Frequency, diagnostic performance of coproantigen detection and genotyping of the *Giardia* among patients referred to a multi-level teaching hospital in northern India

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Giardiasis, a common gastrointestinal parasitic infection in tropics, is diagnosed on stool microscopy (gold standard); however, its sensitivity is low due to intermittent fecal shedding. Coproantigen detection (ELISA) is useful but requires further evaluation. We aimed to study: (a) detection of *Giardia* by stool microscopy and/or coproantigen, (b) diagnostic performance of fecal antigen detection and microscopy, and c) genotypic characterization of *G. lamblia* using PCR specific for triose phosphate isomerase (*tpi*) gene. Stool samples from 2992 patients were examined by microscopy from March 2013 to March 2015 in a multi level teaching hospital in northern India. *Giardia* coproantigen detection was performed by ELISA in a subset of patients. Genetic characterization of *G. lamblia* was performed by PCR targeting *tpi* gene in a subset of microscopy positive stool samples. Of 2992 patients, 132 (4.4%) had *Giardia* by microscopy (cyst/trophozoite) and/or ELISA. ELISA was performed in 264 patients; of them, 127 were positive by microscopy. Sensitivity, specificity, positive and negative predictive values of ELISA were 91, 91, 94, and 91%, respectively, using microscopy as a gold standard. PCR was performed in 116 randomly selected samples having *Giardia* using *tpi* gene. Assemblages A and B were found among 44 (38%) and 72 (62%) patients, respectively. Assemblage B was more often associated with malnutrition and loss of appetite than A (48/72 [67%] vs. 21/44 [48%], $P = 0.044$ and 17/72 [24%] vs. 14/44 [32%], $P = 0.019$). We conclude that 4.4% of studied population had giardiasis. Fecal antigen is a useful method for diagnosis and assemblage B is the most common genotype.

Keywords: Giardiasis, Genotypes, PCR, Triose phosphate isomerase, Enzyme linked immunosorbent assay

Introduction

Giardia lamblia is recognized as an intestinal flagellated protozoan parasite that infects a wide range of mammals including humans.[1] Giardiasis represents a major public health problem and is a common cause of parasitic gastroenteritis. Common clinical manifestations of giardiasis are acute or chronic diarrhea, weight loss, abdominal cramp, and malabsorption.[1,2] It leads to significant morbidity and mortality in both the developing and developed countries with an estimated 2.8×10^8 cases annually worldwide.[3] Community studies show that the frequency of giardiasis varies from 2 to 5% in developed nations[4,5] in contrast to 2–30% among patients with diarrhea in developing countries.[4,6,7] Giardiasis has been reported more commonly in children than in adults.[8] In India, frequency of *Giardia* varies from 2.6 to 20% in children with diarrhea [2,9] and 37% in adults with gastrointestinal complaints (abdominal discomfort, diarrhea, flatulence, weight loss, or anorexia).[2] Most studies reported in India focused on children.[2,9] Till date, there is scanty data on *Giardia* infection in adults from India.

Diagnosis of *Giardia* infection is primarily based on the detection of cysts and trophozoites by direct stool microscopy.[10,11] Microscopy, though gold standard, is less sensitive (50–85.5%) as fecal shedding of parasites is usually intermittent and sometimes load is very low.[12,13] Coproantigen detection by ELISA is a sensitive and specific method, and is useful for screening a large number of specimens in a short time-period.[11,14] Moreover, ELISA can detect the antigen of *Giardia* even in absence of this parasite in stool sample.[11] World Health Organization also approved the antigen detection for detection of *Giardia*.[15] However, till date, only a few studies have been reported on detection of *Giardia* antigen in stool using ELISA.[10,11]

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Polymerase chain reaction (PCR) confers an alternative sensitive and specific method not only for detection of pathogens in stool samples but also their genotypic characterization.[16,17] *Giardia lamblia* is classified into eight assemblages (A–H, genotypes) based on molecular markers like triose phosphate isomerase (*tpi*), glutamate dehydrogenase (*gdh*), and small-subunit (*ssu*) rRNA genes.[18] Among them, assemblages A and B can infect the human beings along with other mammalian hosts.[17,18] The epidemiology of the infection with different *Giardia* genotypes contribute to the understanding of its sources and modes of transmission.[19] PCR is an important and powerful analytical tool to discriminate between the assemblages (A and B) targeting the *tpi* gene as it has high genetic heterogeneity.[17] Till date, there is scanty data on genetic characterization of *Giardia* from India. So, we aimed to study (a) detection of *Giardia* by stool microscopy and/or coproantigen in a multi level teaching center, (b) comparison of fecal antigen detection with microscopy for detection of *Giardia* and (c) genotypic characterization of *G. lamblia* using PCR for *tpi* gene.

