eosinophilic meningitis caused by *Parastrongylus cantonensis*.

Keywords: Eosinophilic meningitis, Dot-blot ELISA, *Parastrongylus cantonensis*

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Angiostrongylus cantonensis, now known as *Parastrongylus cantonensis*, the rat lung worm is the most common infectious cause of eosinophilic meningitis in humans⁽¹⁾. The predominant clinical features of eosinophilic meningitis are neurological sign and symptoms. A case of eosinophilic meningitis was clinically defined as the acute onset of headache, accompanied by at least one of the following symptoms, visual dis-

turbances, photophobia, nuchal rigidity, neck pain, hyperesthesias, or paresthesias⁽²⁾. It is often assumed that the parasite fails to reach the pulmonary circulation, simply perishing in the CNS. Infection is rarely fatal thus, very few autopsy findings have been reported⁽³⁾. All the diagnostic methods have been presumptively supported in some cases by positive with serology as worms had never been recovered. In addition, not realized at that time, was the fact that the parasite under investigation was actually *Parastrongylus cantonensis*. In most cases, the symptoms are not serious. Most cases of *Parastrongylus cantonensis* meningitis have been

Correspondence to: Tomanakan K, Division of Immunology, Department of Clinical Pathology, Khon Kaen Hospital, Khon Kaen 40000, Thailand. Phone: 08-1717-5793, E-mail: kanchanatomanakan@yahoo.com

reported in Southeast Asia and outbreaks have been reported^(4,5). The current method of diagnosis eosinophilic meningitis is based on the clinical symptoms and the elevation of eosinophil in peripheral blood. Eosinophilia was defined by the finding of at least 10 percent eosinophils in the total white cell count in cerebro-spinal fluid or at least 600 eosinophils per cubic millimeter in peripheral blood⁽⁶⁾.

Unfortunately, there is little information on the Parastrongyliasis. This situation should be improved as a result of a recent initiative that is contributing to better surveillance in Thailand. Papas et al developed a visually interpreted dot enzyme-linked immunosorbent assay for the rapid serodiagnosis of Leishmaniasis⁽⁷⁾. This technique was subsequently used for detection of many parasite antibodies in sample dotted directly on the nitrocellulose membrane⁽⁸⁾. Immunoblot analysis revealed that somatic extracts of *Parastrongylus cantonensis* were highly heterogenous with at least 4-25 components, so the 31 kDa components present in the larval and adult worm antigen of *Parastrongylus cantonensis* is a potential candidate for the specific immunodiagnosis of human parastrongyliasis⁽⁹⁾. Eamsobhana P et al developed the multi dot-blot ELISA to differential diagnosis of eosinophilic meningitis⁽¹⁰⁾. Consequently, there is a need to develop a specific immunodiagnostic technique to be used in endemic areas.

Material and Method

This research was approved by the Ethics Committee of Khon Kaen Hospital and the authors will collect 5-10 ml from eligible willing subjects. The authors will make the participants or participant's relatives fully aware of their participation being voluntary and free to withdraw from the study at any time. All elements of the study subjects will be kept confidential.

For the sample size calculation, this study should have 150 samples (From the formula):

$$n = \frac{Z^2 \alpha P (1-P)}{\Delta^2}$$

$Z\alpha = 1.96$ (95% confidence interval)

$P = 0.11$ (from the proportion of positive event)

$\Delta = 0.05$ (from the acceptable error)

$$n = \frac{(1.96)^2 \times 0.11 \times (1-0.11)}{(0.05)^2}$$

Study population

One hundred thirty two serum samples were obtained from the patients who were admitted to Khon

Kaen Hospital during October 2003 through July 2004. The serum samples were divided into five groups, group 1 consisted of 30 cases of Cryptococcal meningitis that were positive Cryptococcal antigen in the CSF and the clinical symptoms as cases of meningitis, group 2 were 22 cases of bacterial meningitis patients who had been clinically diagnosed as a case of bacterial meningitis and proved by CSF culture, group 3 were 32 sera of patients who suffered from severe headache and their blood samples showed an eosinophilia picture (more than 10%), group 4 were other parasitological meningitis proved cases of Cysticercosis (4), Fascioliasis (2) Malaria, (12), and group 5 were the normal control group consisted of 30 healthy Thais in Khon Kaen province who had no clinically diagnosed meningitis in 72 hours. The sera and CSF were collected at the time the clinical sign and symptoms appeared during 3-5 days of admission. Crude extract of *Parastrongylus cantonensis* antigen was kindly provided by Dr. Praphathip Eamsobhana, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University. The Dot-blot ELISA was performed as described previously⁽¹¹⁾. Crude somatic antigen of *Parastrongylus cantonensis* was used in a dot-blot ELISA for detection of circulating antibody in sera of patients. The determination of the bacterial meningitis was performed according to a commercial diagnostic test kit⁽¹²⁾. The authors assessed the diagnosis of cryptococcal meningitis by Crypto-Latex technique⁽¹³⁾. On the other hand, the authors tested the pooled sera of three proven parastrongyliasis cases that were found to have the larvae of this parasite from the patients at the time of collecting their blood as positive control.

