Original Article

Molecular monitoring of the therapeutic effect of Albendazole on *Ascaris lumbricoides* and *Ancylostoma duodenale* infected children using conventional multiplex PCR

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ABSTRACT

Background: Globally, many individuals are infected with soil-transmitted helminths (STHs) with majority occurring in tropical and subtropical regions of the world. Albendazole (ADZ) is a broad-spectrum anthelmintic used efficiently for STHs control. Upgrading the assessment of ADZ efficacy is important to confirm that drug resistance has not emerged.

Objective: To assess the therapeutic efficacy of ADZ in both *A. lumbricoides* and *A. duodenale* infections by conventional multiplex PCR.

Subjects and Methods: Stool specimens from 191 boys and 123 girls, aged from 2 to 13 years were screened by saline wet mount and iodine stained smears and cellophane thick smears (Kato-Katz technique). All positive cases were given a single oral dose of 400 mg ADZ then 2 weeks later new stool samples were collected and subjected to multiplex PCR. Before medication, anemia assessment (finger prick capillary blood sample) was performed.

Results: Prevalence of infection with *A. lumbricoides* and *A. duodenale* was 20.7% and 5.4%, respectively. Parasitic infections were more prevalent in age group 7-13 years (59%) than in 2-6 years (41%) respectively. Boys were slightly more affected than girls (52.6% versus 47.4%). Prevalence of parasitic infections among cases living in rural areas proved to be higher than those living in urban areas (65.4% versus 34.6%). Examination for associated anemia showed that 35.9% of infected cases were anemic (Hb level <11.5 mg/dl) versus 9.7% in non-infected cases. Diarrhea and colic were more prevalent in infected cases (39.7% and 62.8%, respectively) than in those that were non-infected (5.5% and 8.9%, respectively). The therapeutic efficacy of ADZ resulted in a cure rate of 93.8% and 88.2% for *A. lumbricoides* and *A. duodenale* infections, respectively; with an egg reduction rate (ERR) of 96.1% and 91.2%, respectively. The multiplex PCR sensitivity was 100% for detection of both worms; the specificity was 96.8% and 93.8%, respectively, with diagnostic accuracy of 96.9% and 94.1%, respectively.

Conclusion: Multiplex PCR was useful for measurement of the therapeutic efficacy of ADZ as an anthelminthic drug.

Keywords: Albendazole, *A. duodenale, A. lumbricoides*, multiplex PCR, prevalence.

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INTRODUCTION

Soil-transmitted helminths (geohelminths) belong to the group of neglected tropical diseases. This assembly of infectious diseases include parasites namely *A. lumbricoides*, *T. trichiura*, and the two hookworm species, *A. duodenale* and *N. americanus* causing an extensive variety of clinical symptoms and signs. *S. stercoralis* is one of the most important and often neglected additional STH affecting 10-40% of the population mainly in sub-Saharan Africa and Southeast Asia[1]. Geohelminths can affect human health either directly by damaging the internal mucosa of the gut and feeding on host's blood and serum, or indirectly through production of inflammatory cytokines that affect appetite and food intake of hosts especially children[2]. Both mechanisms are a dual burden of parasitic infection leading to malabsorption with drastic deficiencies in macro and micronutrients which in turn leads to a malnutrition state that increases susceptibility to infection by inducing alterations in host immune function. These factors not only affect the physical development of children, but also they impair their cognitive development leading to retarded academic performance[3].

Geohelminths are transmitted by a fecal-oral route, which explains its high rate of prevalence in areas deprived of proper socioeconomic development and/or lack of proper sanitary environmental health conditions; criteria known to be present in low and middle income countries, including Egypt[4]. In developing countries, these worms continue to represent an actual health problem infecting millions of people and exhausting its health budget annually. Globally, the infection with at least one species of geohelminths occurred in more than a billion patients. Most of them are those living in low income countries deprived of education, health

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sanitary conditions and complaining of malnutrition[5]. The majority are also likely to be chronically infected by more than one species of these worms[6]. These children are not only natural victims of geohelminths infection due to their immature immune systems, but they also represent a major source of infection in these communities due to their uncontrolled fecal hand-mouth activities[7,8].

