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Original Article

Efficacy of Nitazoxanide, Nitazoxanide-Garlic Combination and Tinidazole in Treatment of *Giardia duodenalis* and *Blastocystis hominis*: Coproscopic Assessment

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Abstract

Background: *Giardia duodenalis* and *Blastocystis hominis* are among the most common intestinal protozoa worldwide. Treatment of infection by metronidazole (MTZ) has some limitations. The objective of this study was to detect the prevalence of *Blastocystis* and *giardiasis* among school-age children from December 2021 till March 2022 from Motoubes, Kafrelsheikh, Egypt, and determine the efficacy of nitazoxanide (NTZ), NTZ plus garlic and tinidazole (TIN) on *Blastocystis* and giardiasis infection.

Methods: Stool samples were collected from 390 children and microscopically examined using formalin-ethyl acetate concentration and culturing on Jones' medium for *B. hominis*. Those who tested positive for giardiasis (120 children, 30.7%) (Group I) or *Blastocystis* (180 children, 46.1%) (Group II) were equally divided into four subgroups. The first subgroup received NTZ orally, every 12 hours for three successive days. The second subgroup received NTZ in the same dose as the first subgroup plus dry garlic powder every 12 hours for three successive days. The third subgroup received TIN as a single oral dose, and a fourth control subgroup. Successful cure was considered if no *Blastocystis* or giardiasis stages were found in post-treatment faecal specimens.

Results: Cure rate was significantly higher in TIN treated groups (75.5% and 96.6%) than NTZ (57.7% and 40%) or NTZ plus garlic treated groups (55.5% and 43%) in both *Blastocystis* and giardiasis, respectively ($P<0.05$).

Conclusion: TIN, once, is more efficacious than NTZ or NTZ plus garlic in the treatment of *Blastocystis* and giardiasis in children.

Introduction

Enteric protozoa are considered a diverse group of unicellular microparasites inhabiting the human gastrointestinal tract. Worldwide, *Blastocystis hominis* and *Giardia duodenalis* are very common intestinal protozoa. *B. hominis* is prevalent in more than two billion people, while *G. duodenalis* is estimated to infect 280 million people each year (1, 2).

In Egypt, *B. hominis* and *G. duodenalis* are frequently diagnosed in adults (38.9% and 34.6%, respectively) and in children (67% and 64.8% respectively) (3-8). Faecal-oral route is the main route of transmission for both parasites. They are sharing similar ecological factors (9). Although asymptomatic carriers are common in both infections, many symptoms, including bloating, diarrhea, nausea, epigastric pain and vomiting have been reported in giardiasis, while *B. hominis* infection was linked to irritable bowel syndrome (10). Nitroimidazole derivatives (MTZ, TIN and secnidazole) and NTZ are prescribed frequently for treatment of enteric protozoa (3). They were considered the drugs of choice for the treatment of infections caused by *Entamoeba histolytica*, *G. duodenalis*, *B. hominis*, and anaerobic bacteria (11,12).

The main disadvantages for MTZ are its long therapeutic treatment course with decreased compliance and the development of resistance by both *B. hominis* and *G. duodenalis* (13,14). All those MTZ drawbacks encourage seeking different treatment options. The ideal treatment for giardiasis currently includes either the long acting nitroimidazole derivative (TIN) or NTZ. TIN is a suitable treatment option for giardiasis. The short therapeutic course along with the high cure rate is the major advantages for its usage (11). In spite of its high cure rate observed in treating *B. hominis* and *G. duodenalis* (15,16), some studies reported lower cure rates (17,18). Continuous evaluation of drug susceptibility is needed in endemic areas.

NTZ is considered a broad spectrum antiparasitic drug. A course of three days of NTZ is effective in eliminating *G. duedenalis* and *B. hominis* from the stool (11,19). NTZ was recommended as encouraging alternative treatment to MTZ -resistant giardiasis (20).

