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Peruvian Paragonimiasis: Diagnostic Value of the Enzyme-Linked Immunosorbent Assay (ELISA)

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Introduction

The enzyme-linked immunosorbent assay (ELISA) has been applied for the immunodiagnosis of a variety of parasitic infections (Voller *et al.*, 1976). ELISA has several advantages over the radio-immunoassay in simplicity and safety of procedure, while comparable results are obtained by both of the assays in terms of sensitivity and reproducibility in detecting antibodies to parasites (Voller *et al.*, 1977). For the immunodiagnosis of paragonimiasis, a disease due to infection of lung flukes, however, the skin test (ST) and complement fixation test (CFT) have been most widely used (Yokogawa, 1965; Kagan, 1974).

The present paper describes an evaluation of the ELISA for the diagnosis of Peruvian paragonimiasis in comparison with results of the ST, CFT and double diffusion test in agar (DD).

Materials and Methods

Serum samples

Serum samples were collected from students of primary and secondary schools and from inhabitants of the Condebamba district of Cajamarca, Northern Peru. Detailes of epidemiological studies on paragonimiasis carried out in this area in 1979 will be reported elsewhere. Briefly, out of 1,150 individuals examined, 10 cases were found to be positive for *Paragonimus* eggs by the stool examination (AMS III method) or examination of sputum (centrifugation technique with 2% NaOH solution) (Yokogawa *et al.*, 1981).

Antigen

Adult worms of *Paragonimus peruvianus** were obtained from experimentally

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* Recently, Miyazaki (1979), who had described *P. peruvianus* for the first time (Miyazaki *et al.*, 1969), proposed that *P. peruvianus* should be regarded to be a synonym of *P. mexicanus* (Miyazaki and Ishii, 1968) because of the morphological identity of adult worms and metacercariae of these species. In the present paper, however, we use *P. peruvianus* because this species name is now commonly accepted.

infected cats as described (Ibáñez *et al.*, 1974). The worms were lyophilized and used for preparation of veronal buffered saline extracts (VBS antigen) according to a modification (Yokogawa *et al.*, 1955) of the method of Chaffee *et al.* (1954). A preparation of an antigen extracted from the flukes with 0.1% saline (Tsuji, 1974) was also used.

Immunological diagnostic tests

The immediate type of ST was carried out for a total of 1,150 individuals by using VBS antigen (50 μg protein/ml) as described previously (Yokogawa *et al.*, 1955). Among them, 179 individuals (15.6%) showed positive reactions. With respect to the IgE antibody response (see below), the ST score was determined by a calculation based on the diameter of the wheal reaction of the skin. The CFT and double diffusion in agar (DD) were performed on serum samples obtained from these individuals by using 120 μg protein/ml of the VBS antigen or 8 mg/ml of 0.1% saline extract, respectively (Yokogawa and Awano, 1956; Tsuji, 1974).

The ELISA was carried out according to a slight modification of Tanaka *et al.* (1979) as described (Kojima *et al.*, 1983). Briefly, polystyrene microtiter plates (M 129A, Dynatech Laboratories) were coated with 10 μg protein/ml of the VBS antigen diluted with 0.05 M carbonate buffer, pH 9.6. After incubation of 0.3 ml of appropriately diluted serum samples of ST-positive or -negative individuals in each wells of the plates, the same volume of horseradish peroxidase-conjugated rabbit anti-human IgG antibody (Miles-Yeda Ltd.) was added to the wells. For the detection of IgE antibody, β-D-galactosidase-conjugated goat anti-human IgE antibody (Medical and Biological Laboratories Ltd.) was used. Enzyme substrate was 5-aminosalicyclic acid (Tokyo Kasei Kogyo Co.) for the former conjugate and 2-nitrophenyl-β-D-galactopyranoside (Wako Pure Chemical Industries Ltd.) for the latter. Results of the reaction were assessed by absorbance values read in a spectrophotometer (MTP-12, Corona Electric Co., Ltd.). An absorbance of test samples at 450 nm was considered to be an endpoint of positive reactions of IgG antibody when it was four times greater than that of negative control sera at 1:20 dilutions (always less than 0.07). The titer was expressed as a reciprocal of the highest serum dilution. The IgE antibody response was expressed as a score determined from an absorbance at 420 nm. The absorbance of 0.04, corresponding to the score 2, was considered to be the lowest positive limit because the absorbance of normal controls was always less than 0.02.

