The Journal of Infectious Diseases

MAJOR ARTICLE

The Use of Quinacrine in Nitroimidazole-resistant *Giardia Duodenalis*: An Old Drug for an Emerging Problem

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Background. There is little evidence regarding the management of refractory giardiasis after treatment with nitroimidazoles. This study estimates the proportion of persistent giardiasis in 3 hospitals in Barcelona, describes associated risk factors and genotype, and evaluates the efficacy rate of quinacrine in those with persistent giardiasis.

Methods. A clinical, prospective, observational study was conducted in patients with giardiasis treated with nitroimidazoles. Those with persistent giardiasis were provided quinacrine. Molecular characterization of *Giardia* isolates was performed by polymerase chain reaction amplification of a fragment of *tpi* and *bg* genes.

Results. Seventy-seven patients were recruited and treated with nitroimidazoles, and in 14 of 71 (20%) of patients followed up, *Giardia* persisted. Refractory giardiasis was associated with malaise (P = .007) and anorexia (P = .02), with previous giardiasis (P = .03), and with previous antibiotic (P = .02) or antiparasitic(P = .04) use. Quinacrine had an effectiveness rate of 100% in refractory giardiasis (n = 13; 95% confidence interval = 75–100). Molecular characterization showed that 17 (25%) *Giardia* isolates belonged to assemblage A, and 31 (43%) belonged to assemblage B. In refractory giardiasis, assemblage A and B were found responsible in 4 and 6 cases, respectively.

Conclusions. Almost 20% of patients presented persistent giardiasis, belonging to both assemblages A and B, after nitroimidazole. Short course of quinacrine was effective in treating refractory cases. Further controlled studies should evaluate its efficacy and safety.

Keywords. Refractory giardiasis; *Giardia*; treatment; nitroimidazole; PCR; quinacrine; tpi gene; bp gene; genetic characterization.

Giardia duodenalis is one of the most common intestinal parasitic protozoa reported in humans [1]. The protozoon is distributed worldwide [2, 3], and estimates indicate that more than a billion people are at risk of giardiasis infection. It is much more prevalent in developing countries due to its association with poverty [4], where water contaminated with *G. duodenalis* cysts frequently causes travel-related giardiasis [5]. In industrialized countries, the infection is reemerging [6], causing waterborne and, to a lesser extent, foodborne outbreaks [6, 7].

Symptoms range broadly, with some infections asymptomatic and others causing mild to chronic diarrhea [8, 9]. Reasons for the variability of symptoms remain unclear although multiples factors have been proposed. Some findings suggest that age and previous *Giardia* exposure are associated with mucosal inflammation and the intensity of symptoms [10]. Geographic and population variation can also affect the host immune response against *G. duodenalis* [11]. Genetic variability of *Giardia* strains has been also suggested to influence the symptomatology of the disease, although no association has been found between *Giardia* genotypes (assemblage A and B) and clinical symptoms when comparing different studies [1, 12].

Polymerase chain reaction (PCR) techniques, in addition to providing excellent sensitivity and specificity compared with microscopy and antigen-detection methods [13–15], have been extremely useful in recent years to improve knowledge and understanding of *G. duodenalis* genetics. Application of these techniques to characterize the *Giardia* isolates responsible for cases of refractory giardiasis has identified assemblage B as responsible for these cases worldwide. To our knowledge, there have been no reports of refractory giardiasis produced by *Giardia* assemblage A. However, not all reported cases have been typed, and the limited number of those studied makes it difficult to establish a definitive association between assemblage and resistant giardiasis.

Persistence of giardiasis despite nitroimidazole therapy is also relatively common in patients with chronic giardiasis, accounting for approximately 20% of cases [16]. This may be due to reinfection, immunosuppression, or drug resistance [17]. There is no standardized second-line regimen for refractory giardiasis.

Received 22 September 2016; editorial decision 25 January 2017; accepted 27 January 2017; published online February 06, 2017.

Presented in part: 9th European Congress of Tropical Medicine and International Health, Basel, Switzerland, 10 September 2015. A-744-0011-00427.

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The Journal of Infectious Diseases® 2017;215:946–53

DOI: 10.1093/infdis/jix066

A second course of nitroimidazoles given for several consecutive days, changing the type of antiparasitic agent, and combination therapy have been tested in these cases [17]. Quinacrine has been reported to have a high efficacy rate for the treatment of giardiasis [1], although it has been associated with severe side effects and poor adherence. In a previous retrospective study, we demonstrated a 100% efficacy rate in patients treated with this drug using a 7-day short-course regimen, which was not associated with severe adverse effects [5].