The traditional method for the diagnosis of STHs infections is microscopic examination, using either direct fecal smear or various fecal concentration methods, which are time-consuming, and insensitive. For example, although the Kato-Katz technique increases the detection rates of some helminth species and provides quantitative results, the diagnosis of hookworm is difficult due to the rapid disintegration of the eggs. Also, differentiation between hookworm species is difficult, and the detection of *Strongyloides stercoralis* larvae is poor[9]. The use of conventional and real-time PCR can overcome these diagnostic limitations as they have proved to be highly sensitive and specific for detection of many intestinal parasites[10]. In addition, quantitative multiplex PCR shows high sensitivity and specificity, but the high cost and need for special instruments still present obstacles limiting their applications in parasitological routine diagnosis. Therefore, a conventional multiplex PCR assay that has lower cost and greater simplicity, was developed, for the simultaneous detection of STHs in stool samples[11].

Since WHO declaration of its strategy in 2001 to control neglected tropical diseases, including geohelminths, combating these infections became the major concern of many countries including Egypt[12], with the aim of maintaining the individual worm burden below morbidity and mortality levels. In cooperation with WHO and the international health bodies, these countries work hard to implement an integrated plan for improving the quality of the environment by providing clean water supplies, intensifying health education, and economic development in line with mass chemotherapy campaigns as recommended by WHO in a trial to combat these parasites[13,14]. The control of geohelminths infections is particularly challenging because of the high rate of reinfection, particularly in environments where control depends mainly on environmental health sanitation and development which are expensive and need a long time to be implemented representing a real challenge for countries with limited income[15]. Accordingly, WHO adopted worldwide national mass treatment campaigns based on large-scale deworming programs incorporation with international pharmaceutical bodies[16].

Because numbers of infections may sometimes be underestimated, WHO adopted the strategy of giving safe, single-dose, affordable drugs to all at high risk groups at regular intervals. Albendazole (a benzimidazole derivative) is an effective broad-spectrum anthelmintic drug against a wide range of helminths. This drug is the mainstay of the control chemotherapy strategy adopted by the WHO provided as a single-dose of 400 mg given randomly to all high risk groups in endemic areas over the age of 2 years. It recommends periodic mass drug administration of ADZ to school children[13]. Although the treatment proved to be highly effective against geohelminths[14], evidence regarding its effective therapeutic action, even in repeated doses, has been questioned and needs continuous evaluation[17]. Moreover, a major concern is the parasitic resistance to ADZ which represents an adverse impact on the effectiveness and sustainability of chemotherapeutic campaigns to disease transmission and control, leading to its failure[18]. Due to the wide use of anthelminthic drugs globally and the rapid development of resistant parasite strains, ADZ remains a priority for researchers to continuously evaluate and investigate its efficacy[19,20].

The aim of this study is to monitor the effect of ADZ drug on *A. duodenale* and *A. lumbricoides* using conventional multiplex PCR technique as prerequisite for a successful control strategy. Also, the study aims to provide reliable data for the governmental health authorities for decision making regarding the effectiveness of the chemotherapeutic campaigns; whether to continue or shift to another medication.

SUBJECTS AND METHODS

This cross-sectional analytical study was performed on children attending Gastroenterology Unit in Zagazig University Pediatrics Hospital from December 2017 to October 2018. The study was conducted at the Medical Parasitology Department, Faculty of Medicine, Zagazig University.

Subjects: The study was carried out on 314 cases (191 boys and 123 girls); ages ranging from 2 years to 13 years. They complained of different abdominal symptoms as abdominal pain, diarrhea, loss of appetite and weight loss with or without pallor. Children or their parents were asked about socio-behavioral data (i.e. occupation, parent's education, wearing shoes, personal hygiene, food consumption and sources of drinking water). At enrollment, a full clinical examination was conducted, including measurement of weight, height, temperature, anemia and relevant medical history. Children were excluded from the study if they had recent history of anthelmintic treatment or reported hypersensitivity to ADZ.

Samples collection and parasitological procedures: All collected stool samples were examined within 4 h after collection. For each pre- and post-medication specimen, saline wet mount and iodine stained smears[21], and Kato-Katz technique smears[9] were

prepared on microscope slides using standard 50 mg templates. Kato-Katz thick smears were quantitatively examined under a light microscope using 100 x magnification. The number of eggs in one gram of feces was calculated by multiplying the obtained value by 20. It is used as a baseline data for the cure rate and ERR estimation, also to grade the infection as light, moderate, and heavy, according to WHO guidelines for STHs. Infection intensity classifications were as follows: light *A. lumbricoides* 1–4999 eggs/gram stool (EPG), moderate 5000–49999 EPG and heavy ≥ 50000 EPG[22]; *A. duodenale*, light 1–1999 EPG, moderate 2000–3999 EPG, and heavy ≥4000 EPG[23].