Methods

The present study was carried out in the parasitology laboratory, department of microbiology at Sanjay Gandhi Postgraduate Institute of Medical and Sciences, Lucknow, India during a 2-year period (March, 2013 to March, 2015). Patients of all age groups and referred to parasitology services for stool microscopy were included in this study. Patients suffering from other parasitic infections (detected on stool microscopy) were excluded. Demographic details and clinical information for each patient were recorded in a standard questionnaire. Patients were also asked if they had diarrhea, which was defined as passage of more than 3 loose or watery stool per day for at least >3 days[20] and other symptoms such as abdominal pain, nausea or vomiting, loss of appetite, and weight loss. Written informed consent was obtained from each participant. The study protocol was approved by Institutional Ethics Committee (IEC code: 2013-32-IMP-EXP/179).

Sample collection

Three stool samples on three consecutive days were collected from each patient. Microscopy was performed as soon as possible, preferably within one hour. Samples of each patient were pooled in two aliquots. One aliquot was stored at 4 °C for antigen detection on the same day and other was stored at –80 °C for DNA extraction.

Microscopy

Stool samples were examined by direct microscopy (normal saline and iodine wet mount) to look for cysts and trophozoites of *Giardia*.

ELISA

Coproantigen test for detection of *Giardia* was performed by a commercially available ELISA kit (NovaTec Immunodiagnostic GMBH ELISA kit, Germany) in a subset of patients as per manufacturer's instructions.

Table 1 Primers sequences for tpi gene

Primers	Sequence	Annealing temp. (°C)	Expected product size	References
tpiA-F	5-CGAGACAAGTGTTGAGATG-3	57	576 bp	Nora Molina et al. [21]
tpiA-R	5-GGTCAAGAGCTTACAACACG-3			
tpiB-F	5-GTTGCTCCCTCCTTTGTGC-3	54	208 bp	
tpiB-R	5-CTCTGCTCATTGGTCTCGC-3			
tpiA-IF	5-CCAAGAAGGCTAAGCGTGC-3	57	452 bp	
tpiB-IF	5-GCACAGAACGTGTATCTGG-3	54	140 bp	

Extraction of DNA from stool samples

DNA was extracted from pooled stool samples using QIAamp Qiagen mini stool kit (Qiagen Inc., Valencia, CA, USA) following manufacturer's instruction with some modifications. Briefly, the sample was suspended in phosphate buffer saline and subjected to centrifugation. The suspension was heated at 80 °C for 10 min. Concentration of extracted DNA was quantified by NanoDrop ND-2000 spectrophotometer (NanoDrop products, Wilmington, DE, USA).

Polymerase chain reaction

'A' and 'B' genotypes of *Giardia* were identified using *tpi* gene-specific primer sets (Table 1).[21] This is based on heminested PCR (*tpi*-PCR). The PCR conditions were as follows: (i) initial denaturation at 94 °C for 5 min, (ii) 35 cycles of amplification (denaturation at 94 °C for 45 s, annealing at optimal temperature listed in Table 1 for 45 s, extension at 72 °C for 45 s), and (iii) final extension at 72 °C for 5 min. The amplified DNA was observed under 2% agarose gel electrophoresis.

Statistical analysis

Statistical analysis was performed using SPSS, version 15.0 (SPSS, Inc., Chicago, IL, USA). Categorical data were analyzed using Chi-square test. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated as per standard formulae. *P* values less than 0.05 were considered significant for all statistical analysis.

