JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2016. Vol. **10**(2), p. 1015-1020

A First DNA Sequencing of Hydatid Agent Isolated from Human in Iraq

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(Received: 25 March 2016; accepted: 09 May 2016)

A total of 20 human hydatid cysts fluid samples were collected during January, 2015 to July, 2015 from patients attended to Al- Karamah teaching hospital at Wasit Province. Genetic characterization of all isolates of *Echinococcus granulosus* from hydatid cyst was performed by sequencing analysis of fragment of mitochondrial (CO1) cytochrome C oxidase subunit 1 and NADH dehydrogenase subunit1 (ND1) genes to determine genotyping of *E.granulosus* in hyper-endemic areas of Wasit province.This work done by randomly choosing of five samples.The results of DNA sequencing showed genotype 1(G1)and genotype 3 (G3) and the sheep strain G1was more prevalent than buffalo strain G3.

Keywords: Hydatid , Human, DNA sequence, Diagnosis.

Echinococcosis, or Hydatid disease, is an infection caused by tapeworms of the genus *Echinococcus*, a tiny tapeworm just a few millimetres long. Echinococcosis is a zoonosis, a disease of animals that affects humans. This disease is still a major endemic, zoonotic and public health problem also is not yet an organized national control program in Iraq[1]. Outbreak of this disease have occurred in Asian countries such as Lebanon, Jordan, Iraq, Saudi Arabia and Iran leading to substantial health problems and economic losses[2]. This parasite have five medical important species of genus *Echinococcus* are *E.granulosus*, *E. multilocularis* , *E. vogeli* , *E. oligarthrus and E. shiquicus* , these species are morphologically distinction both adult and larval stages[3].

To date ten distinct genotype (G1-G10) have been described in world based on nucleotide sequence analysis of mitochondrial cytochrome oxidase subunit 1(CO1) (4). It has been reported that at least seven of these strains were isolated from human, but the most common one is sheep strain[4,5].

The cysts of *Echinococcus granulosus* found in the most people one cyst , approximately(60-70%)of *Echinococcus granulosus* cysts in the liver and (20-25%) in the lungs[6], also found these cysts in the other organs of human including "the bones, kidneys, spleen, muscles , central nerve system (CNS) and behind the eyes[7].

In human echinococcosis is diagnosed mainly with imaging techniques such as ultrasonic graph, radiology, magnetic resonance imaging (MRI) or (T-scanning) supported by serology tests[8]. Serological tests used in humans include enzyme linked immune-sorbent assays (ELISAs), indirect immune fluorescence, indirect hemagglution, immune blotting and latex agglutination[9,10]. Molecular diagnosis such as DNA sequencing technique to determine the genetic characterization of *E.granulosus* depends on the homology of the sequence of two mitochondrial genes: cytochrome C oxidase subunit 1(CO1) and reduced nicotinamide adenine dinucleotide subunit 1(ND1)[4,11].

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MATERIALS AND METHODS

Samples collection

A total of 20 human hydatid cysts fluid samples were collected during January, 2015 to July, 2015 from patients attended to Al- Karamah teaching hospital at Wasit province and placed in sterile container, then transported to laboratory and stored in freeze (-20C) until genomic DNA extraction step.

Direct examination

The fluid of hydatid cyst was taken from patient after surgical immediately and centrifuged .The precipitate smeared on clean slide and stained by Eosin (0.1%), then examined by microscope under (40x) to show the protoscolices clearly.

DNA Extraction

Genomic DNA from hydatid cyst fluid samples was extracted by using Genomic DNA mini kit extraction Geneaid, USA, and performed according to company's instructions**.**

DNA Profile

The extracted DNA was checked by Nanodrop spectrophotometer (THERMO. USA), which measured DNA concentration (ng/µL) by reading the absorbance at (260 /280 nm).

PCR technique

PCR technique was performed for detection of two mitochondrial genes in *E. granulosus* that using in genotyping study from human samples. This technique was carried out according to method described by[12] and provided by Bioneer company, Korea .

