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Original Article

Serological Screening of Patients Diagnosed with Alveolar Echinococcus Disease in Their Home Regions

***Onder Akkas [1], Hakan Uslu [2], Isa Yilmaz [3], Yener Aydın [4], Ercan Korkut [5], Ahmet Yilmaz [6]**

1. *Department of Medical Microbiology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey*
2. *Department of Medical Microbiology, Faculty of Medicine, Ataturk University, Erzurum, Turkey*
3. *Department of Animal Production and Technology, Faculty of Applied Sciences, Mus Alparslan University, Mus, Turkey*
4. *Department of Thoracic Surgery, Faculty of Medicine, Ataturk University, Erzurum, Turkey*
5. *Department of General Surgery, Faculty of Medicine, Atatürk University, Erzurum, Turkey*
6. *Department of Medical Laboratory Techniques, Erzurum Vocational School of Health, Ataturk University, Erzurum, Turkey*

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***Correspondence Email:**
onder.akkas@erzincan.edu.tr

Abstract

Background: We aimed to determine the prevalence of Alveolar echinococcusis using ELISA in our region, to perform the specific differentiation of species by using the Western Blott method, to diagnose and treat this disease effectively in early periods, and to inform the patients about the results quickly.

Methods: From the provinces of Erzurum, Kars, Ardahan, Iğdır, Ağrı and Erzincan in the Eastern Anatolia Region, Turkey in August-September 2017, blood samples were provided from 305 participants (volunteer patients and their relatives), including 151 females (49.50%), whose ages ranged between 6-85 yr and were diagnosed as Alveolar echinococcosis (AE) and operated in concerned clinics.

Results: EIgG ELISA was determined as positive in 29 (9.5%) participants, including 17 (11.3%) females and 12 (7.8%) males. In addition, Em2-Em18 ELISA was also determined as positive in 15 (4.9%) participants, including 9 (6%) females and 6 (3.9%) males. Tweleve (3.94 %) of these findings were observed as positive in terms of both tests. Through the verification done by Anti-EWB IgG, *Echinococcus multilocularis (EM)* was identified in 7 (21.9%) of the female participants, *E. granulosus (EG)* in 1 (3.1%), and both parasites in 2 (6.2%). For male participants, EM was determined in 3 (9.3%) of them, EG in 1 (3.1%), and both parasites in 1 (3.1%).

Conclusion: AE continues to threaten public health in the region. In families where AE is detected positive, the reason for disease is based on nutrition; thus, it will be proper to check up all family members in the terms of infection.

Introduction

One of the most deadly zoonotic diseases caused by *Echinococcus multilocularis* (EM) metacestodes in humans is Alveolar echinococcosis (AE) (1). Alveolar echinococcosis is an endemic zoonosis that is common in non-tropical regions of the world, especially in the northern hemisphere. It is also seen mainly in the Eastern Anatolia Region in Turkey (2). The disease occurs when people consume food contaminated with eggs excreted in the feces of EM-infected carnivorous final-host animals such as wild cats, foxes, and rodents (3).

It is difficult and important to distinguish AE by cystic echinococcosis (CE) and although its preliminary diagnosis is usually done by using radiological imaging techniques today, it is important to support this preliminary diagnosis with serological diagnostic methods (4). In the serological diagnosis of CE and AE, diagnostic methods such as Western Blot (WB), Enzyme-Linked Immunosorbent Assay (ELISA), Indirect Hemagglutination (IHA), and Indirect Fluorescent Antibody Test (IFAT) are usually used. Radiological imaging techniques are insufficient in patient follow-up after treatment, so it is important to follow up patients with serological diagnostic methods (5).

We aimed to reveal the prevalence of parasitosis by ELISA, to make a specific distinction between species using the WB method on the positive results obtained, to ensure the patients receive fast and effective treatment against the disease, and to inform them about the results.

Materials and Methods

The contact information of people previously diagnosed with AE and had been examined or operated in the General Surgery and Thoracic Surgery clinics of Ataturk University Yakutiye Research Hospital was obtained in April 2017 from the automation system. Determined patients and their relatives were selected as the study group. In the study, the addresses of patients with CE and AE who lived in the Turkey's Eastern Anatolia Region and had been examined or operated on beforehand were visited and blood was drawn voluntarily from 305 people aged 6-85 years.

