Performance of an Enzyme-Linked Immunosorbent Assay for Detection of *Clonorchis sinensis* Infestation in High- and Low-Risk Groups[▽]

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Clonorchis sinensis is still a common parasite in South Korea. An enzyme-linked immunosorbent assay (ELISA) was developed to replace the usual intradermal test, and its performance in an area of endemicity was evaluated. From 2004 to 2006, 182 adults were included. The patient group consisted of 51 adults; 43 patients showed fluke eggs by microscopy, and 8 had their disease diagnosed clinically. The negative-control group included 131 adults: 98 at low risk and 33 at high risk of infestation. Both intradermal testing and ELISA were performed for all patients, and the sensitivity and specificity were evaluated. Statistical analysis for specificity looked at two groups: those at high risk and those at low risk. The sensitivity of the *C. sinensis* ELISA was 80.4%, while that of the intradermal test was 56.9% ($P = 0.019$). The specificities of the *C. sinensis* ELISA in the low-risk and high-risk groups were 93.9% and 33.3%, respectively, while those of the intradermal test were 85.7% and 30.3%, respectively ($P = 0.0968$, $P = 0.9979$). The *C. sinensis* ELISA may be more useful than the intradermal test; however, because of its low specificity, it may not be used independently for the diagnosis of *C. sinensis* infestation or a prevalence survey in a high-risk group.

Clonorchis sinensis is a common parasite found around Nakdong-Gang (river) near Busan, South Korea, a region that has experienced infestation rates of at least 16% and as high as 40% (1, 6, 7). For diagnosis, physicians use imaging, serology, stool microscopy, or an intradermal test, which is simple and requires no special facilities. However, the skin test has several limitations, such as low sensitivity and specificity. Also, interpretation of the results is influenced by subjective reading, and the test cannot distinguish current and past infestations (2).

To overcome these deficiencies, an enzyme-linked immunosorbent assay (ELISA) kit for serum antibody detection was developed. One report described the excellent sensitivity and specificity, 92.5% and 100%, respectively, of the ELISA for a population living in an area of nonendemicity (3). However, as laboratory physicians working for a hospital in an area of endemicity, our experiences were that the ELISA seemed to have a low sensitivity and specificity. Therefore, we compared it with the intradermal test in an area of endemicity.

MATERIALS AND METHODS

Patients. Included were 182 adults who visited Pusan National University Hospital from 2004 to 2006 and who lived in Busan City, South Korea, or the Nakdong-Gang river drainage area, which are areas endemicity for *C. sinensis* infestation. Questionnaires about their residence, recent drug history related to parasite infestation, and consumption of raw freshwater fish were filled out by the visitors, who were divided into patients with *C. sinensis* infestation and a *C. sinensis*-free control group.

A total of 51 adults were included in the patient group, of whom 43 were confirmed to have *C. sinensis* infestation by either the finding of eggs on stool

microscopy (30 of 43 patients), biopsy followed by hepatobiliary operations (7 of 43), or bile cytology (6 of 43) with endoscopic retrograde cholangiopancreatography (ERCP). Eight cases were diagnosed with ultrasonography or computed tomography. These patients had peripheral intrahepatic duct dilation with a recent (within 1 month) history of freshwater fish consumption and showed resolution of the eosinophilia and abnormal liver enzyme concentrations after administration of praziquantel.

The control group included 131 adults who presented with elevations of liver enzymes or eosinophilia or who had confirmed biliary tract disease but in whom *C. sinensis* infestation was excluded by imaging, tissue biopsy, bile cytology, laboratory tests, or some combination thereof. All of the control subjects were free of eggs on stool microscopy. Of those, 33 adults who had frequently eaten raw freshwater fish were subgrouped as high risk. The diagnosis of clonorchiasis in high-risk individuals was excluded as follows: six had liver biopsy and cholecystectomy with bile cytology, eight had ERCP and bile cytology, eight had cholecystectomy with bile cytology, six had stool microscopy more than three times over 3 months, and five had ultrasonography with repeated stool microscopy more than two times over a week. The 98 adults who had no history of consuming raw freshwater fish were categorized as low risk (Table 1).

Intradermal test. The skin test was performed according to the manufacturer's instructions. Briefly, 0.02 ml each of *Clonorchis sinensis* and *Paragonimus westermani* antigens (CS/PW antigen; Green Cross MS, Yongin, South Korea) were injected intradermally on the patients' forearms, at a distance of at least 10 cm. A papule, measured 15 min after injection, which was larger than 60 mm² was considered a positive result.

ELISA test. The ELISA test was performed with the *Clonorchis* Ab (IgG) Micro-ELISA (Genedia Cs/Pw Ab ELISA, Green Cross MS), following the manufacturer's instructions.

Statistical analysis. Statistical analysis was performed with the Medcalc for Windows program (version 9.6.4.0; Frank Schoonjans, Belgium) for comparison of proportions. The specificity analysis used two sets, one for the low-risk group and one for the high-risk group (Table 1). The sensitivity and specificity of the ELISA and intradermal test were compared. A P value of <0.05 was considered significant.

