Short Communication

The 18S Ribosomal DNA Sequence of *Strongyloides stercoralis* in Iran

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Abstract

Background: *Strongyloides stercoralis* is a prevalent parasite in some rural areas in the north of Iran. We decided to investigate whether the 18S ribosomal DNA sequence of the parasite in Iran is similar to the findings of the other researchers.
Methods: We collected 3514 stool samples from Gilan and Mazandaran, northern Iran, during the year 2005-2006, from which 96 were found infected with *S. stercoralis*. Using Bearman method filariform larvae were isolated. The larval DNA was extracted and subjected for PCR amplification and sequencing.
Results: We found 2.73% *S. stercoralis* infection in stool examination. The partially sequence of Iranian S. *stercoralis* 18S rDNA gene was deposited to GenBank at accession number of EF062571.
Conclusion: The 18S rDNA sequence of *S. stercoralis* in Iran is very similar to the related sequences deposited in GenBank (94-93% identification).

Keywords: *18S rDNA,* Strongyloides stercoralis*, Filariform larvae, PCR, Iran*

Introduction

Strongyloides stercoralis is an important human pathogenic parasite. It has the capacity of the internal and external autoinfection and altering from free-living to parasitic life cycle. The ability to multiply in its host and the environment causes *Strongyloides* to survive and persist. It is prevalent in people who live in tropical and subtropical areas with almost heavy rainfall (1) but also occurs at low prevalence (4.9%) in some rural areas in the north of Iran (2). *S. stercoralis* is transmitted by contact with contaminated moist soil. The infection is mostly asymptomatic but when the normal life cycle of the parasite changes; it can have considerable potential for causing disease (especially in certain immunosuppressed patients who usually suffer from severe complicated strongyloidiosis) and death.
Diagnosis is performed by observation of the characteristic larvae in the stools using either direct, concentration or cultivation methods, which are not always reliable (3). Serodiagnosis is another choice for diagnosis (4), and today molecular biological methods are applied to detect specific DNA of *S. stercoralis* with ribosomal DNA sequences used in some studies to design radioactive DNA probes or specific PCR primers (5, 6).

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Due to the lack of any significant molecular study on this parasite in Iran, we decided to investigate if the 18S ribosomal DNA sequence of the parasite in Iran is similar to the findings of the other researchers. We have done a survey in two neighboring endemic provinces in the north of Iran (Gilan and Mazandaran) (7), located beside the Caspian Sea with temperate climate.

Material and Methods

Sampling

Gilan and Mazandaran provinces have 2389195 and 2796120 population, respectively (8). Based on statistician's advice, during years 2005-2006 we chose sixteen primary health care units (7 in Gilan and 9 in Mazandaran) we collected 3514 stool samples by using random stratified sampling. Initially using direct method we examined the samples and then by using the Bearman method we isolated the filariform larvae in the positive samples cultured on charcoal (3).

DNA extraction

The 50 *S. stercoralis* larvae were rinsed by PBS buffer three times to remove remained feces, then suspended with 1 ml of lyses buffer (10mM Tris, 10 mM EDTA, 150 mM NaCl and 2% SDS/2% Triton -X100) and submitted to DNA extraction through phenol-chloroform method. DNA was precipitated with ethanol and dissolved it in sterile deionized water (9).

PCR Amplification

We designed a set of primers for nested PCR based on GenBank accession number AJ47023. The nest I primers (SsF 5`- ACA CGG TAA ATA TTT TAG TTG – 3` and SsR 5`- CTA AAT CAT GAA AGA GCT ATC -3`) amplified a *Strongyloides* 18S rDNA gene specific PCR product of 1092 bp and Nest II primer (SsF2 5`- GCT AAT ACA CGC TAT TTA TAC-3` and SsR2 5`- GTT GAG TCA AAT TAA GCC GC-3`) a specific PCR product of 975 bp. The PCR mixture contained: 0.25 mM dNTP, 1.5 units Taq DNA polymerase (CinnaGen, Iran), 20 Pico moles each of the forward and reverse primers, 1x PCR buffer, 1.5 mM $MgCl_2$, 100ng template DNA (1 micro liter of nest I PCR product for PCR II initiation) and distilled water up to 50µl. PCR cycling parameters included initial predenaturation at 94°C for 5 min. The incubation cycles consisted of denaturation at 94°C for 30 sec, primer annealing at 60°C for 30 sec and extension at 72°C for 30 sec, repeated for 30 cycles with a final incubation at 72°C for 5 min (10).

Electrophoresis

PCR products were submitted to electrophoresis using 1% agarose gel and were stained with ethidium bromide (0.5 µg/ml). The DNA bands were visualized under ultraviolet light (UV transilluminator) (11).

Sequencing

The 975 bp PCR product was purified by DNA purification kit (Fermentas Cat. No. k0513) and was subjected to sequencing by dideoxy chain termination method using nest I forward primer (12).