Dot-Blot ELISA

The membrane was cut into strips (1 x 1 cm). The strips were immersed in Tris-buffer saline tween 20 pH 7.5 for 10 minutes. The nitrocellulose were then left to dry at room temperature. After that, the *Parastrongylus cantonensis* crude antigen were dotted 2 µl on nitrocellulose membranestrip (concentration 2 µg each strip). They were left to dry for 30 minutes at room temperature again, then blocked non specific binding site by blocking solution for 60 minutes then rinsed with working buffer 3-5 times. The strips were soaked for 60 minutes in serum samples (dilute 1:200 with 1% BSA in Tris-buffer saline tween 20). After washing 3-5 times the membrane were incubated with conjugate (dilute 1:2000 in working buffer) for 60 minutes. The strips were washed 3-5 times as above and transferred to a substrate solution (dilute 1:2000

in working buffer) for 10 minutes in a dark condition. The appearance of the purple color was considered positive. The strips were washed with distilled water several times. No color dots were considered negative.

Statistical analysis

Data were analyzed by the diagnostic value as described by Galens⁽¹⁴⁾. Sensitivity was defined as the percentage of laboratory-confirmed cases of eosinophilic meningitis caused by *Parastrongylus cantonensis* whose serum samples showed positive reaction. Specificity was calculated as the percentage of control individuals whose serum samples had a negative reaction.

Results

From 132 serum samples of the present study groups, the results of the Dot-ELISA demonstrated that group 1, which had sera from 30 cases of Cryptococcal meningitis did not react with crude somatic antigen of *Parastrongylus cantonensis*. Group 2, which had the 22 sera of Bacterial meningitis and group 4, which had sera from other parasitic infections (4 of Cysticercosis, 2 of Fascioliasis and 12 of Malaria), also gave a negative result. The authors found the sera of 26 cases group 3, which was collected from 32 patients whose clinical signs and symptoms were as cases of eosinophilic meningitis, demonstrated purple color dots as positive results with this antigen. Likewise, for group 5, none of 30 cases of negative healthy control reacted with crude somatic antigen as shown in Fig. 1 and Table 1.

The seroprevalence of *Parastrongylus cantonensis* infection in the present study group, which focused on the patients who were suffering from meningitis was 19.7% by the Dot-blot ELISA. No false positive were seen with other infections of meningitis. The sensitivity and specificity of this test were 81.3% and 100%, respectively.

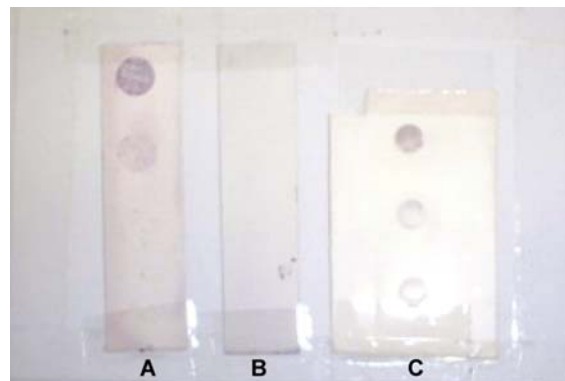


Fig. 1 Characteristic of somatic antigen reacted with serum of patients

Lane A, reaction of positive control

Lane B, reaction of negative control

Lane C, reaction of patients with eosinophilia meningitis

Discussion

The question related to prevalence of *Parastrongylus cantonensis* infection in this area is still not known because of inadequate methods to identify the materials of this parasite. The diagnosis based on histological data and clinical signs and symptoms. However, all these diagnoses have been presumptive. In recent years, there have been few reports of *Parastrongylus cantonensis* infection. This finding has some serious implications in that historically, local research and control activities have no acute headache or eosinophilic meningitis cases to concern on Parastrongyliasis. Even though morbidity and mortalities caused by Parastrongyliasis were low in the local population it is still useful to elucidate the natural pathogen, so that baseline information is available for development of effective control measures and improve laboratory investigation. Therefore, the need for a sensitivity test of diagnosis of eosinophilic meningitis

Table. 1 Detection of circulating antibody react to *Parastrongylus cantonensis* crude somatic antigen in sera of the patients with clinical sign and symptoms of meningitis by Dot-Blot ELISA

