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Evaluation of immunochromatography method in the diagnosis of cystic echinococcosis

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ABSTRACT

Aims: *Echinococcus granulosus* is the causative agent of hydatid cyst, or cystic echinococcosis (CE), with its current name. *Echinococcus granulosus* is a zoonotic cestode; commonly found in humans and farm animals. In cystic echinococcosis infection, transmission occurs by oral ingestion of parasite eggs excreted in infected dog feces. The larval form is responsible for the formation of slowly growing cysts in the organs and tissues of mammals such as humans, sheep, goats and cattle. In this study, it was aimed to compare indirect hemagglutination (IHA) and immunochromatographic (ICT) methods from the sera of patients with suspected CE and to evaluate serological tests based on imaging and clinical diagnosis.

Methods: Between 31 October 2022 and 31 January 2023, blood samples of 95 patients with suspected CE from different units of our hospital and for whom IHA was routinely requested were included in the study prospectively. VIRAPID® Hydatidosis (Vircell, Granada, Spain) test using the immunochromatographic method and ELI.H.A. *Echinococcus* (ELITech Microbio, France) test was studied in accordance with the manufacturer's instructions.

Results: Based on clinical and imaging methods of 95 patients included in our study, 64 (63.1%) were diagnosed with hydatid cyst. Based on imaging and clinical diagnosis; sensitivity, specificity, positive predictive value and negative predictive values were calculated as 81.3%, 96.8%, 98.1%, 71.4% for the IHA test, and were calculated as 75.0%, 93.5%, 96.6%, 64.4% for the ICT test, respectively. Good agreement was found between the two tests (percent agreement=68.0%; kappa value=0.682; p<0.001). Sensitivity (IHA: 87.2%; ICT: 94.9%) and specificity (IHA: 96.8%; ICT: 93.5%) of IHA (positive titer of 1/160 and above) and ICT methods in active cysts with cyst stage (CE) 1-2-3) values were found to be compatible, The sensitivity of the ICT method in inactive cysts with CE 4-5 (IHA: 72%, ICA: 44%) was found to be statistically significantly lower than the IHA method (p<0.001).

Conclusion: Rapid diagnostic tests generally stand out as they do not require personnel training in health institutions and are easy to apply. Especially in the active period of the cysts, the tests show a very good and harmonious performance, and it significantly supports the clinical and radiological findings in the early diagnosis of the disease and in the treatment follow-up, however, they need to be developed in order to be used in the differential diagnosis of inactive cyst stages, especially in cases in between, and performance studies in larger patient groups are required.

Keywords: Cystic echinococcosis, *Echinococcus granulosus*, immunochromatography, indirect hemagglutination

INTRODUCTION

Cystic echinococcosis (CE) is a disease caused by *Echinococcus granulosus* belonging to the Taeniidae family.[1,2] It is a ubiquitous zoonotic agent with worldwide distribution except Antarctica.[3,4] CE is one of the most common parasitic diseases threatening human and animal health in the world and in Turkey.[4,3] Especially the Mediterranean basin is known to be endemic.[5,6] Within the scope of the HERACLES project supported by the 7th Framework Program of the European Union, abdominal CE was detected with ultrasonography (USG) at a rate of 0.6% in Turkey. The Turkey leg of the study was carried out in six provinces (Ankara, Aksaray, Balıkesir, Bitlis, Edirne, Şanlıurfa) in different geographical regions and the prevalence was determined as 6%. According to the data of USG-based CE field studies reported from different provinces in Turkey, the prevalence varies between 0.15% and 1.05%.[7] According to the evaluations of the World Health Organization (WHO), CE was listed as one of the 17 neglected tropical diseases of animal origin and recorded among the most serious parasitic diseases in humans.[6] Infection can occur by ingestion of parasite eggs, which are excreted in the feces of the last host dog, by natural intermediate hosts such as humans, sheep, goats, cattle, through digestion and respiration.[21] Infectious cysts are most commonly located in the liver; It is also seen in various organs and tissues such as lung, spleen and kidney.