DNA sequencing method

DNA sequencing method was performed for genotyping and phylogenetic analysis study of local *Echinococcus granulosus* isolates based mitochondrial cox1 and nad1 gene. The sequencing of the PCR product 450 bp and 400 bp for cox1 and nad1 gene respectively, where the PCR product was purified from agarose gel by using (EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic. Canada). Phylogenetic analysis was performed based on NCBI-Blast Alignment identification and Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version).

RESULTS AND DISCUSSION

Amplification of genes by PCR

Amplification of mitochondrial CO1gene

DNA was amplified by two primers for detection and genotyping of *E.granulosus* based on sequencing of mitochondrial genes by using PCR technique. These primers generated the expected (450 bp) product. The results showed all samples had CO1gene (Figure 1).

Amplification of mitochondrial ND1gene

DNA was showed (NAD1) dehydrogenase subunit 1 gene amplified from all samples of human hydatid cysts by using specific primers . PCR product were purified after a garose gel electrophoresis at (400) bp and visualized under UV transilluminator (Figure 2).

Genotyping of *E.granulosus* isolates

In this study, genetic characterization of 20 isolates of *E.granulosus* from hydatid cyst was performed by sequencing analysis of fragment of mitochondrial CO1 gene and ND1 gene to determine genotyping of *E.granulosus* in hyper-endemic areas of Wasit province. This work done by randomly choosing of five samples. The results of DNA sequencing showed genotype 1 (G1) and genotype 3 (G3) (Figure 3,4). This is the first study at Wasit province, Iraq (Figures 3,4,5).

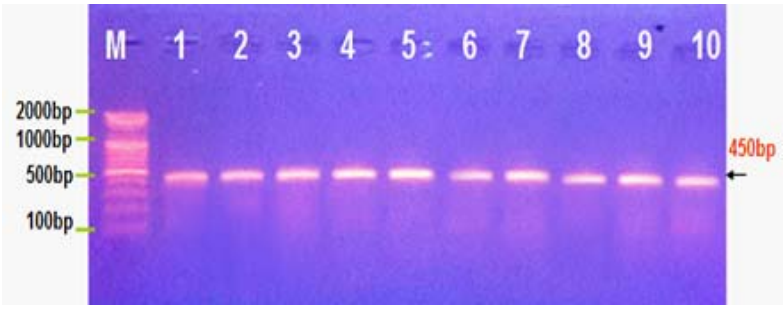

Fig. 1. Agarose gel electrophoresis image shown the PCR product analysis of cox1 gene in *E.granulosus* , Where M marker (2000-100bp), lane (1-10) positive cox1 gene at 450 bp PCR product.

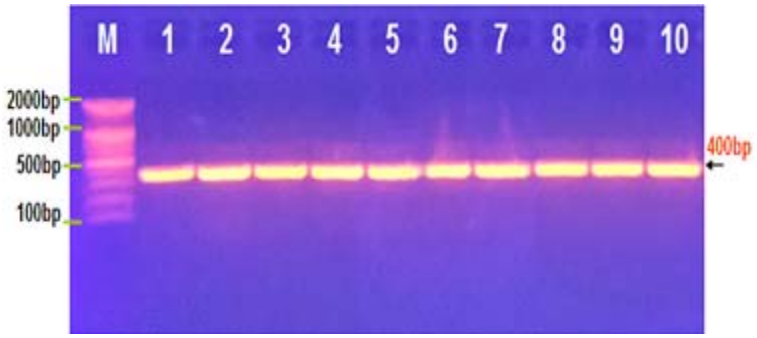

Fig. 2. Agarose gel electrophoresis image shown the PCR product analysis of nad1 gene in *E.granulosus* samples, Where M marker (1500-100bp), lane (1-10) positive nad1 gene at 400 bp PCR product

