

# Investigation of Patients with Suspected Cystic Echinococcosis by ELISA and IHA Methods in Gaziantep, Turkey

 Ahmet Ozkeklkci<sup>1</sup>,  Eylem Akdur Ozturk<sup>2</sup>,  Mesut Akil<sup>2</sup>,  Aysegul Unver<sup>2</sup>,  Nuray Altintas<sup>3</sup>

<sup>1</sup>Dr. Ersin Arslan Education And Research Hospital, Gaziantep, Turkey

<sup>2</sup>Ege University, Faculty of Medicine, Department of Parasitology, Izmir, Turkey

<sup>3</sup>Manisa Celal Bayar University, Faculty of Medicine, Department of Medical Biology, Manisa, Turkey

<sup>4</sup>Ege University, Faculty of Medicine, Department of Parasitology, Izmir, Turkey

## ARTICLE INFO

Received: Jul 29, 2021

Accepted: Aug 19, 2021

Available online: December 09, 2021

## CORRESPONDING AUTHOR

Eylem Akdur Ozturk, Ege University, Faculty of Medicine, Department of Parasitology, Izmir, Turkey, E-mail: [akdureylem@gmail.com](mailto:akdureylem@gmail.com)  
Phone Mobile: + 90 05055116628

## CITATION

Ozkeklkci A, Akdur Ozturk E, Akil M, Unver A, Altintas N. Investigation of Patients with Suspected Cystic Echinococcosis by ELISA and IHA Methods in Gaziantep, Turkey. International Journal of Echinococcoses 2022;1(1):1-6. DOI: 10.5455/IJE.2021.07.05

The journal is the official publication of The Turkish Association of Hydatidology

Copyright@Author(s) - Available online at [www.echinococcoses.org](http://www.echinococcoses.org)  
Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.



## ABSTRACT

**Objective:** Cystic echinococcosis (CE) caused by larval stage of *Echinococcus granulosus* is one of the neglected parasitic disease, of both medical and public health importance. It has a worldwide distribution including Turkey. The current study was designed to determine the seropositivity of *E. granulosus* infection in patients with suspected cystic echinococcosis attending to Dr.Ersin Arslan Training and Research Hospital in Gaziantep, Turkey.

**Materials and Methods:** A total of 545 serum samples were taken from individuals were screened for the presence of *E. granulosus* IgG antibodies using in-house Enzyme-Linked Immunosorbent Assay (ELISA) and the commercial Indirect Hemagglutination Assay kit (IHA-Fumouze kit). Furthermore, socio-demographic characteristics as gender, age, residence were analyzed by SPSS.

**Results:** Out of 545 samples, antibodies to *E. granulosus* were detected in 148 (27.2%) patients with ELISA and 83 patients (15. 2%) were found positive with IHA test. Statistical comparison of two dependent categorical values (ELISA and IHA) showed statistical significance  $p < 0.001$ . Lesion distribution of organ involvement in suspected patients was liver 498 (91.4%), lung 13 (2.4%), spleen 12 (2.2 %), multiple organ involvement 6 (1.1%) and other organ involvement 16 (2.9%). In the distribution according to age groups, the seropositivity of anti-*E.granulosus* IgG antibodies was increased in patients aged 40-49 years. According to ELISA and IHA results, 28 patients (18.9%) and 18 patients (21.7%) were in this age group, respectively.

**Conclusion:** As a result, CE still represents a public health problem in Gaziantep, Turkey. Efforts should be continued, in both animals and humans side, by increasing public awareness.

**Keywords:** Cystic echinococcosis, *Echinococcus granulosus*; serodiagnosis; hydatid fluid; Enzyme Linked Immunosorbent Assay; Indirect Hemagglutination Assay

## INTRODUCTION

Echinococcosis is a chronic disease caused by the larval stage of the tapeworm *Echinococcus spp.* that is a widely endemic helminth disease. *Echinococcus granulosus*, *Echinococcus multilocularis*, *Echinococcus vogeli*, and

*Echinococcus oligarthrus* are the most common species (1).

