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Complete characterization of NADH dehydrogenase subunit 1 gene in human hydatid cysts

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Abstract

Echinococcus granulosus sensu stricto possesses very similar strains G1 and G3. Mitochondrial genes from NADH dehydrogenase (NAD) family have prominent roles in the identification of very similar strains G1 and G3. Thus, the present study mainly aimed to characterize the whole NAD1 gene (from the start codon to the stop codon) of hydatids isolated from humans. Hydatids were surgically isolated from the livers of patients, who are from Basrah and Maysan provinces, at Al-Sadir Teaching Hospital in Basrah province, Iraq. Protoscoleces were aseptically isolated from hydatid cysts and were used to extract DNA. Specific primers were employed to amplify the total NAD1 gene. A consensus sequence of nucleotides was separately generated from Basrah and Maysan sample sequences. Each consensus sequence had 1038 bp and it contained a start codon GTG at residues 87- 89 and a stop codon TAA at residues 978 – 980. The present study results displayed that each deduced amino acid sequence, predicated from a consensus sequence of nucleotides, had 297 amino acids. Furthermore, bioinformatics analysis revealed that each deduced amino acid sequence included a residue isoleucine (I^{188}). This residue is only retained in the NAD1 protein of the G1 strain. The current study results also showed a predicated structural model of Basrah and Maysan NAD1 proteins and both of which had very similar homology models and the important residue I^{188} was located in the α10 helix. In total, for the first time in Iraq, this study characterized the whole NAD1 genes of *E. granulosus* protoscoleces and confirmed these NAD1 genes belonging to the G1 sheep strain.

Keywords: Basrah, *Echinococcus granulosus*, G1 Strain, Maysan, NAD1.

Introduction

The larval stage known as a metacestode of *Echinococcus granulosus sensus lato* is the main causative agent of cystic echinococcosis in the world [1, 2]. The hydatid disease causes great health and economic burdens in developing countries, including Iraq [2, 3]. Lately, World Health Organization (WHO) listed cystic echinococcosis as one of seven neglected zoonotic diseases that need global interaction [4]. The parasite requires a definitive host and an intermediate host to complete its life cycle [5]. The adult parasite lives in the small intestine of the definitive host from canids. Whereas the

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metacestodes find in different organs, in particular the liver and lung, of infected animals (intermediate hosts) such as buffalo, sheep and cattle as well as infected humans [3, 6].

Based on morphological, biochemical and molecular investigations, nine strains (G1-G9) of *E. granulosus* were initially discovered [3, 5, 7, 8]. Recently, molecular identification using mitochondrial genes, in particular COX1 (cytochrome c oxidase subunit 1) and NAD1 (NADH dehydrogenase subunit 1) display ten strains of *E. granulosus* in the world [9]. The molecular analysis of mitogenomes showed that G2 is not a distinct strain and is a part of the strain G3 [10] and G9 is a part of G7 [11]. The *E. granulosus sensu lato* genotypes have a large variation in their host selectivity and pathogenicity [1]. Therefore, they are separated into 5 species [1, 10, 12]. These species are *E. granulosus sensu stricto* (G1/G3), *E. intermedius* (G6/G7), *E. ortleppi* (G5), *E.canadensis* (G8/G10) and *E. equinus* (G4). The first species is widespread worldwide and responsible for the majority of cystic echinococcosis [1]. The partial sequence of NAD1 and NAD5 was utilized to distinguish between very similar strains G1 and G3 [1, 3, 12].In Thi-Qar province of Iraq, the partial sequence of the NAD1 gene showed that eleven isolates out of twelve belonged to strain G1 whereas only one isolate belonged to strain G3 [3]. Mitochondrial genes including NAD1 and NAD5 have prominent roles in the identification of very similar strains (G1/G3). In Iraq, there are no complete sequences available for these genes. Thus, the present study aimed to characterize the NAD1 gene (from the start codon to the stop codon) in human hydatid cysts and to use it in molecular phylogenetic studies.

Materials and Methods

Sample collection

Human livers infected with hydatids were diagnosed based on X-ray and CT scan imaging. The hydatids were surgically isolated from patients in Al-Sadir Teaching Hospital in Basrah province, Iraq. Patients infected with hydatid cysts originally were from two provinces (Basrah and Maysan). Thus, we randomly selected equal samples (6) for each province to focus on the characterization of the NAD1 gene (stare codon – end codon) of *E. granulosus*. The *E. granulosus* protoscoleces were aseptically isolated and the isolated protoscoleces were rinsed 4 – 5 times with phosphate-buffered saline (PBS). The washed protoscoleces were used in DNA extraction.

DNA extraction

Protoscoleces prepared as described above were used in DNA extraction utilizing the tissue protocol of the DNA extraction kit (Geneaid, Taiwan) as a previously used by AL-Asadi *et al.* [3]. DNA was assessed using N50 Implen NanoPhotometer®.

Polymerase chain reaction

PCR (polymerase chain reaction) was applied to amplify the complete mitochondrial NAD1 (mtNAD1) using a specific set of primers. This set of primers was picked up from GenBank (NCBI, https://www.ncbi.nlm.nih.gov/nuccore/EF367317.1) under the GenBank accession number EF367317.1. These primers were forward (5'-GTAGTTACTCTTATGTTGGTG-3') and reverse (5'- CTTGAAGTTAACAGCATCACG-3'). A PCR reaction contained 25 µl of Promega Master Mix (GoTaq® Green), 1 µl of each primer (10 µM, Macrogen), 18 µl of nuclease-free water (Promega) and 5 µl of genomic DNA. The thermal conditions were 95°C for 5 min and 35 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec followed by 72°C for 10 min in an Applied Biosystems thermal cycler. An agarose (1.5% (w/v)) gel was employed to split up PCR products and then VWR International UV transilluminator was utilized to image separated PCR products. The band with the size of 1038 bp was cut from the gel and purified products were gotten using the Promega Gel and PCR Clean-up System.