This study aims to estimate the proportion of nitroimidazole-resistant *G.duodenalis* in the population attending three Tropical Medical Units and attempt to describe the risk factors and the assemblages associated with the persistence of giardiasis. Further, the effectiveness of quinacrine in the treatment of these patients with resistant giardiasis is assessed.

METHODS

Study Design, Participants, and Setting

A prospective, observational study was conducted in 3 specialized Tropical Diseases Units in Barcelona, Spain. From July 2012 to July 2013, all patients diagnosed with *G. duodenalis* attending the Tropical Medicine Unit (TMU) from Hospital Clinic, TMU-Drassanes, and Hospital Sant Joan de Deu in Barcelona were invited to participate in the study. Patients unwilling or unable to give informed consent were excluded. Using a 95% confidence level and a 20% refractory giardiasis rate established by previous studies [16] and considering a total of 71 patients that were finally included and followed-up, the final precision obtained was 10%. Participants were interviewed using a semistructured questionnaire. Particular emphasis was put on previous travel during the last 6 months, previous gastrointestinal diseases, and other factors that could contribute to malabsorption. Sex, age, height (cm), and weight (kg) were recorded. All patients provided a stool sample before they were treated.

Standard formalin-ether concentration of all of the stool samples was microscopically examined for the presence of *Giardia* cysts or trophozoites. In addition, an immunocromatography test was performed in fresh samples with the Stick *Giardia* test (Stick *Giardia* antigen kit, Operon SA). The combined use of these 2 techniques aimed to achieve a greater sensitivity for those cases with low number of cysts and to minimize the possibility of obtaining false negatives. All patients were initially treated with nitroimidazoles (adults and children aged >5 years with tinidazole 2 g for 1 day; children aged <5 years, with metronidazole 30 mg/kg/day for 7 days). Two control stool samples were collected and analyzed by microscopy and immunochromatography (IC) 30 days (+/−10 days) after treatment. Those infections with confirmed persistence of *G. duodenalis* (by detection of cysts in fecal samples or by IC) were defined as refractory giardiasis and were subsequently treated with quinacrine at a dose of 100 mg 3 times a day for 5 days. In children, the dose was adjusted to 8 mg/kg/day for 5 days.

Other routine tests such as stool cultures, microscopic observation, or parasitic serologies were conducted to investigate the presence of other intestinal agents that may cause intestinal symptoms. Human immunodeficiency virus (HIV) and immunoglobulin A deficiency tests were performed in those patients diagnosed with refractory giardiasis.

Molecular Characterization

An aliquot of all fecal samples was refrigerated and sent to the University of Zaragoza, where further molecular analysis was undertaken. Previous to DNA extraction, samples were treated to improve DNA quality. *Giardia* cysts were purified and concentrated using a sucrose gradient flotation method. Thereafter, cysts underwent 5 cycles of freezing (at −80°C for 30 minutes), defrosting (at 100°C for 10 minutes), and a final treatment with proteinase K (final concentration 100 μg/mL, overnight). When no cysts were observed, fresh stool samples were given the same pretreatment with a sucrose gradient flotation method. DNA extraction was carried out after digestion of cysts or on fresh samples using a commercial kit (Stool DNA Isolation Kit, Norgen Biotek Corp., Ontario, Canada) following the manufacturer's instructions.

G. duodenalis assemblage was determined by PCR of the triosephosphate isomerase (*tpi*) and ß-giardin (*bg*) genes following the protocols described by Sulaiman et al [18] and Lalle et al [19], respectively. Polymerase chain reaction products were purified with GFXTM-PCR-DNA Gel Band Purification Kit (GE Healthcare) and then directly sequenced in both directions. The nucleotide sequences obtained were analyzed using BioEdit program (http://www.mbio.ncsu.edu/bioedit/bioedit/html), and they were compared with the sequences deposited in GenBank.

The DNA sequences obtained have been deposited in the genetic sequence database at the National Center for Biotechnical Information under accession numbers JQ782391-JQ782407 and KX468980-Kx469069.

Statistical Methods

Data were double-entered into an EpiData database, checked with EpiData software version 3.1, and analyzed with STATA version 13.

