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Molecular identification of *Enterobius vermicularis* worms isolated from children in Babylon province of Iraq

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Abstract---Background and Objective: The conventional PCR technique was using for study genetic variations for Cytochrome C oxidase subunit 1 (cox1) gene of *E. vermicularis* between local and global to NCBI-BLAST *E. vermicularis*. Materials and methods: Stool samples were collected from children at early morning then transported to the laboratory. The DNA cox1 gene were isolated from stool samples then amplified by PCR technique and its results were analysis via agarose gel electrophoresis. The DNA sequencing method was carried out by sent PCR products to Macrogen Company in Korea. Phylogenetic analysis was conducted by using (UPGMA tree) method in (MEGA 6.0 version). Results: Results appears that Stool samples was positive for infection with E. *vermicularis* worm, DNA for *cox*1 gene was amplified to 407 bp that was positive at agarose gel electrophoresis. The human *E. vermicularis* local isolates showed homology identity to the NCBI-BLAST *E. vermicularis* human isolates from Iran.The phylogenetic analysis was accomplished to constructing the phylogenetic tree. The sequences of local isolates were showed have genetic relationship to NCBI-BLAST *E. vermicularis* with total genetic variations (0.0080-0.0020). Conclusion: PCR technique is valuable technique for isolate and know genetic variations *E. vermicularis COX*1 DNA genes. The local isolates from pinworm infected children showed identity homology to the global NCBI-BLAST *E. vermicularis* human isolates from Iran.

Keywords---*Enterobius vermicularis*, genotype, cytochrome C oxidase, subunit 1 gene, Babylon province, Iraq.

Introduction

Enterobiasis *caused by Enterobius vermicularis* (pinworm) is a common parasitic infection spread throughout the world and usually causes infection in school going children, presenting as peri-anal itching , attributed to the mucoid secretions of the eggs on the skin [1] ,it is remains a public health problem in many countries, it tends to be more common in school children in rural areas and in poorer urban areas [2;3 and4] Humans are the only natural host of *E.vermicularis*, it is very related with high population density, socio-economic factors and the habit of fingers sucking[5;6] . It is a nematode residing in the large intestine of humans[1] , also it is spread by fecal-oral rout when patient ingests eggs that initially present in the perianal area of infested patients [4] . Many cases of *E. vermicularis* infection are asymptomatic but when symptomatic the most common symptoms include intense itching and inflammation of the anal or vaginal areas, intestinal irritation and difficulty sleeping [7;3]. Diagnosis of *E. vermicularis* infection performs by microscopic examination of the characteristic worm eggs, sample collection by swabbing the anal folds using commercially available adhesive cellulose tape (scotch tape) in the morning prior to defecation and before washing the genital area [8;9 and 10].

Molecular methods in the diagnosis of parasites are sensitive and highly accurate [11] . Molecular tools might help to understand transmission routes and distinguish persistent from repeated infections [12] . They can distinguish between strains of a single species, identify pathogens from non-pathogens, study parasitic genetics and virulence factors For pathogens that facilitate the identification and selection of appropriate treatment [11]. *E. vermicularis* DNA extracted from eggs and has been successfully amplified and sequenced to recognize the genetic diversity, phylogeography and host specificity of this global parasite [6] . human *E. vermicularis* has been used in population genetics studies in modern populations especially with the isolation of the mitochondrial *cox* 1gene, and hundreds of prehistoric new World coprolites have been confirmed to harbor this pinworm[13] .

Materials and Methods

Stool samples collection

Stool samples were collected by clean, dry, plastic containers from each child early at the morning by parents for the younger children or by self-collected for older children with heavy infection, then the samples transported to the laboratory and screening for presence of ova or adult stages and considered as an infectious substance. The patients also were asked if they take any antihelminthic therapy within three months before sample collection [14] .

Stool examination

stool samples were examined using wet mount technique and Lugol's iodine smear to detect *E. vermicularis* ova and adults and examine under light microscope with magnifications power 100 X and 400 X. Specimens containing

ova or adults were considered as positive, all positive samples were stored by freezing at -20 °C to preserve them for molecular analysis [15;10] .

Stool DNA Extraction

Extraction of genomic DNA from refrigerated stool specimens was carried out using Presto™ Stool DNA Extraction Kit .Extraction process was done according to kit user manual guidelines. The quantity and purity of extracted genomic DNA which extracted from *E.vermicularis* ova was estimated using agarose gel electrophoresis and Nanodroplite spectrophotometer, then the extracted DNA was stored at -20C until utilized in PCR technique [14].