Nucleotide sequencing and bioinformatics analyses

The purified PCR products from the agarose gel were sequenced in both the upstream and downstream directions using the gene-specific primers. The Sanger sequencing was performed by the Macrogen company (South Korea). For each

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sample, both directions sequences were lined up, treated and then assembled. Based on a province, the assembled sequences were lined up and compared with each other and the consensus sequence of NAD1 was deposited in the GenBank database accessed via the NCBI website. The consensus sequence of NAD1 was translated using a blastx program at BLAST (Basic Local Alignment Search Tool) accessed also through the NCBI website. A phylogenetic tree and a multiple sequence alignment as well as a compute pairwise distance were done using Molecular Evolutionary Genetic Analysis version 11. A molecular model of the *E.granulosus* NAD1 proteins was constructed based on the crystal structure of the *Yarrowia lipolytica* NAD1 protein (PDB: 6yj4.1). The *Yarrowia lipolytica* NAD1 protein was chosen according to searches of HHblits and BLAST databases.

Results and Discussion

Fig. 1 shows the PCR products of the mtNAD1 gene. DNA extracted from protoscoleces of *E. granulosus* obtained from livers (humans) infected with hydatids was amplified using a specific set of mtNAD1 primers. These primers were amplified by two PCR products at 1038 and 210 bp. For Basrah and Maysan samples, the PCR product at 1038 bp in Fig. 1, boxed bands were extracted from agarose gel and sequenced. The nucleotide sequences of Basrah samples were lined up together and the consensus sequence was generated in Fig. 2. Similarly, the consensus sequence of Maysan samples was generated in Fig. 3. Both Basrah and Maysan consensus sequences were placed in GenBank and had accession numbers MN231833.1 and MN231834, respectively.

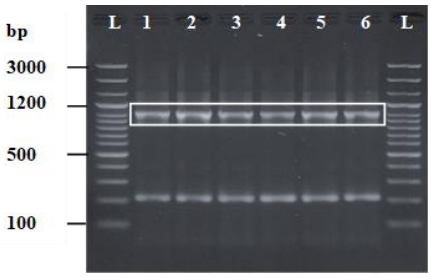

Figure 1. PCR products of the *E. granulosus* NAD1 gene. L represents a DNA marker. Lanes 1-3 represent PCR products derived from hydatid cysts surgically obtained from human livers (Basrah) whereas lanes 4-6 from hydatid cysts surgically obtained from human livers (Maysan).

Fig. 2 and 3 also show the predicted translation of the consensus sequences. In both Basrah and Maysan

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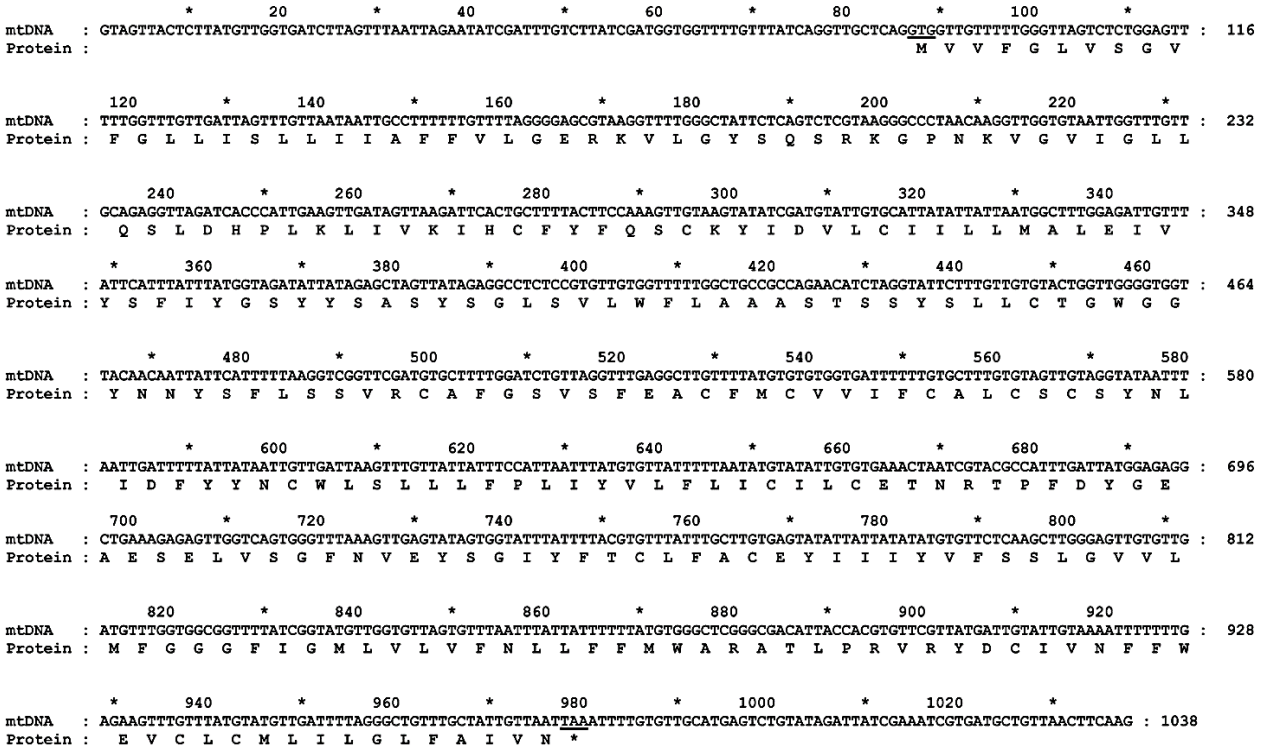

Figure 2. The nucleotide consensus and deduced amino acid sequences of *E. granulosus* protoscoleces obtained from human livers infected with hydatid cysts from Basrah province.