Statistical Analysis

For numerical variables, a normal distribution was tested with the Shapiro-Wilk normality test. Because they did not follow a normal distribution, they were expressed as median (interquartile range [IQR]) and were compared with an unpaired Kruskal–Wallis test.

Categorical variables were described with numbers and percentages. They were compared using Monte Carlo and Fisher exact tests. To show the relation between patients with or without persistence of *G. duodenalis* and the rest of the variables, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated

A backwards stepwise multivariate logistic regression analysis was performed to assess the variation of refractory giardiasis. Independent variables of the model were considered those that showed $P < .20$ with refractory giardiasis in the bivariate analysis. The goodness of fit of this model was calculated using means of the Hosmer-Lemeshow test, requiring a value of $P > .05$. To avoid possible multicollinearity problems, a variance inflation factor <5 in all coefficients of the final model was required. The odds ratio with 95% confidence interval was used as the main estimator obtained from the statistical analyses [20].

Ethics

The study protocol and consent form were approved by the ethics committee of Hospital Clínic and Sant Joan de Deu Hospital and the ethic committee of Gol–i-Gurina.

RESULTS

General Characteristics of the Patients

Seventy-seven patients were included in the study; 41 (53%) were male, and 22 [29] were children (aged <18 years). Twenty-one (27%) were immigrants, 7 (9%) were "visiting friends & relatives," and 41 (53%) were tourist or work travelers who had visited endemic countries. Eight patients (10%) had not travelled outside Spain in the previous 6 months. Patient characteristics by the geographic area where the infection was most probably acquired are summarized in Table 1. Almost half of the patients (37) acquired *Giardia* in Asia (75% of these patients had visited the Indian subcontinent), 26% acquired it in Africa, 14% acquired it in Latin America, and 12% acquired it in Europe. Travelers were the most frequent group for giardiasis acquired in Asia (81%), whereas immigrants were the predominant group for giardiasis from Latin America (55%) and from Africa (40%), and autochthonous patients (89%) were the predominant group for giardiasis acquired in Europe ($P < .001$).

Most patients reported at least 1 risk factor for *Giardia* acquisition. Sixty-eight percent of patients had consumed raw salad or raw meat, and 60% of patients drank tap water on some occasions, although this percentage was higher in people that had been in Latin America or Africa compared with Asia ($P < .001$). Tap-water consumption during travel was also more frequent in migrants (82.35%) and those visiting friends and relatives (85.71%) than travelers (55%) ($P = .02$).

Around a third of patients had been working with patients (hospitals, daycare centers, nurseries, etc), and 4% had a previous episode of giardiasis.

Only eight patients (11%) were asymptomatic, and this was more frequent in migrants ($P = .02$). Asymptomatic infection was more frequent in participants coming from Latin Ameria, followed by Africa and Asia ($P = .009$). In autochthonous infections, all patients were symptomatic. Fever, abdominal pain, vomiting, weight loss, and malaise were more frequent in patients coming from Asia ($P = .004$, $P = .001$, $P = .005$, $P = .04$, and $P = .01$, respectively).

The following coinfections were observed: *Entamoeba histolytica/dispar* [5], *Ascaris lumbricoides* [2], *Strongyloides stercoralis* [3], *Trichuris trichiura* [1], *Blastocystis hominis* [3] *Schistosoma* spp. [2], *Cryptosporidium* spp. [1], and *Salmonella* spp. [1]. However, persistence of giardiasis was not associated with having other concomitant intestinal parasitic infections. For each individual, we also calculated the number of risk factors for *Giardia* acquisition and the number of any other intestinal parasites. No statistically significant differences were found in the distribution of these variables in the geographic areas analyzed (see Table 1).

Refractory Giardiasis

All patients were treated with tinidazole or, in the case of children, metronidazole. Seventy-one patients had further follow-up after the initial treatment, and 20 of them (28%) remained symptomatic (see Figure 1). In 14 patients (20%; 95% CI = 11%–29%), *G. duodenalis* was found in the stool analysis. In the univariate analysis, persistent giardiasis was more frequent when the infection was acquired in Asia (n = 10/35; 29%) compared with Africa (n = 3/18; 17%), Latin-America (n = 1/11; 9%), or Europe (n = 0/7; 0%), but this association was not statistically significant ($P = .23$).