Polymerase chain reaction (PCR)

The PCR primers for detection *Enterobius vermicularis* based on mitochondrial cytochrome oxidase subunit 1 Cox1 gene were designed in this study using NCBI-Genbank (AP017684.1) and primer 3 plus design. These primers was provided from Scientific Reserecher.Co.Ltd, Iraq. The COX1 gene were amplified to 407bp for detection *Enterobius*, forward primer (5' TGTGTTGGCTGGGGCTTTAA 3') and reverse primer (5' GCTGCACAACTAAACGTCCC 3'). The PCR master mix was prepared by using (GoTaq Green PCR Master Mix) and this master mix done according to company instructions as: DNA template (5µl) , Forward primer (2µl), Revers primer (2µl) , Go taq Green Master mix(12.5µL) ,Molecular Grade Water(3.5µl) , Total volume (25µl). These PCR master mix component transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler.

Conventional PCR reaction was performed using the following conditions: Initial denaturation (one cycle of 95°C for 5 min), followed by denaturation (35 cycle of 30 sec at 95°C), annealing (35 cycle of 30 sec at 58°C), Extension (35 cycle of 2 min at 72°C), then final extension (1 cycle of 5 min at 72°C), and hold at 4°C more than 5 min. Analysis of PCR products was performed via agarose gel electrophoresis using 1.5% Agarose gel was prepared in using 1X TBE (Tris-borate-EDTA buffer solution) and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C, Then 3µl of ethidium bromide stain were added into agarose gel solution. Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray. The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer.10µl of PCR product were added in to each comb well and 3µl of (100bp Ladder) in first well. Then electric current was performed at 100 volt and 80 AM for 1hour. PCR products were visualized by using UV Transilluminator [16].

DNA sequence method

The DNA sequencing method was carried out for study the genetic variation of mitochondrial cytochrome oxidase subunit 1 Cox1 gene between local Enterobius vermicularis isolates and NCBI BLAST related *E. vermicularis* isolates . The PCR products were sent to Macrogen Company in Korea in ice bag by DHL for performed the DNA sequencing by AB DNA sequencing system. The DNA

sequencing analysis was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis of the partial Cox1 genes based ClustalW alignment analysis and The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method [14].

Results

Stool sample demonstrated that presence of ova and adults stage of *E. vermicularis* helminths as showing in figer 1,2,3 and 4.

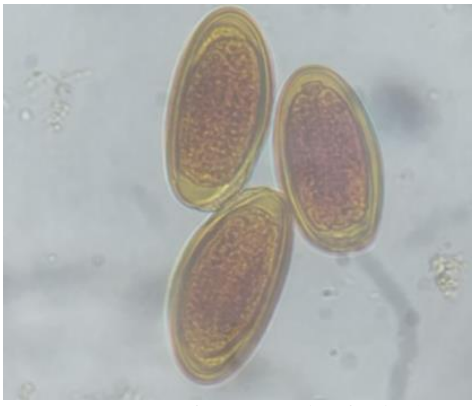

Figure 1. Eggs of E. vermicularis (400X)

Figure 2. Adult female of *E. vermicularis* (40X)

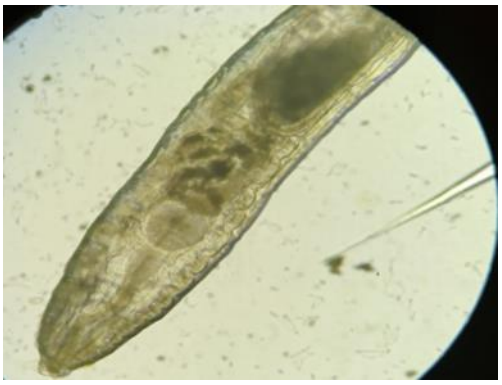

Figure 3. Front portion adult female worm(100X)