Determination of hemoglobin (Hb) levels: Before ADZ administration, anemia was monitored using finger prick capillary blood sample. Child was considered anemic if the Hb level was below 11.5 mg/dl[23].

Drugs administration: ADZ (Alzental, EIPICO, Egypt, 20 mg/ml) 400 mg single oral dose was given for all infected children on an empty stomach. They were asked to come back to the hospital 2 w after administration of medication to repeat stool examination and assess the efficacy of treatment. ERR was calculated using the following formula[24]: ERR = [(Mean EPG pre-treatment - Mean EPG post-treatment)/Mean EPG pre-treatment] X 100. Cure rate was calculated using the following formula[24]: Cure rate = (No. of positive cases pre-treatment who become negative post-treatment/No. of positive cases pre-treatment) X 100.

DNA extraction: Post medication stool samples preserved at –20^{0}C were defrosted at room temperature. The DNA was extracted from stool samples using the QIAamp Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with minor modifications. About 100 mg of stool were suspended in 200 µL of phosphate buffered saline (PBS) that contain 2% polyvinylpolypyrolidone (PVPP; Sigma, Steinheim, Germany); then heated for 10 min at 100°C. ATL buffer containing proteinase K was added to the suspension then left for 3 h at 55^{0}C before performing the DNA extraction. Phocine herpes virus 1 (PhHV-1) 103 PFU/ml was added to the AL lysis buffer to serve as the internal positive control for the extraction process. The extracted DNA samples were stored at -20^{0}C until amplified and analyzed[25].

Multiplex PCR amplification: Specific primers to detect *A. duodenale* and *A. lumbricoides* in stool samples are shown in table (1).

A 50 µl volume was prepared from 2X KAPATaq Ready Mix DNA polymerase (1.25 U KAPATaq DNA polymerase, 0.4 mM dNTP, and reaction buffer with Mg^{2+}), plus 20 pmol of *Ascaris* and *Ancylostoma* specific primers, 1 µl of DNA template, and sterile distilled H2O. PCR thermocycling (C1000TM Thermal Cycler BIO-RAD, Hercules, CA) was performed as follows: 95^{0}C for 3 min then 35 cycles of 95^{0}C for 30 sec then 53^{0}C for 30 sec and 72^{0}C for 1 min; lastly a final step of 72^{0}C for 5 min. The amplified DNA samples were analyzed using 2% agarose gel electrophoresis at 50 volts for 1 h, and then visualized using ethidium bromide and recorded via gel documentation system (G:Box HR; Syngene, Cambridge, UK)[11].

Statistical analysis: Statistical analysis was done *via* the statistical package SPSS version 17 (Chicago, IL, USA). Pre- and post-treatment prevalence were compared using Chi-square test. Anthropometric measures of children were calculated as mean ± SD. Statistical significance was set at $P < 0.05$. Evaluation of multiplex PCR was based on the sensitivity, specificity, positive and negative predictive values and diagnostic accuracy.

Ethical consideration: Ethical approval was obtained from the Committee of Research, Publications and Ethics of the College of Medicine, Zagazig University, Egypt. All procedures were explained to patient's parents and a written or thumb-printed informed consent was obtained.

RESULTS

Stool examination revealed parasitic infection in 78/314 (24.8%) samples, 74 of which showed a single parasitic infection with *A. lumbricoides* or *A. duodenale* and 4 showed mixed infection with both. Prevalence of *A. lumbricoides* infection was 20.7% and that of *A. duodenale* infection was 5.4%. Among the 65 *A. lumbricoides* infected cases, 57 (87.7%) had light infection, 7 (10.8%) had moderate infection and only one (1.5%) had heavy infection. Among the 17 *A.*

Table 1. Specific primers.

Primers	Company	Notes
***A. duodenale* (GenBank accession AJ001594)** Forward 5'- GAA TGA CAG CAA ACT CGT TGT TG -3' Reverse 5'-ATA CTA GCC ACT GCC GAA ACG T-3'	Biolegio, Malden, The Netherlands	Used to amplify a 71-bp fragment of the Internal transcribed spacer 2 (ITS2) sequence[10].
***A. lumbricoides* (GenBank accession EU582499)** Forward 5'- GGA GGT TTT TGG GTC TTT GG -3' Reverse5'- CCA AAC AAG GTA GCC AAC CA -3'	Applied Biosystems, Foster City, CA, USA	Used to amplify 192-bp fragment of the DNA regions of (COI)[11].
***Phocine herpes* virus (GenBank accession 24)** Forward 5'-GGG CGA ATC ACA GAT TGA ATC-3' Reverse 5'-GCG GTT CCA AAC GTA CCA A-3'	Biolegio, Malden, The Netherlands	Used as an internal positive control for the extraction process[26].

duodenale infected cases, 14 (82.4%) had light infection and 3 (17.6%) had moderate infection (Table 2).