Out of the 3 patients with HIV, 2 were cured after tinidazole treatment, and 1 was lost to follow-up. Refractory giardiasis was associated with systemic symptoms (malaise: $P = .014$; anorexia: $P = .049$) and, as expected, with previous antibiotic use ($P = .03$). A relevant, although not statistically significant, association was also found between refractory giardiasis and having traveled to Asia ($P = .08$), suffering diarrhea (0.09), having suffered previous giardiasis ($P = .09$), and having been treated with an antiparasitic ($P = .10$) (see Table 2).

In our model, the peristance of *Giardia* was not significantly associated with the initial treatment for giardiasis (metronidazole vs tinidazole), the origin of *Giardia* acquisition, or having severe symptoms . According to the multivariate analysis, the risk of suffering refractory giardiasis was higher in patients with malaise (OR = 5.02; 95% CI = 1.09–23.03), in patients who had undergone previous treatment (antiparasitic and/or antibiotic; OR = 6.19; 95% CI = 1.41–27.14), and in men (OR = 2.85; 95% CI = .65–12.43) (see Table 3).

Following the study protocol, 13 patients with persistent giardiasis took quinacrine, and tinidazole was prescribed for 1 child who had previously taken metronidazole. This child was the only participant in whom *G. duodenalis* persisted despite the drug treatments, and the child improved clinically and was cured only after receiving quinacrine in a third-line course of treatment (see Figure 1). Another child completed only 3 out of 5 days of quinacrine therapy due to adverse events, presenting irritability and somnolence that disappeared after stopping the

Table 1. Participants' Characteristics by Subgroup

Characteristic	Continent				*P* value	Total (n = 77)
	Latin-America (n = 11)	Asia (n = 37)	Africa (n = 20)	Europe (n = 9)		
Male sex	5 (46)	17 (46)	13 (65)	6 (67)	.40	41 (53)
Age, y						
0–18	2 (18)	4 (11)	9 (45)	7 (88)	**<.001**	22 (29)
19–34	6 (55)	18 (49)	3 (15)	1 (13)		28 (37)
>35	3 (27)	15 (41)	8 (40)	0 (0)		26 (34)
Patients						
Migrants	6 (545)	6 (16)	8 (40)	1 (11)	**<.001**	21 (27)
Travelers	4 (36)	30 (81)	7 (35)	0 (0)		41 (53)
Visited friends or relatives	1 (9)	1 (3)	5 (25)	0 (0)		7 (9)
No travel	0 (0)	0 (0)	0 (0)	8 (89)		8 (10)
Risk factors for giardiasis						
Unwashed raw food	10 (100)	24 (67)	10 (59)	4 (57)	.12	48 (69)
Unsafe drinking water	9 (90)	19 (53)	14 (82)	0 (0)	**<.001**	42 (60)
Working care of patients	3 (30)	15 (42)	9 (47)	6 (86)	.12	33 (46)
Previous giardiasis	1 (10)	1 (3)	0 (0)	1 (13)	.37	3 (4)
Sexual contact with *Giardia*	0 (0)	1 (3)	0 (0)	0 (0)	.78	1 (1)
Previous intestinal disease	1 (9.09)	0 (0)	0 (0)	1 (11)	.12	2 (3)
Symptoms associated with giardiasis						
Asymptomatic	4 (36)	1 (3)	3 (15)	0 (0)	**.009**	8 (11)
Diarrhea	5 (45)	28 (76)	14 (70)	8 (89)	.15	55 (71)
Fever	0 (0)	13 (35)	1 (5)	0 (0)	**.004**	14 (18)
Abdominal pain	6 (55)	30 (83)	11 (55)	1 (13)	**.001**	48 (64)
Vomiting	2 (18)	20 (54)	3 (15)	1 (13)	**.005**	26 (34)
Aerophagia	4 (36)	26 (70)	11 (8)	3 (38)	.12	44 (59)
Weight loss	3 (27)	25 (69)	7 (41)	3 (38)	**.04**	38 (53)
Malaise	4 (36)	24 (65)	6 (30)	1 (13)	**.01**	35 (46)
Anorexia	0 (0)	5 (14)	0 (0)	0 (0)	.13	5 (7)
Previous treatments						
Antiparasitic	0 (0)	1 (3)	0 (0)	1 (13)	.28	2 (3)
Antibiotic	0 (0)	9 (24)	3 (15)	0 (0)	.13	12 (16)
Antidiarrhoeal	1 (9)	4 (11)	0 (0)	1 (13)	.49	6 (8)
Other intestinal parasites						
Entamoeba histolytica/dispar	0 (0)	3 (8)	2 (10)	0 (0)	…	5 (6)
Ascaris lumbricoides	0 (0)	0 (0)	2 (10)	0 (0)	…	2 (3)
Strongyloides stercoralis	3 (27)	0 (0)	0 (0)	0 (0)	…	3 (4)
Trichuris trichiura	0 (0)	0 (0)	1 (5)	0 (0)	…	1 (1)
Blastocystis hominis	2 (18)	0 (0)	0 (0)	1 (13)	…	3 (4)
Schistosoma spp.	0 (0)	0 (0)	2 (10)	0 (0)	…	2 (3)
Cryptosporidium spp.	0 (0)	1 (3)	0 (0)	0 (0)	…	1 (1)
Salmonella spp.	0 (0)	1 (3)	0 (0)	0 (0)	…	1 (1)
HIV	1 (9)	0 (0)	1 (6)	1 (13)	.38	3 (5)
IgA immunodeficiency	…	0 (0)	0 (0)	1 (100)	**.02**	1 (12.5)
Number of risk factors	2.5 (1)	2 (1)	2 (2.75)	2 (2)	.14	…
Number of other intestinal parasites	0 (4)	0 (0)	0 (0)	0 (0)	.14	…

