A family cluster of giardiasis with variable treatment responses: refractory giardiasis in a family after a trip to India

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Abstract

Persistence of giardiasis after some of the recommended drugs is occurring with increasing frequency. We describe the follow-up of four members of a family with giardiasis through microscopic observation, immunochromatography and PCRs of *tpi* and *β-giardin* genes. Three patients did not respond to tinidazole but they were cured after quinacrine. However, PCR became negative at 2 months after negativization of stools in two patients and after 1 year in one patient. In all cases *Giardia* assemblage B was characterized with high homology between all isolates. Further studies are needed to determine the value of PCR in the diagnosis of *Giardia* infections.

Keywords: *Giardia*, nitroimidazole, polymerase chain reaction, quinacrine, refractory giardiasis
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Giardia duodenalis is a common parasitic infection in returning travellers [1]. Nitroimidazoles appear to be the drug of choice [2] but, persistence of the infection in humans after treatment is not uncommon [3]. Resistance of *G. duodenalis* to nitroimidazoles has been demonstrated or induced *in vitro*, although its contribution to the persistence of the parasite after treatment remains unclear [2,4,5]. In addition, it is known that *G. duodenalis* infection can be caused by mixed genotypes [6] and as a result antiparasitic treatment could select resistant strains. Finally, in patients who fail to respond to treatment, a re-infection should also be considered, particularly in areas of high endemicity.

In India, *Giardia* infection with diarrhoea is highly prevalent, ranging from 0.4% to 70%, and asymptomatic cyst passage has been found to be as high as 50% in rural southern areas [7].

We report the case follow-up of a family of four members including the father (59 years), the mother (57 years), son 1 (16 years) and son 2 (14 years) that presented with giardiasis after a trip to India in July 2011. All presented watery diarrhoea, abdominal pain and aerophagia. Stool samples from each one were collected before and after each treatment.

Samples were analysed by microscopy following standard formalin–ether concentration, and by rapid immunochromatography (Stick *Cryptosporidium/Giardia/Entamoeba histolytica*; Operon, Zaragoza, SA, Spain). DNA from all the samples was extracted using a DNA stool kit (IBIAN® DNA Stool Kit; IBIAN Technologies, Zaragoza, Spain) and stored at −20°C until processing. *Giardia duodenalis* assemblage was determined by a PCR of the triosephosphate isomerase (*tpi*) gene [8]. A fragment-nested PCR was also used to amplify a fragment of the *β*-giardin (*bg*) gene according to Lalle *et al.* [9]. Additionally, PCR products were purified with a GFX™ PCR DNA Gel Band Purification Kit (GE Healthcare, Chalfont St Giles, UK) and then directly sequenced in both directions. The nucleotide sequences obtained were analysed using the BioEdit alignment program (http://www.mbio.ncsu.edu/bioedit/bioedit) and compared with randomly obtained assemblage B sequences deposited in GenBank.

The DNA sequences obtained have been deposited in the Genetic sequence database (GenBank) at the National Center for Biotechnical Information under accession numbers JQ782391–JQ782407.

All four patients presented *G. duodenalis* cysts in the microscopic examination of stool samples and received a single dose of 2 g of tinidazole. One of the sons improved significantly—the stool examination and the immunochromatography fecal test were both negative after 1 month. The others remained symptomatic after treatment and a new microscopic examination of stools revealed persistence of *G. duodenalis* cysts. In all three cases, HIV test was negative and the presence of IgA deficiency was ruled out. The three relatives with persistence of symptoms received treatment with quinacrine (100 mg three times a day for 5 days) and showed progressive improvement of symptoms. The control stool examinations of the three patients did not show any

Giardia cyst in any repeatedly control samples but the mother's and father's samples showed *Entamoeba histolytica/dispar* cysts and treatment with paromomycin (500 mg three times daily for 7 days) was prescribed. (Fig. 1 shows the test results and the treatment given to each member of the family).

A total of 18 samples (four from both children and five from the parents) were analysed (see Table 1). Of these, 12 yielded positive amplification for a 140-bp fragment of the *tpi* gene corresponding to Assemblage B whereas none were amplified for Assemblage A. Samples from three family members gave positive amplification for *tpi* gene up to 2 months after the negativization of the stool examination by microscopy but they were negative 1 year after the therapy in the two samples that were analysed. All *tpi* sequences were almost identical, with similarities ranging between 99.2% and 100%. Only five samples gave positive amplification for *bg* gene. With this PCR, the persistence of *Giardia* DNA in the last sample from the father was confirmed and positive amplification was also obtained for the 3-month sample of the asymptomatic son and for the other son after quinacrine treatment. Analysis of the sequences showed a range of similarities between 97.8% and 99.1%. Similarity between β-giardin proteins ranged from 96.4% to 100%. Comparing them with the sequences registered in GenBank, all the β-giardin sequences of this work showed high identity with that of the isolate BAH8 (AY072727), with only one to three single nucleotide polymorphisms, indicating that the present isolates are classified into sub-assemblage BIII.