The use of garlic for the treatment of parasitic diseases has been reported (21-24). Beside its role as anti-parasitic natural product, it has many health protective roles. The antiparasitic activity is due to the enzymatic activity of allinase on alliin producing allicin (21). The commercially available natural product of garlic tablet (Tomex®) was as efficient as MTZ on *Trichomonas vaginalis* in vitro (21). Also, *Blastocystis* spp. demonstrated an increased sensitivity to garlic in vitro (22). Garlic additionally has a therapeutic effect against giardiasis when tested experimentally (23) or even when tested on children with a three days course of therapy (24).

We aimed to detect the prevalence of *B. hominis* and *G. duodenalis* among school-age children and to determine the coproscopic efficacy of NTZ, NTZ plus garlic and TIN in the treatment of *B. hominis* and *G. duodenalis* infection.

Materials and Methods

Study setting

A cross section study was carried out from December 2021 till March 2022, in Motoubes, a town in the northern part of the Kafrelsheikh Governorate, Egypt. Farming and fishing are the main economic activities that employ most of the workforce in the study area. Children aged 6-16 years were recruited.

Parasitological examination

Children were requested to give a fresh stool sample for parasitological examination. About

2 mg from each sample was examined using formalin-ethyl acetate concentration technique. Another part (about 50 mg) was directly cultured in tubes containing 5ml Jones' medium enriched with 10% horse serum then the culture tubes were incubated at 37ºC for 72 h. To detect *Blastocystis* in culture, the samples were examined daily using light microscopy. Parasitological examination was carried out at the Parasitology Department, Faculty of Medicine, Kafrelsheikh University.

Treatment

Children who tested positive for *G. duodenalis* (group I) or *B. hominis* (group II) were equally allocated to one of the following treatment subgroups: the first subgroup received NTZ (Nanazoxid, Utopia, Egypt) as oral tablet (500mg) in case of children above 12 years old and 10 ml suspension (200 mg) in case of children 6-12 years old, every 12 hours for three successive days. The second subgroup received NTZ in the same dose as the first subgroup plus dry garlic powder 200 mg (Tomix, ATOS Pharma, Egypt) every 12 hours for three successive days. The third subgroup received TIN (Protozol, APM Company, Egypt) as a 50 mg/kg single oral dose to a maximum of 2 g once and the fourth was an untreated control subgroup.

The criteria for inclusion were: (a) ability to take oral medication, (b) having no contraindications to NTZ garlic, or TIN, (c) having no history of chronic disease, and (d) receiving no anti-parasitic drugs in the previous 4-weeks. The exclusion criteria were (a) failure to complete the follow up examination, (b) requesting withdrawal from the study by the parents or guardians of children (c) developing sever adverse effects and (d) receiving any medication throughout the study other than the recommended drugs. (e) having mixed parasitic infection.

Treatment was carried out at home under observation of parents or guardians. All parents /guardians were informed to contact with the study conductors at any time during the study period if the enrolled child appeared ill. Participants were asked about any drug adverse effects. Cure was indicated by absence of *B. hominis* or *G. duodenalis* cysts in stool samples collected 7-14 d post treatment.

Ethics statements

The study was approved by the Research Ethics Committee of the Faculty of Medicine, Kafrelsheikh University, Egypt (MKSU 41-11-21). Ethical considerations and confidentiality were assured for all parents/guardians of children who were informed about the objective of the study and were asked to participate voluntarily. Informed written consent was obtained from the legal guardians before data and sample collection.

Statistical analysis

IBM SPSS software suite version 20.0 (IBM Corp., Armonk, NY, USA) was used. Chi-square test was used to compare the study findings. The significance level was set at 5% level.

Results

B. hominis was detected in 180 (46.1%) children. Out of them, 89 were male and 91 were female (Table 1).

The cure rate in TIN treated group was higher (75.5%, 34 children out of 45 children) than NTZ treated subgroup (57.7%, 26 children out of 45 children), NTZ plus garlic treated subgroup (55.5%, 25 children out of 45 children) and the control subgroup (17.7%, 8 children out of 45). The difference in the cure rate in all treated groups was statistically significant compared to control group ($P<0.05$) (Table 2). TIN treated group showed statistically significant difference compared to NTZ plus garlic treated groups ($P<0.04$).

