



Genotype Characterization of Human Hydatid Cyst Isolates From Patients in Karaj, Iran, Using COX1 Gene Sequence

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Abstract

Background: Cystic echinococcosis is a main zoonotic infection. It can cause serious clinical problems for human health around the world. Genotypic specification of *Echinococcus granulosus* in human is important due to control and prevention programs.

Objective: In this investigation, genetic characteristics of human isolates of *E. granulosus* in Karaj, Iran, were studied.

Materials and Methods: In this review, 3 isolates of surgically removed hydatid cysts were obtained from patients in Shahid Madani hospital, Karaj, Iran in 2014. DNA was extracted from the protoscolex of the cyst, and polymerase chain reaction (PCR) assay was done on the COX1 gene.

Results: DNA fragments were sequenced and the results were aligned and analyzed. Among the isolates, 3 (100%) were *E. granulosus* (G1) strain.

Conclusion: The G1 genotype was the most superior strain from human isolates of hydatid cyst in Karaj.

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Background

Hydatid cyst is a significant zoonotic disease all around the world.¹ Cystic echinococcosis is caused by tiny tapeworms (cestoda) belonging to the genus *Echinococcus*.²

Echinococcus granulosus includes different genotypic strains in endemic and hyperendemic regions with global dispersion in definitive and intermediate hosts. Different isolates of *E. granulosus* were defined based on the variation in morphological, biochemical combination, isoenzyme profiles, developmental patterns and features of the intermediate host.^{3,4}

Humans are known as intermediate hosts of *E. granulosus* with various clinical symptoms depending on the infected organs.⁵

Echinococcus granulosus displays a lot of genetic variation with 10 genotypes (G1-G10) which have been molecularly identified so far, mostly based on genetic polymorphism of mitochondrial genes such as COX1.^{6,7} *E. granulosus* has been classified into *E. granulosus sensu stricto* (G1, G2, G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6, G7, G8, G9 and G10).⁸⁻¹¹

The current study was performed using polymerase

chain reaction (PCR) methods and COX 1 sequencing of *E. granulosus* isolates in order to define the genotype of *E. granulosus* collected from humans in Karaj.

Materials and Methods

Sample Collection

Three human isolates derived from *Echinococcus protoscoleces* were collected from the livers (2 isolates) and brain (1 isolate) of infected patients. Samples were frozen at -20°C .

DNA Extraction

Genomic DNA Purification kit (Cinagene, Iran) was used for the extraction of the DNA from hydatid cyst isolates, in accordance with the manufacturer's instructions. The extracted DNA was frozen at -20°C .

DNA Amplification

Genomic fragments of COX1 were amplified from mitochondrial DNA of all isolates. The COX1 gene was amplified with forward primer 5'-TTTTTTGGGCATCCTGAGGTTTAT-3' and reverse

primer 5'-TAAAGAAAGAACATAATGAAAATG-3'.¹² The cycling parameters for PCR were as follows: primary denaturation at 95°C for 5 minutes followed by 30 cycles with final denaturation at 94°C for 1 minute, annealing at 64°C for 1:30 minutes, extension at 72°C for 2 minutes and a final extension at 72°C for 10 minutes. PCR product was electrophoresed on 1% agarose gel and visualized by UV transilluminator.

Sequencing

The amplified DNA fragments were sequenced by BIONEER sequencer (Bioneer 3730). Alignment and Comparison of nucleotide and amino acid sequences of the COX1 with the other sequences in NCBI GenBank were done.

Results

All 3 isolates of surgically removed hydatid cyst were collected from patients in Karaj, Iran. All isolates were positive for *E. granulosus* by microscopical test; COX1 gene was amplified by PCR from genomic DNA obtained from 3 samples. All of the isolates showed a band of 440 bp in PCR assay (Figure 1).

The purified products of all samples were sent to the company and sequenced using ABI 3130 Genetic Analyzer. After sequencing, the initial results of sequences were carefully compared and edited using Chromas and Sequencher software. The sequences were compared with available sequences in GenBank using BLAST program. All samples were reported to belong to G1 genotype (sheep strain).

Discussion

Hydatid cyst infection has been known as one of the most important parasitic diseases in endemic areas in Iran.

The genotype G1 has been presented in sheep, cattle, goat, and human isolates in Iran. By the analysis of the mitochondrial COX1 gene, a significant superiority of the common sheep/dog strain (G1) in human cases was seen.¹³⁻¹⁵

Due to hyperendemicity in Alborz province, researches have been rarely performed to determine the genotype of hydatid cysts; so far, no study has been done on their nucleotide sequencing. In genotyping survey of hydatid cyst isolates, using *cox1* gene sequencing in the current study, the G1 genotype was known as predominant genotype in human samples.

Strain typing studies all around the world such as Australia, New Zealand, Jordan, Kenya, Lebanon, the Netherlands, Spain and China show that common sheep strain (G1) often causes human infection.¹⁶⁻¹⁸

In a study done in Mongolia using SSCP, 34 samples out of 50 human hydatid cysts showed G1-G3 genotype (*E. sensu stricto*) and 16 samples were G6-G10 genotype (*E. canadensis*)¹⁹ and in a study in Bulgaria, G1 genotype was predominant in human and definitive hosts.²⁰

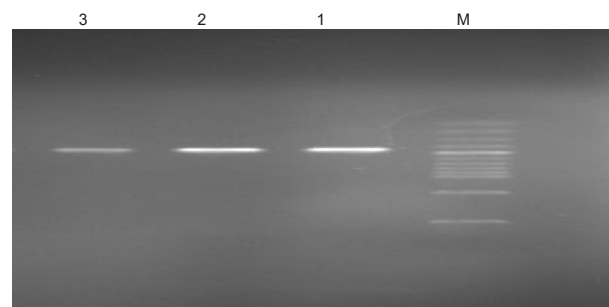


Figure 1. PCR Products of COX1 Gene. Lane M: DNA marker (ladder 100 bp); Lanes 1, 2 and 3: Human Isolates.

G1 is the most common strain in hydatid cyst of human cases in the world.²¹ Some molecular surveys using mitochondrial (COX1) analysis have identified the common sheep strain (G1) in Iran.²²⁻²⁴

Our study and other surveys mentioned above show that dog/sheep strain (G1) as a predominant strain of *E. granulosus* in all parts of Iran including Karaj is circulating in definitive and intermediate hosts.

Conclusion

Results of this study displayed that G1 strain is the significant genotype in humans in Alborz province. This investigation provides support for the need to concentrate on control measures in Karaj to reduce the rates of human diseases.

Authors' Contributions

MS: sampling, processing and performing the conventional and procedures; AM: the study design, management and supervision; MZ and SR: provided advice, read and arranged the final manuscript.

Ethical Approval

This study was approved by Alborz University of Medical Sciences.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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