Cystic echinococcosis (CE) is caused by infection with the larval stage of the cestode *E. Granulosus*. This is one of the neglected parasitic diseases, resulting in economic

losses in both medical and livestock sectors (2,3). CE is responsible for 95% of echinococcosis in human cases, and the worldwide prevalence of CE is about six million (4,5). The disease is common in a place in the world where there is close contact between the intermediate (e.g. human, goats, pigs, cattle) and definitive hosts (carnivores such as dogs, wolves). Humans become infected by accidental ingestion of *E. granulosus* eggs excreted with feces of infected carnivores and do not play a role in the infection cycle (1,6). Oncospheres that are released from the eggs pass to the intestinal wall and are transferred and penetrate to different internal organs by blood or lymph (7). Slowly growing hydatid cyst is most commonly involved in the liver (52% - 77%) but also in the lungs (10%-40%) and rarely, in other organs (10%) (8).

Clinical symptoms and imaging methods are used in the diagnosis of CE, however, simultaneously use of imaging methods and serological tests in early diagnosis of the CE will be a standard approach, also in clinical and epidemiological research (9). Enzyme-Linked Immunosorbent Assay (ELISA) and the commercial Indirect Hemagglutination Assay (IHA) techniques are frequently used in serodiagnosis of CE due to their ease of application and low cost, as well as their high sensitivity and specificity (9).

CE exhibits cosmopolitan distribution in countries on all continents including Turkey, except Antarctica (10). Two forms of echinococcosis occur in Turkey, i.e. *E. granulosus* and *E. Multilocularis*. CE occurs throughout Turkey, whereas alveolar echinococcosis (AE) predominantly occurs in the eastern Anatolian region (9). The prevalence of *E. granulosus* infection in dogs is between 0.32 and 40% and varies widely with geographical location (2). The prevalence of CE in domestic animals ranges from 3.5% to 58.6% has varied widely with geographical location (11). According to the Ministry of Health, 59,808 cases and 939 deaths were recorded between 1987 and 2004 and estimated surgical case rate of CE is 0.87- 6.6 per 100 000 in Turkey (1,2). According to one of the recent publication, age and sex adjusted prevalence of abdominal cystic echinococcosis was 0.59% (0.19–1.85) (12) .

There are many articles published on human and animal origin CE seroprevalence in different regions of Turkey (1,13–20) yet there is limited information about prevalence of CE in Gaziantep city. Having information about the burden of the disease is highly important for eradication and/or control of CE.

The present study was designed to determine specific antibody levels of echinococcosis in patients with suspected CE attending to Dr. Ersin Arslan Training and Research Hospital in Gaziantep city and aimed to contribute to the data on the dissemination of CE in

Gaziantep province in Turkey for establishing control programme.

## MATERIALS AND METHODS

### Study area

Gaziantep is located in the South-eastern Anatolia of Turkey, bordering with the states of Kilis, Hatay, Osmaniye, Kahramanmaraş, Adiyaman and Şanlıurfa cities. The approximated population of Gaziantep is 1.974.836 which is the biggest city in the South-eastern Anatolia and covers an area around 764 km<sup>2</sup>. It is situated between 36 degrees and 38 degrees east longitude and 36 degrees and 37 degrees north latitude (Figure 1). Mediterranean and continental climate are observed in Gaziantep city due to its location. While the southern parts of the province are under the influence of the Mediterranean climate, in general the summers are hot and dry and winters are cold and rainy. Agriculture is very important in Gaziantep's livelihoods. The most important agricultural product of Gaziantep is pistachios. Animal husbandry especially small livestock in Gaziantep is an important source of livelihood in addition to agriculture.



**Figure1:** The study area in map of Turkey, Gaziantep province

### Blood Samples

A total of 545 serum samples were collected from patients with suspicious CE attending to Dr. Ersin Arslan Training and Research Hospital in Gaziantep, Turkey. Out of 545 suspected patients, 371 (68.07%) were female, and 174 (31.92%) were male. The ages were between 5 and 86 years ( $47.45 \pm 17.27$  years).