Statistical analysis was made by Fisher's exact test.

Results

Out of 179 serum samples obtained from individuals positive for the ST, 50 samples (27.9%) were found to be positive for the ELISA with which the IgG antibody to *P. peruvianus* was detected, while all of 75 samples from ST-negative inhabitants were negative for the ELISA. Table 1 summarizes the results of the ELISA in comparison with those of the CFT and DD. Among 21 CFT-positives, 19 samples (90.5%) were positive for the ELISA and 31 (96.9%) out of 32 DD-positives were also positive. On the other hand, positive reactions in the ELISA were observed in 18.5% or 12.3% of negatives for the CFT or DD, respectively. However, a good correlation was observed between the results of the ELISA and those of the CFT and DD when the latter results were combined (Table 1).

Antibody titers determined by the ELISA were also found to correlate well with those determined by the CFT, although 10

Table 1 Comparison of results of the ELISA and those of other immunological tests for Peruvian paragonimiasis

Results of immunological tests*	No. of examined	Results of ELISA	
		No. of positive (%)	No. of negative (%)
ST (+)	179	50 (27.9)	129 (72.1)
ST (−)	75	0	75 (100.0)
ST (+) and			
CFT (+)	21	19 (90.5)	2 (9.5)
CFT (−)	156	29 (18.5)	127 (81.4)
DD (+)	32	31 (96.9)	1 (3.1)
DD (−)	146	18 (12.3)	128 (87.7)
CFT (+) DD (+)	19	19 (100.0)	0
CFT (+) DD (−)	2	0	2 (100.0)
CFT (−) DD (+)	12	11 (91.7)	1 (8.3)
CFT (−) DD (−)	144	18 (12.5)	126 (87.5)

* ST; skin test. CFT; complement fixation test. DD; double diffusion.

Table 2 Correlation between titers obtained with the ELISA and CFT in sera of individuals showing positive skin test against *P. peruvianus* antigen

CFT titer	No. of examined	ELISA titer		
		<20	20–160	≧320
<10	156	127	19	10
10–40	14	2	3	9
≧80	7	0	2	5
Total	177	129	24	24

Table 3 Comparison of results of the ELIS A and DD in sera of individuals showing positive skin test against *P. peruvianus* antigen

ELISA titer	No. of examined	No. of positive for DD (%)
<20	120	1 (0.8)
20–160	24	10 (41.7)
≧320	25	21 (84.0)

samples (6.4%) out of 156 negatives for the CFT (antibody titer less than 1:10) showed relatively high titers (1:320 or more) in the ELISA (Table 2).

As expected, in comparison of the results of the ELISA with those of the DD, there was a tendency that the higher the antibody titers of the ELISA, the higher the percentage of positives for the DD (Table 3).

Table 4 Results of the ELISA, CFT and DD in sera of patients with Peruvian paragonimiasis

Patient No.	ELISA titer	CFT titer	Results of DD*
180	20	10	negative
183	40	10	++
190	320	32.6	+++
213	640	20.0	+++
248	640	12.5	+
360	640	41.0	++
393	320	ND†	++
498	20	10	nagative
1104	640	10	+++
1105	640	32.5	++

* Expressed as relative strength of precipitin bands.

† Not determined.

Table 4 shows results of the ELISA performed for 10 samples which had been obtained from individuals with proven paragonimiasis. One of them (No. 393) was found to be positive for the ELISA and DD, but the CFT titer of the sample was

not determined because of its inhibitory effect on the CFT. However, all of the rest were positive for the CFT, although two of them were negative for the DD.

Results of the ELISA for the determination of the IgE antibody are depicted in Fig. 1, indicating that a good correlation exists between the IgE antibody score and the ST score. If the individuals were divided into two groups, one with the ST score of 100 or more and the other with the score of less than 100, the IgE antibody was detected by the ELISA in 42 (95.5%) out of 44 serum samples of the former group (Fig. 1, from the line A toward the right). Thus, the results of the ELISA for determination of the IgE antibody were well correlated with those of the ST ($p<0.01$). Moreover, if the individuals having the IgE antibody score of 4 or more were considered to be a high responder (Kojima *et al.*, 1983), 31 (96.9%) out of 32 such individuals showed strong positive reactions in the ST (Fig. 1, from the line B toward the right). Again a good correlation was found to exist between the skin test score and the IgE antibody score

