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Original Research Paper

Physiotherapy

GENETIC VARIATION OF *GIARDIA LAMBLIA* LOCAL ISOLATES FROM KOLKATA, INDIA

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KEYWORDS :

INTRODUCTION

Giardia intestinalis, a protozoan parasite is causative agent of diarrheal disease in humans as well as large number of wild and domestic animals. This enteric parasite is considered a species complex having eight distinct assemblages (A-H).[1] They exhibit little differences in their morphology, but possess remarkable genetic variability. Among them members of only assemblage A and B cause human infection. [2] *G. lamblia* assemblage A is divided mainly into three sub-assemblages: AI, AII and AIII. Assemblage B is divided into two sub-assemblages, BIII and BIV. [3] The objective of this study was to identify inter- and intra-assemblage genetic variation in *G. lamblia* among patients in Kolkata and adjacent areas in eastern India. As of now there is very little information available on the diversity of Giardia sub-assemblages and multi-locus genotypes infecting people in this region. The genetic assemblages and subtypes were determined via multilocus sequence typing (MLST) using two house-keeping genes: -giardin and triose phosphate isomerase gene loci.

Materials and Methods

Study population and collection of faecal samples

A total of 413 faecal samples were collected from I.D. Hospital, Beliaghata, Kolkata between January, 2019 to May, 2019. Written consent from patients or guardians according to the availability were collected.

Microscopic examination of the faecal samples

All faecal samples were examined using both Lugol's iodine staining and wet smear technique under a compound microscope in of 400x magnification.

DNA isolation and molecular detection

DNA from the stool samples was extracted directly using Stool DNA Mini Kit (QIAGEN, USA) according to manufacturer's protocol. Molecular detection of *G. intestinalis* was achieved by amplifying 211bp of beta-giardin gene. The primer sequence used is as follows: GldtF: 5'-ATAACGACGCCATCGCGGCTCTCAGGAA-3', GldtR: 5'-TTTGTGAGCGCTTCTGTCGTGGCAGCGCTAA-3' [4]

The PCR mix consisted of 1X buffer containing 1.5 mM $MgCl_2$ (Roche, Germany), 200uM of each dNTP, 10 pmol of each primer (GCC Biotech), 2.5 units of Taq DNA polymerase enzyme (Roche) and 125ng of isolated DNA in final volume of 50ul. PCR cycle condition was: After an initial denaturation step of 5 min at 94°C, a set of 35 cycles was run, each consisting of 30 sec at 95°C, 1 min of annealing at 65°C and 60 sec at 72°C, followed by a final extension of 7min at 72°C and kept for hold at 4°C.

Amplification of beta- giardin and triose phosphate isomerase gene

The samples that were found positive for the presence of *G. intestinalis* in both microscopy and PCR were further used for multilocus genotyping.

A 511bp fragment of beta-giardin (bg) gene was amplified via nested PCR. The required primer pairs are: GlbgF1: 5'-AAGCCCGACGACCTCACCCGCAGTGC-3', GlbgR1: 5'-GAGGCCGCCCTGGATCTTCGAGACGAC-3'; GlbgF2: 5'-GAACGAACGAGATCGAGGTCCG-3', GlbgR2: 5'-CTCGACGAGCTTCGTGTT-3' [4]

A portion of triose phosphate isomerase (tpi) gene with a fragment size of 530bp was amplified via nested PCR using 2 sets of primers viz. GltpiF1: 5'-AAATATGCCTGCTCGTCG-3', GltpiR1: 5'- CAAACCTTTCCGCAAACC-3'; GltpiF2: 5'-CCCTTCATCGGGGTAACTT-3', GtpiR2: 5'-GTGGCCACCACCCCGTGCC-3'.[4]

In both cases, The PCR mix consisted of 1X buffer containing 1.5 mM $MgCl_2$ (Roche, Germany), 200uM of each dNTP, 10 pmol of each primer (GCC Biotech), 2.5 units of Taq DNA polymerase enzyme (Roche) and 125ng of isolated DNA in final volume of 50ul.

PCR cycle condition for *bg* gene amplification was: After an initial denaturation step of 10 min at 94°C, a set of 30 cycles was run, each consisting of 30 sec at 94°C, 1 min of annealing at (65°C for primary, 55°C for nested) and 60 sec at 72°C, followed by a final extension of 5min at 72°C and kept for hold at 4°C.

PCR cycle condition for *tpi* gene amplification was: After an initial denaturation step of 10 min at 94°C, a set of 30 cycles was run, each consisting of 30 sec at 94°C, 1 min of annealing at (52°C for primary, 60°C for nested) and 60 sec at 72°C, followed by a final extension of 5min at 72°C and kept for hold at 4°C.

Agarose Gel Electrophoresis

After completion of PCR, the PCR products were electrophoresed in 1.5% agarose gel to observe specific bands in presence of marker (Figure- 1, 2).

Sequencing Reaction

The purified products of the nested PCR were then sequenced bidirectionally by using ABI PRISM®3100 Genetic Analyzer (Applied Biosystem) with following primers separately: GlbgF2, GlbgR2 for *bg;* GltpiF2, GltpiR2 for *tpi*.