Echinococcus IgG-ELISA (Novalisa *Echinococcus* IgG-ELISA, NovaTec, Germany), *E. multilocularis* Em2-Em18 ELISA (Em2-Em18 plus ELISA; Bordier Affinity Products, Crissier, Switzerland) kits, and Anti-Echinococcus EUROLINE WB IgG (Euroimmun Medizinische Labordiagnostica AG) verification kit were used.

After receiving the contact and address information of 48 patients previously operated on or treated, we tried to contact them or their relatives. Ten patients were contacted and blood was drawn from them and their relatives. The address and contact information of the other 38 patients were either out of date or they did not want to meet with us, or their relatives did not agree to meet with us because the patients had died. In addition, blood was also drawn from 36 people from regions where there was no disease.

After collecting all blood samples included in the study, laboratory studies were started. After the serum samples were removed from -30 and kept at room temperature for 30 min, all samples were first studied with *Echinococcus* IgG-ELISA (Novalisa *Echinococcus* IgG-ELISA, NovaTec, Germany) based on the company's test procedure. Then, by using the *E. multilocularis* Em2-Em18 ELISA (Em2-Em18 plus ELISA; Bordier Affinity Products, Crissier, Switzerland) test kit, all samples were studied with the Micro-ELISA device in accordance with the company's working principles.

With the previously studied *Echinococcus* IgG-ELISA (Eigg ELISA) and *E. multilocularis* Em2-Em18 ELISA (Em2-Em18 ELISA) test kits, IgG antibodies were searched in the blood samples. The positive blood samples (32 samples) obtained in these efforts were studied based on the Anti-*Echinococcus* EUROLINE-WB-IgG (Euroimmun Medizinische Labordiagnostica AG) (Anti-EWB IgG) test procedure for the verification test.

Ethical approval

This study was extracted from the first author's Doctoral Thesis titled "Serological Screening of Patients Diagnosed as Alveolar *Echinococcus* Disease in Their Home Regions". Ethics Committee approval of this study was carried out in accordance with the framework of decision No. B.30.2.ATA.0.01.00/3 and No. 29 of the Ethics Committee for Clinical Research of the Faculty of Medicine of Ataturk University dated 29.01.2016.

Results

According to the ELISA results, EIgG ELISA was positive in 29 (9.51%) participants from whom blood was drawn; while it was positive in 17 (5.57%) of them in terms of EIgG ELISA, it was positive in only 12 (3.94%) of them in terms of both tests. According to the Em2-Em18 ELISA test study, on the other hand, 15 (4.92%) participants had positive results, including only 3 (0.98%) of them in terms of Em2-Em18 ELISA and 12 (3.94%) of them in terms of both tests.

EIgG ELISA results of the blood samples taken from 305 volunteers (151 females: 49.50% and 154 males: 50.50%) were positive in 29 participants, including 17 females (11.3%) and 12 males (7.8%). Em2-Em18 ELISA results were positive in a total of 15 (4.9%) participants. However, the results of both tests were not statistically significant. In the verification conducted with anti-EWB IgG, 7 (21.9%) of the females had EM, 1(3.1%) had *E. granulosus* (EG), and 2 (6.2%) had both parasites. On the other hand, 3 (9.3%) of the males had EM, 1(3.1%) had EG, and 1 (3.1%) had both parasites (Table 1).

Table 1: ELISA and Western Blott positivity rates of the participants in the study group by their gender

Tests	***n/%***	***Gender***		*Total*
		Female n=151	Male n=154	
EIgG-ELISA	n	17	12	29
	%	11.3	7.8	9.5
Em2-Em18 ELISA	n	9	6	15
	%	6	3.9	4.9
WB IgG *granulosus*	n	1	1	2
	%	3.1	3.1	6.2
E. multilocularis	n	7	3	10
	%	21.9	9.4	31.3
Both of them	n	2	1	3
	%	6.2	3.1	9.3
Negative	n	9	8	17
	%	28.1	25	53.1

EIgG ELISA test results were positive in 28% of the participants who consumed green veggies or fruits raw or through cooking without washing thoroughly. The result was positive in only 1 (3.4%) of the participants who did not eat raw. In terms of the incidence of this disease, the difference between participants who eat raw and those who do not eat raw was statistically significant (c^2= 4.754, *P*=0.029). Regarding the relationship between Em2-Em18 ELISA and raw eating habits of people, the results Em2-Em18 ELISA was determined as positive in 14 (93.3%) of the participants who ate raw, and while it was determined positive only in 1 (6.7%) of the participants who did not have a raw eating habit. However, this difference was not statistically significant (c^2= 1.439, *P*=0.230).