Consensus sequences, there were 297 amino acids obtained by translation via a blastx program. The start codon was GTG at residues 87-89 and it codes methionine (M) based on the flatworm mitochondrial genetic code (translation table 9 in NCBI) that differs from the standard genetic code. Whereas the stop codon was TAA at residues 978-980 which is one of three known standard stop codons.

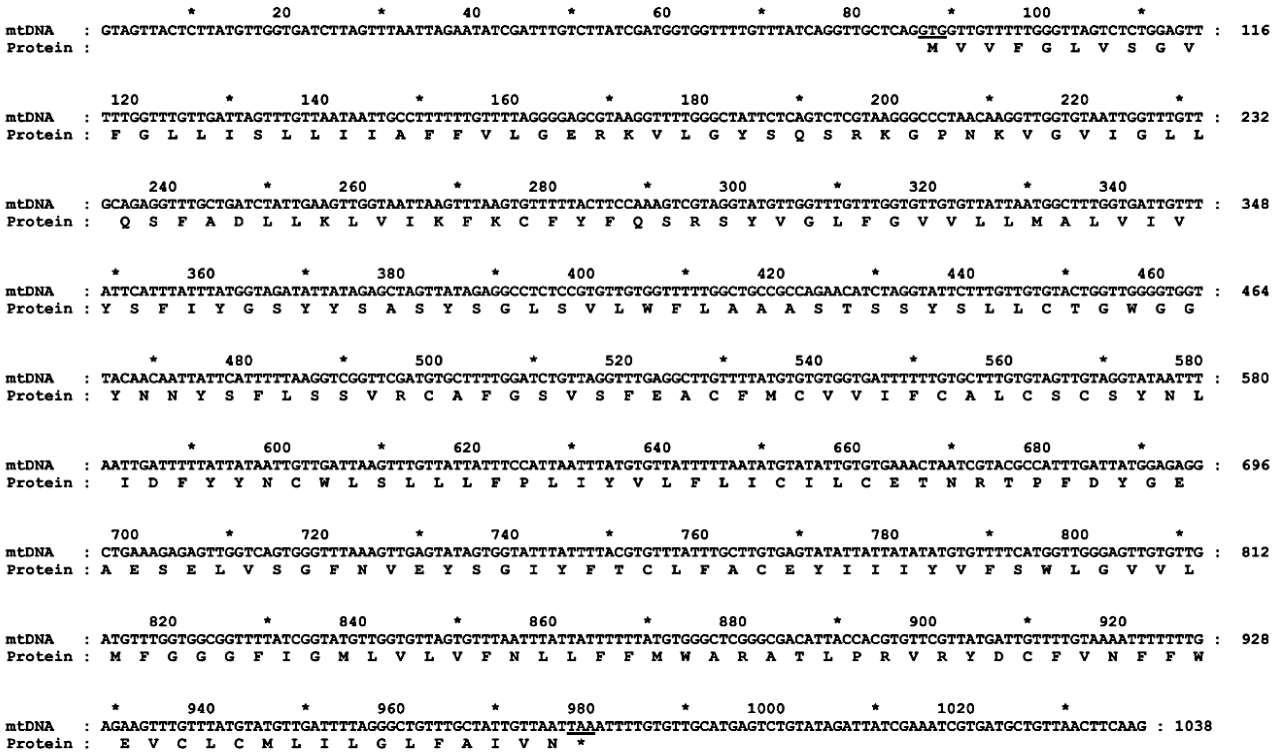

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Figure 3. The nucleotide consensus and deduced amino acid sequences of *E. granulosus* protoscoleces obtained from human livers infected with hydatid cysts from Maysan province.

Phylogenetic analysis

A phylogenetic analysis of the relationships between the *E. granulosus* mtNAD1 proteins obtained from Basrah and Maysan samples in comparison with the *E. granulosus* mtNAD1 proteins (strains G1, G3, G6 and G7) selected from different countries is shown in Fig. 4. These strains in Table 1 were chosen based on the complete gene sequences. A *Fasciola gigantica* mtNAD1 protein was used as the out-group to root a phylogenetic tree.

Table 1. *E. granulosus* strains used in the current study.

Species	Accession No.		Host	Country
	Gene	Protein		
E. granulosus (Strain G1)	MG672280.1:7278-8171	AWD28790.1	Sheep	Italy
	MG672217.1:7278-8171	AWD28034.1	Sheep	Argentina
	MG672129.1:7278-8171	AWD26978.1	Human	Spain
	KY766891.1:7280-8173	ARO49780.1	Buffalo	India
	KY766884.1:7280-8173	ARO49703.1	Human	Finland
	NC_044548.1:16016-16909	YP_009688057.1	Sheep	Australia
	EF367317.1:87-980	ABN12861.1	Sheep	Morocco
	MG672204.1:7278-8171	AWD27878.1	Sheep	Turkey
	MG672131.1:7278-8171	AWD27002.1	Cattle	Romania
	KY766887.1:7280-8173	ARO49736.1	Sheep	Iran
	MG672143.1:7278-8171	AWD27146.1	Sheep	France
E. granulosus (Strain G3)	MG682522.1:7278-8171	AWB97464.1	Sheep	Italy
	MG682528.1:7278-8171	AWB97536.1	Sheep	Spain
	KJ559023.1:5100-5993	AWB97704.1	Buffalo	India
	MG682536.1:7278-8171	AWB97632.1	Sheep	Turkey
	MG682541.1:7278-8171	AWB97692.1	Camel	Iran
	KJ559023.1:5100-5993	AIA24301.1	Human	China
	MG682544.1:7278-8171	AWB97728.1	Human	Algeria
E. granulosus (Strain G6)	MH300933.1:7313-8206	AWW03461.1	Goat	Argentina
	NC_038227.1:7313-8206	YP_009505042.1	Camel	Iran
	MH300954.1:7313-8206	AWW03713.1	Camel	Mauritania
	MH300936.1:7313-8206	AWW03497.1	Human	Kenya
	MH300952.1:7313-8206	AWW03689.1	Camel	Sudan
E. granulosus (Strain G7)	MH301019.1:7311-8204	AWW04493.1	Pig	Italy
	MH300970.1:7311-8204	AWW03905.1	Pig	Argentina
	MH301017.1:7311-8204	AWW04469.1	Pig	France
	MH301020.1:7312-8205	AWW04505.1	Pig	Lithuania
	MH301022.1:7312-8205	AWW04529.1	Pig	Ukraine

