SERODIAGNOSIS OF HUMAN GNATHOSTOMIASIS

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INTRODUCTION

Human gnathostomiasis caused by *Gnathostoma spinigerum* is prevalent in Thailand. Definitive diagnosis can only be made by recovery of the worm from the patient's body which is hardly accomplished. Recent studies in animals and human clinical cases suggested that an enzyme-linked immunosorbent assay (ELISA) could be a useful serodiagnostic test (Dhamkrong-At *et al.*, 1986; Morakote *et al.*, 1987). With sera from parasitologically confirmed cases of ocular and visceral gnathostomiasis and sera from cases with other parasitic infections, the present investigation examined the sensitivity and specificity of the ELISA in comparison to an indirect haemagglutination (IHA) and precipitin test (PPT). In addition, larval somatic and excretory-secretory (ES) antigens were evaluated for their effectiveness in the ELISA.

MATERIALS AND METHODS

Sera : Eight sera were collected from gnathostomiasis cases whose diagnosis was confirmed by recovery of *Gnathostoma* worms. Five of them were ocular gnathostomiasis at Srinakarin Hospital, Khon Kaen; Nong Kai Hospital, Nong Kai, and Maharaj Nakorn Chiang Mai Hospital, Chiang Mai. Three sera of visceral gnathostomiasis cases were kindly provided by Dr. Prasert Setasuban, Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok.

Angiostrogyliasis sera were obtained from two parasitologically confirmed and one clinical cases. Trichinosis sera were obtained from cases at Srisangwan Hospital, Mae Hong Son, following the outbreak of trichinosis. Amoebiasis and cysticercosis sera were obtained from clinical cases at Maharaj Nakorn Chiang Mai Hospital. All of the above sera were positive for antibodies against homologous antigens by an indirect haemagglutination test (IHA) and counter-immunoelectrophoresis (CIEP). Other parasitosis sera were from cases who were positive for parasites or their products.

Blood donor sera were obtained from the Blood Bank Unit, Maharaj Nakorn Chiang Mai Hospital, and the Central Blood Transfusion Center, Faculty of Medicine, Khon Kaen University.

Serological tests : Details of antigen preparations and serological tests have been described previously (Morakote *et al.*, 1987). Somatic antigen was prepared by homogenizing advanced L3 with an aid of a tissue grinder. The suspension was next sonicated at 250 watts for 3 min with Braunsonic 1510 (Braun, Melsungen, Hamburg). The suspension was left overnight in the refrigerator with continuous stirring and then centrifuged at 10,000 x g for 30 min. The supernatant fluid was collected, aliquoted and stored at -40° C. The protein content was estimated according to Lowry *et al.*, (1951). Excretory-secretory antigen was prepared from medium used in maintaining the larvae. The spent medium was collected, pooled, dialyzed against distilled water, and concentrated by vacuum dialysis. The solution was clarified by centrifugation at 10,000 x g for 30 min, aliquoted, and stored at -40° C. An IHA titer of 1 : 1,024 or more was set to be positive based on the frequency distribution of blood donor serum titers. For ELISA, the enzyme conjugate used was peroxidase-conjugated goat anti-human IgG (Cappel Laboratories, Westchester, PA). Sera were tested at 1 : 400 dilutions for ES antigens and 1 : 200 for somatic antigens. A pooled serum of 8 confirmed gnathostomiasis cases was used as a reference positive serum and a pooled blood donor serum was a reference negative serum. An ELISA value of tested serum was determined according to Voller *et al.*, (1979).

RESULTS

Using an absorbance of 0.2 as a cut off level, the ELISA antibody titers of gnathostomiasis sera found to range from 1 : 400 to 1 : 51,200 against somatic antigens and 1 : 200 to 1 : 25,600 against ES antigens (Table 1). This demonstrated a high level of antibodies detectable by the ELISA in most of sera.

When sera were tested at a single optimal dilution, i.e. 1 : 400 for somatic and 1 : 200 for ES antigens, means ($\bar{x}$) and standard deviation (SD) of ELISA values derived from 200 blood donors sera were 0.6 and 0.6 for somatic antigens and 0.4 and 0.6 for ES antigens. In confirmed gnathostomiasis cases, $\bar{x}$ + SD were 4.64 ± 1.47 for somatic and 4.11 ± 1.63 for ES antigens respectively. Using $\bar{x}$ + 3SD of ELISA values derived from blood donor sera as a cut off level for diagnostic purpose, 7 of confirmed cases were positive by ELISA (Table 1). With IHA and PPT, only 5 and 3 were positive respectively. From the above data, ELISA has a sensitivity superior to IHA and PPT.

When other parasitosis sera were examined by the ELISA, the positivity rate ranged from 0 to 8 % using the somatic antigens and 0 to 14 % using the ES antigens (Table 2). In the blood donor group, 2 and 2.5 % were positive with ES and somatic antigens respectively. Combining the data from parasitosis and blood donors, the specificity of 96.9 and 97.4 % were established for somatic and ES antigen respectively.