Proportions are expressed as cases/total number of patients (percent). Numerical values are expressed as median (interquartile range). Continuous variables were analyzed by using an independent Kruskal–Wallis test, and categorical variables were analyzed with Monte Carlo and Fisher exact tests. Bolding indicates *P* values statistically significant.

Abbreviations: HIV, human immunodeficiency virus; IgA, immunoglobulin A.

treatment. However, *Giardia* was not found in subsequent serial stool examinations, and he was considered cured.

Giardia Analysis and Characterization

Molecular analysis was conducted for 76 patients. A total of 105 sequences from 54 patients were obtained. Amplification was positive for 58 samples from 40 patients in whom the *tpi* gene was analyzed and for 47 samples from 39 patients in whom the *bg* gene was analyzed. Amplification of the 2 genes simultaneously was positive for only 24 samples from 20 patients. The predominant genotype was assemblage B in 31 patients (42%), followed by assemblage A in 17 (25%) patients. A mixed

Table 2. Factors Associated With Refractory Giardiasis

	Refractory giardiasis, no. (%)	Nonrefractory giardiasis, no. (%)	OR (95% CI)	*P* value
Sex				
Male	8/14 (57)	29/57 (51)	1.28 (.40–4.18)	.77
Female	6 /14 (43)	28/57 (49)		
Age				
<18 y	3/14 (21)	18/57 (32)	0.59 (.16–2.24)	.55
>18 y	11/14 (79)	39/57 (68)		
Patient type				
Migrant	3/14 (21)	15/57 (26)	0.76 (.20–2.94)	1.00
Travelers	11/14 (79)	29/57 (51)	3.54 (.95–12.99)	.19
Visited friends or relatives	0 (0)	7/57 (12)	…	…
No travel	0 (0)	6/57 (11)	…	…
Continent				
Latin-America	1 /14 (7)	10 /57 (18)	0.78 (.21–3.01)	.68
Asia	10 /14 (71)	25 /57 (44)	3.2 (.94–10.80)	.08
Africa	3/14 (21)	15/57 (26)	0.76 (.20–2.94)	1.00
Europe	0 /14 (0)	7/57 (12)	…	…
Risk factors for giardiasis				
Unwashed raw food	4/12 (33)	41/53 (77)	0.15 (.04–.57)	**.005**
Unsafe drinking water	7/12 (58)	32/54 (59)	0.96 (.28–3.25)	1.00
Working care of patients	4 /14 (29)	28/54 (52)	0.37 (.10–1.33)	.14
Previous giardiasis	2/13 (15)	1/54 (2)	9.63 (.82–115)	.09
Sexual contact with *Giardia*	0/14 (0)	1/57 (2)	…	…
Previous intestinal disease	0/14 (0)	2/57 (4)	…	…
Symptoms of *Giardia*				
Asymptomatic	0/14 (0)	7/57 (12)	…	…
Diarrhea	13/14 (93)	39/57 (68)	6.00 (.73–49.5)	.09
Fever	4/14 (29)	10/57 (18)	1.88 (.49–7.22)	.45
Abdominal pain	9/13 (69)	36/57 (63)	1.31 (.36–4.8)	.60
Vomiting	4/14 (29)	21/57 (37)	0.69 (.19–2.46)	.76
Weight loss	8/13 (62)	28/55 (51)	1.54 (.45–5.10)	.56
Malaise	11/14 (79)	22/57 (39)	5.83 (1.46–23.30)	**.01**
Anorexia	3/14 (21)	2/57 (4)	7.45 (1.12–50.30)	**.049**
Previous treatment				
Antiparasitic	2/14 (14)	1/57 (2)	9.33 (.78–111.50)	.10
Antibiotic	5/14 (36)	6/57 (11)	4.7 (1.19–18.81)	**.03**
Assemblage				
A	3/14 (21)	13/49 (27)	0.76 (.20–2.97)	1.00
A+B	0/14 (0)	1/49 (0)	…	…
B	6/14 (43)	23/49 (47)	0.85 (.27–2.72)	1.00
Not typable	4/14 (29)	13/49 (27)	1.11 (.31–4.00)	1.00