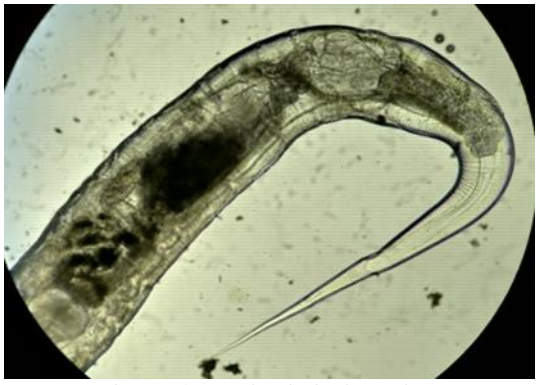

Figure 4. End portion of adult female worm (100X)

The Agarose gel electrophoresis results for PCR products in *E. vermicularis* worm showed appears *cox1 gene* bands at 407 bp PCR product size as showing in Figure 5. The DNA sequencing results for 6 human *E. vermicularis* local isolate (IQH , No.1 - IQH,No.6) for cox1 gene in Babylon province revealed that there were some nucleotide substitution in these local isolates. The linear DNA 298bp of *E . vermicularis* isolate IQH No.1 sequence showed that the bases count were 63 A, 27C, 75 G, and 133T,a nucleotide substitution analysis revealed that there were three substitutions as showing in figure 6 .

Linear DNA 294 bp *E . vermicularis* isolate IQH No.2 sequence showed that the bases count were 62 A, 25C, 74 G and 133T, the nucleotide substitution analysis revealed that there were two substitutions as showing in figure 7.The linear DNA295 bp of *E . vermicularis* isolate IQH No.3 sequence showed that the bases count were 62 A, 26C, 74 G, and 133 T,the nucleotide substitution analysis revealed that there were two substitutions as showing in figure 8.The linear DNA 296 bp of *E . vermicularis* isolate IQH No.4 sequence showed that the bases count were 63 A, 26C, 74 G, and 133T, the nucleotide substitution analysis revealed that there were one substitutions as showing in figure 9 .The linear DNA 294 bp of *E . vermicularis* isolate IQH No.5 sequence showed that the bases count were 62 A, 25C, 74 G, and 133 T ,a nucleotide substitution analysis revealed that there were two substitutions as showing in figure10.

The linear DNA 296 bp of *E . vermicularis* isolate IQH No.6 sequence showed that the bases count were 63 A, 26C, 74 G, and 133T ,a nucleotide substitution analysis revealed that there were two substitutions as showing in figure11. All these six local isolates for *E. vermicularis* were recorded in NCBIBLAST GenBank as a global scientific website (Table 1).Phylogenetic analysis showed that The local *E. vermicularis* (IQH-No.1-IQH-No.6) isolates were showed genetic related to NCBI-BLAST *E. vermicularis* at total genetic changes (0.0080-0.0020) as in Figure 12.

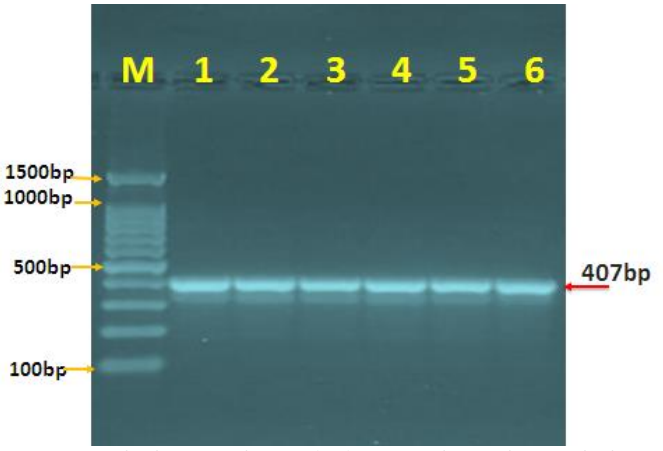

Figure 5. Agarose gel electrophoresis image that showed the PCR product analysis of mitochondrial cytochrome oxidase subunit 1 Cox1 gene in Enterobius vermicularis from Human stool samples. Where , the Lane (M): DNA marker ladder (1500-100bp) and the Lane (1-6) were showed positive *Enterobius vermicularis* cox1 gene at 407bp PCR product size

Score	Expect	Identities	Gaps	Strand
534 bits(289)	5e-156	295/298(99%)	0/298(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGTTAGTCA
T 60
Sbjct 1 .....**T**..... 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATGATTTATG
CT 120
Sbjct 61 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTACTATTG
GT 180
Sbjct 121 **T**.....**G**..... 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTGTGCCAA
CT 240

Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTTGTGCAG
C 298
Sbjct 241 298

Figure 6 Multiple sequence alignment analysis of mitochondrial Cox1 gene partial sequence in local E.vermicularis_IQH_No.1 isolate with NCBI-BLAST Enterobius vermicularis related Genotypes isolates by using (Multiple Sequence Alignment by CLUSTALW). The multiple alignment analysis were showed the similarity (*) and genetic variation (Substitution mutation) at mitochondrial Cox1 gene nucleotide sequences.