Results

Ninety six positive samples were detected and cultured for recovery from *S. stercoralis'* larvae (2.73%). Genomic DNA was extracted from lysed filariform larvae and subjected to 18S rDNA gene amplification using PCR method. The nested-PCR product was electrophoresed on agarose gel shown in Fig. 1. PCR product of *S. stercoralis* 18S rDNA was purified, sequenced and deposited to GenBank at accession number EF062571. With Blast software it was compared with the other deposited 18S rDNA genes of *S. stercoralis* from the other countries (Fig. 2). There were lots of similarities (94-93% homology) between 18S rDNA sequence of *S. stercoralis* in Iran and the corresponding sequences of the other geographical areas.

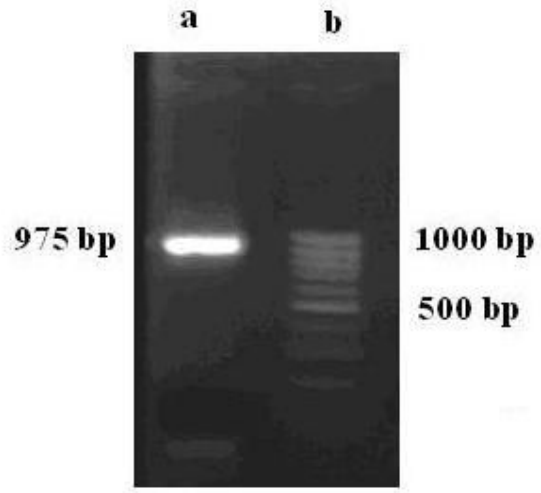

Fig 1. 1% agarose gel electrophoresis
Lane a: 975 bp as PCR product of 18S rRNA *Strongyloides stercoralis*
Lane b: 100 bp DNA ladder marker

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     1     AACTTGTTGGTAAAGGAAAGGGCAAGTCTGGTGCCAGCACCCGCGAGTAATACCAGCTTT  60
AB453314  367   .....................................G.....-...........-..  424
AF279916  464   .....................................G.....-...........-..  521
AJ417023  432   .....................................G.....-...........-..  489
AB453316  367   .....................................G.....-...........-..  424
AB453315  368   .....................................G.....-...........-..  425
M84229    530     ...................................G.....-...........-..  585

EF062571   61   TCCAATGTGCATAAAATGCATTGTTGAGGTTAAAAAGCTCGTAGTTGGCCTTATGAAGAT  120
AB453314  425   .....-...........-.......T.....................-A....A.....  481
AF279916  522   .....-...........-.......T.....................-A....A.....  578
AJ417023  490   .....-...........-.......T.....................-A....A.....  546
AB453316  425   .....-...........-.......T.....................-A....A.....  481
AB453315  426   .....-...........-.......T.....................-A....A.....  482
M84229    586   .....-...........-.......T.....................-A....A.....  642

EF062571  121   TGTATAATGAGCATCTTGGATGTTATTTAATCATTATCATCANAATATTTTTATTATATT  180
AB453314  482   ........................................-TT.................  540
AF279916  579   ........................................-TT.................  637
AJ417023  547   ........................................-TT.................  605
AB453316  482   ........................................-TT.................  540
AB453315  483   ........................................-TT.................  541
M84229    643   ........................................-TT.................  701

EF062571  181   AGAAATAATATCATAACTGTTC-CTTTGAATAAATCAGAGGGTATAAACCAGACATTATA  239
AB453314  541   ...........A.........A-.....................T.................  599
AF279916  638   ...........A.........A-.....................T.................  696
AJ417023  606   ...........A.........A-.....................T.................  664
AB453316  541   ...........A.........A-.....................T.................  599
AB453315  542   ...........A.........A-.....................T.................  600
M84229    702   ...........A........-..A.....................T.................  760
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Fig. 2: Comparison between the 18S rDNA sequence of Iranian *Strongyloides stercoralis* with the high similarities accession numbers in Genbank

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EF062571  240   CGTTTGTATGGTATAGCATGGAATAACACTATAGAAAAATTTAGTGTGGTGTCCCTTAAT  299
AB453314  600   T..........C.....................................T..A......  659
AF279916  697   T..........C.....................................T..A......  756
AJ417023  665   T..........C.....................................T..A......  724
AB453316  600   T..........C.....................................T..A......  659
AB453315  601   T..........C.....................................T..A......  660
M84229    761   T..........C.....................................T..A......  820

EF062571  300   TTTTCATGATTTATAGGAACAAACGGGGGCATTCGTATCGCTACGTTAGAGGTGAAAATM  359
AB453314  660   ..........A.............................................T.C  719
AF279916  757   ..........A.............................................T.C  816
AJ417023  725   ..........A.............................................T.C  784
AB453316  660   ..........A.............................................T.C  719
AB453315  661   ..........A.............................................T.C  720
M84229    821   ..........A.............................................T.C  880

