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Full Length Research Paper

Genotypic characterisation of *Echinococcus granulosus* isolated from human in Turkey

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Cystic echinococcosis is one of the most important zoonotic diseases in Turkey. The aim of this study was for the molecular analysis of *Echinococcus granulosus* isolates from different regions of Turkey. For this purpose, 46 hydatid cyst samples collected from humans during the surgery were included. The partial sequences of mitochondrial cytochrome c oxidase subunit 1 (CO1) gene was used for identification and molecular analysis of *E. granulosus* strains. Molecular analysis showed that all of the human cysts belonged to the G1 genotype (common sheep strain) of *E. granulosus*. According to the results of our study, the sheep strain (G1) is the predominant genotype of *E. granulosus* in humans in our country.

Key words: *Echinococcus granulosus,* human, hydatid cyst, genotype, Turkey.

INTRODUCTION

Echinococcus granulosus is the causative agent of cystic echinococcosis (CE) which has serious impacts on human and/or animal health resulting with significant economic losses (Eckert et al., 2001; Sarıözkan and Yalçın, 2009; Snabel et al., 2009). The disease is endemic in the Middle East, Central Asia and Nothern and Eastern Africa (Eckert et al., 2001; Sadjjadi, 2006; Azlaf and Dakkak, 2006).

A number of genetic variants of *E. granulosus* have been described. This genetic variations may determine phenotypic characteristics, host specificity, antigenicity, transmission dynamics, infection route, pathology, control, antimicrobial susceptibility and vaccine development strategies (Thampson and Mc Manus, 2001). So far, based on the partial sequences of mitochondrial cytochrome coxidase subunit 1 (CO1) and NADH dehydrogenase 1 (ND1) genes, ten distinct genetic types (G1-G10) of *E. granulosus* have been identified (Bowles and Mc Manus, 1993a; Bowles et al., 1994; Snabel et al., 2000). Recently, *E. granulosus* was divided following groups; *E. granulosus sensu stricto* (G1; sheep strain, G2; Tasmanian sheep strain, G3; buffalo strain), *E. equinis* (G4; horse strain*), E. ortleppi* (G5; cattle strain) *ve E. canadensis* (G6; camel strain, G7; pig strain, G8; cervid strain, G9; human strain, G10; Fennoscandian cervid strain) (Nakao et al., 2007). Among the genotypes in the *E. granulosus sensu strico* group, the sheep strain (G1 genotype) has the most wide geographic distribution around the world. The sheep strain is also dominant in the Mediterranean area (Euzeby, 1991; Breyer et al., 2004; Eckert and Thampson, 1997; Mc Manus and Thampson, 2003; Romig et al., 2006; Varcasia et al., 2007; Busi et al., 2007). Some of the other genotypes of *E. granulosus* have been reported to cause occasionally infections in human, but some genotypes are not involved in human infections (Bowles and Mc Manus, 1993b; Dinkel et al., 2004). Although cystic hydatid disease continues to be endemic in our

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Table 1. The distribution genotypes of *E. granulosus* according to the origin of the patients and the localisation of the cysts.

Origin of the patients	Genotype	Liver	Lung
Black sea region	G1	2	-
Marmara region	G1	3	1
East Anatolia region	G1	14	-
Southeast Anatolia region	G1	13	-
Mediterranean region	G1	2	1
Central Anatolia region	G1	10	-

country, large molecular epidemiologic data regarding the genotypes of *E. granulosus* strains infecting humans are still limited. The aim of this study was for the molecular characterisation of *E. granulosus* strains obtained from human originating from different geographic regions of Turkey.

MATERIALS AND METHODS

Collection of cysts

Between March 2008 - December 2009, cyst contents of 46 patients (28 males and 18 women) were collected during the surgical procedures performed in different hospitals in Istanbul. Demographic, epidemiologic and clinical data were recorded (Table 1). The ages of the patients were between 16-74 years old. Hydatid cyst diseases diagnosis was based on clinical, radiological and serological findings. The samples were checked for the presence of protoscoleces microscopically for the assessment of fertility states. Protoscoleces and cyst walls (germinal and laminar layers) were preserved in 70% ethanol until DNA purification. The samples were rinsed several times with sterile distilled water to remove the ethanol prior to DNA purification.

DNA purification

The hydatid cyst walls and protoscoleces were minced using surgical scalpales under sterile conditions. Genomic DNA was purified from 25-30 mg of minced tissue using a commercial DNA purification kit (Nucleospin tissue kit, Macherey Nagel, Germany) in accordance with the manufacturer's instructions and were stored at -80°C until use.