Sample group	Sample size	Dot-Blot ELISA No. positive / No. test (%)
Cryptococcal meningitis (group 1)	30	0/30 (0)
Bacterial meningitis (group 2)	22	0/22 (0)
Eosinophilic meningitis (group 3)	32	26/32 (81.3)
Other parasitic infection (group 4)	18	0/18 (0)
Negative healthy control (group 5)	30	0/30 (0)

caused by *Parastrongylus cantonensis* was important in this endemic area. The meaning of in vitro sensitivity and specificity of Dot-Blot ELISA was to classify the disease etiology from other meningitis. In the present study, the authors found that there was no cross reaction from Cryptococcal, Bacterial and other parasitic meningitis whose sera we tested. However, during the present study, the authors did not have Gnathostoma infection serum that gave a false positive with crude somatic antigen as in the report of Yen CM et al⁽¹⁵⁾. In conclusion, the data obtained Dot-blot ELISA has a potential in the diagnosis of eosinophilic meningitis caused by *Parastrongylus cantonensis*.

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การตรวจวินิจฉัยโรคเยื่อหุ้มสมองอักเสบจากการติดเชื้อพยาธิหอยโข่งโดยวิธี Dot-blot ELISA

กาญจนา โถมนาคาร, นัตถิยา ศรีสุราช, สุรินทร์ แซ่ตั้ง, ชูรัตน์ เพ่งพินิจ

โรคเยื่อหุ้มสมองอักเสบที่เกิดจากการติดเชื้อพยาธิหอยโข่งที่มีภาวะ eosinophil สูง เกิดจากสาเหตุที่รับประทานอาหารสุก ๆ ดิบ ๆ ซึ่งมีตัวอ่อนพยาธิอยู่เป็นปัญหาสาธารณสุขอีกโรคหนึ่งในประเทศไทย วิธีการตรวจวินิจฉัยการติดเชื้อพยาธิชนิดนี้ค่อนข้างยาก เพราะไม่สามารถที่จะได้ตัวพยาธิเพื่อนำมาจำแนกชนิดได้ จากตัวอย่างชิ้นเนื้อหรือสิ่งส่งตรวจอื่น ๆ วัตถุประสงค์ของการวิจัยครั้งนี้ เพื่อต้องการพัฒนาแอนติเจนของพยาธิหอยโข่ง *Parastrongylus cantonensis* เพื่อนำมาใช้ ตรวจวินิจฉัยการติดเชื้อพยาธิหอยโข่ง โดยวิธี Dot-blot ELISA การเตรียมแอนติเจน และการทดสอบกับตัวอย่างน้ำเหลืองของผู้ป่วยเริ่มดำเนินการตั้งแต่เดือนตุลาคม พ.ศ. 2547 จนถึงเดือนกรกฎาคม พ.ศ. 2548 ที่จังหวัดขอนแก่นซึ่งเป็นแหล่งที่มีการระบาดของพยาธิชนิดนี้ จำนวนตัวอย่างซีรัมจากผู้ป่วย 132 ราย เป็นชาวบ้านในพื้นที่จังหวัดขอนแก่น และใกล้เคียง แบ่งออกเป็น 5 กลุ่ม กลุ่มที่ 1 ประกอบด้วยซีรัมจากผู้ป่วย 30 ราย ที่ได้รับการวินิจฉัยว่าเป็นโรค Cryptococcal meningitis กลุ่มที่ 2 ประกอบด้วยซีรัมของผู้ป่วย bacterial meningitis 22 ราย กลุ่มที่ 3 เป็นซีรัมจากผู้ป่วยที่มีอาการทางคลินิกสงสัยว่ามีการติดเชื้อพยาธิหอยโข่ง eosinophilic meningitis 32 ราย กลุ่มที่ 4 เป็นซีรัมจากผู้ที่มีการติดเชื้อพยาธิอื่น ๆ 18 ราย ส่วนกลุ่มที่ 5 เป็นซีรัมจากกลุ่มควบคุมที่สุขภาพสมบูรณ์แข็งแรงดี 30 ราย ผลการทดสอบกับแอนติเจนโดยวิธี Dot-blot ELISA พบว่าซีรัม 26 ราย จาก 32 ราย ในกลุ่มที่ 3 ทำปฏิกิริยาให้ผลบวก คิดเป็น 81.3% ส่วนซีรัมจากผู้ป่วยกลุ่มอื่น ๆ และกลุ่มควบคุมไม่ทำปฏิกิริยากับแอนติเจนนี้ จากผลการวิจัยบ่งชี้ว่าแอนติเจนที่เตรียมใช้ในวิธี Dot-blot ELISA มีประสิทธิภาพที่สามารถช่วยวินิจฉัยโรค eosinophilic meningitis ที่มีสาเหตุจากการติดเชื้อพยาธิหอยโข่งได้