Regarding *G. duodenalis*, it was detected in 120 (30.7%) children. Out of the 120 infected children 55 were males and 65 were female (Table 1). TIN gave the best result with 96.6% cure rate (29 children).There was slight in-

crease in the cure rate in NTZ plus garlic treated subgroup (43%, 13 children out of 30) than NTZ treated subgroup (40%, 12 children out of 30). The lowest cure was observed in the control subgroup (20%, 6 children out of 30). The difference in the cure rate in TIN treated group was statistically significant compared to all groups (P <0.001) (Table 3).

Table 1: Giardia duodenalis and Blastocystis hominis infection among the examined children according to age and gender

Variable	***Categories***	***G. duodenalis*** *(n=120)*		***B. hominis*** *(n=180)*	
		No.	**%**	**No.**	**%**
Age (yr)	6-12	65	54.2	99	55
	12-16	55	45.8	81	45
Gender	Male	62	51.6	89	49.5
	Female	58	48.4	91	50.5

Table 2: Comparison between the efficacy of NTZ, NTZ plus garlic and TIN in *Blastocystis hominis* infected children (n= 180)

Drug	***Cured***		***Not cured***		***P***
	No.	%	No.	%	<0.001*
NTZ	26	57.7	19	43.3	
NTZ plus garlic	25	55.5	20	44.5	
TIN	34	75.5	11	24.5	
Control	8	17.7	37	82.3	
P	P_1= 0.8 & p_2= 0.07 & p_3< 0.001** & p_4= 0.04*& p_5= < 0.001** & p_6=< 0.001**				

*each group contained 45 children
* P value <0.05 is significant, ** P value <0.01 is highly significant
P1 between NTZ and NTZ plus garlic treated groups
P2 between NTZ and TIN treated groups
** P3 between NTZ and control groups
* P4 between NTZ plus garlic and TIN treated groups
** P5between NTZ plus garlic and control groups
** P6 between TIN and control groups

Table 3: Comparison between the efficacy of NTZ, NTZ plus garlic and TIN in *Giardia duodenalis* infected children (n=120)

Drug	***Cured***		***Not cured***		***P***
	No.	**%**	**No.**	**%**	
NTZ	12	40	18	60	<0.001*
NTZ plus garlic	13	43	17	57	
TIN	29	96.6	1	0.4	
Control	6	20	24	80	
P value	P_1= 0.7& p_2< 0.001** & p_3=0.09 & p_4< 0.001** & p_5= =0.052 & p_6=< 0.001**				

*each group contained 30 children
* P value <0.05 is significant, ** P value <0.01 is highly significant
P1 between NTZ and NTZ plus garlic treated groups
** P2 between NTZ and TIN treated groups
P3 between NTZ and control groups
* *P4 between NTZ plus garlic and TIN treated groups
P5between NTZ plus garlic and control groups

* *P6 between TIN and control groups treated groups

Although TIN gave the highest cure rate in both parasites, it was more effective in treating *G. duodenalis* than *B. hominis* (96.6% vs.75%, respectively). The addition of garlic to NTZ did not increase the cure rate in *Blastocystis* infected subgroup but a slight increase was observed in *G. duodenalis* infected subgroup (Table 2 and 3). No major side effects were observed in all subgroups.

Eight (17.7%) and six (20%) cases of *B. hominis* and giardiasis infected cases, respectively cured spontaneously

Discussion

B. hominis is a very prevalent protozoan parasite. The prevalence of *B.hominis* in the present study was 46.1%. Its prevalence ranges from 7 to 20% and from 30–100% in developed and developing countries, respectively (9). A higher prevalence of *B. hominis* was reported in Egypt among 54.5% of immunocompromised children and 67% in their control asymptomatic group (5). In some cases, it may be self-limiting infection, however, treatment is necessary in many patients due to persistence of symptoms (13,25). MTZ was considered the main line of treatment of *B. hominis* with success rate reaching 100% (13). Its multiple side effects and long course with multiple doses are responsible for low drug compliance especially in children (25,26).