DISCUSSION

The results clearly demonstrated that antibodies reactive to *Gnathostoma* antigens were present in sera of gnathostomiasis cases and detected by ELISA. Interesting observation was the apparently lower titers in ocular in comparison to visceral gnathostomiasis cases. This situation was similar to that of ocular toxocariasis and local antibody pro-

Table 1

Serological results of human gnathostomiasis sera.

Case No.	gnathostomiasis	ELISA titre somatic	ES	ELISA	IHA	PPT
1	Ocular	1:1600	1:1600	+	+	−
2	Ocular	1:6400	1:3200	+	−	−
3	Ocular	1:1600	1:800	+	+	−
4	Ocular	1:400	1:200	−	+	+
5	Ocular	1:25600	1:12800	+	−	−
6	Visceral	1:51200	1:25600	+	+	−
7	Visceral	1:6400	1:6400	+	−	+
8	Visceral	1:12800	1:25600	+	+	+
Total No. positive (%)				7 (87%)	5 (62%)	3 (38%)

Table 2

Specificity of ELISA in serodiagnosis of human gnathostomiasis.

Group of sera	Total No. examined	No. of positive (%) somatic antigen	ES antigen
Angiostrongyliasis	3	0	0
Taeniasis	2	0	0
Bancroftian filariasis	6	0	0
Strongyloidiasis	12	0	0
Paragonimiasis	2	0	0
Trichinosis	20	0	0
Thelazia infection	1	0	0
Cysticercosis	5	0	0
Amoebiasis	17	0	1 (5.8)
Opisthorchiasis	50	4 (8)	3 (6)
Plasmodium falciparum infection	14	0	2 (14)
Mixed heminthiasis*	55	3 (5.4)	0
Blood donors	200	5 (2.5)	4 (2)
Total	387	12 (3.1)	10 (2.6)

* Total of 55 cases, 31 had *Opisthorchis* and hookworm, 11 had *Opisthorchis* and *Fasciola*, and 11 had mixed infection of 3 helminths including *Opisthorchis*, hookworm and *Fasciola*.

duction has been proposed to be the reason behind this (Badley *et al.*, 1987).

The sensitivity of the ELISA was found to be 87 % as 7 of 8 cases were positive. The case which was negative became positive at $\bar{x}$ + 2SD cut off level. In addition, this sera was positive by IHA and PPT. This observation necessitate the use of more than one assay for the serodiagnosis of gnathostomiasis, when the ELISA result was doubtful.

The specificity of the ELISA in the present study was greater than that observed by Suntharasamai *et al.*, (1985). Of noteworthy was that none of nematodiasis sera was ELISA-positive demonstrating the high specificity of ELISA. It was noted however, that at $\bar{x}$ + 2SD cut off level, 2 of 3 angiostrongyliasis cases became positive. This supported the findings by Dhamkrong-At *et al.*, (1986) that there was some degree of cross-reactivity between *Gnathostoma* and *Angiostrongylus* antigens. However, the false positivity could be eliminated by either setting the cut off level at $\bar{x}$ + 3SD or finding higher level of homologous antibodies.

High false positivity rate in opisthorchiasis sera could be due to either cross-reactivity or subclinical *Gnathostoma* infection. The first possibility was difficult to accept as most of opisthorchiasis sera produced strong precipitin bands with *Opisthorchis* antigens, yet only 4 sera were found to be positive by *Gnathostoma*-ELISA. The second possibility, as suggested by Suntharasamai *et al.*, (1985) was more likely as opisthorchiasis and gnathostomiasis have similar mode of transmission, i.e., consuming raw or insufficiently cooked fish.

In ELISA, somatic and ES antigens gave similar results. Thus either antigen could be employed in the diagnostic test. Common antigens may be presented in both preparations as demonstrated in *Toxocara* (Maizels *et al.*, 1984) and *Trichinella* (Silberstein and Despommier, 1984). Antigenic analysis will confirm this possibility.

SUMMARY

An enzyme-linked immunosorbent assay (ELISA) was evaluated for serodiagnosis of human ocular and visceral gnathostomiasis in comparison to an indirect haemagglutination (IHA) and precipitin (PPT) tests. The ELISA antibody titers were found to range from 1 : 400 to 1 : 51,200 against somatic and 1 : 200 to 1 : 25,600 against excretory-secretory (ES) antigens. When sera were tested at single dilutions, the ELISA was positive in 7 of 8 gnathostomiasis cases while only 5 and 3 were positive by IHA and PPT respectively. The overall specificity of the ELISA was 96.7 % and 97.4 % with somatic and ES antigens respectively. Since somatic and ES antigens produced similar ELISA results, either can be used for diagnostic purpose. It was suggested that the ELISA was a reliable serodiagnostic test for human gnathostomiasis.

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