Proportions are expressed as cases/total number of patients. Categorical variables were analyzed with Monte Carlo and Fisher exact tests. Odds ratios and confidence intervals were calculated by Woolf´s method. Bolding indicates *P* values statistically significant.

Abbreviations: CI, confidence interval; OR, odds ratio.

infection of the 2 assemblages was observed in 6 patients, and no amplification despite the observance of cysts was observed in 18 (25%) patients. A negative PCR test was observed in 5 cases (7%).

Notably, out of 44 nonrefractory cases, 15 were PCR-positive 1 month after the antiparasitic therapy (12 presented *Giardia* assemblage B, 2 presented assemblage A, and 1 was a mixed infection A+B), although only 3 of the patients were still symptomatic. These nonrefractory cases were followed for 1 month after treatment as planned.

Table 3. Multivariate Logistic Regression Model of the Independent Association of Various Variables With Refractory Giardiasis

Variable	OR (95% CI)	*P* value
Sex: Man	2.85 (.65–12.43)	.06
Malaise: Yes	5.02 (1.09–23.03)	.04
Previous treatment: Yes	6.19 (1.41–27.14)	.02

Test for goodness of fit for logistic regression model: Hosmer-Lemeshow test = 1.18. *P* = .76. R^2 Nagelkerke = 0.30.

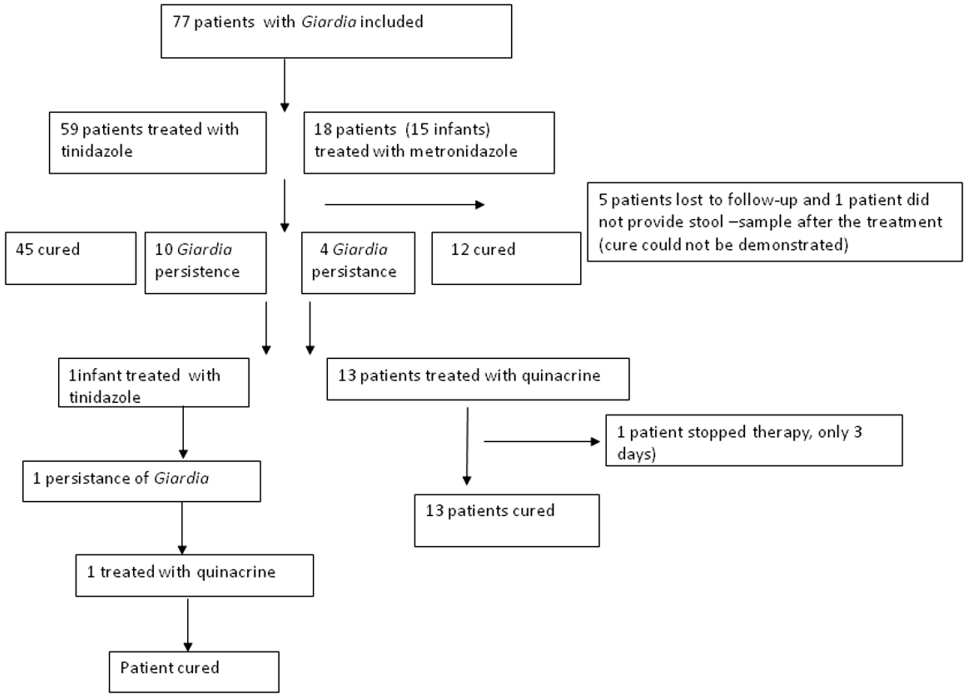

Figure 1. Diagram of the clinical and treatment outcome of *Giardia* cases.