The phylogenetic analysis clearly generated two separate clusters. One of these clusters contained the mtNAD1 proteins of strains G1 and G3 whereas another one contained the mtNAD1 proteins of strains G6 and G7. In the cluster of strains G1 and G3, the Maysan mtNAD1 protein was grouped in a clade with mtNAD1 proteins of strain G1 obtained from Australia, India, Morocco, Romania, Iran, Turkey, Spain, Italy, France, Argentina and Finland. However, the Basrah mtNAD1 protein was not grouped in a clade with mtNAD1 proteins of strain G3.

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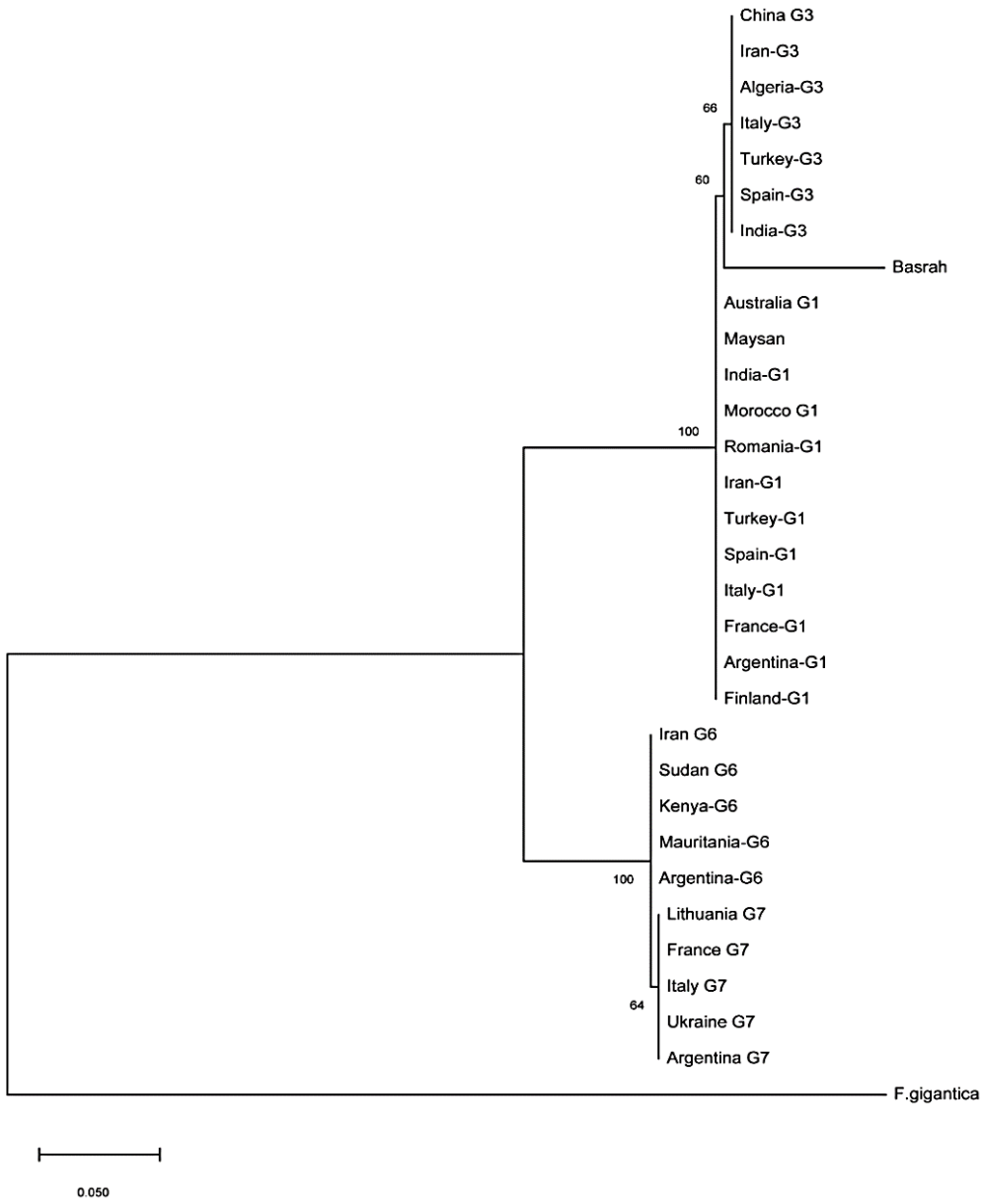

Figure 4. A phylogenetic analysis shows the relationships between the *E. granulosus* mtNAD1 proteins obtained from Basrah and Maysan samples with the *E. granulosus* mtNAD1 proteins (strains 1, 3, 6 and 7) selected from different countries.

Percent identity matrix of *E.granulosus* mtNAD1 proteins

Table 2 displays the percent identity of *E.granulosus* mtNAD1 proteins. The *E.granulosus* mtNAD1 proteins of strain G1 shared 100% identity with each other. Similarly, the *E.granulosus* mtNAD1 proteins of strain G3 shared 100% identity with each other. The Maysan mtNAD1 protein shared 100 and 99.3% identity with the mtNAD1 proteins of strains G1 and G3, respectively. Unlike the Maysan mtNAD1 protein, the Basrah mtNAD1 protein shared 93.3% amino acid identity with the mtNAD1 proteins of strains G1 and G3.