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The clinic is usually asymptomatic; It occurs depending on the size and localization of the cysts.[4]

The diagnosis of cystic echinococcosis is mainly made by imaging methods. While computed tomography and magnetic resonance are used in almost all organ locations, USG has been the first choice for liver cysts and direct radiography for lung cysts.[1] It is recommended to use radiological diagnosis methods together with serological diagnosis methods in cases such as differential diagnosis of the cyst with other space-occupying lesions, determination of postoperative recurrences and the absence of a clear clinical picture. In addition, serological tests are used not only in the diagnosis of CE, but also in determining its prevalence in the community and in identifying asymptomatic individuals. In the serological diagnosis, indirect hemagglutination (IHA), enzyme-linked immunosorbent (ELISA), latex agglutination, indirect fluorescent antibody (IFA) and immunoblotting (IB) methods, in which specific immunoglobulin G antibodies are detected, are frequently used.[5,8]

Recently, the use of the immunochromatographic (ICT) method, which gives rapid results for the diagnosis of CE, has become quite common. This study, it was aimed to compare IHA and ICT methods from sera of patients with suspected CE and to evaluate serological tests based on clinical and imaging diagnoses.

METHODS

The study was carried out with the permission of Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date: 24.11.2022, Decision No: 21/14). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Between 31 October 2022 and 31 January 2023, blood samples of 95 patients with suspected CE from different units of our hospital and whose IHA tests were routinely requested were included in the study. The clinical diagnoses and radiological reports of the patients were obtained through the hospital information system (LIS) and epicrisis. in our study, cyst stages (CE) were determined by imaging methods according to the criteria of the WHO Echinococcosis Informal Study Group (WHO-IWGE).9 Accordingly, cyst stages are defined in three groups: "active" cysts that are usually viable, unilocular (CE-1) and multivesicular (CE-2) with daughter vesicles, "transitional" with separation of endocyst (CE-3a), and predominantly with daughter vesicles (CE-3a) solid cysts (CE-3b) and "inactive", nonviable, solid and calcified cysts (CE-4 and CE-5). While evaluating the results of our study, CE-1-2-3 stages were classified as active cysts and CE-4-5 stages were classified as inactive cysts.

Test procedures

Serum samples were studied simultaneously with the *Echinococcus* assay (ELI.H.A.; ELITech Microbio, France) with IHA method and the VIRAPID® Hydatidosis (Vircell, Granada, Spain) immunochromatographic assay with the ICT method. By the manufacturer's recommendations, sera were diluted in eight-well microplates for the IHA method. Serum dilutions with antigen erythrocyte suspension (reagent) added were evaluated after 2 hours of incubation. Ringing at the bottom of the well was considered negative, a red/brown cloudy image was considered positive, and the last well dilution seen was recorded.

In the kit package insert, serums with a titration value below 1/160 are indicated as the possible absence of hydatid disease and the need to repeat the test after 2-3 weeks. Suspected infection for serum samples with a titration value of 1/160; Titrations of 1/320 and above were indicated as an important reaction in favor of progressive hydatid cyst. For the ICT method, the serum sample and developer solution were dropped into each cassette and incubated for 30 minutes. The results were evaluated visually according to the interpretation chart in **Figure 1A**. According to the test procedure, samples with a control line but not a test line and a density value less than 0.5 were evaluated as negative. Those with a test line intensity value of 0.5 and above were considered positive and 0.5, 1, 2, and 3 according to their intensity. Images of some patient samples are shared in Picture 1B. It is stated in the package insert that the internal quality control studies were carried out by the manufacturer before the kit was put on the market, and the sensitivity and specificity were reported as 94.74% and 99.5%, respectively. The VIRAPID® Hydatidosis test interpretation chart and ICT images of some patient samples (patients 4 and 5 were evaluated as negative, other patients as positive) were presented in **Figure 1**.

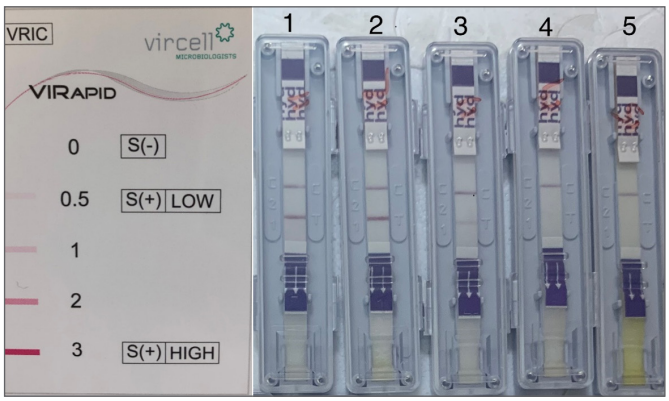

Figure 1. A. VIRAPID® Hydatidosis test interpretation chart B. ICT images of some patient samples (patients 4 and 5 were evaluated as negative, other patients as positive).

