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A pilot comparative study between serological and genetic investigations in relationship to clinical outcomes on patients with cystic echinococcosis

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Summary

The aim of this study was to investigate whether Enzyme-Linked Immunosorbent Assays (ELISA) and Western Blotting (WB) methods could contribute to the assessment of clinical outcomes in genotype-defined cystic echinococcosis (CE) patients. Twenty-nine human isolates and blood samples have been taken from patients who underwent surgery or percutaneous aspiration (PAIR) for therapeutic purposes at Ege University and Manisa Celal Bayar University Hospitals. All sera of patients were screened for the presence of *E. granulosus* IgG antibodies using in-house approved ELISA and WB methods. According to the ELISA results, five patients had high, thirteen patients had medium and eight patients had low specific antibody level response which ranged 1/640 -1/5000. Despite confirmed WB positivity three patients were found to be negative by ELISA. Immunoblot analysis of EgAg showed many protein bands with size of 8, 12, 20, 22, 24, 36, 75 and 90 kDa. Among of them, 8 – 12 kDa bands (90 %), 20 – 22 kDa and 36 kDa bands presented strong reactivity against human serum specimens. No serum samples from healthy control reacted with EgAg. Phylogenetic analysis of resulting COX1 and NAD1sequences has revealed that all patients in our study were infected with the *E. granulosus* G1-G3 genotype. There was no consistent correlation between results of ELISA and WB, the number or size of cysts and genotype. Our study brings a unique contribution in terms of relationship between serological investigation, disease genotypes and clinical outcomes.
Keywords: Cystic echinococcosis; ELISA; western blotting; serology; sequence analysis

Introduction

Cystic echinococcosis (CE) is a parasitic disease spread worldwide. It establishes a major public health problem in the regions where sheep and cattle are bred and used for the consumption. The CE is endemic and occurs frequently in Mediterranean regions, Middle East, Asia, North and East Africa, Australia and South America, and some European countries (McManus *et al*., 2003). In Turkey, the CE has a negative impact on the national economy as well as the health, and still remains one of the most important and serious helminthic diseases. It is quite common due to the wide prevalence of stray dogs and the lack of necessary sanitary precautions (Altintas, 2003; Šnábel, 2009).

In humans, CE may be represented by wide spectrum of clinical manifestations. Clinical signs and symptoms of the disease are not specific and depend on the location of the cyst. The growth rates of cysts may vary between cysts in the same organ or within the same individual and between individuals in various regions. Early diagnosis and prompt treatment are essential to treat disease efficiently. Both, imaging techniques (US, CT) and serology provide useful and complementary information about the character of the cyst that may be relevant for therapeutic intervention. Sero-

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logical tests provide an extremely important information related to the diagnosis and prognosis of disease (Yolasigmaz *et al*., 2006; Manzano-Roman *et al*., 2015).

The CE genotype could affect the biological outcome and disease management In human G1 genotype is the most common (88.44 %) while the G6/G7 genotypes which are closely related strains are less frequent it (11.07 %). On the other hand the G5, G8, and G10 genotypes occur in human rarely (Alvarez Rojas *et al*., 2014). So far only one human case with G4 genotype was identified (Kim *et al*., 2017). However, for many genotypes of *E. granulosus* sensu lato we still have insufficient information regarding e.g. geographical distribution, host specificity, morphology and infectivity. In a study which compared the cysts locations, their size and other features isolated from various strains (*E.granulosus* G1, *E.ortleppi* and *E.canadensis* G6) was noted that G6 genotype showed accelerated growth rate (Guarnera *et al*. 2004, Alvarez Rojas *et al*., 2014).

Studies on the genotyping of *E. granulosus* isolates obtained from different geographic regions of Turkey and intermediate hosts (sheep, cattle, goat, camel, buffalo, horses, mules, and mouflon) confirmed the occurrence of G1-G3, G4 genotypes. Meanwhile in another group of intermediate hosts (sheep, cattle, goat, camel, buffalo, horses, mules) and human regarding the *E. granulosus* G1,G3 and G7 genotypes were confirmed (Simsek & Eroksuz 2009; Šnábel *et al*., 2009; Eryıldız *et al*., 2012; Altintas *et al*., 2013; Utuk *et al*., 2013; Gokpinar *et al*., 2017).