RESULTS

The sensitivity of the ELISA was 80.4%, significantly higher than that of the intradermal test at 56.9% ($P = 0.019$). The specificities of the *C. sinensis* ELISA in the low-risk and high-risk groups were 93.9% and 33.3%, respectively, while those of

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TABLE 1. Composition of subject groups according to diagnostic method and risk

Group	No. of patients examined (no. of males/no. of females)
Patients	
<i>C. sinensis</i> egg positive.....	43 (34/9)
Clinically diagnosed.....	8 (8/0)
Negative controls.....	
Low risk.....	98 (67/31)
High risk.....	33 (27/6)
Total.....	182 (136/46)

the intradermal test were 85.7% and 30.3%, respectively. The specificity of the *C. sinensis* ELISA was not significantly higher than that of the intradermal test in either group ($P = 0.0968$, $P = 0.9979$) (Table 2).

DISCUSSION

The purpose of this study was to assess the performance of a commercial *C. sinensis* ELISA test kit in comparison with that of the intradermal test. The ELISA test utilizes parasite antigen to detect specific antibody in serum, whereas the intradermal test detects the cell-mediated immune response to the antigen in the skin. The sensitivity of the *C. sinensis* ELISA test in this study (80.4%) was similar to that in previous studies (3, 10, 12) and was significantly higher than that of the intradermal test. The specificity in the low-risk group (93.9%) was also similar to that in previous studies (3, 10, 12). However, the specificity in the high-risk group (33.3%) was not consistent with the earlier findings. One reason for this difference may be the use of different antigens, but most likely, it reflects the involvement in previous studies of people who had never been exposed to *C. sinensis* as the control subjects. In areas of endemicity, patients visiting a physician's office to rule out *C. sinensis* infestation most likely have been exposed to the parasite before. Therefore, in a real situation, many patients who do not have the parasite currently might show false-positive results. Thus, the specificity can be overestimated if the test is used to assess exposure-naïve people. The specificity for our low-risk healthy control group was 93.9%, which is consistent with the findings of previous studies. Such differences in the performance of a diagnostic test according to populations are also observed in other infectious diseases. For example, a prospective study of the gamma interferon release assay (IGA) for tuberculosis in South Korea, which is an area with a moder-

ately high prevalence of tuberculosis, showed a low specificity (49%), whereas similar studies in Italy and Denmark showed high specificities (99% to 100%) (5, 9, 11). Another reason for the low specificity in the high-risk group may be cross-reactivity with other parasites. Eosinophilia and elevation of liver enzyme levels can also be caused by other parasite infections. Infections with other flukes, including *Paragonimus westermani* and *Metagonimus yokogawai*, are also found in the Nakdong-Gang (river) area. However, according to a 2009 report from the South Korea Centers for Disease Control and Prevention, *Clonorchis sinensis* accounts for 92.2% of all parasite infections found in the Nakdong-Gang (river) area (http://www.cdc.go.kr/kcdchome/jsp/home/common/brd/COMMBRD0200Detail.jsp?boardid=1002&boardseq=22145&menuid=100039&appid=kcdchome&contentid=null&pageNum=2&pageNo=17&q_value=&q_name=&sub=1). Subjects who showed the eggs of flukes other than those of *C. sinensis* were excluded from this study. Further study of the influence of cross-reactivity between various parasites would be necessary. However, considering the relatively low specificity of the intradermal test in the low-risk group, the *C. sinensis* ELISA, with its relatively high specificity, could be useful in areas of nonendemicity.

One of the possible causes of the low specificity in the high-risk group is past infection. The antibody remains after cure and causes positive results in serologic tests for decades (4). Just being exposed to the antigen may also cause antibody formation. The intradermal test using parasite antigen as the test reagent has been utilized in this area. The injected antigen may be the cause of postexposure antibody formation, which would induce a false-positive reaction in the ELISA test. For this reason, a survey that utilizes the ELISA as a single criterion may overestimate the prevalence rate (8), especially in areas of endemicity.

We did our best to exclude clonorchiasis patients from our disease-negative group. For selection of a low-risk control group, a negative fluke egg examination, diagnosis of a disease other than *C. sinensis* infestation, and no history of raw freshwater fish consumption were required. For selection of a high-risk control group, we enrolled patients who had a history of surgical biopsy or some other invasive procedure of the liver or biliary tract or who had repeated stool examinations to rule out current clonorchiasis. Therefore, the possibility that clonorchis-infested patients were enrolled in these patient groups was extremely low or absent.

Physicians utilize laboratory tests to make strategic therapy decisions. To be used to determine therapy, the *C. sinensis* ELISA should have a high sensitivity. In view of our results, the

TABLE 2. Performance comparison of ELISA and intradermal skin tests, depending on statistical set

Parameter	ELISA (%)	Intradermal test (%)	Difference in percentages	95% CI ^a		Chi-square value	Significance (P)
				ELISA	Intradermal test		
Sensitivity	80.4	56.9	23.5	5.48	39.59	5.498	0.019
Specificity in low-risk group	93.9	85.7	8.2	-0.44	17.1	2.758	0.0968
Specificity in high-risk group	33.3	30.3	3.0	-18.7	24.4	0	0.9979

^a CI, confidence interval.

ELISA, which showed a higher sensitivity than the intradermal test and which does not expose patients to the parasite antigen, can be more useful than the intradermal test. However, considering its low specificity in a high-risk group, the *C. sinensis* ELISA may not be used alone for the diagnosis of infestation in individuals or for prevalence surveys in areas of endemicity.

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