### Serological tests

All serum samples of patients were screened for the presence of *E. granulosus* IgG antibodies by in-house

ELISA using hydatid cyst fluid antigen and the commercial Indirect Hemagglutination Assay kit (IHA Fumouze Diagnostic, France).

#### a. Antigen

Liver hydatid cysts from naturally infected sheep were collected from slaughterhouses. Each hydatid cyst was pierced using a 22 GA needle and drained of the hydatid fluid. One drop of fluid from each specimen was placed on a slide with a cover-slip in order to identify protoscolices for fertility under a light microscope. After centrifugation at 1000x g for 15 minutes at 4°C, the supernatant was stored at -20°C as hydatid fluid (HF) antigen for subsequent use.

Protein concentrations were estimated using the Bradford protein assay kit (Bio-Rad) and bovine albumin as a standard.

#### b. Enzyme-Linked Immuno Assay (ELISA)

HF-ELISA was carried out on polystyrene microtiter plates with 96 wells (F-Form; Maxisorp, Nunc, Denmark) as previously described (21) Plate was coated with 100 µl/well of crude sheep HF (at a concentration of 5 µg/well) in phosphate-buffered saline (PBS) buffer, overnight at +4 °C. Plates were then washed three times in 0.5% PBS with Tween 20 (PBS-T) and blocked with milk with PBS-T for 1 h at room temperature. Then, 100 µl of serum samples diluted 1:640 in 5% non-fat milk with PBS-T added and incubated for 1 h at room temperature. After washing, plates were treated with conjugate of alkaline-phosphatase-conjugated anti-human IgG (Sigma) in optimal dilution 1:5.000 for 1 h. After repeated washing, the reaction was stopped about 20 min of incubation in the dark at room temperature by 100 µl of 1µg/ml p-Nitrophenyl Phosphate (N4645 SIGMA) in dietanolamin buffer (DEAB Merck 8.03116.1000), and the optical density was measured spectro-photometrically at 405 (Bio-Tec/µ QUANT, MQX200). For each sample, S/P % was calculated according to 'S/P%=(OD value of the sample)/(OD value of

positive control) x100' equation.

#### c. Indirect Hemagglutination Assay (IHA)

The commercial Indirect Hemagglutination Assay kit (IHA-Fumouzekit) was performed according to the manufacturer's recommendations (Fumouze Diagnostic, France).

#### Statistical Analysis

All statistical data analyses were implemented using SPSS for windows version 16.0. Statistical evaluation was produced by Chi-square test and P < 0.05 was considered to be significant.

#### Ethical Approval and/or Informed Consent

Informed written consent was obtained from each participant. The study was approved by the local Clinical Research Ethical Committee.

## RESULTS

Out of 545 samples, antibodies to *E. granulosus* were detected in 148 (27.2%) patients with ELISA, 83 patients (15.2%) with IHA test and 69 patients(12.6%) were positive with both tests.

Statistical comparison of two dependent categorical values (ELISA and IHA) showed statistical significance p <0.001 (Table 1).

According to nationality information; 17 of 545 patients were Syrian citizen and 6 of them were seropositive with both ELISA and IHA.

Seropositivity of ELISA rate was 27.8% (103/371) among female, and 25.9% (45/174) among male patients. Seropositivity of IHA rate was 15.6 % (58/371) among female, and 14.4% (24 /174) among male patients. There were no statistically significant differences between male and female individuals and positivity, P=0.701 (Table 2).

Table 1: Comparison of ELISA and IHA results

			IHA results		
			Negative	Positive	Total
ELISA results	Negative	Count	383	14	397
		% within ELISA results	96.5%	3.5%	100%
	Positive	Count	79	69	148
		% within ELISA results	53.4%	46.6%	100%

In the distribution of age groups, the seropositivity of anti-*E. granulosus* IgG antibodies was increased in patients aged 40-49 years (Table 3). According to ELISA and IHA results, 28 patients (18.9%) and 18 patients (21.7%) were in this age group, respectively. CE is a chronic disease, and hydatid cysts grow very slowly, this causes to delayed

presentation, suggesting it is a disease of middle-aged people.

