Tehran University of Medical Sciences Publication
http://tums.ac.ir

Iran J Parasitol

Open access Journal at
http://ijpa.tums.ac.ir

Iranian Society of Parasitology
http://isp.tums.ac.ir

Original Article

Serological Follow-up of Human Cystic Echinococcosis in the Thrace Region, Turkey

Canan Eryıldız [1], Taner Tarladaçalısır [1], Gulcan Kuyucuklu [2], Berrak Çakmakçı [1], *Nermin Sakru [1]

1. *Department of Medical Microbiology, Faculty of Medicine, Trakya University, Edirne, Turkey*
2. *Department of Medical Services and Techniques, Health Services Vocational School, Trakya University, Edirne, Turkey*

Received 15 Feb 2022
Accepted 19 Apr 2022

Keywords:
Echinococcus granulosus;
Cystic echinococcosis;
Indirect hemagglutination test;
Turkey

***Correspondence**
Email:
nsakru@yahoo.com

Abstract

Background: Parasites of the genus *Echinococcus* are common worldwide and are important cestodes that cause serious infections in humans and animals. This retrospective study evaluated the indirect hemagglutination (IHA) test results of serum samples obtained from patients with a pre-diagnosis of cystic echinococcosis (CE) within ten years. In addition, the role of the IHA test results of the patients in the follow-up of the treatment and determining possible recurrences was investigated.

Methods: The IHA test results of 2426 serum samples of patients with a pre-diagnosed CE admitted to Trakya University Health Center for Medical Research and Practice in Edirne, Turkey, between January 2011 and December 2020 were evaluated retrospectively. The data of 53 patients with CE who had medical treatment and/or postoperative follow-up serological records were evaluated.

Results: Of 2426 IHA tests, 376 (15.5%) were seropositive, and 2050 (84.5%) were seronegative. It was determined that 376 serum samples detected as positive belonged to 207 patients with CE. Of 207 CE patients, 109 (52.7%) were female and 98 (47.3%) were male. The most common organ involvement was the liver in 186 (89.9%) cases. Of 53 patients, 16 were considered relapse cases. The median follow-up period for 16 recurrent cases was 31.8 (1-77) months. Our results showed a statistically significant correlation between long-term serological follow-up and recurrence detection (P=0.034).

Conclusion: Long-term serological follow-up after treatment is considered useful in determining possible recurrent cases. CE is an important public health problem for endemic regions, including our country, and we think our study results will contribute to the status and follow-up of the disease.

Introduction

Hydatid cysts of *Echinococcus granulosus* develop as fluid-filled unilocular cysts in the internal organs of humans and other intermediate hosts, primarily the liver and lungs (1). In regions where cystic echinococcosis (CE) is endemic, such as Central Asia, South America, and the Mediterranean countries, the incidence is approximately between 1-200/10^5, while the mortality rate (2-4%) is generally low (2). The WHO states that human incidence rates for CE may reach >50/10^5 in endemic regions, and the prevalence may even be as high as 5-10% in some regions of Argentina, Peru, East Africa, Central Asia, and China (3). In Turkey, while the morbidity rate was reported as 0.57/10^5 in 2008, it was reported as 2.25 in 2019 (4). Diagnosis of CE depends on clinical findings, imaging techniques, and serological methods (5). All imaging methods can show some stages of hydatid cysts, but generally, cross-sectional imaging methods (ultrasonography, computerized tomography, or magnetic resonance imaging) provide the best images. The use of imaging methods alone or in combination with laboratory test results can provide a 100% positive diagnosis of hydatid disease in most cases (6). Enzyme-Linked Immunosorbent Method (ELISA), indirect haemagglutination (IHA) test, latex agglutination, and immunoblotting are the most widely used serological methods (5).

In this retrospective study, IHA test results of serum samples obtained from patients with a pre-diagnosis of CE within ten years were evaluated. In addition, we aimed to evaluate the role of IHA test results of patients diagnosed surgically and/or pathologically in determining treatment follow-up and possible recurrence in CE.

Materials and Methods

This study was approved by Trakya University Faculty of Medicine Scientific Research Ethics Committee (TÜTF-BAEK-2021/441).