Even after administration of triple antibiotic therapy (diloxanide furoate, trimethoprim/sulfamethoxazole and secnidazole), the eradication of *B. hominis* was achieved in only 60% (six out of 10 cases) of irritable bowel syndrome patients (27). TNZ has an antimicrobial spectrum comparable to that of MTZ. A single dose of TIN achieved a significantly higher cure rate than six doses of NTZ (26). TIN had a better inhibiting effect on *B.hominis* than MTZ and azithromycin in vitro (28). In the present study, TIN had the highest cure rate (75.5%, 34 children out of 45) with a statistically significant difference.

The broad spectrum anti-parasitic NTZ was reported to be effective in treating *Blastocystis* with cure rates ranging from 97-100% in children (13). Another study reported 86% cure rate (36 out of 42 patients) compared to 38% (16 out of 42 participants) in the control subgroup (29). In the present study NTZ cure rate was 57.7%. A previous study reported treatment failure of NTZ in treating five *B. hominis* infected cases (18). This relatively lower cure rate may be due to the development of drug resistance or subtypes variability in the treatment response.

NTZ alone cured one more *B.hominis* case than NTZ plus garlic (2.2%). The effect of garlic on *B. hominis* may be subtype dependent. *B. hominis* isolates, particularly subtype1, from irritable bowel syndrome patients have increased susceptibility to garlic in vitro (22).

In developing countries, the prevalence of *G. duodenalis* ranged from 10 - 50% (9). In Egypt, the prevalence of *G. duodenalis* among children diverges from 10 to 36.6% (3, 8-9). In Beni-Suef, Egypt a previous in study conducted among children suffering from acute diarrhea the prevalence *G. duodenalis* was 27.2% which is lower than the prevalence detected in the present study (30.7%) (9). The higher prevalence detected in the present study may be due to the endemicity of giardiasis in that area (7).

5-nitroimidazoles are the most frequent drugs used to treat this infection. However, nitroimidazole-refractory giardiasis has been documented in 5.7% up to 70% of cases (14).

The results of in vitro and clinical studies have already confirmed the efficacy of NTZ against giardiasis. NTZ was 2.5 and 50 fold more potent than albendazole and MTZ, respectively when used against giardiasis in vitro (16).

NTZ cure rate in giardiasis ranged from 70% to 80% (26,30,31). The efficacy of NTZ was 43% in the present study. The lower cure rate of NTZ in the present study may be due to drug resistance. NTZ resistance has been identified in treating *G. duodenalis* as the effica-

cy rates after three successive courses of NTZ were 69%, 79% and 81% (32) moreover, NTZ resistance can be induced in vitro (33). In folk medicine garlic has been used for many years as a panacea. It has bactericidal, anti-protozoan and anti-viral properties (34). The physiology of G. *duodenalis* trophozoites was affected by whole garlic extract and allyl alcohol in vitro (35). Garlic has demonstrated an anti-*Giardia* effect when used twice per day for three days to treat giardiasis in children (24). The addition of garlic to NTZ in the present study slightly improved the cure rate compared to NTZ alone (43% vs. 40%) but with non-significant difference in cure rates.

The efficacy of TIN in treating giardiasis was 96.6% in the present study. This result is consistent with a TIN cure rate of 97.5% and 96.1% reported previously in adults and children, respectively (15, 36). In other studies, lower cure rates of 72% and 81.9% were reported in *G. duodenalis* infected children (17, 37). TIN is generally more tolerable than NTZ, easy to be administrated with a good compliance thus reducing patient's exposure to drug selective pressure (26).

In the present study 17.7% and 20% of *B.hominis* and giardiasis infected cases, respectively cured spontaneously. Recovery and improvement in *B.hominis* infected patients has been observed without treatment (13).

Giardiasis infection may become self-limited however the fear of complication encourages the treatment option (38, 39).

The present community based study depended on only parasitological assessment of drug efficacy as most enrolled participants didn't seek medical advice. However clinical evaluation of symptoms is valuable and should be done to ensure patient relief.

Conclusion

A single oral dose of TIN is more efficacious than a course of three successive days of NTZ or NTZ plus garlic in the treatment of *B. hominis* and *G. duodenalis* in children.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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