In terms of the relationship between the presence of EgIgG ELISA and AE awareness, 21 of the participants who had positive test results had information about AE, while 8 of them had no information about AE. For the first time, EIgG ELISA test results were positive. In terms of Em2-Em18 ELISA positivity, there was a statistically significant difference between having knowledge of AE and having no knowledge (c^2 = 6.361, *P*=0.012). In the majority of those whose tests were positive, the AE information sources were the 1st and 3rd-degree relatives. As a result of the verification done by the Anti-EWB IgG test method, out of 29 participants examined by the EIgG-ELISA method and whose test results were positive, nine were verified only as *E. multilocularis*, two were verified only as *E. granulasus*, and three were verified as both *E. multilocularis* and *E. granulasus*. One of the three samples that found negative with the EIgG-ELISA test was verified only as EM by the Anti-EWB IgG method, and the remaining two were verified as negative. Fifteen blood serum samples with a positive Em2-Em18 ELISA test result were studied with the Anti-EWB IgG test. Out of the positives, eight were confirmed as *E. multilocularis* only and two were confirmed as *E. granulosus* only. Out of the other five, three were identified as both *E. granulasus* and *E. multilocularis*. On the other hand, two samples with negative Em2-Em18 ELISA test results were determined as *E. multilocularis* only by the Anti-EWB IgG test. The remaining two were verified as negative (Table 2).

Table 2: The distribution of Western blot positivity in patients in whom ELISA EIgG and Em2-Em18 were detected positive serologically

WB Test Result	***n/%***	***ELISA Results***			
		EIgG (n=29)		Em2-Em18-IgG (n=15)	
		Negative	Positive	Negative	Positive
E. multilocularis	n	1	9	2	8
	%	3.1	28.1	6.2	25
E. granulosus	n	0	2	0	2
	%	0.0	6.3	0.0	6.2
Both of them	n	0	3	0	3
	%	0.0	9.4	0.0	9.4
Negative	n	2	15	15	2
	%	6.2	46.9	46.9	6.2
Total	n	3	29	17	15
	%	9.3	90.7	53.1	46.9

When the verification result (obtained by the Anti-EWB IgG test) of the blood samples of 10 people diagnosed with AE was examined, four of them were negative, four of them were *E. multilocularis*, and the remaining two were positive in terms of both parasites. *E. multilocularis* was detected in four people among first-degree relatives and it was detected in only one person among the third-degree relatives, while it was not found in the second-degree relatives. *E. granulosus*, on the other hand, was detected only in one person in each of the second and third-degree relatives. One of both parasites was detected in the second-degree relatives (Table 3).

Table 3: Distribution of AE information source according to WB test result

Description		***N/%***	***AE Information Source***						***Total***
			No AE Information	**Him/herself**	**First-degree relative**	**Second-degree relative**	**Third-degree relative**	**Other intimates**	
Negative		N	128	3	57	7	27	51	273
		%	46.9	1.1	20.9	2.5	9.9	18.7	100.0
WB (n=32)	*E. multilocularis*	N	1	4	4	0	1	0	10
		%	10.0	40.0	40.0	0.0	10.0	0.0	100.0
	E. granulosus	N	0	0	0	1	1	0	2
		%	0.0	0.0	0.0	50.0	50.0	0.0	100.0
	Both of them	N	0	2	0	1	0	0	3
		%	0.0	66.7	0.0	33.3	0.0	0.0	100.0
	Negative	N	8	1	3	0	1	4	17
		%	47.1	5.9	17.6	0.0	5.9	23.5	100.0
Total		N	137	10	64	9	30	55	305
		%	44.9	3.3	21.0	3.0	9.8	18.0	100.0

E. multilocularis was not encountered in samples taken from volunteers living in areas where the disease was not observed. Moreover, in the verification test of 32 positive blood samples done with the Anti-EWB IgG technique, *E. Multilocularis* IgG antibodies were detected in the blood samples of three participants for the first time. They were referred to the appropriate clinic and their radiological examinations were positive.

Discussion

According to our results, EIgG-ELISA positivity was detected in 9.51% of 305 participants from whom blood was drawn and Em2-Em18-ELISA positivity was detected in 4.92% of the participants, while both ELISA positivity was detected at a rate of 3.94%

There are differences between studies in terms of the distribution of echinococcosis positivity by gender. Overall, 162 cases of AE were detected in 24 different studies published in Turkey between 2000 and 2010, and 62.20% of these were observed in females (6).