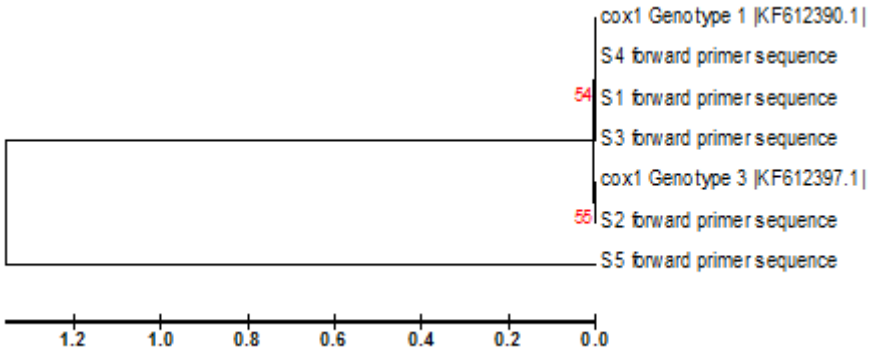

Fig. 3. Phylogenetic tree analysis based on the cox1 gene of *E.granulosus*. The isolates (1, 3, 4, 5) were shown closely related to NCBI-Blast *E. granulosus* isolate (Genotype 1) (KF612390.1), whereas the isolate (2) was shown closely related to NCBI-Blast *E. granulosus* isolate (Genotype 3) (KF612397.1)

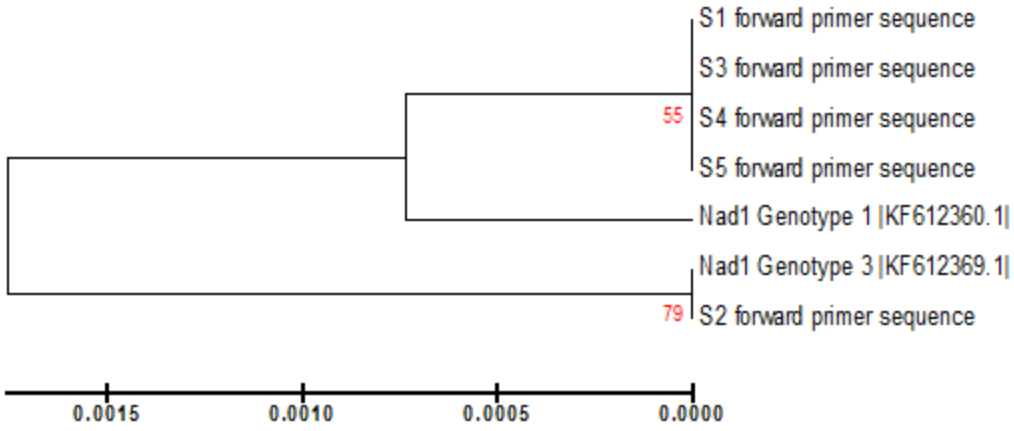

Fig. 4. Phylogenetic tree analysis based on the nad1 gene of *E.granulosus* . The isolates (1,3,4,5) were shown closely related to NCBI-Blast *E. granulosus* isolate (Genotype 1) (KF612360.1), whereas the isolate (2) was shown closely related to NCBI-Blast *E. granulosus* isolate (Genotype 3) (KF612369.1)

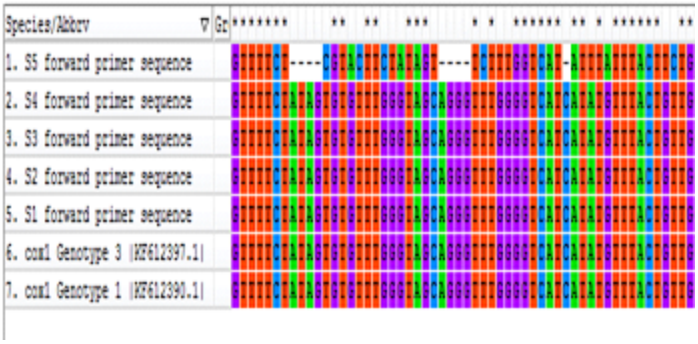

Fig. 5. Multiple sequence alignment analysis of the partial cox1 gene sequence for local *E. granulosus* isolates and NCBI-Genbank *E. granulosus* isolates. The multiple alignment analysis similarity (*) and differences in cox1 gene nucleotide sequences

Fig. 6. Multiple sequence alignment analysis of the partial nad1 gene sequence for local *E. granulosus* isolates and NCBI-Genbank *E. granulosus* isolates. The multiple alignment analysis similarity (*) and differences in nad1 gene nucleotide sequences