Lesions distribution of organ involvement in suspected patients is in order to liver 498 (91.4%), lung 13 (2.4%), spleen 12 (2.2%), multiple organ involvement 6 (1.1%) and other organ involvement 16 (2.9%) .

Table 2: Comparison of ELISA and IHA results by gender

	ELISA (%)		IHA (%)	
	Negative	Positive	Negative	Positive
Male	129 (74.1%)	45 (25.9%)	149 (85.6%)	25 (14.4%)
Female	268 (72.2%)	103 (27.8%)	313 (84.4%)	58 (15.6%)
Total	397 (72.8%)	148 (27.2%)	462 (84.8%)	83 (15.2%)

Table 3: Comparison of ELISA and IHA results by age

	ELISA (%)		IHA (%)	
0-19	18 (4.5%)	18 (12.2%)	28 (6.1%)	8 (9.6%)
20-29	29 (7.3%)	23 (15.5%)	39 (8.4%)	13 (15.7%)
30-39	76 (19.1%)	24 (16.2%)	86 (18.6%)	14 (16.9%)
40-49	79 (19.9%)	28 (18.9%)	89 (19.3%)	18 (21.7%)
50-59	82 (20.7%)	23 (15.5%)	93 (20.1%)	12 (14.5%)
60-69	69 (17.4%)	17 (11.5%)	78 (16.9%)	8 (9.6%)
70 and Older	44 (11.1%)	15 (10.1%)	49 (10.6%)	10 (12.0%)
Total	397	148	462	83

## DISCUSSION

Human echinococcosis is an endemic parasitic disease in many areas of the world, including Turkey. Information on the frequency of CE is mostly available and can be found from the hospital and the Ministry of Health records in Turkey (2). According to the Ministry of Health sources, the number of human cases with CE surgery is reported to be 0.8–2 per 10 5 population in each year (9).

The seroprevalence rate of human CE is reported that 0.94 %- 14.6% in different regions of Turkey (9,22).

Despite the fact that CE is one of the most important zoonotic diseases in a different regions of Turkey, there is limited information about its epidemiology (1).

In this study, we aimed to detect seropositivity of patients with suspicious CE in Gaziantep Province and contribute to control program with our data.

Seropositivity of ELISA rate was 27.8% (103/371) among female, and 25.9% (45/174) among male patients and seropositivity of IHA rate was 15.6 % (58/371) among female and 14.4% (24 /174) among male patients and no significant correlation between gender and positivity was found,  $P=0.701$ . In published papers mostly have reported that CE is more common in women than man. It has been stated that this situation may be due to the fact that the woman is responsible for animal care, eating and cleaning and the possibility of exposure to parasite eggs in this process(19,23).

Cystic echinococcosis is diagnosed at almost all ages, and the possibility of infection with age is increasing (1)). In results of our research, the positive patients in the > 30 years age groups was remarkably higher than younger age groups. The seropositivity rate reached a peak in the 40–49 age groups. CE is a chronic disease, and hydatid cysts grow very slowly, this causes to delayed presentation, suggesting it is a disease of middle-aged people. In published papers, CE has been shown to increase with age, as in our results (1,13,24)

It has been declared that hydatid disease may seen in every organ in the human body but it is most commonly seen in the liver, then lungs (25–27). In our study, lesions distribution of organ involvement in suspected patients is liver 498 (91.4%), lung 13 (2.4%), spleen 12 (2.2 %), multiple organ involvement 6 (1.1%) and other organ involvement 16 (2.9%). The results of our study are consistent with the information on organ distribution.

According to our results, the overall seropositivity of the ELISA test was higher than the IHA test (ELISA and IHA; 27.2% and 15.2 % respectively). Delibaş et al. published that 17% of 465 patients were positive by ELISA and 14% by IHA, and only 12% of the patients were found positive with both tests (28). In another study, the diagnostic value of ELISA, IFAT and IHA method was investigated, and 35 (87.5%) of 40 patients with CE were positive by ELISA, 33 (82.5%) by IFAT and 36 (90%) by IHA (29). The diagnostic performance of serological methods varies considerably in published studies. It has been reported that the sensitivity and specificity of the serological tests vary according to the characteristics of the antigen , the host from which the antigen was obtained, the patient's immune response and used method. (30).