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Table 2. Percent identity matrix of *E. granulosus* mtNAD1 proteins.

No.	Species/Strain	This study	G1												G3						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Eg_Basrah-This study	100	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3
2	Eg_Maysan-This study		100	100	100	100	100	100	100	100	100	100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
3	Eg_Italy-G1			100	100	100	100	100	100	100	100	100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
4	Eg_Argentina-G1				100	100	100	100	100	100	100	100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
5	Eg_Spain-G1					100	100	100	100	100	100	100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
6	Eg_India-G1						100	100	100	100	100	100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
7	Eg_Finland-G1							100	100	100	100	100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
8	Eg_Australia-G1								100	100	100	100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
9	Eg_Morocco-G1									100	100	100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
10	Eg_Turkey-G1										100	100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
11	Eg_Romania-G1											100	100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
12	Eg_Iran-G1												100	100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
13	Eg_France-G1													100	99.3	99.3	99.3	99.3	99.3	99.3	99.3
14	Eg_Italy-G3														100	100	100	100	100	100	100
15	Eg_Spain-G3															100	100	100	100	100	100
16	Eg_India-G3																100	100	100	100	100
17	Eg_Turkey-G3																	100	100	100	100
18	Eg_Iran-G3																		100	100	100
19	Eg_China-G3																			100	100
20	Eg_Algeria-G3																				100

Amino acid sequence comparison of the *E.granulosus* mtNAD1 proteins

A multiple sequence alignment of the *E.granulosus* mtNAD1 proteins of strain G1 with the Basrah and Maysan mtNAD1 proteins is revealed in Fig. 5. The Maysan mtNAD1 proteins totally shared the same amino acids with the mtNAD1 proteins of The Basrah mtNAD protein shared highly conserved regions with the *E.granulosus* mtNAD1 proteins of strain G1 and the Maysan mtNAD1 protein, except for some regions. It retained a residue I, conserved in the mtNAD1 proteins of strain G1, at a position 188.

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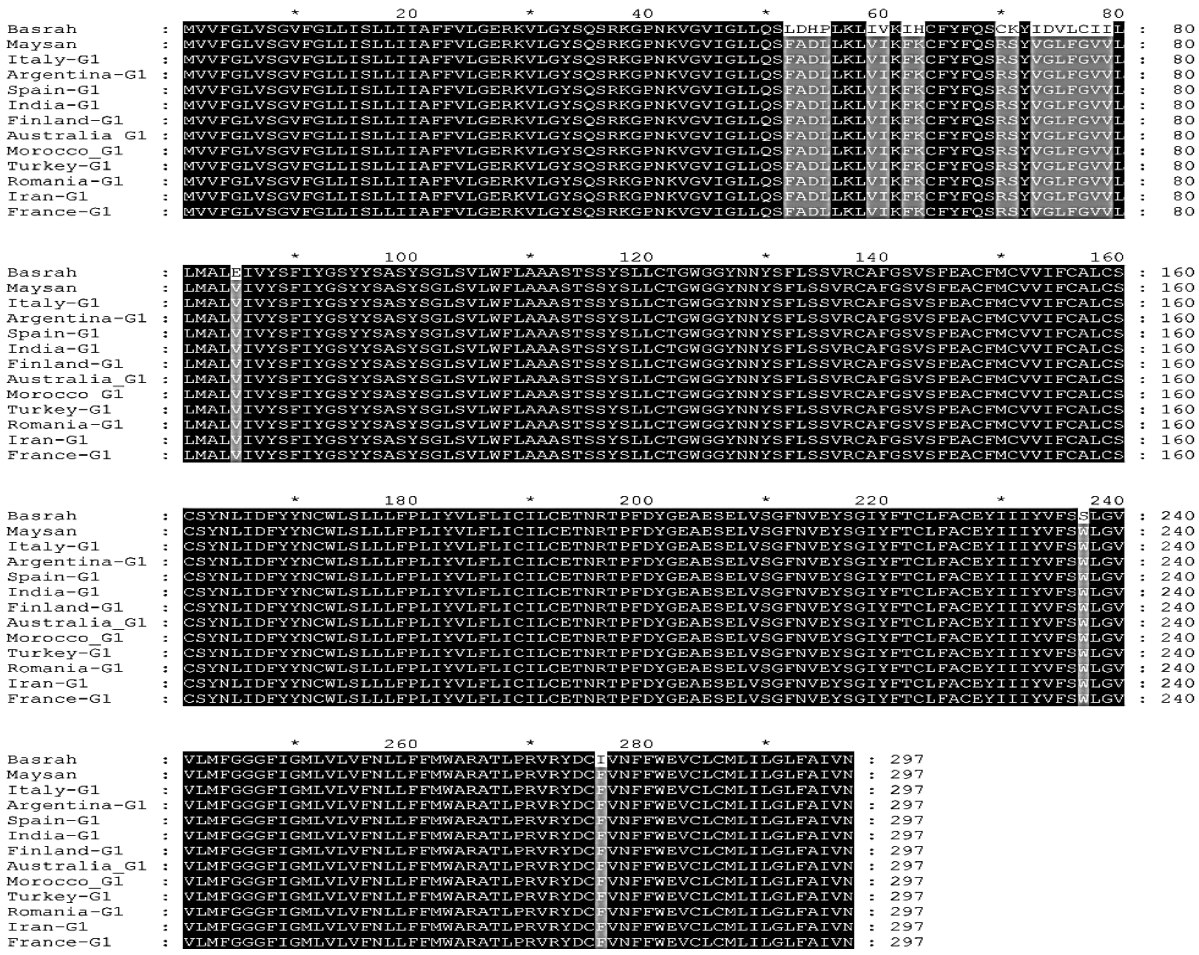

Figure 5. A multiple sequence alignment of the *E.granulosus* mtNAD1 proteins of strain G1 with the Basrah and Maysan mtNAD1 proteins.