Results

Frequency and demography of patients with giardiasis

Of 2992 patients screened over a two-year period, 132 (4.4%) were diagnosed having *Giardia* either on stool microscopy or ELISA. Of them, 87 (65%) were male

with mean age of 29 ± 17 years (range: 2–78 years) and 45 (34%) were female with mean age of 25 ± 16 years (range: 1–85 years).

Comparative evaluation of microscopy and ELISA

Both microscopy and ELISA were performed among 264 patients. Microscopy and ELISA were positive in 127/264 (45.5%) and 132/264 (50%) samples, respectively. Using microscopy as gold standard, sensitivity, specificity, positive and negative predictive values of ELSA were 91, 91, 94, and 91%, respectively.

Genotypic characterization and association between clinical symptoms and assemblages

PCR was performed in 116 microscopy positive stool samples using *tpi* gene. Assemblages A and B were found among 44/116 (38%) and 72/116 (62%) patients, respectively. The frequency of assemblages A and B was comparable in both male and female (Table 2). Assemblage B was more often associated with malnutrition and loss of appetite (Table 2). Clinical symptoms like diarrhea, daily stool frequency and consistency, dehydration, abdominal pain, seizure, headache, nausea or vomiting and pallor were comparable between patients with assemblages A and B (Table 2).

Discussion

In the present study, 4.4% of patients referred to parasitology laboratory for stool microscopy had giardiasis. Most patients infected with *Giardia* were male (221/314, 76%) with mean age of 28 ± 17 years. Coproantigen detection using ELISA proved to be a highly sensitive and specific test for diagnosis of giardiasis. Assemblage B was most common among patients with giardiasis and was associated with malnourishment and loss of appetite.

Recently, a study reported *Giardia* prevalence among patients with diarrhea to be 2% in a tertiary care hospital in New Delhi, India.[7] Another study showed that frequency of giardiasis was 5% among patients with malabsorption syndrome.[22] Likewise, studies from Norway and Spain showed that the frequency of giardiasis were 3.2 and 5.8% among patients with gastrointestinal symptoms and diarrhea, respectively.[23,24] Our results are not consistent with other community-based studies conducted in different regions. Prevalence rates of *Giardia* vary from 0.8 to 31% among patients with diarrhea worldwide.[4–6] Studies from southern India showed high prevalence of *Giardia* infection varying from 2.1 to 53%.[2,25,26] Such high prevalence of giardiasis might be attributed to exposure to poor sanitation, overcrowding, contaminated drinking water, and poor personal hygiene.[27] Since, the population studied in the current report included patients from tertiary level care, many of them might have received prior empirical treatment; an underestimation of frequency of giardiasis is quite expected.

We found that a large proportion of patients with giardiasis were young males. Recent studies reported that males and people of younger age were at higher risk for both symptomatic and asymptomatic giardiasis.[8,28] Another study showed that the rate of *Giardia* infection among males was 37.1% in the age group of 15 to 26 years, which is in accordance with the present study. Higher rate of *Giardia* infection among males might be due to higher outdoor activity and more environmental exposure than females.[29]

Microscopy is considered as gold standard for diagnosis of giardiasis. Microscopy, however, requires trained personnel and has low sensitivity. It may be due

Table 2 Association between assemblage A and B and clinical parameters

	Assemblage A (*N* = 44)	Assemblage B (*N* = 72)	Total patients (*N* = 116)	
	N (%)	*N* (%)	*N* (%)	**P*-value
Gender (male)	30 (68%)	49 (68%)	79 (68%)	0.989
Age (mean, SD)	33 ± 18	25 ± 17	28 ± 18	0.176
Diarrhea	38 (86%)	58 (80%)	96 (83%)	0.422
Type of diarrhoea				
Acute	20/38 (52%)	27/58 (46%)	47/96 (49%)	0.609
Chronic	18/38 (47%)	31/58 (53%)	49/96 (51%)	
>3 stool per day	28/38 (73%)	45/58 (77%)	73/96 (76%)	0.842
Loose and watery stool	39 (88%)	55 (76%)	94 (81%)	0.103
Abdominal pain	36 (81%)	49 (68%)	85 (73%)	0.104
Fever	27 (61%)	36 (50%)	63 (54%)	0.233
Nausea or vomiting	21 (48%)	25 (35%)	46 (40%)	0.165
Loss of appetite	14 (32%)	39 (54%)	53 (46%)	0.019
Malnourishment	21 (48%)	48 (67%)	69 (59%)	0.044
Headache	6 (14%)	8 (11%)	14 (12%)	0.685
Seizure	0	2 (3%)	02 (2%)	0.265
Pallor	5 (11%)	14 (19%)	19 (16%)	0.254

