

Comparative Evaluation of *Echinococcus* Serology with Cytology for the Diagnosis of Hepatic Hydatid Disease

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J Lab Physicians:2020;12:98–102

Abstract

Objectives The purpose of this study is to determine the diagnostic efficacy of enzyme-linked immunosorbent assay (ELISA) in radiologically confirmed liver mass lesions for the diagnosis of hepatic hydatid disease (HHD) and to compare the diagnostic performance of ELISA with fine needle aspiration cytology (FNAC) (taken as standard) for HHD diagnosis.

Materials and Methods This retrospective study included blood samples of 223 patients with radiologically confirmed liver mass lesions in which immunoglobulin G (IgG) anti-*Echinococcus* antibodies were tested using a commercial IgG ELISA (RIDASCREEN, R-Biopharm AG, Darmstadt, Germany). Results of ELISA, ultrasonography, FNAC, and liver function tests were obtained from the hospital information system. ELISA results were compared with those of FNAC to analyze the diagnostic efficacy of ELISA for HHD diagnosis.

Statistical Analysis Comparison of the results obtained from ELISA was performed with respect to FNAC results (taken as standard) to analyze the diagnostic efficacy of ELISA for HHD detection. Data has been represented as median (range) or in frequencies. Wilson score was used to assess 95% confidence interval of diagnostic parameters. The analysis was performed using SPSS Version 22.0 (IBM Corp.) and Open Epi (version 3.01).

Results Out of 223 cases with liver mass lesions, *Echinococcus* IgG was reactive in 62 (28%) cases and FNAC was positive in 16 (7.2%) cases. Since two cases were FNAC-positive but IgG-nonreactive, total HHD cases were 64 (28.7%). *Echinococcus* IgG reactive cases were seen more in the extremes of age group, that is, 1 to 10 years and 81 to 90 years. Taking FNAC as the standard, the sensitivity, specificity, positive predictive value, and negative predictive value of ELISA were 87.5, 76.8, 22.6, and 98.7%, respectively. Cytology-positive cases demonstrated a mean ELISA optical density/cut-off (OD/CO) of 4.2 ± 3 standard deviation.

Conclusion ELISA in radiologically confirmed liver mass cases is highly sensitive in detecting HHD and hence should be used along with ultrasonography for the screening of HHD followed by confirmation with cytology even in cases with a higher OD/CO of ELISA.

Keywords

- hepatic hydatid disease
- ELISA
- fine needle aspiration cytology
- liver mass lesions

Introduction

Echinococcosis or hydatid disease (HD) is a parasitic illness caused by infection with dog tapeworms (*Echinococcus granulosus* and *Echinococcus multilocularis*) in its larval stage.¹ HD global prevalence is estimated at 2 to 3 million human cases, with an estimated mortality rate of 2 to 4% per 100 inhabitants.^{2,3} HD is endemic in more than 100 countries globally, with the highest prevalence found in Mediterranean regions, parts of Russia, central Asia, China, Australia, parts of South America, and Africa. It has been included in the list of neglected tropical diseases and is considered to be one of the six priority neglected zoonotic diseases.⁴ Although HD is endemic in many regions in India, detailed epidemiological studies from India are scanty.^{4,5} The parasite exhibits a predilection for the liver (70–80%) followed by the lungs (20–30%), with the spleen, kidney, heart, brain, bone, and breasts being involved less commonly.^{6,7} The definitive host for the parasite is dogs, whereas animals such as sheep, cattle, goats, and camels serve as intermediate hosts.⁸ Humans are dead-end occasional intermediate hosts and acquire the infection through accidental ingestion of *Echinococcus* eggs excreted with feces in dogs and transmitted through a close contact with infected animals.^{9,10}

Most HD patients either remain asymptomatic for several years or exhibit nonspecific symptoms due to which diagnosis is often made incidentally.¹¹ Infection with *Echinococcus* induces an antibody response, most commonly immunoglobulin (Ig) G followed by IgM, IgA, and IgE.¹² Hence, the main serological methods used for human HD diagnosis and follow-up are based on the detection of specific IgG antibodies. The usual diagnostic approach to HD involves a combination of both imaging (ultrasonography [USG], computed tomography [CT], magnetic resonance imaging [MRI]) and serological techniques (IgG enzyme-linked immunosorbent assay [ELISA], indirect fluorescent antibody [IFA], indirect hemagglutination [IHA], or latex agglutination [LA])⁴ in conjunction with a history of exposure or immigration from an endemic area.^{12,13} The definitive diagnosis of HD can be achieved by the demonstration of scolices, hooklets, or protoscolices in aspirated fluid by fine needle aspiration cytology (FNAC), but it is typically not performed in cases with a suspicion of hydatid cyst due to the perceived risk of anaphylactic shock.^{4,11,14} In routine clinical practices, most clinicians usually rely on the modern imaging techniques for HD diagnosis^{8,9} because although serology is a helpful diagnostic adjunct, its efficacy as a screening assay for HD diagnosis is still debatable.^{15,16}