However, the excepted motifs were LDHP at residues 42-45, IV at a residue 59, IH at residues 62-63, CK at residues 70-71and IDVLCII at residues 73-79. Moreover, the other exception was a residue E at a position 85, a residue S at a position 237 and a residue I at a position 276.

Fig. 6 displays a multiple sequence alignment of the *E.granulosus* mtNAD1 proteins of strain G3 with the Basrah and Maysan mtNAD1 proteins. The Maysan mtNAD1 proteins shared most of amino acids with the mtNAD1 proteins of strain G3, except for two residues. The exception was a residue I at position 60 and a residue I at position 188, conserved in the mtNAD1 proteins of strain G1. Similarly, the Basrah mtNAD1 proteins shared most of amino acids with the mtNAD1 proteins of strain G3, except for some regions. It retained a residue I at position 188, conserved in the mtNAD1 proteins of strain G1. However, the excepted motifs were LDHP at residues 52-55, IV at residues 59-60, IH at residues 62-63, CK at residues 70-71 and IDVLCII at residues 73-79. Moreover, the other exception was a residue E at position 85, a residue I at position 188, a residue S at position 237 and a residue I at position 276.

In total, the Basrah and Maysan mtNAD1 proteins retained an isoleucine (I) amino acid at a residue 188. This amino acid was only found in the mtNAD1 proteins of strains G1. Thus, both Basrah and Maysan proteins belonged to the strain G1.

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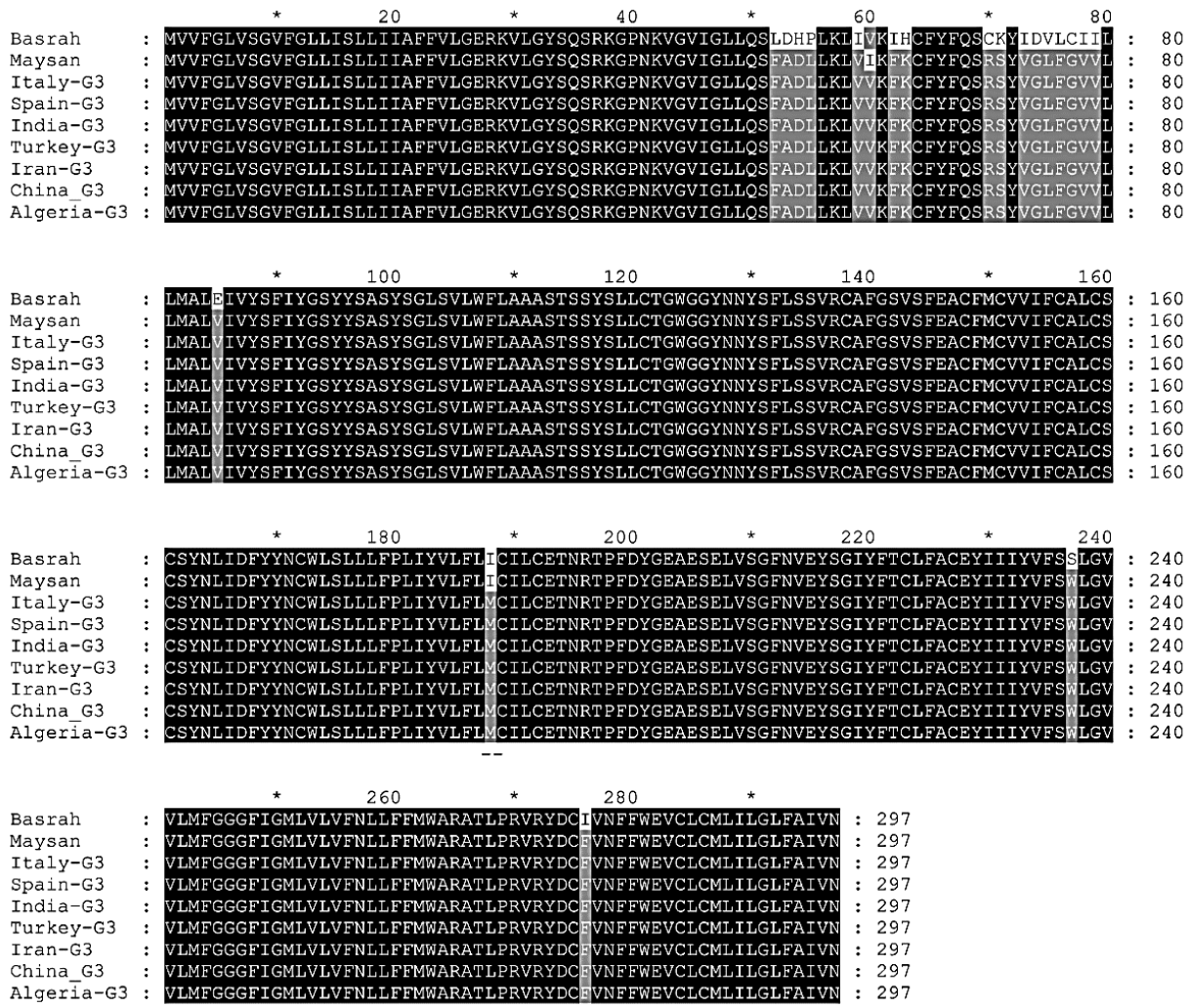

Figure 6. A multiple sequence alignment of the *E.granulosus* mtNAD1 proteins of strain G3 with the Basrah and Maysan mtNAD1 proteins.