*Between assemblage A vs. B patients.
$P < 0.05$ was considered as significant.
SD: Standard deviation.

to intermittent excretion of *Giardia*.[11] Previous studies showed that sensitivity of ELISA varied between 95 and 100% and specificity over 90% using microscopy as gold standard, which is in accordance with the result of the current study.[10,11,14] In the present study, ELISA detected *Giardia* in 12 additional samples that were negative on microscopy. Low sensitivity of microscopy might be due to intermittent excretion of *Giardia* cyst or trophozoites in stool.[11] However, seven samples were negative by ELISA though positive on microscopy. This might be related to the fact that *Giardia* antigen can detect only when the cyst or trophozoites concentration is above 5×10^3 and 2×10^4 per ml of diluted stool samples, respectively. Another possibility is degradation of epitope region or elution of *Giardia* antigen as ELISA detects only soluble or free-floating antigen, not associated with *Giardia* cyst wall or trophozoites.[30]

Assemblage B (62%) was the most common genotype of *Giardia duodenalis* in stool samples determined by PCR using *tpi* gene. This result is in accordance with the previous studies reported from India (87–100%).[17,21] Recently, a study showed assemblage B was frequently associated with giardiasis ($N = 82$, 100%) using *tpi* as target gene.[31] Moreover, same result has also been reported from other countries like United Kingdom ($N = 21/33$, 64%), Thailand (31/61, 51%), and Spain (61/108, 56.5%).[3,32,33] In contrast, two studies from Egypt showed predominance of assemblage A (65–75%).[34,35] The variations in predominance of assemblages A and B of *Giardia* species in different geographical regions might be due to mode of transmission. Previous studies showed that assemblage A is predominantly associated with zoonotic transmission, while assemblage B was associated with anthroponotic (from human to human) transmission. Recent evidences have shown assemblage B is responsible for zoonotic transmission as well.[19,36]

Association between clinical symptoms and *Giardia* assemblages is still controversial. In the present study, assemblage B was associated with malnourishment and loss of appetite. Likewise, one study from Germany showed that assemblage B was associated with severe malnutrition.[37] Previous studies reported that infection with assemblage A was associated with intermittent diarrhea and assemblage B was associated with chronic diarrhea.[38] Nevertheless, some studies found no relationship between acute or chronic diarrhea and genotypes,[39,40] which is in accordance with the results of our study. Therefore, longitudinal studies on larger sample of patients are required to assess the potential role of different assemblages of *Giardia duodenalis* in contributing to gastrointestinal symptoms in hyper-endemic region.

In conclusions, detection of *Giardia duodenalis* infection in the study population is 4.4%, ELISA proved to be a useful diagnostic tool, assemblage B of *Giardia duodenalis* was most common and associated with malnourishment and loss of appetite. However, further studies on larger number of samples from humans and animals including association between risk factors and genotypes of *Giardia* might be helpful in understanding the epidemiology of giardiasis and its control strategies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