PCR amplification

Primers choosen from the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene of *E. granulosus* were used for the PCR amplification as previously described (Bowles et al., 1992) (Table 2). The PCR mixture contained 0.5 µM each primer, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl(pH 8.3), 0.2 mM each deoxynucleoside triphosphate (Fermentase®, Lithuania), and 1.25 U of *Taq* DNA polymerase (Fermentase®, Lithuania). The final reaction volume was 50 µl containing 10 µl of DNA sample. The thermal cycling conditions were as follow: the first denaturation cycle was performed at 95°C for 2 min followed by 30 cycles dena-turation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. The final extension step consisted of 5 min at 72°C. The amplification products were run on a 1.5% agarose gel electrophoresis and visualised on an UV transilluminator. The expected size of the PCR product was 446 bp. (Figure 1). For the exclusion of the PCR inhibitors, all samples were tested in a separated run using beta-globulin primers.

DNA sequencing

The PCR products were purified by using a commercial PCR product purification kit (High Pure PCR Product Purification kit, Roche Diagnostic, GmbH, Germany), and subjected to the cycle sequencing using big-dye terminator kit (ABI®, USA). Following the cleaning-up procedure through sephadex G-50 fine columns the cycle sequencing products were run on an automated DNA sequencer (ABI®, 310). The obtained sequences were edited and aligned, using the Bioedit software (Hall, 1999) then compared against the data available in GenBank™.

RESULTS

All samples were positive for *E. granulosus* DNA and none of them contained PCR inhibitors. The patients originating from different geographical regions of Turkey had either history of previous rural life or were still living in rural aereas. Microscopically all human cysts were found to be fertile. According to the results of the comparison of the obtained the partial mitochondrial cytochrome c oxidase subunit 1 (CO1) gene sequences with the similar sequences deposited at the GenBank™ using BLAST (http://www.ncbi.nlm.nih.gov./BLAST/BLAST-databases.html) software program, all sequences were G1 genotype (sheep strain). The alignment of the partial mitochondrial cytochrome c oxidase subunit 1 (CO1) gene sequences and prototype strains is given in Figure 2. The sheep strain (G1 genotype) of *E. granulosus* was predominant genotype in humans in the study.

DISCUSSION

The sheep strain (G1 genotype) of *E. granulosus* is the most widely distributed strain around the world. It has been found to be dominant strain both in human and animals (Thampson and Mc Manus, 2001; Ahmadi and Dalimi, 2006; Varcasia et al., 2006; Bart et al., 2006b; Li et al., 2008).

Despite geographically, Turkey is localised in a high endemicity region, data about the prevalence of CE are still limited. The prevalence rates among cattle and sheep are 39.7 and 58.6% respectively (Altıntaş, 2003; Ulutaş et al., 2007). Although, there is no community based large screening studies in Turkey, based on hospital records the prevalence rate has been estimated to be between 0.8-2.0/100 000 (Yolasığmaz et al., 2006; Altıntaş, 2008). The major risk groups for CE are people living in rural areas especially stockbreeders and farmers and abottoir workers. One of the major hinderances in the prevention of CE in Turkey is in house slaughtering

Table 2. The primer set used in this study.

Primer set	Direction	Sequence (5'-3')	Position	Product size
JB 3	Sense CO1	ttt ttt ggg cat cct gag gtt tat	2575	
JB 4.5	Antisense CO1	taa aga aag aac ata atg aaa atg	3021	446 bp.