For the 14 patients with persistence of *Giardia* cyst in stool examinations after nitroimidazole therapy (13 symptomatic and 1 asymptomatic), 6 presented *Giardia* assemblage B (5 Asia-acquired cases, 1 Latin America–acquired case). Three cases presented assemblage A (2 Africa-acquired cases, 1 Asia-acquired case), and in 4 cases there was no amplification, and the PCR results were negative. A specific assemblage was not associated with the persistence of *Giardia* ($P > .05$). Homology of sequences of different samples of the same patient ranged 97%–100% for assemblage B (analyzed by amplification of *tpi* gene) and was 100% for assemblage A. When comparing sequences of *bg* gene, there were not any single nucleotide polymorphims. However, identity among sequences from different patients and different geographical locations was observed. One month after quinacrine therapy, all 14 patients improved clinically, and fecal antigen detection and stool examinations were negative for all patients but PCR was still positive in 3 cases. Two of these patients were further followed up, and they were still PCR-positive at least 2 months after the quinacrine therapy. Both of these patients presented assemblage B.

DISCUSSION

This work highlights an elevated proportion of refractory giardiasis after treatment with nitroimidazoles. It was observed in almost 20% of patients treated with nitroimidazoles, which is similar to the reported efficacy of 80%–90% elsewhere [17, 21, 22]. Our study was not able to demonstrate any statistically significant association with refractory giardiasis and the acquisition of giardiasis in Asia, as has been reported elsewhere [16, 23].

The differences in treatment failure among geographic areas could be attributable to acquired drug resistance, explained by several mechanisms. First, it has been proposed that that there is a geographical distribution of *Giardia* assemblages around the world, although a particular assemblage of *G. duodenalis* has not been demonstrated to be associated with refractory giardiasis [24]. In our study, assemblage B was predominant in refractory cases, consistent with other reports [25], although the study lacked the statistical power to demonstrate this effect. To our knowledge, only cases of refractory giardiasis produced by *Giardia* belonging to assemblage B have been described. Therefore, this work is the first to describe refractory giardiasis by *Giardia* assemblage A, proving its plausibility and suggesting the importance of carrying out molecular characterization of *Giardia* populations concerning refractory giardiasis.

Second, because the levels of drug pressure may be variable in different parts of the world where *G. duodenalis* is endemic, this could also be a key factor in the development of drug resistance in *Giardia* strains [26].

Being a traveler or a migrant was significantly associated with the geographic origin where the *Giardia* was acquired. Accordingly, for most travelers, *Giardia* was acquired when travelling to Asia, particularly to India, whereas for migrants, the geographic origin of *Giardia* was mainly either Africa or Latin America. These findings could be explained because in the 3 sites where the study was carried out, >40% of travelers go to Asia, and migrants are predominantly coming from Africa and Latin America (Travel Clinic Unit, Hospital Clinic, Barcelona, unpublished data).

Our data are in concordance with other published data of imported giardiasis, in which the risk of giardiasis in returning traveler was higher for the Indian subcontinent and Arab countries [27]. Although our study could not demonstrate that giardiasis acquired in Asia is more likely to be refractory to first-line therapy, a greater proportion of refractory giardiasis was found in cases coming from this continent compared with the other regions.

Having severe symptoms such as malaise or anorexia before initial therapy was associated with refractory giardiasis and could be suggestive of higher parasitic-load infections. If this could be demonstrated, offering another treatment scheme, such as a multiple dose of nitroimidazoles, could be considered in these cases [1].