Score	Expect	Identities	Gaps	Strand
532 bits(288)	2e-155	292/294(99%)	0/294(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGTTAGTCA
T 60
Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATGATTTATG
CT 120
Sbjct 61 .....**A** 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTACTATTG
GT 180
Sbjct 121 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTGTGCCAA
CT 240
Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTTGTG
294
Sbjct 241 .....**T**..... 294

Figure 7 Multiple sequence alignment analysis of mitochondrial Cox1 gene partial sequence in local E.vermicularis_IQH_No.2 isolate with NCBI-BLAST Enterobius vermicularis related Genotypes isolates by using (Multiple Sequence Alignment by CLUSTALW). The multiple alignment analysis were showed the similarity (*) and genetic variation (Substitution mutation) at mitochondrial Cox1 gene nucleotide sequences.

Score	Expect	Identities	Gaps	Strand

534 bits(289)	5e-156	293/295(99%)	0/295(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGTTAGTCA
T 60
Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATGATTTATG
CT 120
Sbjct 61 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTACTATTG
GT 180
Sbjct 121 .....**C**..... 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTGTGCCAA
CT 240
Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTTGTGC
295
Sbjct 241 .....**T**..... 295

Figure 8 Multiple sequence alignment analysis of mitochondrial Cox1 gene partial sequence in local E.vermicularis_IQH_No.3 isolate with NCBI-BLAST Enterobius vermicularis related Genotypes isolates by using (Multiple Sequence Alignment by CLUSTALW). The multiple alignment analysis were showed the similarity (*) and genetic variation (Substitution mutation) at mitochondrial Cox1 gene nucleotide sequences

Score	Expect	Identities	Gaps	Strand
542 bits(293)	3e-158	295/296(99%)	0/296(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGTTAGTCA
T 60
Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATGATTTATG
CT 120
Sbjct 61 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTACTATTG
GT 180
Sbjct 121 .....**C**..... 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTGTGCCAA
CT 240
Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTTGTGCA
296
Sbjct 241 296

Figure 9 Multiple sequence alignment analysis of mitochondrial Cox1 gene partial sequence in local E.vermicularis_IQH_No.4 isolate with NCBI-BLAST Enterobius vermicularis related Genotypes isolates by using (Multiple Sequence Alignment by CLUSTALW). The multiple alignment analysis were showed the similarity (*) and genetic variation (Substitution mutation) at mitochondrial Cox1 gene nucleotide sequences.

Score	Expect	Identities	Gaps	Strand
532 bits(288)	2e-155	292/294(99%)	0/294(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGTTAGTCA
T 60
Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATGATTTATG
CT 120
Sbjct 61 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTACTATTG
GT 180
Sbjct 121 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTGTGCCAA
CT 240
Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTTGTG
294
Sbjct 241 .....**T**.....**A**.... 294

Figure 10 Multiple sequence alignment analysis of mitochondrial Cox1 gene partial sequence in local E.vermicularis_IQH_No.5 isolate with NCBI-BLAST Enterobius vermicularis related Genotypes isolates by using (Multiple Sequence Alignment by CLUSTALW). The multiple alignment analysis were showed the

similarity (*) and genetic variation (Substitution mutation) at mitochondrial Cox1 gene nucleotide sequences.

Score	Expect	Identities	Gaps	Strand
536 bits(290)	1e-156	294/296(99%)	0/296(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGTTAGTCA
T 60
Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATGATTTATG
CT 120
Sbjct 61 .....**C**..... 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTACTATTG
GT 180
Sbjct 121 .....**A**..... 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTGTGCCAA
CT 240
Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTTGTGCA
296
Sbjct 241 296

Figure 11 Multiple sequence alignment analysis of mitochondrial Cox1 gene partial sequence in local E.vermicularis_IQH_No.6 isolate with NCBI-BLAST Enterobius vermicularis related Genotypes isolates by using (Multiple Sequence Alignment by CLUSTALW). The multiple alignment analysis were showed the similarity (*) and genetic variation (Substitution mutation) at mitochondrial Cox1 gene nucleotide sequences.