Parasitic infections were more prevalent in 7-13 years age group (59%) than in that of 2-6 years (41%) with a significant statistical difference ($P = 0.028$). Boys (52.6%) were slightly more affected than girls (47.4%) without statistical significant difference. Prevalence of parasitic infection among cases living in rural areas (65.4%) was higher than in those living in urban areas (34.6%) with a statistically significant difference ($P = 0.007$) (Table 3).

The recorded mean weight (24±1.8 kg) and height (129±12.7 cm) of infected group was statistically insignificant; and in non-infected group they were 27±2.3 kg and 131±10.2 cm, respectively. Statistically significant anemia was recorded in 35.9% of infected cases versus 9.7% of non-infected cases ($P = 0.000$). Diarrhea was documented in 39.7% of infected cases and 5.5% of non-infected cases with significant statistical difference ($P = 0.000$). Colic complaint in 62.8% infected cases was statistically significant than in 8.9% of non-infected cases. (Table 4).

Evaluation of the therapeutic efficacy of ADZ showed that the cure rates for *A. lumbricoides* and *A. duodenale* were 93.8% (95% CI: 91.14-96.46%) and 88.2% (95% CI: 84.64-91.76%), respectively. The ERR for *A. lumbricoides* and *A. duodenale* were 96.1% (95% CI: 93.96- 98.24%) and 91.2% (95% CI: 88.07-94.33%), respectively (Table 5). Multiplex PCR detected 2 post treatment cases of *A. lumbricoides* (192 bp) and one case of *A. duodenale* (71 bp) which were negative by microscopy. Multiplex PCR sensitivity was 100% in detection of both worms with a specificity of 96.8% for *A. lumbricoides* and 93.8% for *A. duodenale*, with diagnostic accuracy 96.9% for *A. lumbricoides* and 94.1% for *A. duodenale* (Figure 1).

Table 2. Frequency and intensity of *A. lumbricoides* and *A. duodenale* among infected children.

	No. (%)					Total
	Light	Moderate	Heavy	Single	Mixed	
A. lumbricoides	57 (87.7)	7 (10.8)	1 (1.5)	61	4	65 (20.7%)
A. duodenale	14 (82.4)	3 (17.6)	0 (0)	13	4	17 (5.4%)

Table 3. Demographic data of infected children.

	Infected cases		Statistical analysis	
	No.	%	Chi-squares (X^2)	*P* value
2-6 years	32	41	4.84	0.028*
7-13 years	46	59		
Boys	41	52.6	2.97	0.085
Girls	37	47.4		
Rural	51	65.4	7.23	0.007*
Urban	27	34.6		
Total	**78**	**24.8**		

*Significant differences at P value < 0.05.

Table 4. Anthropometric measures and clinical problems of examined groups.

	Infected	Non infected	Statistical analysis	*P* value
Weight (Kg) (Mean ±SD)	24 ± 1.8	27 ± 2.3	*t* test=0.78	0.227
Height (cm) (Mean ±SD)	129 ± 12.7	131 ± 10.2	*t* test=0.92	0.188
Anemia no. (%)				
Positive	28 (35.9)	23 (9.7)	$X^2 = 29.5$	0.000 *
Negative	50 (64.1)	213 (90.3)		
Diarrhea, no. (%)				
Positive	31 (39.7)	13 (5.5)	$X^2 = 57.02$	0.000 *
Negative	47 (60.3)	223 (94.5)		
Colic, no. (%)				
Positive	49 (62.8)	21 (8.9)	$X^2 = 98.4$	0.000 *
Negative	29 (37.2)	215 (91.1)		

*Significant differences at P value < 0.05, Anemia positive if Hb < 11.5 mg/dl.

Table 5. ERR and cure rate of ADZ for *A. lumbricoides* and *A. duodenale* infections.

