Molecular identification of Giardia duodenalis; is there any correlation between diarrhea and genotyping in Turkish population?

Z. Gulden Sonmez Tamer* Bayram Pektas**

* Kocaeli University Faculty of Medicine, Department of Medical Microbiology, Kocaeli, Turkey

** Katip Celebi University Faculty of Medicine Atatürk Education and Research Hospital; Department of Medical Microbiology, Izmir, Turkey

Introduction

Giardiasis is a major diarrheal disease found throughout the world. *Giardia duodenalis (G. duodenalis)* highly prevalent intestinal parasite in Turkey. However, molecular characterization of *G. duodenalis* in Turkey is relatively scarce. To date, eight main assemblages of *G. duodenalis* have been described, but only genotype A and B the main causes of human infections(1-4). The aim of this study is to investigate the molecular identification of *G. duodenalis* in patients with diarrhea.

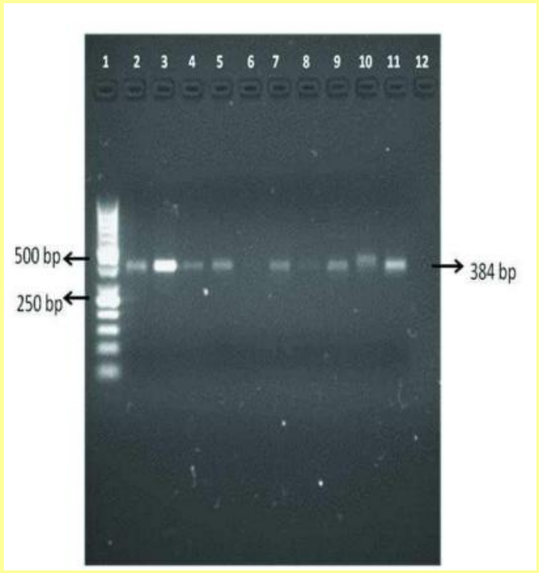

Figure 1. A representative 2% agarose gel to visualize PCR amplified fragment of the *β-giardin* gene. Lane 1, 50 bp marker; lane 2, positive control; lane 12, mixture control; otherlines, clinical samples.

Materials and Methods

Sixty five microscopically positive *G. duodenalis* samples were collected from Kocaeli University Hospital over a 12 month period (2014). To the microscopically positive samples, PCR was used to generate a 384 bp fragment for*β-giardin.* The PCR products were sequenced and the sequences were subjected to phylogenetic analysis by using PHYLIP.

DNA extraction and PCR amplification: All samples were used for DNA isolation. DNA was extracted from 200 mg stool samples using QIAmp DNA Stool Mini Kit (Qiagen, GmbH. Germany)following the manufacturer's instructions. Elution step was accomplished by adding 30 µl elution buffer (Qiagen, GmbH. Germany). Both positive (DNA isolated from the Portland-1 strain (ATCC 30888D TM LGC Promochem) and negative controls (no template added) were included in each series of PCR reactions. A 384 bp fragment of the *β-giardin* gene was amplified using the forward primer G376(5'CATAACGACGCCATCGCGGCTCTC AGGAA-3') and the reverse primer G759 (5'GAGGCCGCCCTGGATCTTCGAGACGAC -3') [11]. A ready-to-use PCR mixture, FastMix/Frenche i-Taq (iNtRON Biotechnology, Korea) was used to set up PCR reactions. Samples were analyzed in 2% agarose gels stained with ethidium bromide to confirm the amplification of expected product size. PCR samples that gave 384 bp band on agorose gel (Figure 1.) were purified by using PCR purification kit (Qiagen, GmbH. Germany) and sequenced from both strands (Iontek Inc., Turkey).

DNA sequencing and phylogenetic analyses: Bands were excised from agarose gels and purified using QIAquick Gel Extraction Kit (Qiagen, GmbH. Germany), according to the manufacturer's instructions. DNA sequencing was conducted in both directions using the PCR primers (Iontek Inc., Turkey). The sequences (23 of them) were contig assembled in vector NTI (Life Tech, USA) edited in BIOEDIT and used in BLAST search for identification of assemblages [12]. The results revealed that all sequences belonged to *ß-giardin*. The sequences were then aligned and examined. Examination of the alignment revealed the presence of partial sequences and the sequences which generated dubious quality. These sequences or sequence parts were systematically eliminated. The edited sequences were then aligned with Clustal W using default parameters and were subjected to phylogenetic analysis using the freely available PHYLIP package. In short, the sequence data were bootstrapped for 1000 times by randomly choosing columns from the original alignment by using the program SEQBOOT. The input order of the sequences was randomized with a jumble number of 10. Then NJ (NEIGHBORJOINING) trees were built by using the generated bootstrapped data. The majority rule consensus trees were created by using CONSENSUS and the tree was drawn with DRAWTREE and edited in Adobe Illustrator 10.

Statistical analysis: All statistical analyses were performed using IBM SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA). Kolmogorov-Smirnov tests were used to test the normality of data distribution.

Results

Based on the phylogenetic analysis of the sequences, assemblage A, B and mixed (AB) were determined. Of 65 isolates, 41 were identified to be assemblage A (63%), 22 were assemblage B (34%) and 2 were assemblage AB (3%). No association between epidemiological-clinical data and assemblages was detected.

Conclusions

Our findings are important in understanding of distribution of assemblages and their phylogenetic relationships in the Kocaeli region of Turkey. We suggest that using sensitive techniques and larger sample for detection of *G. duodenalis* genotypes and their subtypes would be necessary for investigation the immune system respond and correlation with diarrhea in the future studies in Turkey.

References

1.Feng Y, Xıao L: Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev, 2011; 24: 110-140

2.Laishram S, Kannan A, Rajendran P, Kang G, Ajjampur SS. Mixed *Giardia duodenalis* assemblage infections in children and adults in South India. 3.Epidemiol Infect, 2012; 140 (11): 233-244

3.De Martinez-Ruiz R, Merino FJ, Bailo B, Aguilera M, Fuentes, Carmena D, Molecular Genotyping of Giardia duodenalis Isolates from Symptomatic Individuals Attending Two Major Public Hospitals in Madrid, Spain. PLos One, 2015; 10: 140-147

4.Skhal D, Aboualchamat G, Al Nahhas S. Giardia duodenalis in Damascus, Syria: Identification of Giardia genotypes in sample of human fecal isolates using polymerase chain reaction and restriction fragment length polymorphism analyzing method. Acta Trop, 2016; 1-5