All these six local isolates for *E. vermicularis* were recorded in NCBI BLAST GenBank as a global scientific website (Table 1).

Table 1
The NCBI-BLAST Homology Sequence identity (%) between local and global *E. vermicularis* isolates

Isolate No.	Genbank accession no.	Homology sequence identity			
		Identical isolate	Country	Mutation %	Identity %
IQH-No.1	MZ505531.1	MH802611.1	Iran	1.1%	98.99%
IQH-No.2	MZ505532.1	MH802611.1	Iran	0.68%	99.32%

IQH-No.3	MZ505533.1	MH802611.1	Iran	0.68%	99.32%
IQH-No.4	MZ505534.1	MH802611.1	Iran	0.34%	99.66%
IQH-No.5	MZ505535.1	MH802611.1	Iran	0.68%	99.32%
IQH-No.6	MZ505536.1	MH802611.1	Iran	0.68%	99.32%

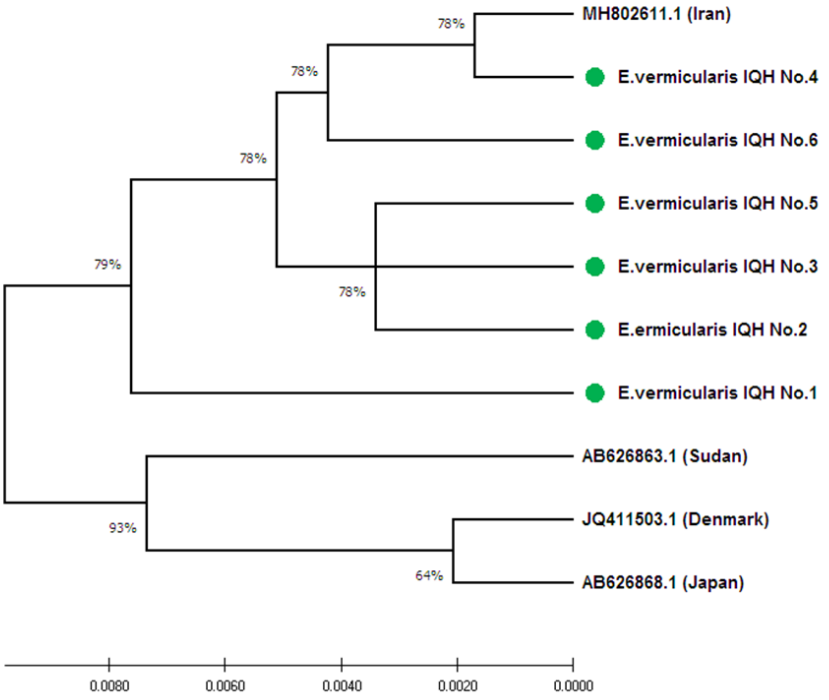

Figure 12 Phylogenetic tree analysis based on the Mitochondrial Cox1 gene partial sequence that used for *E. vermicularis* genetic relationship analysis. The phylogenetic tree was constructed using The evolutionary distances were computed using the Maximum Composite Likelihood method (UPGMA tree) in (MEGA 6.0 version), the local *E. vermicularis* isolates (IQH-No.1-IQH-No.6) were show closed related to NCBI-Blast *E . vermicularis* Iran isolate (MH802611.1). at total genetic change (0.0080-0.0020).

Discussion

great studies have been made using various kinds of data including molecular sequences to clarify the phylogenetic relationships of nematodes, In the phylum Nematoda, inferring accurate phylogenies can provide significant insights into the evolution and diversity of this remarkable group [17] . Molecular techniques have been developed to enhance diagnosis of pathogens, validating of PCR technique when it is using for diagnosis is challenging. The using of computer modeling for primer designing to enhance PCR conditions, sensitivity, and specificity. The diagnostic purposes require using of primers that target housekeeping genes or

ribosomal ribonucleic acid that common to numerous species to identify the pathogens at genus level [18]. Analysis of DNA sequences is importance in confirmation of classical morphology-based identifications of nematode species and in reconstruction of their phylogenetic relationships with available sequences in GenBank [19] .The phylum Nematoda, inferring accurate phylogenies can provide significant insights into the evolution and diversity of this remarkable group. Nematodes constitute one of the most common, ecologically diverse, and speciose animal groups in the world [17] .