Prediction of the structure of the *E. granulosus* mtNAD1 Proteins

The predicted structural model of *E. granulosus* mtNAD1 proteins (obtained from Basrah and Maysan) is generated from the crystal structure of the *Yarrowia lipolytica* NAD1 protein (Protein Data Bank accession number, PDB: 6yj4.1). The monomer form of both Basrah and Maysan mtNAD1 proteins was predicted to have fourteen α-helices whereas there were no β-sheets in these proteins in Fig. 7A and B. Like the Basrah mtNAD1 protein, the Maysan mtNAD1 protein contained the isoluesine residue^{188} (I^{188}) located in the α10 helix near to the C-terminus. In total, the homology model of the Basrah and Maysan mtNAD1 proteins was very similar and the isoluesine residue found only in the strain G1 was located in the α10 helix in both mtNAD1 proteins.

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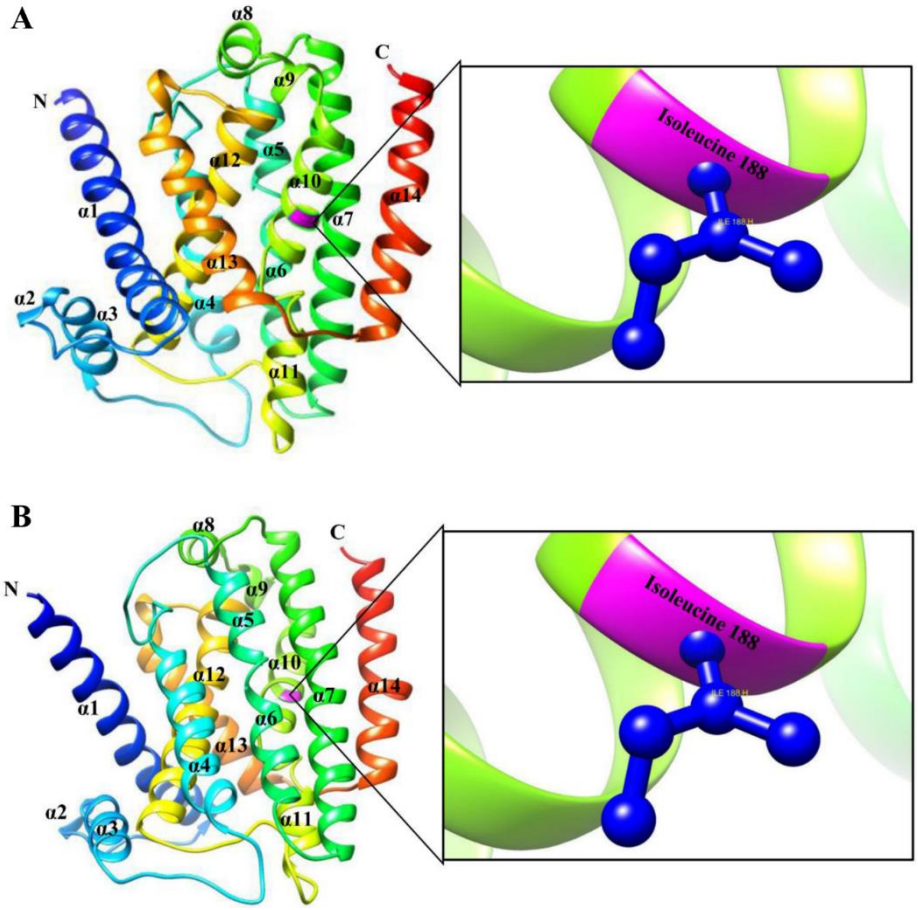

Figure 7. Structural models of the *E. granulosus* NAD1 proteins were built from the crystal structure of the *Yarrowia lipolytica* NAD1 protein (Protein Data Bank accession number, PDB: 6yj4.1). A represents the structural model of the *E. granulosus* NAD1 protein for the Basrah strain whereas B represents the structural model of the *E. granulosus* NAD1 protein for the Maysan strain.

Discussion

E. granulosus is known to have up to eight strains and these strains are not easy to distinguish using morphological ways [9, 12]. In recent years, mitochondrial genes, NAD1 and NAD5, have had prominent roles in the identification of very similar *E. granulosus sensu stricto* strains, G1 and G3 [1, 3, 12]. Based on the partial sequence of the NAD1 gene, it was reported that the G1 strain was the common strain in the Thi-Qar province of Iraq [3]. As a result of this, the current study focused on the characterization of the NAD1 gene from the start codon to the stop codon in hydatids isolated from humans that originally were from two Iraqi provinces, Basrah and Maysan.

The whole mtNAD1 gene of *E. granulosus sensu stricto* was amplified and sequenced for the first time in Iraq. To distinguish between very similar G1 and G3 strains and to determine informative positions in nucleotide sequences and amino acid sequences of NAD1, for each province, the consensus sequence was generated from six sequenced samples using BioEdit Sequence Alignment Editor. The current study results showed that the Basrah and Maysan mtNAD1 genes had 1038 bp and they encoded 297 amino acids. This agrees with data published on the NCBI website under specific accession numbers in Table 1. In contrast to this, the phylogeny indicated the Maysan consensus sequence belonged to the G1 strain but the Basrah consensus sequence needed to be investigated further. In Basrah and Maysan consensus sequences, a codon ATA at a position 648-650 in the gene encoding important isoleucine

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(I) amino acid at a position 188 in the protein. Moreover, the alignment of Basrah and Maysan mtNAD1 proteins with their relevant proteins from the G1 and G3 strains (from GenBank) showed the presence of the I^{188} residue in both Basrah and Maysan proteins. The I^{188} residue was a unique residue in the sequence of the *E. granulosus sensu stricto* G1 strain whereas the methionine (M) amino acid at position 188 was a unique residue in the sequence of the G3 strain [3]. Bioinformatics analyses demonstrated that both Basrah and Maysan consensus sequences belonged to the *E. granulosus* G1 strain. This corresponds with previous studies that showed the most common strain in Iraq is G1 [3, 13-15].