Hence, the aim of this study was to determine the diagnostic efficacy of ELISA in radiologically confirmed liver mass lesions for the diagnosis of hepatic HD (HHD) and to compare the diagnostic performance of ELISA with FNAC (taken as standard) for HHD diagnosis.

Materials and Methods

Study Population

This is a retrospective study that included 223 patients with radiologically confirmed liver mass lesions whose

blood samples were received, serum separation was performed, and *Echinococcus* IgG serology was performed in the Department of Clinical Virology in a tertiary liver-care hospital in Delhi from August 2014 to July 2017. The Institutional Ethics Committee (IEC) of the Institute of Liver and Biliary Sciences approved the study protocol.

Serology

Patients were tested for IgG anti-*Echinococcus* antibodies by commercial IgG ELISA (RIDASCREEN, R-Biopharm AG, Darmstadt, Germany) as per the manufacturer's instructions. This test is an enzyme immunoassay for the qualitative determination of IgG antibodies against *Echinococcus granulosus* and *Echinococcus multilocularis* in human serum. Results of *Echinococcus* IgG serology was obtained from the hospital information system (HIS).

Data Gathering

The following other parameters were retrieved from HIS: USG findings, FNAC reports, and liver function tests (LFTs).

Statistical Analysis

Comparison of the results obtained from ELISA was performed with respect to FNAC results (taken as standard) to analyze the diagnostic efficacy of ELISA for HHD detection. Data have been represented as median (range) or frequencies. The Wilson score was used to assess 95% confidence interval of diagnostic parameters. The analysis was performed using SPSS Version 22.0 (IBM Corp.) and Open Epi (version 3.01).

Results

A total of 223 cases having a median age of 50 years (range: 4–85 years) with radiologically confirmed liver mass lesions were included in the study. Median LFTs were found to be normal in the study population, as shown in ►Table 1, with cystic lesions of the liver being the most predominant (48.4%) among the liver mass lesions.

Out of 223 cases with liver mass lesions, *Echinococcus* IgG was found to be reactive in 62 (28%) cases and nonreactive in 161 (72%) cases. LFTs were deranged in only 17/62 (27.4%) serologically confirmed HHD cases, with a median ALT (alanine aminotransferase) of 26 IU/mL (range: 9–660 IU/mL) and a median AST (aspartate aminotransferase) of 29 IU/mL (range: 11–295 IU/mL). Among the radiologically confirmed liver mass lesions in the 62 *Echinococcus* IgG reactive cases; the most predominant was hepatic cyst (63%) followed by liver abscess (22.5%) and solid mass lesion (14.5%).

The study group showed the maximum number of liver mass lesions in the age group of 51 to 60 years. *Echinococcus* IgG reactive cases were seen more in the extremes of age-group, that is, 1 to 10 years and 81 to 90 years. Both the radiologically confirmed liver mass lesions and serologically confirmed liver hydatid cases demonstrated male preponderance except IgG reactive cases in the age group of 41 to 50 years, which demonstrated female preponderance (►Table 2).

Of the 223 cases with liver mass lesions, FNAC was positive in 16 (7.2%) cases, and of the 62 serologically confirmed

HHD cases, FNAC was positive in only 14 (22.6%) cases. There were two cases that were FNAC-positive but IgG-nonreactive. Hence, the total HHD cases in this study were 64/223 (28.7%).

Out of 16 FNAC-positive cases, 14 (87.5%) were found to be IgG-reactive. Overall, both FNAC and ELISA were positive in 14/223 (6.3%) cases and negative in 159/223 (71.3%) cases. Hence, the concordance rate between ELISA and FNAC results for the diagnosis of HHD cases was 77.6% (► **Tables 3 and 4**). ► **Table 5** depicts the diagnostic performance of ELISA as compared with FNAC for HHD diagnosis.

Among the 62 ELISA reactive cases, a maximum number of samples demonstrated a low optical density/cutoff (OD/CO) in the range of 1 to 2 (► **Table 6**), with a mean OD/CO of 3.28 ± 2 standard deviation (SD). Maximum cytology-positive

cases were seen in cases with OD/CO > 10 followed by those in the range of 4 to 5. The 16 cytology-positive cases demonstrated a mean ELISA OD/CO of 4.2 ± 3 SD.