These reports also showed that both, the early CE diagnosis and appropriate hospitalization are very important for lessening the number of fatal cases and serious health outcome (Khachatryan, 2017). In addition, a good symptoms distinction and adequate information about the disease play an important role in death cases prevention (Belhassen-García, M, 2014). The, molecular identification of human CE cases should be suggested for better understanding of pathology, the disease outcome and epidemiology. The essential question which needs to be addressed is whether the link between clinical outcomes and distinct CE genotypes exists.

The aim of this study was to investigate the association between antigenic presentation and antibody response in CE genotype defined patients where the clinical outcomes based CE genotype were compared and analyzed.

Materials and Methods

Patient samples

Twenty-nine human isolates (germinal layer and/or protoscoleces) and blood samples have been taken from CE patients just before the surgery (22 patients) and the application of puncture-aspiration-injection-reaspiration (PAIR) (7 patients) for diagnostic purposes at Ege University Hospital and Celal Bayar University Hospital were collected. The livers cysts were classified according to the classification determined by WHO Informal Working Group on Echinococcosis (WHO-IWGE). According the USG results eight patients were classified as CE1, six patients were CE2, four patients were CE3 and three patients were CE4/CE5 by USG. Remaining cysts were determined by CT. In total, 29 hepatic CE cyst fluid and germinal layer isolates were obtained and examined under microscope for the presence of protoscoleces or hooklets. All samples were kept at -20 °C until further used. The details of demographic and clinical data obtained from the patients (age, sex, geographical area, cyst type, cyst location, size of cyst) were recorded. Regarding the control group, only the blood serum from healthy individuals of which the fecal examinations for the other parasitic infections including the CE were negative was used.

Serological Analysis

All serum samples of patients were screened for the presence of *E. granulosus* IgG antibodies using in-house approved ELISA and WB tests. In ELISA and WB tests, sheep hydatid fluid (HF) was used as the antigen. HF was collected from fertile cysts obtained at slaughterhouse in city Izmir, Turkey. After centrifugation at 10,000 g for 30 min at 4 °C, the antigen was concentrated by Amicon ultrafiltration with YM2 membrane (Amicon Corp., Danvers, MA, USA) and kept at -20 °C for subsequent use. Protein concentrations were determined by the Bradford protein assay kit (Bio-Rad) and bovine albumin used as a standard. Based on the results of ELISA tests, patient outcomes were interpreted taking into account the negative and positive serum readouts and cut-off values.

Enzyme-Linked Immunosorbent Assays (ELISA)

ELISA was carried out on polystyrene microtiter plates with 96 wells (F-Form; Maxisorp, Nunc, Fisher Scientific, USA) coated with 100 µl/well (at a concentration of 5 µg of proteins per well) of HF diluted in phosphate-buffered saline (PBS) buffer and incubated at +4 °C overnight. Plates were 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. Serum samples (100 µl) diluted 1:640 in 5 % non-fat milk with PBS-T were added and incubated for 1 hour at RT. After washing, plates were treated for 1 hour with alkaline-phosphatase anti-human IgG (Sigma) conjugate diluted at 1:5.000. After repeated washing, the reaction was stopped after about 20 min of incubation in dark by 100 µl of 1µg/ml p-Nitrophenyl Phosphate (pNPP) in dietanolamine buffer (DEAB). The optical density at 405 nm (OD405) was determined by ELISA plate reader (Thermo Labsystems Opsys MR, USA). Cut off values were determined by taking the average OD of negative control sera plus 3 standard deviations (SD).

Western Blotting Assay (WB)

Patients blood serum positive for the CE determined by ELISA were also confirmed by Western-blot technique. Electrophoresis (ELFO-SDS PAGE) was performed with Bio-Rad Mini Protein Slab Cell (Bio-Rad Laboratories, CA, USA) on a 12 % SDS-polyacrylamide gel and 4 % stacking gel under reducing conditions (Laemmli,

Table 1. Oligonucleotide primers used in PCR and DNA Sequencing for typing of *Echinococcus granulosus*.