Statistical analysis

SPSS (Statistical Packages for the Social Sciences) software version 22.0 (SPSS Inc., Chicago, USA) program was used for statistical analysis of the study. The results obtained with two different tests were recorded as categorical variables. The agreement between these results was analyzed by calculating Cohen's kappa value and percent agreement. Kappa value: <0.20 poor, 0.21-0.40 near medium, 0.41-0.60 moderate, 0.61-0.80 good, and 0.81-1.00 almost perfect fit. Shapiro-Wilk test was applied to check the normality assumption of continuous variables such as age. Kruskal-wallis analysis of variance was used to determine whether there was a significant difference in age distribution between the different groups. Clinical diagnosis and imaging methods were accepted as the gold standard; Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of IHA and ICT methods were calculated using the Chi-Square test. With the Receiver Operating Characteristic (ROC) analysis, the threshold values at which IHA and ICT methods gave high sensitivity and specificity positivity were calculated according to the method accepted as the gold standard method (separately as cyst active and cyst inactive). Statistically significant p value was determined as <0.05.

RESULTS

Blood samples of 95 patients aged between 10 and 83 (mean: 50.16), 59 (62.1%) female, 36 (37.9%) male, suspected of CE and routinely requested IHA test were included in the study. Of the patients (n:64) who were positive according to imaging and clinical diagnosis, 5 (7.8%) were aged 16 years or younger, 18 (28.2%) were aged 16-44, and 41 (64.0%) were aged 44 years or older determined. Considering the distribution in age groups, a statistically significant difference was found between patients aged 44 years and older and other age groups (p<0.001).

Of the patient samples, 51 (53.7%) were in general surgery, 17 (17.9%) in gastroenterology, 6 (6.3%) in infectious diseases, 6 (6.3%) in internal medicine, 6 (6.3%) in pediatrics, 2 (2.1%) were sent from intensive care units and 7 (7.4%) were sent from different clinical branches.

Liver in 56 (87.5%), lung in 3 (4.6%), kidney in 2 (3.2%), and spleen and liver in 2 (3.2%) of patients (n:64) who were positive according to imaging and clinical diagnosis However, it was determined that 1 (1.5%) had intra-articular localization.

All of the patients (n:64) who were positive according to imaging and clinical diagnosis were found positive at 1/80 titer in the IHA method, and 53 (82.8%) were positive at 1/160 and higher titer. Fifty (52.6%) of these patients were found to be positive with an intensity value of 0.5 and above on the test line by the ICT method. The IHA and ICT results of the patients who were positive according to imaging and clinical diagnosis are presented in **Table 1**. Based on imaging and clinical diagnosis, the sensitivity, specificity, PPV, and NPV were 81.3%, 96.8%, 98.1%, and 71.4% for the IHA method, and 75.0, 93.5%, 96.6%, and 64.4% for the ICT method, respectively. One (4.2%) of 24 samples found to be negative by indirect hemagglutination method was found to be 0.5 positive by ICT. 9 (16.9%) of the 53 samples found to be 1/160 or more positive with IHA were found to be negative with ICT. The number of samples with positive results with both tests was calculated as 44 (68.7%) and the number of samples with negative results as 36 (56.2%), and a good level of agreement was found between the two tests (percent agreement=68.0%, kappa value=0.682, p <0.001). **Table 2** shows the results of IHA and ICT methods according to titer and test line intensities.

Table 1. IHA and ICT results by imaging and clinical diagnosis

	Imaging and Clinical Diagnosis				
		Positive			
	Negative (n: 31)	Active CE-1-2-3 (n: 39)	Inactive (CE-4-5) (n: 25)	Total CE-1-2-3-4-5 (n: 64)	Total (n: 95)
IHA (1/160)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
positive	1 (3.2)	34 (87.2)	18 (72.0)	52 (81.2)	53 (55.7)
negative	30 (96.8)	5 (12.8)	7 (28.0)	12 (18.8)	42 (44.3)
ICT	n (%)	n (%)	n (%)	n (%)	n (%)
positive	2 (6.5)	37 (94.9)	11 (44.0)	48 (75.0)	50 (52.6)
negative	29 (93.5)	2 (5.1)	14 (56.0)	16 (25.0)	45 (47.4)

Table 2. Results of IHA and ICT methods according to titer and test line intensities.