In a study conducted to design the best serological strategy for the diagnosis of human AE cases in routine operations of the medical laboratory, 46 out of 47 (24 females and 23 males aged 23-79) AE patient serum samples as positive by using IHA and 2 screening techniques such as Em2plus-ELISA and/or recEm18-ELISA (7). They used Immunoblot (recEm18-IB) to verify the results. After all, based on the data obtained in their study, they strongly recommended the use of IHA associ-

ated with at least 1 ELISA method, and then the use of 1 or 2 Immunoblots based on the patient's origin for verification purposes. In similar studies (8, 9), there was AgB-WB with reliable precision to separate both AE and KE-originated *Echinococcus*, the most specific one for AE was Em18–WB, and Em2plus-ELISA was reliable for the specific diagnosis of AE.

There was conformity between the results of our verification studies conducted with anti-EWB IgG and previous studies and their results (7-16). In our study, 3 participants whose tests were found positive for both EG and EM received a diagnosis of both KE and AE in the hospital records. A similar situation was found in another study (16). Overall, 31 AE patients were treated with albendazole and then with Mebendazole therapy after liver resection (12). As a result, no recurrence was observed in patients, and the results of RecEm18 WB and RecEm18-ELISA were also negative. A similar situation was also observed in our study. Anti-EWB IgG verification of 4 out of 10 patients was found negative. Based on the hospital records, we determined that these patients were people who had been receiving long-term treatment. Antigen titers decrease in patients who have been taking medication for a long time (12, 17-19). Since Anti-EWB IgG verification could not be made on all blood samples in our study, specificity and sensitivity applications could not be performed on the results. Nevertheless, as a result of verification carried out with Anti-EWB IgG, serious results were detected. Therefore, we consider the routine application of the Em2-Em18 ELISA or Anti-EWB IgG test, especially in areas where AE is often encountered, as an important requirement

In our study, EIgG-ELISA was positive in 28 (96.60%) of the participants who consumed green vegetables or fruits raw without thoroughly washing or cooking them. On the other hand, only 1 (3.40%) of the participants who did not eat raw food had a positive result. As a result, in terms of the incidence of this disease, the difference between people who eat raw and those who do not eat raw was statistically significant. In terms of the relationship between Em2-Em18 ELISA and people's raw eating habits, Em2-Em18 ELISA results were positive in 14 (93.3%) of the participants who ate raw, while they were positive only 1 (6.7%) of the participants not have a raw eating habit. The majority of AE cases are observed in people who live in rural areas and spend their livelihood in agriculture and animal husbandry, there is a cycle of wolves, foxes, dogs, and wild animals in the habitats of most of these people, and transmission occurs when local plants found in nature are collected by humans and eaten without washing or cooking.

Overall, 137 (44.9%) of 305 people who participated in the study had answered "no" to the question of whether they had information about AE previously, while 168 (55.1%) answered "yes". EIgG/ Em2-Em18 ELISA was detected as positive in the blood samples of 9 (28.1%) of the participants without AE knowledge. As a result of verification performed on nine blood sera by the anti-EWB IgG test, 1 (3.1%) of them was verified as *E. multilocularis*, while the remaining 8 (25%) were determined as negative. Overall, 168 participants with AE knowledge were asked about the source of this information. 10 (3.3%) of these participants were people who had been personally diagnosed with AE and had been treated with medication or surgery. Because the test result of 3 (1.1%) of these 10 participants was determined negative as a result of the EIgG and Em2-Em18 ELISA test studies of the blood samples, Anti-EWB IgG verification was not performed. As a result of the verification done for the remaining 7 participants by the Anti-EWB IgG test, 4 (4%) of them had *E. multilocularis* and 2 (2%) had both parasites, while the remaining 1 (1%) was negative. Whereas 4 (4%) of the first-degree relatives and only 1 (1%) of the third-degree relatives

had *E. multilocularis*, it was not encountered in the second-degree relatives. *E. granulosus*, on the other hand, was detected in only one (1%) person in each of the second and third-degree relatives. One of both parasites was detected in second-degree relatives.

Conclusion

E. Multilocularis was not detected in samples taken from volunteers living in areas where the disease was not observed. Anti-EWB IgG was positive at a rate of 4.3% in the places where AE was observed. Three of these cases were undiagnosed cases, and their treatment was started by referring them to the relevant clinics. This disease can also be transmitted to close people in the environment where AE is observed, so parasitosis continues to pose a threat to public health in the region.

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Conflicts of interest

The authors declare there are no conflicts of interest.

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