Primers	Gene Regions	Nucleotide Sequences	Sources
MS1	NAD1	CGTAGGTATGTTGGTTTGTTTGGT	Sharbatkhori *et al*., 2009
MS2	NAD1	CATAATCAAATGGCGTACGAT	Sharbatkhori *et al*., 2009
JB3	CO1	TTTTTTGGGCATCCTGAGGTTTAT	Utuk *et al*., 2008
JB4.5	CO1	TAAAGAAAGAACATAATGAAAATG	Utuk *et al*., 2008
BD1	ITS-1	GTCGTAACAAGGTTTCCGTA	Bowless & McManus, 1993
4S	ITS-1	TCTAGATGCGTTCGAA(G/A)TGTCGATG	Bowless & McManus, 1993

1970). Antigens were electrophoresed at 60 V for approx. 2 h at room temperature. Low molecular weight markers (prestained SDS-PAGE standards, Bio-Rad) were included into each electrophoretic run. Following electrophoresis, proteins were transferred on nitrocellulose (NC) membrane in Tris-glycine buffer (pH 8.8) for 1h using a Bio-Rad Trans-Blot Cell. After blotting, the NC membrane was cut into 2 mm wide strips and blocked with 5 % (w/v) dry milk in Tris-Borate-Saline solution containing 0.1 % Tween 20 (TBS-T) (pH 7.2) for 1 h at room temperature. All serum samples of patients were diluted 1:100 with 0.5 % (w/v) dry milk in TBS-T and incubated in shaker for 1 h RT. The strips were than washed three times with TBS-T and reacted with alkaline-phosphatase-conjugated anti-human IgG (Sigma) at dilution 1:5000 for 1 hour at RT. Subsequently, the strips were washed again three times in TBS-T, and bands were visualized by incubating 5 min with 33 µl 5-bromo-4-chloro-3-indolyl phosphate and 330 µl nitro blue tetrazolium chloride (BCIP/NBT) in 10 ml alkaline phosphatase (ALP) buffer distributed evenly (1 ml) to the wells.

Molecular Analysis

For molecular evaluation the DNA was extracted from both, germinal layer and protoscoleces. The total genomic DNA was extracted with RTA-DNA Isolation Kit (Gebze/Kocaeli, Turkey) according to the manufacturer instructions. The amount of DNA in samples was determined in ng/µl by a spectrophotometry (NanoDrop ND-1000 Spectrophotometer) at a wavelength ratio of 260/280 nm. Kit-isolated DNAs were PCR-primed with primer sets specific for NAD1, COX1 and ITS-1 (Table 1). The extracted DNA was kept at -20 ° C until further analysis. All PCR products were run on a gel and gel images were imaged and photographed with UV gel imaging system (SYNGENE, Cambridge, UK) located at the Molecular Biology Laboratory of Medical Biology Department Faculty of Medicine of Manisa Celal Bayar University. After the PCR treatment, the resulting products were run on 3 % agarose gel and amplified with PCR using primer sets specific for typing of *E. granulosus*. Post-PCR specimens were cut with RsaI (Fermentas), MspI (Fermentas), and CfoI (Fermentas) ve AluI (Fermentas) enzyme for the determination of ribosomal ITS's-1 gene (PCR-RFLP). For genetical characterization of all isolates these procedures were applied to all patients and control group of individuals. Subsequently ribosomal ITS-1 gene region in all samples were digested with restriction endonucleases and deoxyribonucleic acid sequencing of the mi-