Overall, 2426 serum samples from patients with suspected CE admitted to Trakya University Health Center for Medical Research and Practice in Edirne, Turkey, between January 2011 and December 2020 were included. IHA test results were analyzed retrospectively from laboratory records. According to the manufacturer's recommendations, the IHA test (Fumouze Diagnostics, France) was performed. Serum samples with IHA titers equal to or greater than 320 were considered positive. Serum samples with IHA titers of 160 were classified as an equivocal reaction.

The data of 53 patients with CE whose medical treatment and/or postoperative follow-up (radiological and/or serological) records were evaluated. Recurrence is defined as the emergence of new active cysts after therapy. It may occur due to the continued growth of viable cysts at the site of a previously treated cyst or the emergence of a new distant disease resulting from spillage (7).

Statistical analysis

The normality of continuous variables was checked by the Shapiro-Wilk test. The data of the two groups were compared using the Mann-Whitney U test, and the relationship between the two categorical variables was examined using Pearson's Chi-square test. Data were presented as mean and standard deviation, median, minimum and maximum values, while percentages were given for categorical variables. Statistical analyzes were performed using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp.). A $P < 0.05$ was considered statistically significant.

Results

Overall, 376 (15.5%) of 2426 CE suspected serum samples were found to be IHA positive and 2050 (84.5%) negative. 57.5% of the samples belonged to female patients and the mean age was 49.3±17.9 years. There was no statistically significant difference in seropositivity by gender (*P*=0.367). The 376 seropositive samples belonged to 207 patients. Of these patients, 75 (36.2%) were under 40, 132 (63.8%) were over 40 (Table 1).

Table 1: Distribution of indirect hemagglutination test results by age and gender

Variable	*Total*	*Seropositive*	*Seronegative*	*p*
Mean age (yr)	49.3±17.9, 51 (0-98)[a]	45.2±18.0, 46.5 (8-95)[a]	50.0±17.9, 52 (0-98)[a]	
Gender				0.367
Female	1394 (57.5)	224 (59.6)	1170 (57.1)	
Male	1032 (42.5)	152 (40.4)	880 (42.9)	
Total	2426 (100)	376 (100)	2050 (100)	
207 patients with CE				
Mean age (years)		45.1±19.2, 47 (8-95)[b]		
Gender				
Female		109 (52.7)		
Male		98 (47.3)		
Total		207 (100)		

[a, b] Quantitative data were shown as mean±SD, median (min-max), qualitative data as n (%)

The most common organ involvement in 207 CE patients was the liver (186 cases, 89.9%), followed by the lung (11 cases, 5.3%). The other organ involvements (two bones, one peritoneum, one common bile duct) were detected in four (1.9%) cases. In addition, more than one organ involvement was observed in 6 (2.9%) cases. Liver and lung involvements were found in five of these cases, and liver and peritoneal involvement were found together in one.

Medical treatment and/or postoperative follow-up records of 53 patients with CE were evaluated. There was liver involvement in 42 (79.2%) cases, bone involvement in one (1.9%) case, and paravertebral region involvement in one (1.9%) case of these patients. Eight (15.1%) patients had liver and lung involvement, and one (1.9%) patient had liver and peritoneal involvement. Of 53 patients, 41 (77.4%) were treated surgically, 5 (9.4%) were treated medically, and 7 (13.2%) were treated both surgically and medically. Seropositive IHA titers ranged from 1/320 to 1/40960.

Sixteen (30.2%) of 53 patients in our study were considered a recurrence. In the follow-up of the treatment of 16 patients with recurrence, the IHA test detected a mean increase of 3.4 (0-8) twofold dilutions. In three patients treated for liver and lung cysts, recurrence was found only in the liver. Other recurrent cases occurred in the same organs (Table 2). The median value for the follow-up period of 53 patients was 10 (1-114) months. Of these, the median follow-up period for 16 recurrent cases was 31.8 (1-77) months, and for the other 37 patients, the median follow-up period was 9 (1-114) months. These findings showed a statistically significant correlation between long-term serological follow-up and recurrence detection (*P*=0.034).