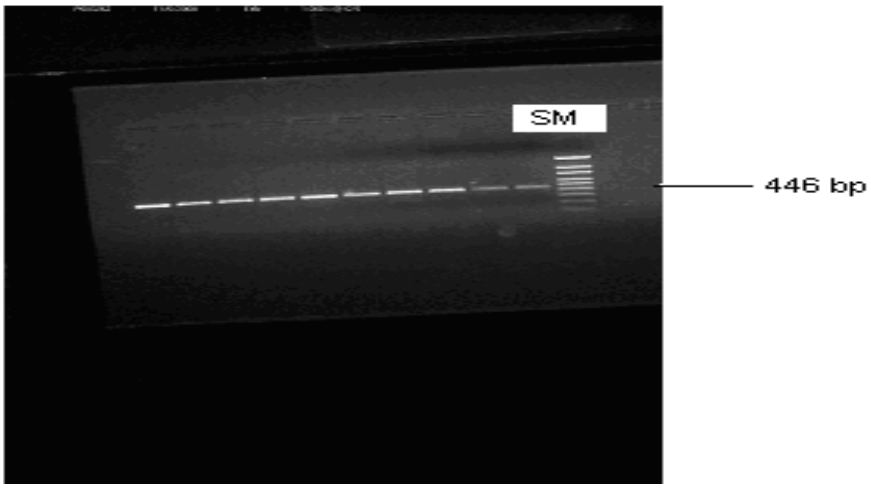

Figure 1. PCR products of some positive cases.
SM: Size marker

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                 '''|''''|''''|''''|''''|''''|''''|''''|''''
                    40         50         60         70
GI               ATTAGTGCTAATTTTGATGCGTTTGGGTTCTATGGGTTGTTGT
G2               ....................T.........T.............
G3               ..............................T.............
G4               ..............G....T.........T.............
G5               ..C............G....TT........T...........A.
G6               ......T.......G....TT........T.............
G7               ......T.......G....TT........T.............
H-1              ..........................................
H-5              ..........................................
H-6              ..........................................
H-8              ..............................T.............

                 |''''|''''|''''|''''|''''|''''|''''|''''|'
                 240       250       260       270       280
GI               GAATTCGAGTGTTAATGTTAGTGATCCGGTTTTGTGATGGGT
G2               ..................C.........................
G3               ..................C.........................
G4               A.......A.......AAG.........T.............
G5               ......T.A.......CG..........T.........G.....
G6               ......T.A.........C.........T.........G.....
G7               ......T.A.........C.........T.........G.....
H-1              ..........................................
H-5              ..........................................
H-6              ..........................................
H-8              ..........................................
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Figure 2. Some of the study strains and prototype strains (CO1 partial sequences).

practises and adequacy of the slaughtering facilities. Although, it has been tougth that CE considerable impacts on both human and animal health in Turkey, and any nation wide preventive programs have not been implemented yet (Vural et al., 2008). The production and offal losses in animals have been calculated as 12-13% and 1.5-3.2% respectively (Umur, 2003; Sarıözkan and Yalçın, 2009).

Genotype of infecting strain affects the fertility rate of the cysts in the intermediate hosts and thereby the infectivity of strain for the subsequent hosts. In addition, the size and localisation of the cysts may be different among the genotypes which may be relevant regarding examination of the organs with naked eye for the cysts. Also the prepatent periods may vary between genotypes which can be important for the programs based on regular antiparasitic treatment of the terminal host, before the parasite produces eggs (Thompson and Lymbery, 1988). The genotypes are also important regarding the host specificity and life cycle of the *E. granulosus* (Bowles and Mc Manus, 1993b; Dinkel et al., 2004).

The occurrance and host preference of the sheep strain (G1 genotype) of *E. granulosus* in different countries have been shown by previous molecular epidemiologic studies based on mitochondrial gene sequences (Bowles et al., 1992). Although, G1 genotype strains may also infect other intermediate host such as cattle, goats, dog, fertility rate of the cysts in these animals low is or it dosen't produce fertile cysts at all (Eckert and Thompson, 1997; Mwambate et al., 2004). To our knowledge this is the most comprehensive genotyping study in humans performed in Turkey and involve 46 cases originating from all geographic regions, except Aegean region. In this study, all of 46 strains were G1 genotype (sheep strain) based on the partial sequences of mitochondrial cytochrome c oxidase subunit 1 (CO1).

In a study conducted by Snabel et al. (2009) in Aegean region which is not included in our study, five of ten human hydatid cyst samples were G1 genotype and only one was G7 genotype. In the same study also sheep cyst samples were genotyped as G1, G3 and G7 Utuk et al. (2008) investigated 179 sheep, 19 cattle, 7 goat, 1 camel, 1 dog and 1 human cyst and including the human strain all were G1 genotype. Among animal cysts investigated Vural et al. (2008) again G1 genotype was dominant genotype.

From many countries in the Mediterranean area including our country, G1 genotype has been reported as the most prevalent genotype (Mwambete et al., 2004; M'rad et al., 2005; Bart et al., 2006a; Schneider et al., 2008) in both of human and animals. Based on the results of this study and other studies reported from our country and Mediterranean area it can not be suggested that G1 genotype is predominant genotype both in human and animals. This finding should be taken into account during the planing and implementing *E. granulosus* control programs.

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