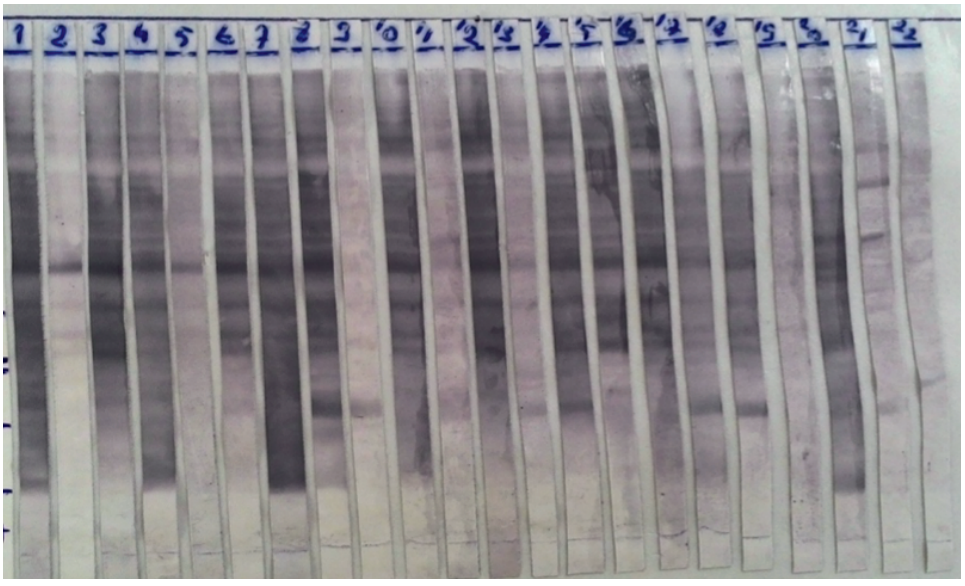

Fig. 1. Western Blot analysis of patient sera using HF (HF-WB). The lines represent: 1-Positive Control, 2-Negative Control, 3-22 Sera with confirmed CE.

Table 2. Gender and age, ELISA and Western Blot results, organ localization, molecular identification and clinical symptoms, drug used and dog owner informations of the 29 CE cases.

No of patient	Gender	Age	Province	ELISA Od value / Evaluation	WB bands (kDa)	Organ localisation	Genotype of *E. granulosus s.s.*	Drug Used /NA	Dog owner	Clinical symptoms
1	M	29	Kütahya	2,403/H	12, 20-22, 36, 75, 90	Liver right lobe	*E. granulosus s.s.* (G1-G3)	Y	Y	Mild pain
2	M	36	İzmir	2,859/H	8 12, 20-22, 36, 75, 90	Liver right lobe seg. 6-7	*E. granulosus s.s.* (G1-G3)	Y	N	Pain, palpable mass, headache
3	M	12	Izmir	1,047/M	8, 12, 20-22, 36, 75, 90	Liver right lobe posterior	*E. granulosus s.s.* (G1-G3)	Y	N	Pain
4	F	31	Bornova/İzmir	0,792/M	12, 20-22, 36, 75, 90	Liver right lobe seg. 6-7	*E. granulosus s.s.* (G1-G3)	Y	N	Pain, vomiting
5	M	36	Bergama/İzmir	0,865/M	8, 12, 20-22, 36, 75, 90	Liver left lobe seg. 7	*E. granulosus s.s.* (G1-G3)	Y	Y	Palpable mass, pain, nausea, vomiting,
6	M	63	Karabağlar/İzmir	2,069/H	8, 12, 20-22, 36, 75, 90	Liver right 4A-B	*E. granulosus s.s.* (G1-G3)	Y	N	Pain
7	M	53	Izmir	0,498/L	12, 36 (low), 75, 90	Liver seg. 4-5-6	*E. granulosus s.s.* (G1-G3)	Y	N	Palpable mass, pain, weakness, nausea
8	M	23	Manisa	0,974/M	20-22, 36, 75, 90	Liver right lobe	*E. granulosus s.s.* (G1-G3)	Y	Y	Palpable mass, pain
9	M	42	İzmir	0,469/L	12, 20-22, 36 (low), 75, 90	Liver right 4A-B	*E. granulosus s.s.* (G1-G3)	Y	N	Pain
10	M	40	Buca/İzmir	1,032/M	20-22, 36, 75, 90	Liver hilum	*E. granulosus s.s.* (G1-G3)	N	Y	Pain
11	F	30	İzmir	0,831/M	12, 36 (low), 90	Liver right 4B	*E. granulosus s.s.* (G1-G3)	Y	Y	Pain, nausea, vomiting
12	F	13	Merkez/Aydın	1,569/H	12, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	N	Pain, nausea, vomiting
13	F	61	Alaşehir/Manisa	0,552/L	20-22, 36, 75, 90	Liver right lobe	*E. granulosus s.s.* (G1-G3)	N	Y	Ağrı,çarpıntı
14	F	49	Denizli	1,058/M	8, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	N	Y	Pain, nausea
15	M	49	Söke/Aydın	0,936/M	8, 12, 20-22, 36	Liver	*E. granulosus s.s.* (G1-G3)	Y	N	Pain, palpitations
16	M	10	Gömeç/Balıkesir	0,099/N	12, 20-22 (low)	Liver	*E. granulosus s.s.* (G1-G3)	Y	Y	Palpable mass, pain, hepatomegaly, dizziness
17	F	62	Balıkesir	0,454/L	12, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	Y	Severe pain, nausea, vomiting, jaundice
18	F	19	Aydın	0,142/N	20-22 (low)	Liver	*E. granulosus s.s.* (G1-G3)	Y	Y	Palpable mass, severe pain
19	M	59	Akhisar/Manisa	0,944/M	36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	Y	Back stiffness, severe pain, hepatomegaly
20	M	30	Balıkesir	1,018/M	20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	N	Severe pain, nausea, vomiting, jaundice
21	F	15	Bornova/İzmir	0,243/N	12, 36, 75	Liver	*E. granulosus s.s.* (G1-G3)	Y	N	Severe pain, nausea
22	M	15	Izmir	0,551/L	8, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	N	Palpable mass, pain
23	F	52	Menemen/İzmir	1,063/M	8, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	Y	Pain
24	M	8	Ayvalık/Balıkesir	0,649/L	8, 12, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	Y	Palpable mass, fever, vomiting
25	M	26	Söke/Aydın	0,649/L	8, 12, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	Y	Severe pain, swelling
26	M	19	Akhisar/Manisa	1,066/M	8, 12, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	N	Pain
27	F	10	Manisa	0,498/L	12, 20-22	Liver	*E. granulosus s.s.* (G1-G3)	Y	N	Pain, loss of appetite, anemia
28	M	48	Muğla	1,262/H	8, 12, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	Y	Vomiting, weakness, hepatomegaly
29	M	10	Izmir	1,047/M	8, 12, 20-22, 36, 75, 90	Liver	*E. granulosus s.s.* (G1-G3)	Y	Y	Palpable mass, nausea, vomiting