Discussion

Echinococcosis is among the most neglected parasitic diseases, and most clinicians usually rely on the modern imaging techniques for its diagnosis, with USG being the method of choice for the detection of both hepatic and extrahepatic echinococcosis.^{8,9} Although HD serology is a helpful diagnostic adjunct, its efficacy as a screening assay for HD diagnosis is still debatable.^{15,16} This may be because in developing countries like India, serological tests for HD, which are still considered as alternatives to imaging modalities, are not routinely employed in clinical settings.¹² Hence, this study aimed at determining the diagnostic efficacy of ELISA as a screening assay in radiologically confirmed liver mass lesions for the diagnosis of HHD and to compare the diagnostic performance of ELISA with FNAC (taken as standard) for HHD diagnosis.

In the study, ELISA was reactive in 62 (28%) USG-positive HD cases, which is in accordance with a study from Chandigarh in North India¹⁷ but in contrast to studies from China and Egypt where seropositivity was higher.^{18,19} On the other hand, FNAC in the study was positive in 16 (7.2%) cases, which is similar to two other studies from Chandigarh.^{20,21}

In the study, *Echinococcus* IgG reactive cases were seen more in the age group of 1 to 20 years with a male preponderance, whereas liver mass lesions on USG were predominant in the age group of 51–60 years. This result is in accordance with a seroepidemiological study performed in Kashmir (North India) where age < 15 years and male gender were considered as significant factors associated with *Echinococcus* seropositivity,⁵ thus supporting the worldwide earlier well-reported observation that infection is usually

Table 1 Baseline characteristics of the study population

Total number of samples with radiologically confirmed liver mass lesions (n)	223
Age (years), median (range)	50 (4–85)
Male, n (%)	146 (65.5%)
Female, n (%)	77 (34.5%)
Male:female	1.9:1
ALT (IU/L), median (range)	25.5 (7–717)
AST (IU/L), median (range)	29 (8–671)
Bilirubin (total) (mg/dL), median (range)	0.6 (0.2–25.3)
Total number of radiologically confirmed liver cystic lesions	108/223 (48.4%)
Total number of radiologically confirmed liver abscess lesions	79/223 (35.4%)
Total number of radiologically confirmed liver solid mass lesions	36/223 (16.14%)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 2 Age- and sex-wise distribution of radiologically confirmed liver mass lesions (n = 223) and serologically confirmed liver hydatid cases (n = 62)

Age (years), n	Total no. of males with liver mass lesions, n (%)	Total no. of females with liver mass lesions, n (%)	Total no. of IgG-reactive cases, n (%)	Total no. of IgG-reactive (males), n (%)	Total No. of IgG-reactive (females), n (%)
1–10 (5)	3 (60)	2 (40)	3 (60)	2 (66.7)	1 (33.3)
11–20 (11)	6 (54.5)	5 (45.4)	6 (54.5)	3 (50)	3 (50)
21–30 (20)	10 (50)	10 (50)	6 (30)	3 (50)	3 (50)
31–40 (35)	26 (74.3)	9 (25.7)	14 (40)	8 (57)	6 (43)
41–50 (44)	28 (63.6)	16 (36.4)	15 (34%)	7 (46.7)	8 (53.3)
51–60 (55)	34 (61.8)	21 (38.2)	8 (14.5)	4 (50)	4 (50)
61–70 (22)	17 (77.3)	5 (22.7)	1 (4.5)	0 (0)	1 (100)
71–80 (28)	20 (71.4)	8 (28.6)	7 (25)	5 (71.4)	2 (28.6)
81–90 (3)	2 (66.7)	1 (33.3)	2 (66.7)	2 (100)	0 (0)
Total = 223	146 (65.5)	77 (34.5)	62 (28)	34 (55)	28 (45)

Table 3 Comparative analysis of FNAC and ELISA results for the diagnosis of hepatic hydatid cases

		FNAC		Total
		Positive	Negative	
ELISA	Reactive	14	48	62
	Nonreactive	2	159	161
	Total	16	207	

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FNAC, fine needle aspiration cytology.