Molecular studies for the genotype characterization of *E. vermicularis* are limited [20]. The application of molecular and analytical tools from the fields of population genetics and systematics enable a reconstruction of evolutionary relationships between parasites over a wide range of temporal and spatial scales, improving our ability to identify parasites, Molecular characteristics of *E. vermicularis* isolates are the starting point for defining the genetic diversity of human pinworm [7]. Molecular study findings that conducted by using polymerase chain reaction technique for amplification Cox1 mitochondrial genes with 407 bp of *E. vermicularis* revealed that six sample of adult worm and ova is positive ,these mean founding of *E. vermicularis* worm in a sample , these results agreement with findings of [21] , their results appear a bands of Cox1genes by 333 nucleotide long fragments, also with results of [22] , their results clarify a bands of Cox1genes by 333 nucleotide long fragments, as with a study by [23] , in Indonesia on *cox*1 gene ,as well as with findings of [24] , they were revealed that the all samples belonged to haplogroup B depend on Cox1 genes ,also with results of [7] , their findings appears existence of a three different haplotypes of pinworm by using Cox1 genes , as with [6] they were get on a bands of cox1 gene , also with [20] , they were amplified 390 bp piece from cox1 gene, as well as with findings of [16] her results appear bands of COI gene of *E.vermicularis* parasite based on the 301pb, also with results of [14] her findings appears bands of COI gene of based on the 566 pb, PCR products of *cox*1 gene in *E. vermicularis* showed that 28 of samples were positive.

The *cox1* gene used for detected many of living organism because a *cox1* gene has been reported to display high variability than the other regions and proposed to phylogenetic studies [24] , also can be coded on one strand [25] , as well as a mitochondrial genes is a useful tool for nematode and has been widely applied to resolve uncertainty within this group [17] . Some authors argue that an evolution of a cox1 gene is sufficiently rapid to discriminate between closely related species and investigate intraspecific diversity [26] . The cox1 region is highly efficient for discriminating vertebrate and invertebrate species [27;28] . The DNA barcoding system using the cytochrome c oxidase subunit 1 Cox1 that appears to have a better phylogenetic signal than the other mitochondrial genes [29] .

Studying and analysis of pinworms mtDNA genes showed to be a powerful tool in identification, studying evolutionary history, geographical variations among same species and population differences [7;22] . In present molecular study tests and from a cox1 genes sequences analysis by PCR technique that confirmed a diagnosis of *E. vermicularis* ,a local *E. vermicularis* isolates (IQH-No.1-IQH-No.6) were show closed related to NCBI-Blast *Enterobius vermicularis* of Iran isolate

(MH802611.1),at total genetic changes or variations (0.0020-0.0080),these findings were. These results were agree with results of [22] they were shows pinworms in Denmark closely related to samples collected in Germany, Greece and Japan cox1 mitochondrial gene,also agree with results of [7] their results appears a sequences homogeneous together with human *E. vermicularis* isolates from Denmark, Germany, Greece, and Japan with genetic variation 0.01,as well as with findings of [6] , they were found a genetic variation in phylogenetic analysis and similarity in Thailand isolates with isolates from Japan and Korea, Iran, Czech Greece, Denmark and Sudan ,as well as with findings of [20] , their results showed 0.02% a genetic variation and homology with isolates from Greece , also with results of [16] she was found genetic homology of her study isolate with isolate from Iran and Hunan and she was found a genetic variation. , as well as [14] her results were showed a genetic related to NCBI-BLAST *E. vermicularis* at total genetic changes(0.0020%)and a local isolates were showed 99%homology identity to the NCBI-BLAST *E. vermicularis* isolates.

The use of whole mitochondrial genome sequences is one alternative that has been widely applied to many nematode branches where relationships were unclear [17] , cox1 gene is the more conserved gene within Oxyurids [11] ، mt DNA genes proved to be good genetic targets to identify and study pinworms of medical importance [17;30],amplification of partial specific mtDNA genes confirmation a species and a draw its relationships with other Oxyurids [30] , the mitogenome can provide resolution for an enormously broad range of phylogenetic tree from shallow divergence times among populations of a single species to deep divergence within an entire phylum [17].

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