	VIRAPID® Hydatidosis Test Results					
	3+ n (%)	2+ n (%)	1+ n (%)	0.5+ n (%)	Negative n (%)	Total n (%)
ELI.H.A. *Echinococcus* Test Results						
1/1280 titer	11 (30.6)	8 (22.2)	10 (27.8)	4 (11.1)	3 (8.3)	36 (100)
1/640 titer	- (0)	1 (25)	1 (25)	1 (25)	1 (25)	4 (100)
1/320 titer	- (0)	- (0)	1 (25)	1 (25)	2 (50)	4 (100)
1/160 titer	- (0)	- (0)	- (0)	6 (67)	3 (33)	9 (100)
1/80 titer	- (0)	- (0)	- (0)	5 (27.8)	13 (72.2)	18 (100)
Negative	- (0)	- (0)	- (0)	1 (4.2)	23 (95.8)	24 (100)
Total	11 (11.6)	9 (9.5)	12 (12.6)	18 (18.9)	45 (47.4)	95 (100)

When patients who are positive according to imaging and clinical diagnosis are divided into active and inactive cysts; The sensitivity, specificity, PPV and NPV of the IHA method (1/160 titer and above) in cyst active patients (CE 1-2-3) were; as 87.2%, 96.8%, 97.1% and 85.7%; If the cyst is in inactive patients (CE 4-5); It was calculated as 72%, 96.8%, 94.7% and 81.1%.

When patients who are positive according to imaging and clinical diagnosis are divided into active and inactive cysts; When the ICT method (0.5 line intensity and above line intensity) is evaluated, sensitivity, specificity, PPV and NPV in cyst active patients (CE 1-2-3); 94.9%, 93.5%, 94.9%, 93.5% and in inactive patients (CE 4-5); It was calculated as 44%, 93.5%, 84.6% and 67.4%.

ROC analysis and comparison results of ICT and IHA method results of cyst active and inactive patients are shown in **Table 3** and **Figure 2**.

Table 3. ROC analysis and comparative results between methods in cyst active and inactive patients

	AUC			
	ICT		IHA	
Cyst active	0.964 (0.918-1.000)	p<0.001	0.981 (0.958-1.000)	p<0.001
Cyst inactive	0.695 (0.551-0.840)	p=0.013	0.963 (0.922-1.000)	p<0.001

AUC=Area Under Curve [95% CI].

DISCUSSION

Diagnosis of cystic echinococcosis usually requires the use of imaging methods. However, the radiological diagnosis should be supported by laboratory diagnostic methods to make the differential diagnosis of cyst with other space-occupying lesions such as tumor, abscess, and simple cyst, and to evaluate recurrences after surgery in a healthier way.[1,10,11] In addition, since laboratory test results are required in the follow-up of the treatment for CE, it is extremely important to know the sensitivity and specificity values of these laboratory tests and the factors affecting the test results. For this purpose, standard diagnostic tests with high sensitivity and specificity are still being investigated in the diagnosis of CE.

Immunological methods used for screening and follow-up of CE are ELISA, ICT, and IB test which is commonly used as the confirmatory method due to its higher sensitivity-specificity values. Other less frequently used methods can be listed as IFA, IHA and