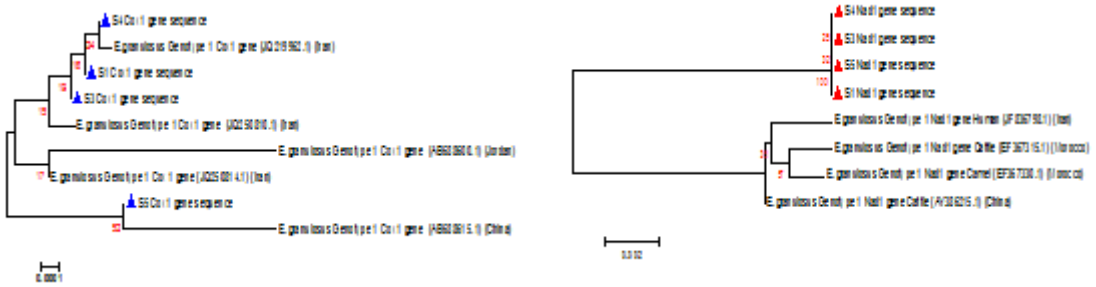

Fig. 7. A,B. Phylogenetic tree analysis based on the Cox1 gene (A) and nad1 gene (B) partial sequence used for *E. granulosus* country relationship. The local *E. granulosus* isolates (S1, S3 and S4) shown closely related to neighboring countries available NCBI-Blast *E. granulosus* genotype1 Iran isolates (JQ250810.1) and (JQ219962.1), Whereas other local *E. granulosus* isolate (S5) shown closely related to NCBI-Blast *E. granulosus* genotype1 China isolate (AB688615.1)

DISCUSSION

This is the first study to determine the genetic characterization of the strains of *E.granulosus* from humans at Wasit province. Two genotypes G1 and G3 were identified among our DNA samples based on (CO1) and (ND1) sequences data, genotype G1was the most common available genotype in the studied population. The phylogenetic analysis of concatenated sequences

of COX1 and NAD1 showed 4 samples (s1,s3,s4,s5) represent all strains related to G1genotype and sample(s2) represents strain of G3 genotype.

The preliminary studies by[13], the G1 was found in all humans isolated, also confirmed by study of[14] , in Ilam province,Iran which reported that all of humans isolates belonged to G1genotype. The disagreement of these results could be explained by type of studied samples, difference in geographic area and exploited method for genotyping[15].

Numerous studies have been carried out on genetic characterization of hydatid cyst,using hydatid fluid, cystic membrane and scolices[16].Various PCR techniques have been introduced to detect *E. granulosus* genome in biological specimens[17]. PCR is currently developed as acomplementary diagnostic tool for species differentiation for echinococcosis,using biopsy samples and tissue specimens[18,19].

The DNA sequencing of specific region of NADH Nicotinamide adenine dinucleotide subunit1gene confirmed the presence of sheep strain G1and buffalo strain G3 at Wasit Province. When comparing the results of sequencing of ND1 in G1 and G3 by Gen-Bank were showed identity (100%) in Wasit province and identically with Iraqi also have identity (100%) for ND1of G1 and G3. These result were comparatively closer to similar studies conducted by many authors in Iran[20], France[21] and Romania[22]. However, different results were obtained by other authors worldwide[23].

Abdullah *et al*., 2012 [24] showed that sheep strain G1 is predominant in Kurdistan-Iraq and it was mostly responsible of human hydatid disease and Kia *et al*., 2010 [25], confirmed that G1 was the predominant genotype of hydatidosis in human in central Iran. As well as Eryildis and Sakra, 2012[26], proven that G1 was the predominant in Turkey and is the principle agent of human and animals hydatidosis. These agreed with our results that confirmed G1 predominant than G3 at Wasit Province .

The previous studies in the world were used mitochondrial and nuclear DNA markers in molecular characterization of *E.granulosus*, but have no adequate studies to determine and identify *E.granulosus* strains that are cause infection of human[27-29]. The recent studies reported that at least seven of these strains are infective to human but the sheep strain and the G1 were the most common of these strains[30].

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