Table 4 Results obtained from ELISA and cytology in radiologically confirmed liver mass lesions (n = 223)

	Total, n (%)
Total ELISA-reactive	62 (28)
Total FNAC-positive	16 (7.2)
ELISA-reactive and FNAC-positive	14 (6.3)
ELISA-reactive and FNAC-negative	48 (21.5)
ELISA-nonreactive and FNAC-positive	2 (0.9)
Total cases of hepatic hydatid disease by either ELISA or FNAC or both	64 (28.7)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FNAC, fine needle aspiration cytology.

Table 5 Diagnostic performance of ELISA as compared with FNAC for the diagnosis of liver hydatid cases

Sensitivity (95% CI)	87.5% (63.98–96.50)
Specificity (95% CI)	76.8% (70.61–82.04)
Positive predictive value (95% CI)	22.6% (13.96–34.41)
Negative predictive value (95% CI)	98.7% (95.58–99.66)
Diagnostic accuracy (95% CI)	77.58% (71.66–82.56)
False-positive rate	21.5%
False-negative rate	0.89%

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; FNAC, fine needle aspiration cytology.

Table 6 Correlation of FNAC results with ELISA OD/CO results in radiologically confirmed liver mass lesions (n = 223)

ELISA result interpretation	ELISA OD/CO (n)	FNAC-positive, n (%)	FNAC-negative, n (%)
Nonreactive	<0.9 (160)	2 (1.3)	158 (98.7)
Reactive	1–2 (22)	1 (4.5)	21 (95.5)
	2–3 (14)	2 (14.3)	12 (85.7%)
	3–4 (9)	2 (22.2)	7 (77.8)
	4–5 (8)	5 (62.5)	3 (37.5)
	5–6 (3)	1 (33.3)	2 (66.7)
	6–7 (1)	0 (0)	1 (100)
	7–8 (3)	1 (33.3)	2 (66.7)
	8–9 (2)	1 (50)	1 (50)
	9–10 (0)	0 (0)	0 (0)
	>10 (1)	1 (100)	0 (0)
Total	223	16 (7)	207 (93)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FNAC, fine needle aspiration cytology; OD/CO, optical density/cutoff.

acquired in childhood, remaining asymptomatic for long period, with symptoms manifesting in adult age depending upon the organ involved.²²

In this study, ELISA demonstrated a fairly good sensitivity and negative predictive value of 87.5 and 98.7%, respectively, but a moderately low specificity of 76.8% when FNAC was taken as the standard in all radiologically confirmed liver mass lesions. Other studies have usually evaluated the diagnostic efficacy of ELISA using imaging techniques especially USG, with a sensitivity of ELISA ranging from 64.8 to 100%.^{2,18} Another study found that the sensitivity of ELISA was 100% when compared with IHA, IFA, and Casoni's intradermal test.²³ To the best of our knowledge, this is the first study that has evaluated the diagnostic

efficacy of ELISA with FNAC for HHD diagnosis taking into consideration the fact that FNAC is a confirmatory test for HHD diagnosis^{4,24} in contrast to USG, which although is a convenient tool for HHD diagnosis and detects the location, number, and size of the cysts with relative ease^{22,25–27} but has poor sensitivity in detecting small-sized cysts,^{11,15} and USG findings may mimic other pathologies presenting as liver mass lesions,⁴ which has also been found in this study as only 62/223 (28%) of the liver mass lesions on USG demonstrated IgG reactivity.

Interestingly, when OD/CO results of ELISA were compared with FNAC results, it was found that as the OD/CO increases from 1 to 5, FNAC positivity demonstrated 58% increase from 4.5% to 62.5%, but as the OD/CO increased beyond 5, FNAC

positivity showed a sudden decline. The 16 cytology-positive cases demonstrated mean ELISA OD/CO of 4.2 ± 3 SD with maximum cytology-positive cases seen with OD/CO > 10 followed by those in the range of 4 to 5. This is the first study that has compared the OD/CO of ELISA with FNAC results for the diagnosis of HHD. Since no OD/CO ranges taken in the study showed 100% FNAC positivity, it can be speculated that all ELISA results of USG-positive cases must be confirmed by FNAC irrespective of OD/CO range.

Limitations of the Study

Follow-up of the patients could not be performed as this was a retrospective study.

Conclusion

In conclusion, the diagnostic approach of HHD should involve a combination of imaging techniques, serological tests, and cytology, as no single diagnostic modality is enough to arrive at a confirmation of the presence of HHD. ELISA in radiologically confirmed liver mass cases is highly sensitive in detecting HHD and hence should be used along with USG for the screening of HHD followed by confirmation with cytology even in cases with a higher OD/CO of ELISA.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or profit sectors.

Conflicts of Interest

There are no conflicts of interest to report.

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