Table 2: Demographic and medical data of recurrent cases

Patient	*Age (yr)*	*Gender*	*Follow-up interval (months)*	*Follow-up date*	*IHA Test result*	*Organ involvement*	*Operation date*	*Medical treatment*
1	83	M	0	07.2017	1/1280	Liver	2014	Yes
			4	11.2017	1/10240			
2	49	F	0	10.2011	1/2560	Liver	2012/2013 2014/2017	Yes
			7	05.2012	1/1280			
			23	09.2013	1/2560			
			63	01.2017	1/2560			
			75	01.2018	1/10240			
			76	02.2018	1/20480			
			77	03.2018	1/40960			
3	51	F	0	03.2017	1/2560	Liver, Lungs	2017	No
			6	09.2017	1/640			
			7	10.2017	1/320			
			11	02.2018	1/160			
			13	04.2018	Negatif			
			18	09.2018	1/1280			
4	29	F	0	10.2016	1/640	Liver	2012	No
			8	06.2017	1/20480			
5	39	M	0	02.2012	1/640	Liver	No	Yes
			5	07.2012	1/1280			
			7	09.2012	1/160			
			8	10.2012	1/320			
			12	02.2013	Negatif			
			19	09.2013	1/2560			
			21	11.2013	1/2560			
			24	02.2014	Negatif			
			27	05.2014	1/640			
6	64	F	0	08.2013	1/160	Liver	2011/2013	Yes
			6	02.2014	Negatif			
			36	08.2016	1/1280			
7	37	F	0	11.2012	1/2560	Liver	2015	Yes
			37	12.2015	1/1280			
			40	03.2016	Negatif			
			73	12.2018	1/10240			
			76	03.2019	1/20480			
8	37	F	0	11.2011	1/2560	Liver	2011/2014	No
			4	03.2012	1/640			
			5	04.2012	Negatif			
			14	01.2013	1/1280			
			29	04.2014	1/1280			

			53	04.2016	1/2560			
9	60	F	0	11.2018	Negatif	Liver	2008	Yes
			4	03.2019	1/1280			
10	35	F	0	05.2012	1/160	Liver	2012/2016	No
			3	08.2012	1/1280			
			20	01.2014	Negatif			
			22	03.2014	1/2560			
11	48	M	0	03.2015	1/2560	Liver	2008/2018	No
			33	12.2017	1/2560			
12	49	F	0	03.2011	1/160	Liver	2008	No
			1	04.2011	1/320			
13	27	M	0	12.2017	Negatif	Liver	2016	No
			15	03.2019	1/320			
14	45	M	0	02.2015	1/2560	Liver, Lungs	2015/2020	No
			66	08.2020	1/2560			
15	26	F	0	03.2015	1/2560	Liver	2009/2014	No
			4	07.2015	1/320			
			34	01.2018	1/5120			
16	60	M	0	02.2016	Negatif	Liver, Lungs	2015	No
			36	02.2019	1/320			

Discussion

Echinococcus species are important cestodes that cause zoonotic infections in humans and economic losses in the livestock sector. Although some species have limited geographical distribution, *Echinococcus* is found worldwide. (1,3,8). Hydatid cysts of *E. granulosus* develop as fluid-filled unilocular cysts in the internal organs of humans and other intermediate hosts (most commonly the liver and lung). Diagnosis in CE depends on clinical findings, imaging techniques, and serological methods (5).

There are many studies on the IHA test; one of the most commonly used serological methods in diagnosing cystic echinococcosis (9-17). Gureser et al. (9) studied 253 patients with a pre-diagnosis of CE, including 148 women and 105 men. In the study, 32 (12.7%) patients, 23 (15.5%) females, and nine (8.6%) male cases were found to be seropositive, but no statistically significant difference was found in terms of gender. The age range of seropositive patients was 16-90 years (a mean of 51), and it was statistically significant that 24 (75%) of patients were over 40 years old. Beyhan et al. (10) examined 2921 serum samples, and the difference between seropositivity and gender was found statistically significant. Selek et al. (11) evaluated 299 patients with a CE diagnosis. 44.5% of the cases were male, 55.5% were female. 5% in the 0-15 age group, 31.8% in the 16-30 age group, 29.4% in the 31-45 age group, 24.4% in the 46-60 age group, and 9.4% were 61 and over.