EF062571  360   ATGGACCGTAGCGAGACGTCTTACTGCGAAAGCATTTCCCAAGAATGTTTTCATTAATCA  419
AB453314  720   T.................C.............G.......................  779
AF279916  817   T.................C.............G.......................  876
AJ417023  785   T.................C.............G.......................  844
AB453316  720   T.................C.............G...........-...-.....  777
AB453315  721   T.................C.............G...........-...-.....  778
M84229    881   T.................C.............G.......................  940

EF062571  420   AGAACGAAAGTTAGAGGTTMGAAGGCGATCAGATACCGCCCTAGTTCCAAGCCGTAAACT  479
AB453314  780   .................C.........................T..-.........  838
AF279916  877   .................C.........................T..-.........  935
AJ417023  845   .................C.........................T..-.........  903
AB453316  778   .................C.........................T..-.........  836
AB453315  779   .................C.........................T..-.........  837
M84229    941   .................C.........................T..-.........  999

EF062571  480   ATGCCTTCTMGATGTATGAATTATTAGTTATAATAATTTAAGCATCTTCTCGGAAAGCGA  539
AB453314  839   ......A..A.............................T..............-...  897
AF279916  936   ......A..A.............................T..............-...  994
AJ417023  904   ......A..A.............................T..............-...  962
AB453316  837   ......A..A.............................T..............-...  895
AB453315  838   ......A..A.............................T..............-...  896
M84229    1000  ......A..A.......................T.....T..............-...  1058

EF062571  540   AAGTCCCTCGGTTCCGGGGGAAGTATGGTTGCAAACGCAGAACACTTAAAGGAATTGACG  599
AB453314  898   .....TT.............................-..T...-.................  955
AF279916  995   .....TT.............................-..T...-.................  1052
AJ417023  963   .....TT.............................-..T...-.................  1020
AB453316  896   .....TT.............................-..T...-.................  953
AB453315  897   .....TT.............................-..T...-.................  954
M84229    1059  .....TT.......................A...-..T...-.................  1116

EF062571  600   GAAGGGCACCACCAGGAGTGGAGC  623
AB453314  956   .........................  979
AF279916  1053  .........................  1076
AJ417023  1021  .........................  1044
AB453316  954   .........................  977
AB453315  955   .........................  978
M84229    1117  .....-...................  1139
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Fig. 2: Continued …

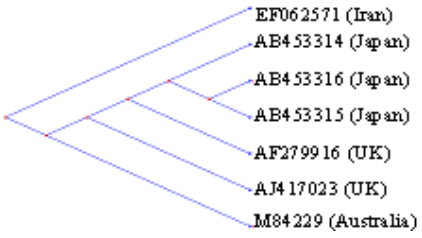

Fig. 3: Slanted distance tree of the 18S rDNA sequence of Iranian *Strongyloides stercoralis* and the high similarities accession numbers in Genbank (using blast pairwise alignments)

Discussion

The prevalence of *S. stercoralis* infection in tropical and subtropical areas with its alarming health and economic consequence has led researchers to perform studies on epidemiologic aspects, diagnostic methods, definition of gene markers and the control of the infection (13). Moreover, in molecular biology and genetic research it has been found advantageous to use the genus *Strongyloides* because of its altering parasitic and free-living generations and vice versa (14). But since morphologically it is not possible to make distinction among different *Strongyloides* species' larvae, some of which may cause disease in human beings, DNA sequence analysis has been used to make accurate diagnosis (15). Consequently, sequencing of small subunit ribosomal DNA genes has become the ″gold standard″ (16), and the 18S rDNA has a variation in nucleotide sequences among *Strongyloides* spp. which is a ″suitable species marker″ (15) for genetic variability analysis in molecular epidemiology.

This is the first report of *Strongyloides* 18S rDNA gene sequence from Iran. But there are some GenBank accession numbers on it from Iran, with other fragments of rDNA gene (ITS1 region, accession numbers: EF653264-66 and EF545004) (17). The parasite 18S rDNA gene sequence at GenBank accession numbers AF279916, AJ417023 (from UK) (18, 19), AB453314, AB453316, AB453315 (from Japan) (15) and M84229 (from Australia) (20) were used for comparison in this study (Fig. 2). The highest degree of similarity between the gene sequence in this article and the already mentioned ones are 94% for the first threes and 93% for the others.

As the slanted distance tree shows, all the sequences branched from a unique source but the second branch indicates that with the Iranian sequence as an exception, the others all originated from the same source (Fig. 3). Probably these results are due to their different geographical regions. ″The nucleotide arrangement presumably reflects the process of geographical dispersal and adaptation to the host″ (15). It seems that it is required to perform further investigations on 18S rDNA gene sequence of *S. stercoralis* to find out more about this gene in other parts of Iran.

In conclusion, the sequence of Iranian *S. stercoralis* 18S rDNA gene has a high degree of similarity (94-93%) with the related sequences previously deposited in GenBank.

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