1 Halliez MC, Buret AG. Extra-intestinal and long term consequences of *Giardia duodenalis* infections. World J Gastroenterol. 2013;19(47):8974–8985.
2 Laishram S, Kang G, Ajjampur SS. Giardiasis: a review on assemblage distribution and epidemiology in India. Indian J Gastroenterol. 2012;31(1):3–12.
3 Tungtrongchitr A, Sookrung N, Indrawattana N, Kwangsi S, Ongrotchanakun J, Chaicumpa W. *Giardia intestinalis* in Thailand: identification of genotypes. J Health Popul Nutr. 2010;28(1):42–52.
4 Solaymani-Mohammadi S, Singer SM. *Giardia duodenalis*: the double-edged sword of immune responses in giardiasis. Exp Parasitol. 2010;126(3):292–297.
5 Yoder JS, Gargano JW, Wallace RM, Beach MJ. Giardiasis surveillance – United States, 2009–2010. MMWR Surveill Summ. 2012;61(5):13–23.
6 Muhsen K, Levine MM. A systematic review and meta-analysis of the association between *Giardia lamblia* and endemic pediatric diarrhea in developing countries. Clin Infect Dis. 2012;55(Suppl 4): S271–S293.
7 Yadav P, Tak V, Mirdha BR, Makharia GK. Refractory giardiasis: a molecular appraisal from a tertiary care centre in India. Indian J Med Microbiol. 2014;32(4):378–382.
8 Painter JE, Gargano JW, Collier SA, Yoder JS. Giardiasis surveillance – United States, 2011–2012. MMWR Surveill Summ. 2015;64(Suppl 3): 15–25.
9 Jethwa DK, Chaudhri U, Chauhan D. Prevalence of giardia infection in paediatric age group. Int J Curr Microbiol App Sci. 2015;4(8):907–911.
10 Jahan N, Khatoon R, Ahmad S. A comparison of microscopy and enzyme linked immunosorbent assay for diagnosis of *Giardia lamblia* in human faecal specimens. J Clin Diagn Res. 2014;8(11):DC04–06.
11 Singhal S, Mittal V, Khare V, Singh YI. Comparative analysis of enzyme-linked immunosorbent assay and direct microscopy for the diagnosis of *Giardia intestinalis* in fecal samples. Indian J Pathol Microbiol. 2015;58(1):69–71.
12 Stark D, Al-Qassab SE, Barratt JL, Stanley K, Roberts T, Marriott D, et al. Evaluation of multiplex tandem real-time PCR for detection of cryptosporidium spp., dientamoeba fragilis, entamoeba histolytica, and *Giardia intestinalis* in clinical stool samples. J Clin Microbiol. 2011;49(1):257–262.
13 Nazeer JT, El Sayed Khalifa K, von Thien H, El-Sibaei MM, Abdel-Hamid MY, Tawfik RA, et al. Use of multiplex real-time PCR for detection of common diarrhea causing protozoan parasites in Egypt. Parasitol Res. 2013;112(2):595–601.
14 Schunk M, Jelinek T, Wetzel K, Nothdurft HD. Detection of *Giardia lamblia* and entamoeba histolytica in stool samples by two enzyme immunoassays. Eur J Clin Microbiol Infect Dis. 2001;20(6):389–391.
15 Al-Saeed AT, Issa SH. Detection of *Giardia lamblia* antigen in stool specimens using enzyme-linked immunosorbent assay. East Mediterr Health J. 2010;16(4):362–364.
16 Minvielle MC, Molina NB, Polverino D, Basualdo JA. First genotyping of *Giardia lamblia* from human and animal feces in Argentina, South America. Mem Inst Oswaldo Cruz. 2008;103(1):98–103.
17 Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, et al. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg Infect Dis. 2003;9(11):1444–1452.
18 Tamer GS, Kasap M, Er DK. Genotyping and phylogenetic analysis of *Giardia duodenalis* isolates from Turkish children. Med Sci Monit. 2015;21:526–532.