In Logar et al. study (17), 127 of 1323 serum samples were seropositive by the IHA test, and no significant difference was found between gender and seropositivity. In Jordan, 512 serum samples were evaluated with the IHA test, and 21 (4.1%) were seropositive. No statistically significant relationship was found between seroprevalence, age, and gender in the evaluation (16). In a study performed in Turkey, IHA test results were positive in 143

(15.2%) of 938 patients with at least one radiological imaging report. In 130 (90.9%) of these 143 patients, CE findings were recorded in at least one radiological imaging report. In that study, no statistically significant difference was found in seropositivity and gender (12). This situation was compatible with other studies (9, 16, 17), while it was different from the study of Beyhan et al. (10). In our study, no significant difference was found in *Echinococcus* IHA test positivity according to gender (p=0.367), and 63.8% of 207 patients with CE were over the age of 40.

Cystic echinococcosis most commonly localizes to the liver and lungs while less frequently developing in the bones, kidneys, spleen, muscles and central nervous system (3). In a study (9), liver involvement was detected in 31 (96.9%) seropositive patients. In another study, cysts were mostly localized in liver, lungs and peritoneal cavity, 71.9%, 11.4% and 4.7%, respectively (11). Tasbent et al. (12) found the liver as the highest organ involvement in 311 (85.9%) patients. In the study by Yolasigmaz et al. (18), the majority of the patients had echinococcal cysts in the liver (88.9%). In our study, organ involvement was concordant with the literature.

Recurrence in CE is defined as the emergence of new active cysts after therapy, including the re-emergence of viable cysts at the site of a previously treated cyst or the emergence of a new distant disease. However, the WHO-IWGE Expert Consensus does not clearly define relapse, recurrence, and reinfection (7,19,20). Recurrence after treatment is still a severe problem for endemic regions such as our country. It may take 3-4 years for recurrent cases to become symptomatic (21). Many factors such as medical treatment before and after surgery, cyst localization, the connection of the cyst with the biliary tract, and insufficiency of scolicidal agents are associated with the development of recurrence (22).

Recurrence after treatment is a frequently encountered condition in patients with CE; therefore, patients are followed up for recurrence. The IHA test is one of the tests used in the follow-up of recurrence after treatment in patients with CE. However, false negative results in immunodiagnostic tests for CE may be seen in some patients. It has also been shown that the different antibody levels of the patients depend on the individual immune response. The multiple localization and stages of CE cysts demonstrate the need for a complex approach to confirm the diagnosis. The IHA titers increases during follow-up are significant in terms of recurrence, and in favor of recurrence, positive test results must be confirmed by radiological methods (7,18,23). In our study, in the follow-up of the treatment of 37 patients without recurrence, a mean decrease of 2.6 (0-5) twofold dilutions was detected in the IHA titers. When the IHA test results of the recurrent patients were evaluated, an increase in the IHA titers was detected. In addition, a statistically significant correlation between long-term serological follow-up and recurrence detection was found in our study.

Conclusion

Increases in IHA test titers were observed in recurrent cases. It was determined that long-term serological follow-up after treatment provided significant benefits, especially in determining possible recurrent cases. In conclusion, CE is an important public health problem for endemic regions, including our country, and we think that our study results will contribute to the status and follow-up of the disease.

Acknowledgements

Some part of the data in this study were previously presented in 2 congresses: 1 st Croatian Congress on Travel, Tropical, Migration Medicine & HIV, Dubrovnik, Croatia, 2015 and Sixth International Meeting on Emerging Diseases and Surveillance, IMED 2016.

Conflict of interest

There are no conflicts of interest.