ELISA: H: High, M: Medium, L: Low, N: Negative. Cut off value: 0,382
Drug used and dog owner: Y: Yes, N: No.
Drug used: NA (duration is not available)

tochondrial COX1 and NAD1 genes with ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Forward and reverse sequences of amplicons were examined with Sequencing Analysis software and their alignment analyses were performed with SeqScape V2.6 software (Applied Biosystems, Foster City, CA). Finally, the alignment analyses of all samples were accomplished with NCBI BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare the other *E. granulosus* sequence data. Phylogenetic analysis of strains was done by Genius software (Biomatters, New Zealand).

Statistical Analysis

Sensitivity, specificity, positive and negative predictive values were calculated using SPSS program (IBM Corporation, Chicago, USA).

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

Antibody responses caused by E. granulosus

All patients had from 1 to 3 of hydatid cysts in the liver (100 %). According to the ELISA results the samples of these patients were grouped as negative (-), low positive (+), medium positive (++) and high positive (+++). Five patients had high specific antibody response, thirteen patients had medium specific antibody response, and eight patients had low level of specific antibody. The response ranges were between 1/640 and1/5000. Three patients were found to be specific antibody negative. However, those three patients which were negative by ELISA were found to be positive by Western Blotting (Table 2). Immunoblot analysis of EgAg showed protein bands of 8, 12, 20, 22, 24, 36, 75 and 90 kDa size. Among of them, 8 – 12 kDa bands, 20 – 22 kDa and 36 kDa bands displayed strong reactivity against human serum specimens. No serum samples from healthy control reacted with EgAg (Fig.1 and Table 2). The most common clinical manifestation in case of hepatic cyst was abdominal pain which was present in 96 % of patients. The other complaints were palpable abdominal mass, hepatomegaly, nausea, vomiting etc. In all cases the USG and/or CT examinations confirmed the CE.