19 Sprong H, Cacciò SM, van der Giessen JW. Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl Trop Dis. 2009;3(12):e558.
20 WHO. Persistent diarrhoea in children in developing countries: memorandum from a WHO meeting. Bull World Health Organ. 1988;66(6):709–717.
21 Molina N, Polverino D, Minvielle M, Basualdo J. PCR amplification of triosephosphate isomerase gene of G*iardia lamblia* in formalin-fixed feces. Rev Latinoam Microbiol. 2007;49(1–2):6–11.
22 Ghoshal UC, Mehrotra M, Kumar S, Ghoshal U, Krishnani N, Misra A, et al. Spectrum of malabsorption syndrome among adults & factors differentiating celiac disease & tropical malabsorption. Indian J Med Res. 2012;136(3):451–459.
23 Lopez-Velez R, Batlle C, Jimenez C, Navarro M, Norman F, Perez-Molina J. Short course combination therapy for giardiasis after nitroimidazole failure. Am J Trop Med Hyg. 2010;83(1):171–173.
24 Mørch K, Hanevik K, Robertson LJ, Strand EA, Langeland N. Treatment-ladder and genetic characterisation of parasites in refractory giardiasis after an outbreak in Norway. J Infect. 2008;56(4):268–273.
25 Shetty N, Narasimha M, Raghuveer TS, Elliott E, Farthing MJ, Macaden R. Intestinal amoebiasis and giardiasis in Southern Indian infants and children. Trans R Soc Trop Med Hyg. 1990;84(3):382–384.
26 Kang G, Mathew MS, Rajan DP, Daniel JD, Mathan MM, Mathan VI, et al. Prevalence of intestinal parasites in rural Southern Indians. Trop Med Int Health. 1998;3(1):70–75.
27 Suman MSH, Alam MM, Pun SB, Khair A, Ahmed S, Uchida RY. Prevalence of G*iardia lamblia* infection in children and calves in Bangladesh. Bangl. J. Vet. Med. 2013;9(2):177–182.
28 Abbas NF, El-Shaikh KA, Almohammady MS. Prevalence of *Giardia lamblia* in diarrheic children in Almadinah Almunawarh, KSA. J Taibah Univ Sci. 2011;5:25–30.
29 Abbas NF. Prevalence of G*iardia lamblia* in diarrheic children in Almadinah Almunawarh, KSA. J Taibah Univ Sci. 2011;5:25–30.
30 Wilson JM, Hankenson FC. Evaluation of an inhouse rapid ELISA test for detection of *Giardia* in domestic sheep (Ovis aries). J Am Assoc Lab Anim Sci. 2010;49(6):809–813.
31 Tak V, Mirdha BR, Yadav P, Vyas P, Makharia GK, Bhatnagar S. Molecular characterisation of *Giardia intestinalis* assemblages from human isolates at a tertiary care centre of India. Indian J Med Microbiol. 2014;32(1):19–25.
32 Amar CF, Dear PH, Pedraza-Diaz S, Looker N, Linnane E, McLauchlin J. Sensitive PCR-restriction fragment length polymorphism assay for detection and genotyping of *Giardia duodenalis* in human feces. J Clin Microbiol. 2002;40(2):446–452.
33 Sahagun J, Clavel A, Goni P, Seral C, Llorente MT, Castillo FJ, et al. Correlation between the presence of symptoms and the *Giardia duodenalis* genotype. Eur J Clin Microbiol Infect Dis. 2008;27(1):81–83.
34 Helmy MM, Abdel-Fattah HS, Rashed L. Real-time PCR/RFLP assay to detect *Giardia intestinalis* genotypes in human isolates with diarrhea in Egypt. J Parasitol. 2009;95(4):1000–1004.
35 Abdel-Moneim SM, Sultan DM. Genetic characterization of *Giardia lamblia* isolates from Egyptian patients with relation to clinical giardiasis. J Egypt Soc Parasitol. 2008;38(2):547–560.
36 El-Tantawy NL, Taman AI. The epidemiology of *Giardia intestinalis* assemblages A and B among Egyptian children with diarrhea: a PCR-RFLP-based approach. Parasitol United J. 2014;7(2):104–109.
37 Ignatius R, Gahutu JB, Klotz C, Steininger C, Shyirambere C, Lyng M, et al. High prevalence of *Giardia duodenalis* assemblage B infection and association with underweight in Rwandan children. PLoS Negl Trop Dis. 2012;6(6):e1677.
38 Homan WL, Mank TG. Human giardiasis: genotype linked differences in clinical symptomatology. Int J Parasitol. 2001;31(8):822–826.
39 Jerez Puebla LE, Nunez FA, Martinez Silva I, Rojas Rivero L, Martinez Gonzalez M, Mendez Sutil Y, et al. Molecular characterization and risk factors of *Giardia duodenalis* among school children from La Habana, Cuba. J Parasitol Res. 2015;2015:378643–378648.
40 Al-Mohammed HI. Genotypes of *Giardia intestinalis* clinical isolates of gastrointestinal symptomatic and asymptomatic Saudi children. Parasitol Res. 2011;108(6):1375–1381.