References

1. Craig PS, Hegglin D, Lightowlers MW, et al. Echinococcosis: Control and prevention. Adv Parasitol. 2017;96:55-158.
2. Anvari D, Rezaei F, Ashouri A, et al. Current situation and future prospects of *Echinococcus granulosus* vaccine candidates: A systematic review. Transbound Emerg Dis. 2021;68(3):1080-96.
3. WHO. Echinococcosis. Available from: https://www.who.int/news-room/fact-sheets/detail/echinococcosis#:~:text=Key%20facts,(hydatidosis)%20and%20alveolar%20echinococcosis. [cited: 2022 March 5].
4. Altıntaş N, Topluoğlu S, Yıldırım A, et al. Current situation report of cystic echinococcosis in Turkey (in Turkish). Turk Hij Den Biyol Derg. 2020; 77(3):1-52.
5. Brunetti E, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Trop. 2010;114(1):1-16.
6. Sever A, Elmas N. Imaging methods in Echinococcosis (in Turkish). In: Altıntaş N, Tinar R, Coker A, editors. Echinococcosis. İzmir: Hidatidoloji Derneği; 2004. p. 203-18.
7. Prousalidis J, Kosmidis C, Anthimidis G, et al. Postoperative recurrence of cystic hydatidosis. Can J Surg. 2012;55(1):15-20.
8. Deplazes P, Rinaldi L, Alvarez Rojas CA, et al. Global distribution of alveolar and cystic echinococcosis. Adv Parasitol. 2017;95:315-493.
9. Güreser AS, Özcan O, Özünel L, et al. Evaluation of the radiological, biochemical and serological parameters of patients prediagnosed as cystic echinococcosis in Çorum, Turkey (in Turkish). Mikrobiyol Bul. 2015;49(2):231-9.
10. Beyhan YE, Babür C, Mungan M, et al. Evaluation of cystic echinococcosis suspected patients applied to National Parasitology Reference Laboratory of Public Health Institution of Turkey between 2009-2013 (in Turkish). Turkiye Parazitol Derg. 2015;39(1):17-21.
11. Selek A, Selek MB, Karadayı N. Evaluation of the cystic eccinococcosis cases diagnosed in Dr. Lütfi Kırdar Kartal Education and Research Hospital Pathology Laboratory between 2007 and 2013 (in Turkish). Turkiye Parazitol Derg. 2015;39:112-6.
12. Taşbent FE, Yağcı B, Kadıyoran C, et al. Comparative evaluation of the efficacy of indirect hemagglutination test and radiological methods in the pre-diagnosis of cystic echinococcosis (in Turkish). Turkiye Parazitol Derg. 2021;45(1):22-7.
13. Akkaya Işık S, Seyman D, Zerdali E, et al. Evaluation of 170 followed-up cases treated for hydatid disease: a multicentre study (in Turkish). Turkiye Parazitol Derg. 2020;44(4):197-202.
14. Ok ÜZ, Kilimcioğlu AA, Özkol M. Cystic echinococcosis in humans in Turkey (in Turkish). Mikrobiyol Bul. 2020;54(3):510-22.
15. Taş Cengiz Z, Yılmaz H, Beyhan YE, et al. Cystic echinococcosis seropositivity in the blood samples sent to Parasitology Laboratory of Yüzüncü Yıl University Medical Faculty between 2005 and 2013: Retrospective assessment (in Turkish). Turkiye Parazitol Derg. 2015;39(3):209-11.
16. Himsawi N, Hijjawi N, Al-Radaideh A, et al. Seroprevalence of cystic echinococcosis in a high-risk area (Al-Mafraq Governorate) in Jordan, using indirect hemagglutination test. Parasite Epidemiol Control. 2019;5:e00104.
17. Logar J, Soba B, Kotar T. Serological evidence for human cystic echinococcosis in Slovenia. BMC Infect Dis. 2008;8:63.
18. Yolasigmaz A, Reiterová K, Turk M, et al. Comparison of serological and clinical findings in Turkish patients with cystic echinococcosis. Helminthologia. 2006;43(4):220-5.
19. Velasco-Tirado V, Romero-Alegría Á, Belhassen-García M, et al. Recurrence of cystic echinococcosis in an endemic area: a retrospective study. BMC Infect Dis. 2017;17:455.
20. Meeting of the WHO Informal Working Group on Echinococcosis (WHO-IWGE), Geneva, Switzerland, 15–16 December 2016. Geneva, Switzerland: World Health Organization; 2017

(WHO/HTM/NTD/NZD/2017.01). Licence: CC BY-NC-SA 3.0 IGO.

21. Akyıldız HY, Akcan A, Karahan İ, et al. Recurrent liver hydatid disease: when does it become symptomatic and how does one diagnose it? Clin Imaging. 2009;33(1):55-8.
22. Secchi MA, Pettinari R, Mercapide C, et al. Surgical management of liver hydatidosis: a multicentre series of 1412 patients. Liver Int. 2010;30(1):85-93.
23. Sarkari B, Rezaei Z. Immunodiagnosis of human hydatid disease: Where do we stand? World J Methodol. 2015;5(4):185-95.