Molecular identification of Echinococcus species causing CE

Using the DNA Sequence Analysis Technique of CE patients, it was determined that the patients were infected with *Echinococcus granulosus s.s.* (Genotype G1-G3) (Fig. 2). Nineteen of the 29 cases were male (65.5 %) and 10 were female (34.5 %). Their ages ranged from 8 to 63. When the gender and age range of the patients were taken into account, CE occurrence and clinical outcomes were found to be in accordance with the literature information that can be seen in both genders and all age groups (Table 3). Most of them lived in rural areas without significant differences ($P > 0.05$) or kept a dog (55 %) for a long time. Data on patient gender, age and residence is available in Figure 3.

The NADH1 gene sequence profiles of all samples were detected as the same and compatible with sequence data with the GenBank

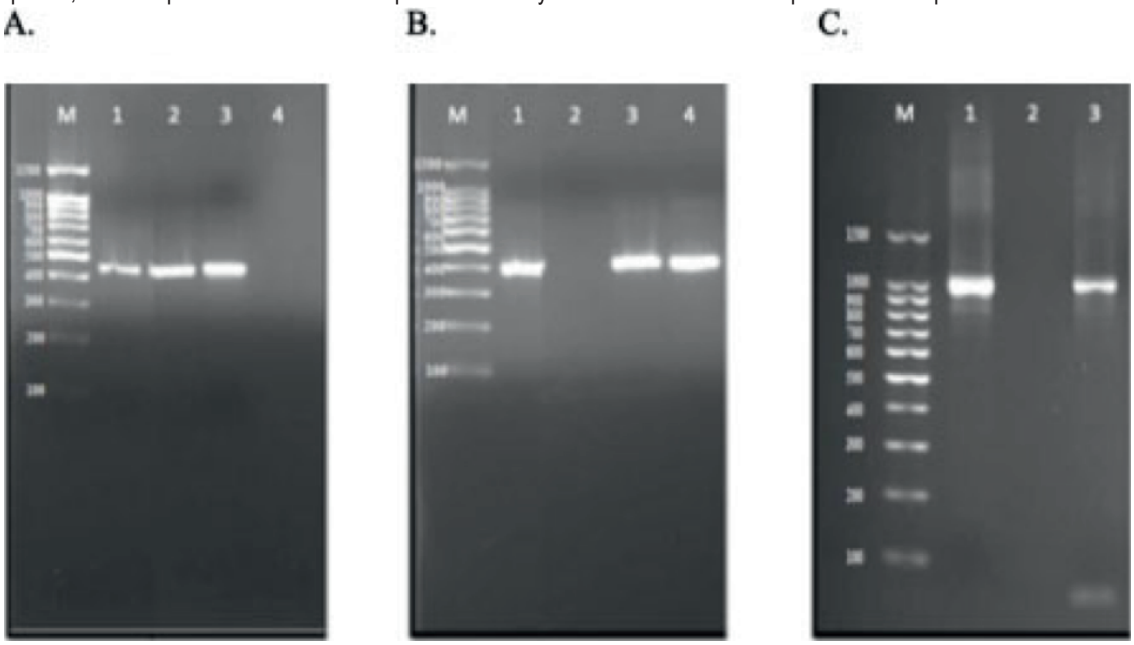

Fig. 2. CO1, NAD1 and ITS-1 Gene Amplicons. A. CO1 gene amplicons (446 bp). M:DNA Marker, 1:Positive Control, 2-3:Human Isolates, 4:Negative Control (Distilled water) B. NAD1 gene amplicons (378 bp). M:DNA Marker, 1:Positive Control, 2:Negative Control (Distilled water), 3-4:Human Isolates. C. ITS-1 gene amplicons. M:DNA Marker, 1:Positive Control, 2:Negative Control (Distilled water), 3:Human.