The evaluation of treatment efficacy in *G. duodenalis* cases is rather complicated using the current diagnostic methods. Polymerase chain reaction could be helpful to identify low parasitic-load infections. However, our data suggest that almost 35% of patients presented positive PCR results, despite being considered cured according to the clinical and stool-tests findings, which is an unexpectedly high percentage compared with other studies that show a 100% negativization of PCR 1 week after a 3-day course of metronidazole therapy [28]. One possible explanation is that PCR sensitivity may be higher due to the molecular techniques undertaken for the genotypic characterization (in our study several PCR tests were undertaken on the same sample, which may have increased the global sensitivity). On the other hand, as reported by Martinez-Gordillo et al, the intraepithelial presence of a low number of *Giardia* trophozoites is possible, resulting in a positive PCR result [29]. Additionally, the variability obtained for sequences of *tpi* gene and the low variability of *bg* gene raises questions about what differences exist among *Giardia* assemblages and the usefulness of genotyping methods for their characterization. The significance of these results should be clarified in future studies.

Quinacrine demonstrated a 100% effectiveness rate, as has been previously described [5, 30, 31], and only 1 case of adverse events was observed, which led to stopping the treatment. This case was a child weighing <10 kg, making the optimal dose difficult to obtain due to a lack of pediatric formulation of quinacrine. However, even with a shorter 3-day regimen, quinacrine was effective to eradicate the parasite.

Thus, quinacrine should be considered as a second-line drug treatment when available. Quinacrine is currently a medicinal product on a compassionate-use basis, and therefore advocacy is needed to include giardiasis as one of its uses. However, more controlled studies such as randomized trials are required to better evaluate the efficacy rate of quinacrine for giardiasis and particularly to monitor its potential side effects.

Besides the skin discoloration that has been reported, one of the main concerns when prescribing quinacrine is the occurrence of psychiatric adverse events, particularly in those people predisposed to such [32]. In a study conducted during the World War II in 7604 patients with malaria, quinacrine was shown to induce psychosis in 0.46% of patients [33]. However, the mean total cumulative dose was 2100 mg, which is higher than the 1500-mg total dose recommended in our study. Moreover, the role of preexisting malarial infection and previous combat experience that could have contributed to the clinical spectrum of the psychiatric disorders was not properly evaluated. Another prospective case series reported evidence of pronounced psychological stimulation in 5 cases after quinacrine administration with cumulative dose in the first day of 1200 mg and a total cumulative dose >4000 mg, proving that quinacrine acts as a cortical stimulant [34]. All patients had a rapid resolution of symptoms, and there were no permanent sequelae noted. Finally, another more recent prospective study described neurologic and psychiatric adverse events in 3 patients with refractory giardiasis treated with quinacrine in combination with metronidazole, but again all patients were treated at a dose of 100 mg 3 times a day for 3 weeks, which is equivalent to a cumulative dose of >6300 mg [35], much higher than the dose recommended in our study. In another clinical trial conducted in children comparing mebendazole and quinacrine for the treatment of giardiasis, adverse events were frequently described in those treated with quinacrine. However, all adverse events were graded as mild; they did not affect the central nervous system nor warrant the discontinuation of the treatment in any patient [36].

In our study, all patients were cured with a 5-day schedule at a dose of 100 mg 3 times a day. Only 1 of 14 patients suffered an adverse event that caused discontinuation of the therapy. The patient was an 11-month-old baby that presented irritability and sleeplessness, and it was not possible to evaluate the existence of a neuropsychiatric disorder. Further controlled studies are warranted to evaluate the efficacy and also the adverse effects of 3–5-day regimens of quinacrine.

CONCLUSIONS

Almost 20% of patients presented persistence of giardiasis despite nitroimidazole therapy. This outcome was not associated with a particular assemblage, as *Giardia* assemblage B was predominant, but some cases produced by *Giardia* assemblage A were observed. A short course of quinacrine had 100% effectiveness, although the optimal dosage remains unknown, and the drug has not been approved to be used in giardiasis. Randomized controlled trials are needed to evaluate the efficacy and safety of quinacrine as a second-line therapy. Finally, positive PCR results may persist in asymptomatic patients apparently cured according to clinical and stool-test criteria.

Notes

Financial support. This work was partially supported by the Ministerio de Sanidad y Consumo Project Formación en Investigación en Salud (FIS) Project PI 09/1585 (IACS) +DGA – FSE B124.

The Barcelona Institute for Global Health (ISGlobal) Research group was supported by Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) (2014SGR26) and Red de Investigación cooperativa en enfermedades tropicales (RICET) (RD12/0018/0010) within the Spanish National plan of Recerca, Desenvolupament, Innovació (R+D+I), which is cofunded by Instituto de Salud Carlos III (ISCIII)-(Fondo Europeo de Desarrollo Regional [FEDER]).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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