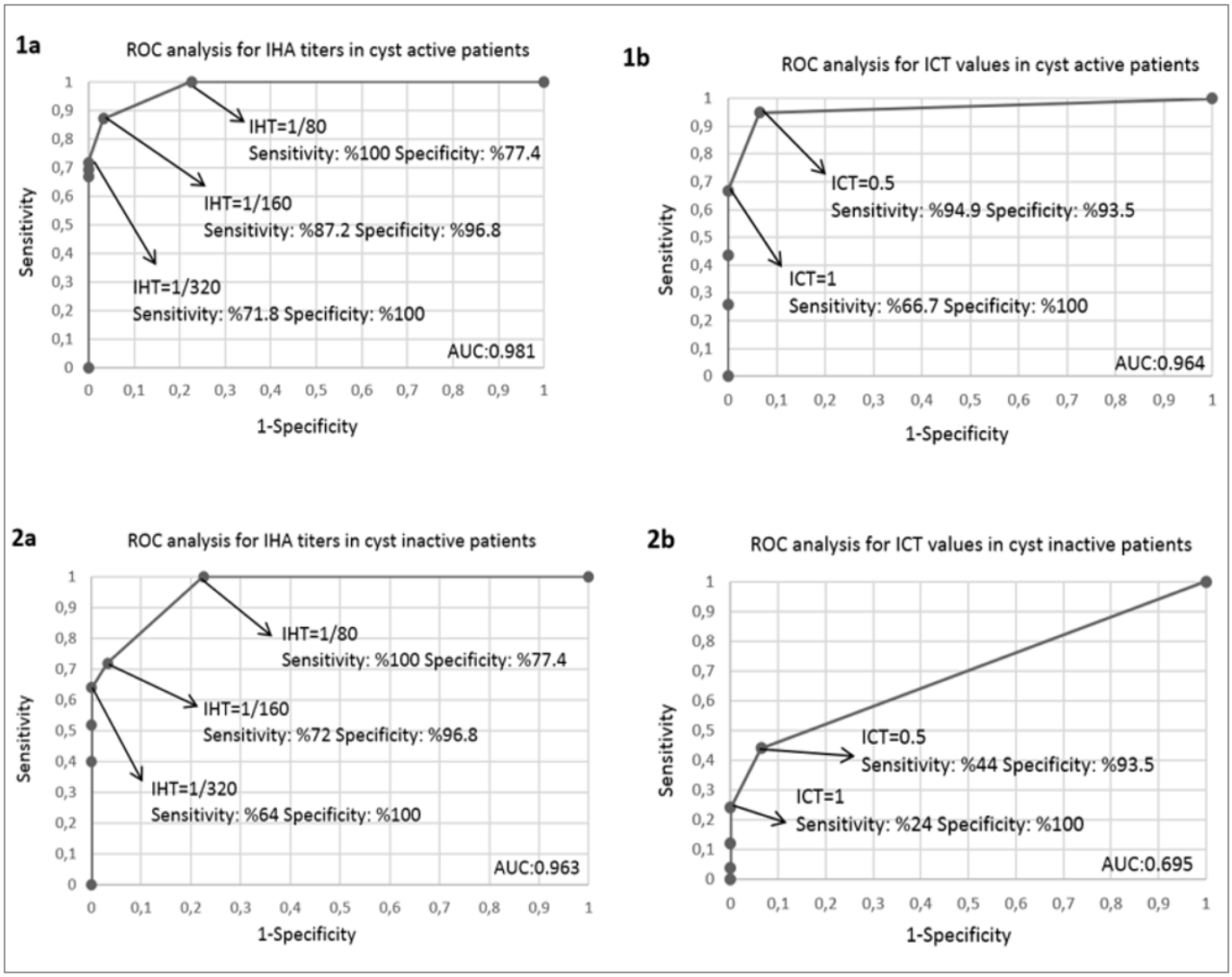

Figure 2. 1a-1b ROC curves of IHA and ICT methods in cyst active patients.2a-2b ROC curves of IHA and ICT methods in cyst inactive patients. AUC: Area Under Curve

point immunogold filtration (DIGFA).[12] Among the serological methods, IHA methods provide advantages such as low cost and high specificity and sensitivity.[6] The IHA method, which is routinely used for diagnostic purposes in our laboratory, has been reported to have a sensitivity of 65-90% and a specificity of 97.5%-100% in different studies.[3] In two studies reported from our country; The specificity and sensitivity of the hemagglutination method were determined as 94.59% and 88.76% by Akgün et al.[10] and 97.0% and 86.2% by Zait et al.[11] respectively.

VIRAPID® Hydatidosis, a commercially available rapid diagnostic test for the diagnosis of cystic echinococcosis using the ICT method, is widely used in laboratories and is being investigated for ease of use, specificity and sensitivity. Ertuğ et al. studied the VIRAPID® Hydatidosis test using the ICT method on 50 clinically and pathologically positive samples and reported the specificity and sensitivity as 100% and 96%, respectively. As a result of their studies, they predicted the rapid diagnostic test as practical and easily applicable in the diagnosis of CE.[8]

In the study conducted by Tamer et al.[5] the specificity and sensitivity of the VIRAPID® Hydatidosis (Vircell, Granada, Spain) ICT test was 87.5% and 96.8%, respectively, according to clinical and radiological diagnosis. They showed advantages such as low cost, long shelf life, fast results, no special equipment, easy readability and usability by non-experts.

Tamarozzi et al.[13] compared three rapid identification tests with a commercial ELISA test that they routinely use in their laboratories, and reported that the VIRAPID® Hydatidosis rapid diagnostic test showed the best diagnostic accuracy but the sensitivities of these three tests were lower than the ELISA (R-Biopharm, Darmstadt, Germany) test. However, they found that the sensitivities of all three tests were lower than the ELISA (R-Biopharm, Darmstadt, Germany) test. They found the specificity and sensitivity of the VIRAPID® Hydatidosis test to be 74% and 96%, respectively, and predicted that this test could be used in environments where there are insufficient resources to complete USG diagnosis in patients with suspected hydatid cysts.