There are several tests that can be used in the serodiagnosis and follow-up of CE. Among these tests, IHA and ELISA tests are mostly preferred, and there is also a need for confirmation tests such as Western Blot(28). Therefore, it has been reported that using more than one test in serological diagnosis will increase sensitivity and specificity (28–32).

Efforts of preventive measures should be continued to

control echinococcosis, in both animals and humans side, by increasing people awareness of the disease with training programmes and health education.

## CONCLUSION

In conclusion, serological tests provide an extremely important information related to the diagnosis and prognosis of disease. The present study prove the importance of diagnosing human CE and indicates that CE still is important public health problem in Gaziantep, Turkey as well as other parts of the country. Therefore, these areas of interest need to be investigated more comprehensively on larger groups of patients.

**Competing interests:** The authors declare that they have no competing interest.

**Financial Disclosure:** There are no financial supports.

**Ethical approval:** Approved by Ege University Faculty of Medicine Clinical Research Ethics Committee. Protocol No: 16-9.1/13

## REFERENCES

1. Akalin S, Kutlu SS, Caylak SD, et al. Seroprevalence of human cystic echinococcosis and risk factors in animal breeders in rural communities in Denizli, Turkey. *J Infect Dev Ctries*. 2014;8:1188–94.
2. Altintas N. Past to present: Echinococcosis in Turkey. *Acta Trop*. 2003;85:105–12.
3. Cengiz ZT, Yilmaz H, Beyhan YE, et al. [Cystic Echinococcosis Seropositivity in the Blood Samples Sent to Parasitology Laboratory of Yuzuncu Yil University Medical Faculty between 2005 and 2013. 2015;39:209–11.
4. Fotiou V, Malissiova E, Minas A, Petinaki E, Hadjichristodoulou C. Seroprevalence of IgG antibodies against *Echinococcus granulosus* in the population of the region of thessaly, Central Greece. *PLoS One*. 2012;7:7–9.
5. Siracusano A, Teggi A, Ortona E. Human Cystic Echinococcosis : Old Problems and New Perspectives. *Interdiscip Perspect Infect Dis*. 2009;2009:1–7.
6. Lawn SD, Bligh J, Craig PS, Chiodini PL. Human cystic echinococcosis: Evaluation of post-treatment serologic follow-up by IgG subclass antibody detection. *Am J Trop Med Hyg*. 2004;70:329–35.
7. Engström ELS, Salih GN, Wiese L. Seronegative, complicated hydatid cyst of the lung: A case report. *Respir Med Case Reports*. 2017;21:96–8.
8. Akcam AT, Ulku A, Koltas IS, et al. Clinical characterization of unusual cystic echinococcosis in