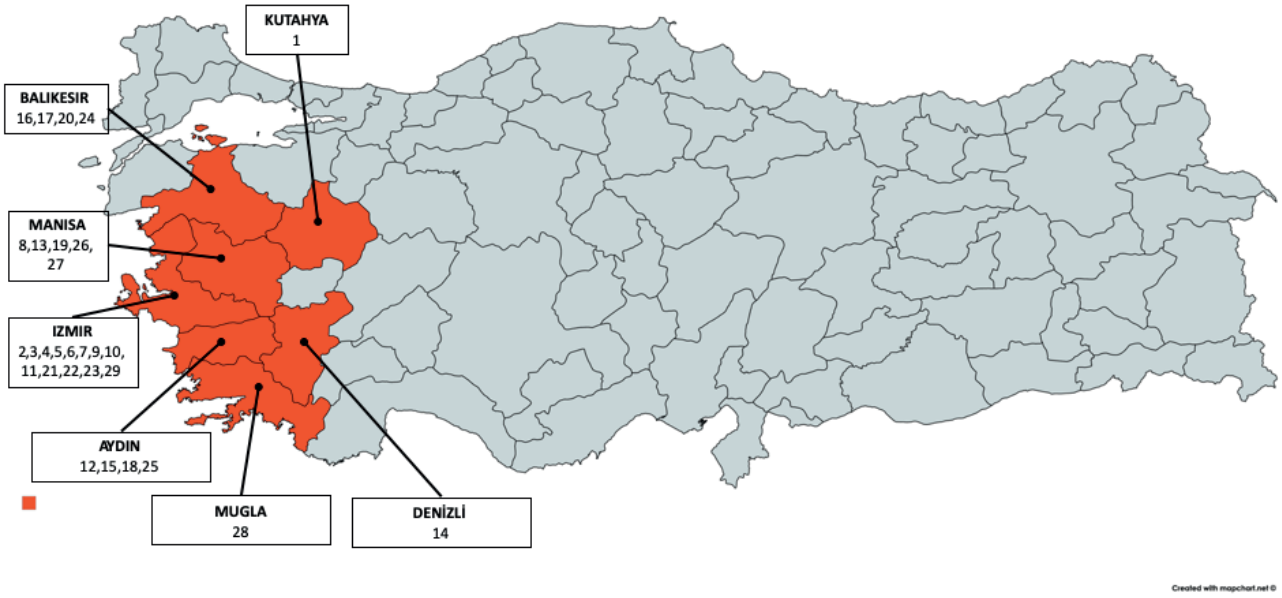

Fig. 3. A map of Turkey showing the distribution of 29 CE cases province caused by *E. granulosus*.

accession number MN270000, and accordingly, COX1 gene sequence profiles of all samples were compatible with KT001403 GenBank accession number (both sequences are attributable to *E. granulosus s.s.* G1-G3).

The forward and reverse sequences of all samples were analyzed and compared with the BLAST program. As a result of our study all patients found to be infected with the *E. granulosus s.s.* (G1-G3) genotype. There was no consistent correlation between results of ELISA and Western Blotting, the number or size of cysts and genotype.

Discussion

Human CE caused by the tapeworms *Echinococcus granulosus s.s* is among the most pathogenic helminthic zoonoses. The larval stages of the tapeworm *Echinococcus granulosus sensu lato* are the causative agent of CE, one of the most important cestodes infections responsible for the morbidity and mortality in humans and significant economic losses in livestock (Salamatin *et al.,* 2017). Around one million or more people are currently suffering from CE globally. The financial burden with up to $2 billion lost annually on the livestock industry is substantial (Torgerson & Macpherson, 2011).

In Turkey, as determined by DNA sequence analysis of COX1 region, the G1 is the dominant genotype in human and the other intermediate hosts (Utuk & Simsek, 2008). The current study which supported this interpretation examined the CE agents responsible for causing 29 cases of liver CE and all were found to be G1. It would also be interesting to see and compare results from different localities and CE patients with different genotypes and with clinical outcomes from various Turkey regions.

For clinical practice it should be noted that the ELISA utilizing crude hydatid cyst fluid has a high sensitivity (over 95 %) but its specificity is often unsatisfactory. It should be remembered that approximately 10 to 20 % of patients with hepatic cysts and about 40 % with pulmonary cysts do not produce detectable specific serum antibodies (IgG) and therefore give false-negative results

Table 3. Age and gender distribution of CE patients.

Patient Age	Male	Female	Total
0 – 12	4	1	5
13 – 20	2	3	5
21 – 35	3	2	5
36 +	10	4	14
Total	19	10	29

(Pawlowski *et al*., 2001). Cysts in the brain, bone, or eye and calcified cysts often induce no or low antibody responses. In routine laboratory practice, usually at least two different tests should be used to obtain the most accurate results (Eckert & Deplazes, 2004). So, in this study ELISA and WB tests were used together to get reliable results. Three patients which were negative by ELISA were found to be positive by Western Blotting in which the USG and CT examinations were also positive.