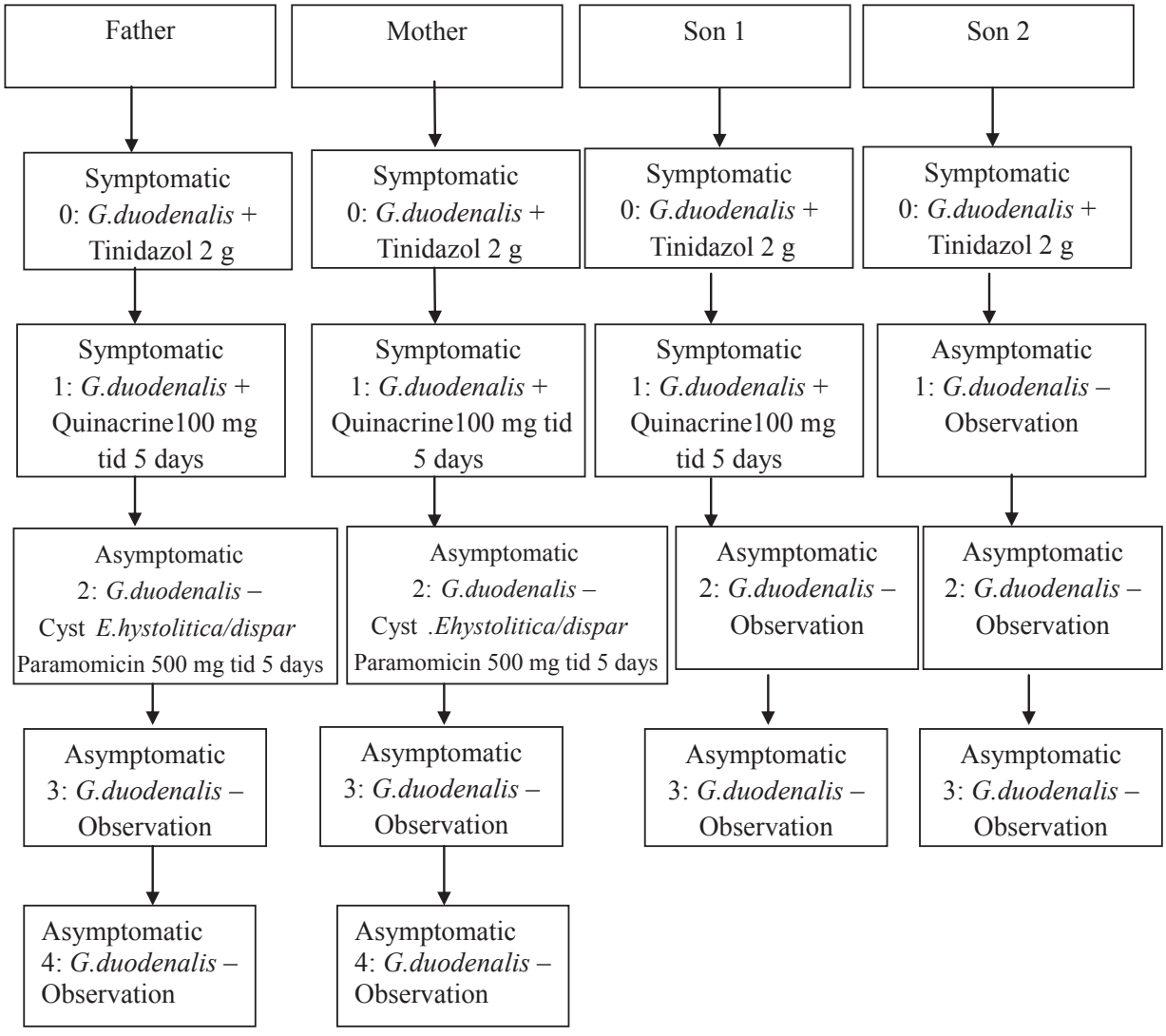

FIG. 1. Clinical management of cases with imported giardiasis. Samples numbered as 0, 1, 2, 3, 4 correspond to 0: before treatment, 1, 2, 3 and 4: month 1, 2, 3 and 12 after treatment); +: Stool examination positive for cyst of *Giardia duodenalis*; –: Stool examination negative for cysts of *G. duodenalis*.