Previous studies around the world have either partially or totally used the mtNAD1 sequences of *E. granulosus* isolated from different intermediated hosts including humans in the diagnosis of strains (G1 - G10) or/and the determination of mutations between them based on nucleotide sequences [16]. Unlike the previous studies, this study reported the genetic variation of the *E. granulosus* G1 strain in human samples according to the whole sequences of mtNAD1 and looked for to the main differences between very similar G1 and G3 strains and within the G1 strain based on the complete deduced amino acid sequences. Within the G1 strain, the Maysan mtNAD1 protein had completely no mutations based on the deduced amino acids when compared with those relevant sequences from GenBank. This situation was different with the Basrah mtNAD1 protein which had 20 (6.7%) amino acid differences compared with the Maysan and GenBank deduced amino acids. Within the G3 strain, the Maysan mtNAD1 protein had 2 (0.7%) amino acid differences and the Basrah mtNAD1 protein had 20 (6.7%) amino acid differences when compared with the G3 strain sequences from GenBank. Although these variations were between our samples and the G1 and G3 strains from GenBank, the I^{188} residue and the M^{188} residue were unique in the G1 and G3 strains, respectively [3]. This indicates that these two amino acid residues are the most important in distinguishing between very similar G1 and G3 *E. granulosus* strains.

Based on the differences between the amino acids, some motifs that are conserved in the G1 strain were only replaced in the Basrah mtNAD1 protein. From these motifs, there are nine amino acids in the Basrah mtNAD1 protein that should participate in hydrophobic interactions because the replaced amino acids such as valine (V), leucine (L), proline (P), phenylalanine (F), and isoleucine (I) are all non-polar and most of them have aliphatic R groups, except the F amino acid has aromatic R groups [17]. From these observations, it can be predicted that the Basrah and Maysan mtNAD1 proteins have only 11 amino acid differences. In similar circumstances, the previous study on human complex I subunits showed that an increase in the number of point mutations had negative effects on the efficacy of mitochondrial import to protein [18]. The most popular disorder in the order of oxidation phosphorylation is due to the amino acid mutations in complex I subunits [19-21]. This suggests that the amino acid differences in the Basrah mtNAD1 protein might affect the survival and fertility of the parasite. Thus, this needs to be investigated whether or not this is the case.

According to our search, there are no studies focusing on the experimental structural model of mtNAD1 protein in *E. granulosus*. Thus, the present study showed the predicted structural model of *E. granulosus* mtNAD1 proteins. Both Basrah and Mysan mtNAD1 proteins had very similar homology models and the important isoleucine residue (I^{188}) was located in the α10 helix in both mtNAD1 proteins. Similar to the study performed by AL-Asadi *et al.* [3], this indicates these proteins with a unique I^{188} residue could be used as a strong tool in diagnosis between very similar *E. granulosus* G1 and G3 strains.

Conclusion

In conclusion, this study presented for the first time in Iraq the total characterization of *E. granulosus* mtNAD1 gene. The isoleucine residue (I^{188}) is unique for the G1 strain while the methionine residue (M^{188}) is unique for the G3 strain. The current study also emphasizes the importance of the

mtNAD1 gene as a very useful tool in the diagnosis of very similar *E. granulosus* strains G1 and G3.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Basrah.

Authors' Contribution Statement

S. A. M. AL. designed, performed and analysed experiments as well as wrote the paper. A. H. A. collected the samples and commented on the paper.

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التوصيف الكامل لجين NADH dehydrogenase subunit 1 في الاكياس العدرية البشرية

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الخلاصة

يمتلك طفيلي الاكياس العدرية سلالاتين متشابهتين (G1 و G3) ونظرا للدور البارز الذي تلعبه عائلة جينات نازعات الهيدروجين في التميز بين هاتين السلالاتين، لذا هدفت الدراسة الحالية الى اجراء التوصيف الجزيئي الكامل لاحد ابرز انواع هذه العائلة وهو الجين NAD1 (من شفرة البدء الى الي شفرة التوقف) للاكياس العدرية المعزولة جراحيا من اكباد المرضى (ولمحافظتي البصرة وميسان) في مستشفى الصدر التعليمي في محافظة البصرة، العراق. عزلت الرؤيسات الاولية من الاكياس تحت ظروف معقمة واستخدمت في استخلاص الحامض النووي منقوص الاوكسجين. اذ ضخم التتابع الكامل للجين في عينات الدراسة وانجز التتابع (السكونس) لهما باستخدام بادئات متخصصه. اظهرت نتائج تحليل السكونس المتكرر Consensus sequence امتلاك كل من عينات البصرة و ميسان 1038 زوجا من القواعد وتقع شفرة البدء GTG في الموقع 87-89 وشفرة التوقف TAA في الموقع 978-980. كما اظهرت استدلالات الاحماض الامينية الى وجود 297 حامض اميني لبروتينات عينات البصرة وميسان. فضلا عن ذلك بينت تحاليل المعلومات الحياتية احتواء هذه البروتينات على الحامض الاميني Isoleucine في الموقع 188 (I^{188}) وهو موجود فقط في سلالة الاغنام G1. وان النموذج التركيبي لبروتينات عينات البصرة وميسان اظهر تشابها كبيرا و يقع I^{188} في الحلزون 10 (α10). بينت الدراسة ولاول مرة على مستوى العراق التوصيف الكامل للجين NAD1 والعائد الى سلالة الاغنام G1 المسببه لمرض الاكياس العدرية البشري.

الكلمات المفتاحية: بصرة، المشوكة الحبيبية، سلالة G1، ميسان، NAD1.