Study covering Turkey's east and southeast Anatolian 179 sheep, 19 cattle and 7 goats were examined by the PCR-RFLP method for 205 ribosomal ITS-1 gene region of the *E. granulosus* isolate. It has been reported that all isolates were of G1 genotype (Utuk *et al.,* 2008). Another study in the western region of Turkey in which 22 *E. granulosus* isolates (12 sheep, 10 humans) were analyzed by DNA sequencing methods for mitochondrial COX1 and NAD1, and found that G1 genotype were confirmed in 17 isolates, while G1-G3 strain was found in sheep isolate. In Turkey, for the first time G7 strains were detected in two sheep and one human isolate (Šnábel *et al*., 2009). Analysis of 58 samples (42 human, 13 cattle, and 3 sheep) in Çukurova region of Adana found that the active genotype is solely G1 strain (Eroglu *et al*., 2016).

The purpose of our study was to determine the cysts genotype obtained from 29 individuals who were diagnosed with CE and compared them with ELISA and Western Blot results. In our recent study, we found that common genotype in human is *E. granulosus s.s.* G1-G3. There was no consistent correlation between ELISA and Western Blotting results, the number or size of cysts and CE genotype.

Regarding molecular analysis, the COX1 and NAD1 genes and the ITS-1 gene region were amplified in all isolates obtained. When the amplicons of the COX1 and NAD1 genes (COX1:446 bp and NAD1:378 bp) were electrophoresed on agarose gel, a single band was observed. There was no difference in the size of the amplicon's bands obtained from cysts taken from the same host. The amplicons of the COX1, NAD1 and ITS-1 genes were similar to those obtained from previous studies (Xue *et. al* 1993; Mwambete *et al.,* 2004; Utuk *et al.,* 2008; Ergin *et al.,* 2010; Eryildiz & Sakru, 2012; Parsa *et al.,* 2011; Mogoye *et al.,* 2013; Adwan, 2013; Yan *et al.,* 2013; Ahmed *et al.,* 2013; Altintas *et al.,* 2013). According to the ELISA results, antibody titers varied broadly and were found low in some patients or very high in others. Immunoblot analysis of EgAg showed many protein bands of with size 8, 12, 20, 22, 24, 36, 75 and 90 kDa. Among of them, 8 – 12 kDa bands, 20 – 22 kDa and 36 kDa bands presented strong reactivity with human serum specimens. Obviously none of the blood serum samples from healthy individuals reacted with EgAg. All patients in our study were found to be infected with the *E. granulosus* G1-G3 genotype. In conclusion; this was the first study that investigates the correlation between clinical outcomes with specific species or genotypes and serological results using CE parasitic material derived from Turkish human hosts. Our results showed that the *E. granulosus* s.s. (G1-G3) is predominant in the Aegean Region of Turkey where G7 strain is also present as it was determined in the study published earlier (Šnábel *et al.,* 2009). In order to investigate whether the specific immunoreactions in CE patients is related to the species by DNA sequence analysis and with ELISA and WB results it is necessary to compare different types or genotypes with infected patients antigen and antibody outcomes. In our study it was not possible because all our patients were infected with *E. granulosus s.s.* (G1-G3 genotype). Unfortunately, the limitation of this study was that there is limited number of patients to search for different genotypes. As reported by Grubor *et al*., (2017), future studies will give us opportunity to investigate the role of determined genotypes on immunomodulatory effects on parasite infections in humans. However, it might be difficult to understand the host-parasite relationship because of development of CE takes long-time. These findings are extremely important for the better development and improvement of CE diagnosis and treatment, as well as for control strategies and vaccine development.

For many of the genotypes we still have insufficient information. In particular regarding geographical distribution, host relationships in humans and animals, clinical outcomes and pathology. Therefore, these areas of interest need to be investigated more comprehensively on larger groups of patients and hosts. It will be interesting to compare results from different Turkey regions where *E. granulosus* is quite common. We understand that further studies and collaborations utilizing larger sample sizes are required to examine the links between different genotypes (if possible), localizations, cyst stages as well as clinical and immune parameters outcomes.

Conflict of Interest

The authors do not have a conflict of interest.

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