TABLE I. Diagnostic results obtained using different techniques and single nucleotide substitutions in triosephosphate isomerase (*tpi*) and β-giardin (*bg*) gene sequences from the isolates of *Giardia* assemblage B studied

Family Member	Month of follow-up	Microsc. exam.	IC	*tpi* gene					*bg* gene						
				PCR	SNP Pos 21	Pos 54	Pos 84	Pos 98	PCR	SNPs Pos 73	Pos 91	Pos 194	Pos 217	Pos 257	Pos 271
Father (1002)	0	+	+	+	A	C	T(H)	A	–	–	–	–	–	–	–
	1	+	+	+	A	C	T(H)	A	–	–	–	–	–	–	–
	2	–	–	+	A	C	T(H)	A	+	T	A	G	T	C	T
	3	–	–	+	A	G	G (Q)	C	+	C	G	G (A)	T	C	T
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Son 1 (1003)	0	+	+	+	G	C	T(H)	A	–	–	–	–	–	T	–
	1	+	+	+	A	C	T(H)	A	+	C	G	G	C	C	C
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Son 2 (1004)	0	+	+	+	A	C	T(H)	A	–	–	–	–	–	–	–
	1	–	–	+	G	C	T(H)	A	–	–	–	–	–	–	–
	2	–	–	+	A	C	T(H)	A	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	+	T	A	G	C	C	T
Mother (1005)	0	+	+	+	A	C	T(H)	A	–	–	–	–	–	–	–
	1	+	+	+	A	C	T(H)	A	–	–	/	A	T	C	T
	2	–	–	+	A	C	T(H)	A	+	/	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–

IC, immunochromatography: H, histidine; A, alanine; Q, glutamine; *tpi*, triosephosphate isomerase; SNPs, single-nucleotide polymorphisms; –, negative; +, positive; /, there is not substitution.
Month of follow-up: samples numbered as 0, 1, 2, 3, 4 correspond to 0: before treatment, 1,2, 3 and 4: month 1, 2, 3 and 12 after treatment'; +: stool examination positive for cyst of *Giardia duodenalis*; – stool examination negative for cysts of *G. duodenalis*.

There are increasing reports of refractory giardiasis after treatment with nitroimidazoles [10]. This might be a result of re-infection, immunosuppression or drug resistance [11]. To our knowledge, there are few reported cases of refractory giardiasis monitored over several months and with PCR. Genotypic analysis of *Giardia* was performed using *tpi* and *bg* genes because the combination of the two loci provides a higher discriminatory power for subgenotyping the parasite [12]. Twelve samples were amplified using *tpi* gene and only five with the *bg* gene.

The high identity between isolates, which is seen in the study of the two genes (*tpi* and *bg*), suggests the presence of the same isolate in all samples, the small variations observed over time probably being a result of the high genetic variability of the parasite. However, the techniques available for genotyping *Giardia* have limitations and this is the closest approximation that can be made. Therefore, new target genes are needed for genotyping to allow a higher certainty in the differentiation.

All parasites were characterized as *Giardia* Assemblage B. Both Assemblage A and B isolates, have been described as causing different grades of infection in healthy individuals, and it has been proposed that a particular genotype of *G. duodenalis* could be associated with refractory giardiasis [10]. It has also been described at least in one case, that when mixed assemblage giardiasis becomes refractory, Assemblage B remains [13]. In the family studied here, although an apparently identical genotype was successfully treated with tinidazole in one of the sons, the nitroimidazole therapy was ineffective for the rest of the family. In this regard, other unknown factors, including host–parasite interactions [10], patient-intrinsic factors or the amount of inoculum, are probably relevant [11].

Another interesting issue is the appearance of *E. histolytica*/*dispar* after nitroimidazole and quinacrine therapy in both the mother and father. Although the possibility of a new acquired infection after these therapies is plausible, it is more likely that this infection was acquired in India but remained undetected. Unfortunately, in our laboratory we cannot differentiate between *E. histolytica* and *E. dispar* and it was decided to treat *E. histolytica* in both cases.

Surprisingly, in three family members, PCR remained positive after an effective treatment, even though immunochromatography and stool examination were negative. It is possible that damaged and unrecognizable cysts are eliminated in the faeces for a long time. Indeed, in son 2, the PCR result persisted for at least for 3 months after tinidazole therapy despite the son being asymptomatic and his stool examinations being repeatedly negative.

Conversely, it may be that patients partially respond to the infection, leading to a situation where patients remain

asymptomatic but the parasite persists with a low parasitic load that is undetectable by the conventional techniques (stool examination or the immunochromatography–faecal test) but measurable by the more sensitive PCR. Unfortunately we could not quantify the amount of parasitic DNA by real-time PCR to check if low parasitic DNA loads are associated with negative stool examination or immunochromatography. In our study, the negativization of the stool samples, collected 2–3 months after treatment with persistent positive PCR, reinforces this hypothesis. Moreover, we do not know if had the quinacrine therapy been provided to the second son, whether the PCR would had become negative earlier.

Whether an alternative therapeutic option can be offered to patients like those described here remains unclear and further research is required to interpret the value of PCR in these cases.

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Transparency Declaration

We declare no conflict of interest.

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