Phylogenetic analysis

The sequences were validated using the BLAST database search and then aligned using ClustalW. Afterwards, a rooted phylogenetic tree was created via UPGMA method with clustalW. Representative sequences of *Giardia intestinalis* isolates from human were obtained from the NCBI database and were used to prepare the tree (Figure-3, 4).

RESULTS

In this study, 25 samples (6%) were positive for *G. intestinalis* using either of the parasitological procedures. Of these, 18 samples were found positive in both microscopy and PCR. Out of those only 7 samples were successfully amplified and sequenced for both beta- giardin and triose phosphate isomerase loci. Sequence results showed that 3 local isolate belonged to assemblage A while 3 local isolates were of assemblage B. One possible mixed infection (A+B) was also obtained. Further investigation into sub-assemblage level revealed that 2 local isolates (Sample ID- 9993, 11576) along with laboratory strain Portland 1 (ATCC-30888) belonged to sub-assemblage AI. Another local isolate (Sample ID- 10339) showed similarity to sub-assembalge AI for beta-giardin gene whereas for triose phosphate isomerase locus it showed similarity towards AII sub-assemblage.

Incongruent genotyping results were also obtained for the 3 local isolates (Sample ID- 10069, 10378, 10428) of assemblage B. All of them showed same percentage of sequence similarity towards both sub-assemblage BIII and BIV for *bg* locus, whereas they were classified as BIII at *tpi* locus (Table- 1).

CONCLUSION

Genetic heterogeneity of *Giardia intestinalis* causes major inconvenience in multi-locus genotyping for assemblage assignation. [5] Previous to this study, mixing between two different assemblages i.e. presence of both assemblage A and B together had been reported in this limited geographical boundary. The long-standing explanations to this phenomenon are: 1) occurrence of mixed infection 2) possible allelic sequence heterogeneity in a single parasite and 3) meiotic recombination. [6] In this case, we have found 'mixed template' situation between subtypes within the assemblages. Heterogeneous sequences in assemblage B parasites has already been reported and is a common occurrence, whereas heterogeneity within assemblage A is less frequent. [7] Our present study also corroborates to these reports.

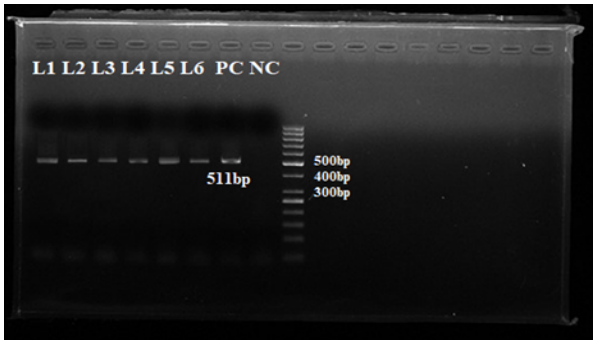

Figure-1: Amplification of *Giardia intestinalis* beta-giardin (*bg*) gene fragment 511bp

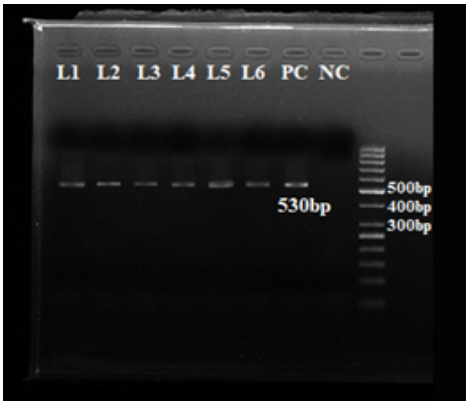

Figure-2: Amplification of *Giardia intestinalis* triose phosphate isomerase (*tpi*) gene fragment 530bp

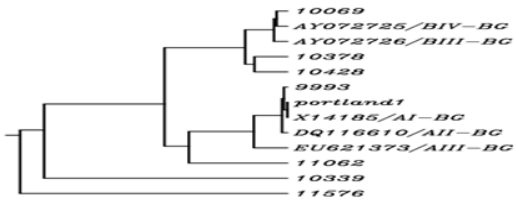

Figure-3: Phylogenetic tree of *G. duodenalis* based on nucleotide sequences of *bg* gene

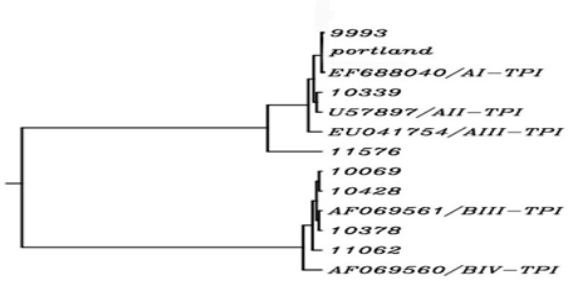

Figure-4: Phylogenetic tree of *G. duodenalis* based on nucleotide sequences of *tpi* gene

Table 1: Genotyping results of the 7positive stool samples for *G. intestinalis* based on two different loci using sequence analysis

SAMPLE NO.	BETA GIARDIN	TRIOSE PHOSPHATE ISOMERASE
10069	BIII, BIV	BIII
9993	AI	AI
11576	AI	AI
10378	BIII, BIV	BIII
10428	BIII, BIV	BIII
10339	AI	AII
11062	AI	BIII
PORTLAND1	AI	AI

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