At a veterinary faculty in Italy, Peruzzu et al.[12] evaluated the diagnostic performance of four commercial test kits in the serum of 259 patients with positive (n:74) and negative (n:185) CE. They specified the IB test method as the best in terms of sensitivity-specificity and diagnostic performance, and the VIRAPID® Hydatidosis test as the second ICT method. They stated that in endemic areas, these tests can be considered as support for clinical evaluation.

In our study, IHA and ICT methods were compared using ELI.H.A. *Echinococcus* (ELITech Microbio, France) test and VIRAPID® Hydatidosis (Vircell, Granada, Spain) test to detect E. granulosus antibody in 95 serum samples. In our study, based on imaging and clinical diagnosis, the sensitivity and specificity of the IHA method at titrations of 1/160 and above were calculated as 81.3% and 96.8%, respectively, and the sensitivity and specificity of the ICT method at 0.5 and above were determined as 75% and 93.5%, respectively. Of 64 patients diagnosed by imaging methods and clinically, 53 (55.7%) were found to be positive with IHA method and 50 (52.6%) with ICT method, and good agreement between the two methods (percent agreement=68%, kappa value= 0.682, $p<0.001$).

Studies report that the specificity and sensitivity of the tests used in serological diagnosis may vary depending on the characteristics of the antigen, the organ where the cyst is localized, and the host immune response.[14] Similar to our results in studies conducted in our country, it was found that the most common liver-localized CE cases were, and the rate of seropositivity between liver and other organ involvements was not found statistically significant in many studies.[6,10,12] In our study, hepatic involvement of CE was detected in 56 (87.5%) of 64 patients based on imaging methods and clinic. The number of cases with extrahepatic involvement is not sufficient to statistically compare organ involvement in terms of seropositivity. Extra-hepatic CE cysts should be evaluated with a larger cohort in terms of the use of serological diagnostic methods.

Some researchers may also recommend testing the same serum with more than one method in order to increase the sensitivity and specificity of laboratory diagnosis and to obtain the most reliable results.[1,15,16] However, in recent years, studies have been started to determine the diagnostic efficacy of rapid diagnostic tests in different stages of the disease instead of working with all laboratory and imaging methods in each patient.[12,17] Although clinical and imaging methods are used in the diagnosis, laboratory tests should have a confirmatory role in cases where the diagnosis is in between. However, in the test performance studies conducted by Tamarozzi et al.[17] serological test results were generally evaluated as variable and insufficient for the diagnosis of inactive CE4 and CE5 stages. In our study, cyst stages were evaluated according to the criteria determined by the World Health Organization (WHO) Echinococcosis Informal Study Group (WHO-IWGE), and CE1-2-3 stages were classified as active cysts and CE4-5 stages as inactive cysts.[9] In active cysts (CE 1-2-3), the sensitivity (IHA: 87.2%; ICT: 94.9%) and specificity (IHA: 96.8%; ICT: 93.5%) were found to be compatible with the IHA

test (at a titer of 1/160 and above), while inactive The sensitivity of the ICT method in cysts (IHA: 72%, ICT: 44%) was statistically significantly lower than the IHA method.

Our results show that IHA and ICT methods have overall comparable performance based on clinical and radiological diagnoses. However, especially the VIRAPID® Hydatidosis test shows poor sensitivity in the presence of inactive (CE-4-5) cysts with cyst stages, which may cause important problems in differential diagnosis. Although it is a common approach by clinicians to use rapid diagnostic tests and imaging methods together to monitor and confirm each other, determining their effectiveness in diagnosis in different stages of the disease and choosing the right method will be a cost-effective and rational diagnostic strategy for patients and health institutions in terms of reducing workload and saving time.

CONCLUSION

In the present study, it can be predicted that rapid diagnostic tests, which we have evaluated in general, do not require personnel training in health institutions where opportunities are limited, they are easy to apply and give fast results, as well as their low cost and long shelf life. Especially in the active phase of the cysts, rapid diagnostic tests show a very good and harmonious performance, support the clinical and radiological findings in the early diagnosis of the disease and in the treatment follow-up, however, they need to be developed and performance studies in larger patient groups are needed in order to be used in differential diagnosis, especially in the inactive cyst stages.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date 24.11.2022, Decision No: 21/14).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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