- southern part of Turkey. *Ann Saudi Med.* 2014;34:508–16.
9. Altintas N, Yazar S, Yolasigmaz A, et al. A sero-epidemiological study of cystic echinococcosis in İzmir and its surrounding area, Turkey. *Helminthologia.* 1999;36:19–23.
  10. Manciuilli T, Mariconti M, Vola A, et al. Cystic Echinococcosis in the Mediterranean. *Curr Trop Med Reports.* 2017;4:235–44.
  11. Altintas N. Control / Elimination Programme of Cystic Echinococcosis in Turkey. 28th World Congress of Echinococcosis "Toward the Control and Elimination of Echinococcosis", 29-31 October 2019, Lima, Peru.
  12. Tamarozzi F, Akhan O, Cretu CM, et al. Prevalence of abdominal cystic echinococcosis in rural Bulgaria, Romania, and Turkey: a cross-sectional, ultrasound-based, population study from the HERACLES project. *Lancet Infect Dis.* 2018;18:769–78.
  13. Fotiou V, Malissiova E, Minas A, Petinaki E, Hadjichristodoulou C. Seroprevalence of IgG antibodies against *Echinococcus granulosus* in the population of the region of thessaly, Central Greece. *PLoS One.* 2012;7:7–9.
  14. Ertabaklar H, Dayanir Y, Ertug S. Research to Investigate the Human Cystic Echinococcosis with Ultrasound and Serologic Methods and Educational Studies in Different Provinces in Aydın/Turkey. *Turkish J Parasitol.* 2012;36:142–6.
  15. Hakverdi S, Sayar H, Yaldiz M, et al. Unusual Localization of Echinococcosis in Cukurova (134 Case). *Turkish J Parasitol.* 2009;33:77–81.
  16. Kilimcioğlu AA, Girginkardeşler N, Korkmaz M, et al. A mass screening survey of cystic echinococcosis by ultrasonography, Western blotting, and ELISA among university students in Manisa, Turkey. *Acta Trop.* 2013;128:578–83.
  17. Özkol M, Kilimciolu AA, Girginkardeşler N, et al. A discrepancy between cystic echinococcosis confirmed by ultrasound and seropositivity in Turkish children. *Acta Trop.* 2005;93:213–6.
  18. Utuk AE, Simsek S, Koroglu E, McManus DP. Molecular genetic characterization of different isolates of *Echinococcus granulosus* in east and southeast regions of Turkey. *Acta Trop.* 2008;107:192–4.
  19. Yazar S, Ozkan AT, Hokelek M, et al. Cystic Echinococcosis in Turkey from 2001-2005. *Turkish J Parasitol.* 2008;32:208–20.
  20. Yazar S, Yaman O, Cetinkaya F, Sahin I. Cystic echinococcosis in Central Anatolia, Turkey. *Saudi Med J.* 2006; 27:205-9.
  21. Yolasigmaz A, Reiterová K, Turk M, et al. Comparison of serological and clinical findings in Turkish patients with cystic echinococcosis. 2006;43:220–5.
  22. Yazar S. Cystic echinococcosis in Kayseri during the last six years. *Turkish J Parasitol.* 2005;29:241–3.
  23. Mor N, Diken Allahverdi T, Allahverdi E, Tekdoğan ÜY. Retrospective evaluation of patients diagnosed with cystic Echinococcosis at Kafkas University Faculty of Medicine's Surgical outpatients unit. *Turkish J Parasitol.* 2018;42:196–201.
  24. Tiaoying L, Jiamin Q, Wen Y, et al. Echinococcosis in Tibetan populations, western Sichuan Province, China. *Emerg Infect Dis.* 2005;11:1866–73.
  25. Beyhan YE, Cobanoglu U, Celik S, et al. Molecular characterization of human lung and liver cystic Echinococcus isolates in Van province, Turkey. *Acta Trop.* 2020;206-1054512.
  26. Doghan A, Hussein K, Jasim A. Laparoscopic treatment of hepatic hydatid cyst. *Mustansiriya Med J.* 2017;16.
  27. Tuxun T, Zhang J hui, Zhao J ming, et al. World review of laparoscopic treatment of liver cystic echinococcosis-914 patients. *Int J Infect Dis.* 2014;24:43–50.
  28. Delibas S, Ozkoc S, Sahin S, et al. Evaluation of patients presenting with a suspicion of cystic echinococcosis to the serology laboratory of the Parasitology Department of Dokuz Eylul University Medical Faculty. *Turkish J Parasitol.* 30:279-81.
  29. Sari C, Ertuğ S, Karadam SY, et al. The comparative evaluation of Enzyme Linked Immunosorbent Assay (ELISA), Indirect Hemagglutination Test (IHA) and Indirect Fluorescent Antibody Test (IFAT) in the diagnosis of cystic echinococcosis. *Turkish J Parasitol.* 2009;33:73-76.
  30. Gottstein B. Molecular and immunological diagnosis of echinococcosis. *Clin Microbiol Rev.* 1992;7:248-61.
  31. Cetinkaya U, Hamamci B, Kaya M, et al. Investigation of anti-*Echinococcus granulosus* antibodies in patients with suspected cystic echinococcosis. *Turkish J Parasitol.* 2012;36:57–60.
  32. Kilic S, Dogruman Al F, Celebi B, Babür C. The Investigation of the Seroprevalence of Cystic Echinococcosis in Veterinary Surgeons. *Turkish J Parasitol.* 2007;31:109-11.