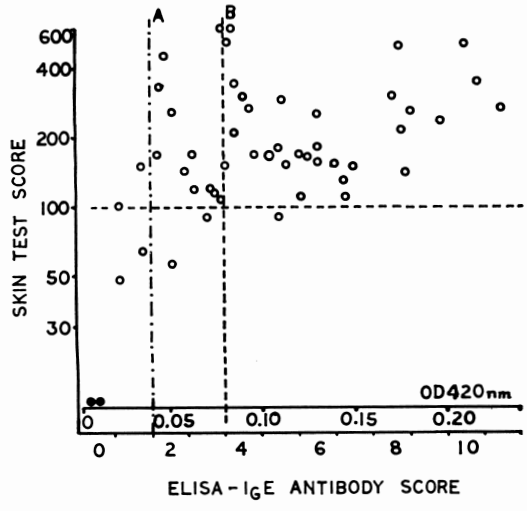

Fig. 1 Correlation between the skin test score and the IgE antibody score determined with the ELISA. Lines A and B; see text.

Table 5 Correlation between the skin test score and the IgE antibody score determined by the ELISA

IgE antiboby score	No. of individuals showing the skin test score		Total
	≧100	<100	
≧4	31	1	32
<4	13	4	17
Total	44	5	49

($p<0.025$, Table 5).

Discussion

The results described in the present paper indicate that there exists a good correlation between the results of the ELISA and those of other immunological tests such as the CFT and DD (Tables 1–3). The IgG antibody to *P. peruvianus* was demonstrated with the ELISA among all of the individuals who had been found to be positive for *Paragonimus* eggs either by the stool examination or by the examination of the sputum, even when the antibody was not always detected by the CFT or DD, indicating that the ELISA was sensitive enough to detect the IgG antibody as compared with the CFT or DD (Table 4).

Moreover, the IgE antibody was also detected with the ELISA in 7 out of 8 serum samples obtained from these individuals with proven paragonimiasis (data not shown). The ST score of the exceptional individual, whose serum IgE antibody was not detected, was found to be 64 which corresponded to the lowest positive limit in the ST score. Indeed, there was a good correlation between the IgE antibody score and the ST score (Fig. 1 and Table 5).

It is known that the ST remains positive for as long as 10–20 years after a successful treatment of paragonimiasis, while the CFT turns negative within 3–9 months

after the treatment (Yokogawa, 1965). Therefore, it is interesting to note that none of serum samples obtained from ST-negative individuals show positive reactions in the ELISA for the IgG antibody, confirming the effectiveness of the ST as a screening method for *Paragonimus* infections.

In conclusion, the present results indicate the diagnostic value of the ELISA in paragonimiasis. This is consistent with previous observations made by others concerning the immunodiagnosis of parasitic diseases with the ELISA (Voller *et al.*, 1976, 1977; McLaren *et al.*, 1978; Tanaka *et al.*, 1979). However, further studies will be necessary to clarify whether the ELISA, like the CFT (Yokogawa *et al.*, 1962; Yokogawa, 1965), serves as a criterion of cure in the treatment of paragonimiasis.

Summary

The ELISA was used for the detection of the IgG and IgE antibodies to *P. peruvianus* adult worm antigen in serum samples obtained from inhabitants of the Condebamba district of Cajamarca, Northern Peru. A good correlation was found to exist between the results of the ELISA for the IgG antibody and those of the CFT or DD. The ELISA was more sensitive than the latter immunological tests. The IgE antibody score was also well correlated with the ST score. From these observations, the ELISA was found to be useful for the immunodiagnosis of paragonimiasis as previously shown in other parasitic diseases.

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ペルー肺吸虫症：ELISA の診断的価値について

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著者らは，肺吸虫症の流行地として知られているペルー北部のカハマルカ州コンデバンバ地区の住民に対して，皮内反応 (ST)，補体結合反応 (CF)，ゲル内二重拡散法などを実施し，肺吸虫症の流行状況を調査した．この際，一部の血清については ELISA を実施することが出来た．本篇ではこの ELISA の成績と前記諸種反応成績との比較を行った．その結果は，ELISA によるペルー肺吸虫成虫抗原に対する IgG 抗体の検出状況と，補体結合反応およびゲル内二重拡散法による抗体価の検出成績との間には密接な関係が存在することが明かにされ，また ELISA の方がより鋭敏であることが示された．また，ELISA による IgE antibody score も skin test score によく相関していた．以上の結果から，ELISA は肺吸虫症の免疫診断法として有用であることが明かにされた．