	A. lumbricoides	*A. duodenale*
Mean egg count/gm stool		
Pre treatment	4173	982
Post treatment	163	86
ERR% (95% CI)	96.1 (93.96 - 98.24)	91.2 (88.07 - 94.33)
No. of infected cases		
Pre treatment	65	17
Post treatment by microscopy	2	1
Post treatment by multiplex PCR	4	2
Cure rate% (95% CI)	93.8 (91.14 - 96.46)	88.2 (84.64 - 91.76)
Multiplex PCR		
Sensitivity	100%	100%
Specificity	96.8%	93.8%
PPV	50%	50%
NPV	100%	100%
Diagnostic accuracy	96.9%	94.1%

ERR: Egg reduction rate, **95% CI:** (95% confidence interval), **PPV:** Positive predictive value, **NPV:** Negative predictive value.

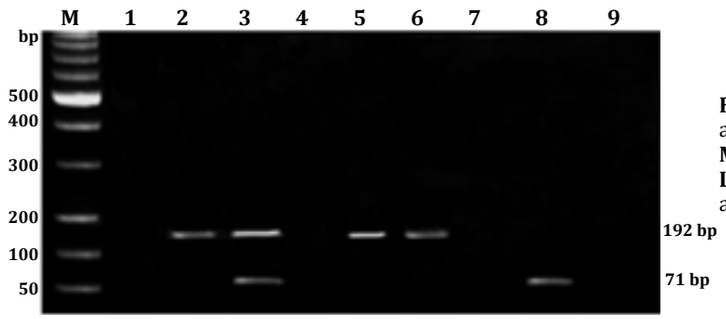

Fig. 1. Gel electrophoresis analysis of multiplex PCR-amplified *A. lumbricoides* and *A. duodenale* DNA.
M: Molecular weight DNA ladder.
Lanes 1- 9 represent parasites DNA bands at 192 bp and 71 bp, respectively in stool samples.

DISCUSSION

Parasitic infections mainly STHs remain a major public health problem in developing countries. Regular deworming with anthelmintic drugs mainly ADZ and mebendazole is the current global control strategy[23]. Our study aimed to assess the therapeutic efficacy of a single oral dose of 400 mg ADZ on both *A. lumbricoides* and *A. duodenale* infections by conventional multiplex PCR.

Concerning the prevalence and intensity of parasitic infections according to area, age and sex, controversial recorded differences could be generally attributed to the geographical distribution of the patients and conditions of exposure to infection. In our study the prevalence of parasitic infection was 24.8% (78/314). Prevalence of *A. lumbricoides* infection was 20.7% while, that of *A. duodenale* was 5.4% (Table 2). These results are a higher record than that reported in 2015 by Abd Ella *et al.*,[27] who reported a prevalence of 6.64% and 1.75% respectively in Qena province in Egypt. Also, Vaz Nery *et al.*,[24] reported 2.6% prevalence of *Ancylostoma* spp. in Timor-Leste Susana. Exceeding our results for A. lumricoides Krücken *et al.,*[28] reported a pre-ADZ treatment prevalence of 33.3% for Ascaris in Rwandan schoolchildren, and Augusto *et al.,*[29] detected 65.8% rate in children and young adults in Mozambique.

Among the 65 *A. lumbricoides* infected cases, 87.7% had light infection, 10.8% had moderate infection and only 1.5% had heavy infection; while among the 17 *A. duodenale* infected cases, there were only light infections (82.4%) and moderate (17.6%) infections (Table 2). Similarly, Soukhathammavong *et al.*,[23] who tested both ADZ and mebendazole effect on hook worms and *Ascaris* infection, reported that most of the cases had light infection, few had moderate infection and heavy infection was rare. Also, Smith *et al.*,[30] in their study found that overall infections of Ascaris were mostly moderate and light.

According to age, we recorded a significant statistical prevalence of parasitic infections in age group 7-13 years (59%) than 2-6 years (41%). Hegazy *et al.,*[31] had reported an apparently higher prevalence of 51.8% in children aged between 2-6 years in Damanhur province, Egypt; while El-Masry *et al.*,[32] detected 60.2% rate among Egyptian school children in Sohag governorate villages. Controversially, Ibrahium[33] found that the prevalence among Egyptian school children in a village in El-Minia governorate was 29.3%. Regarding sex, boys (52.6%) appeared to be insignificantly more affected than girls (47.4%). In agreement Abd Ella *et al.*,[27] found parasitic infection more common in males 49.5 % than females 40.25%.

Also, Dyab *et al.*,[34] reported that parasitic infection was more prevalent in boys 53.8% than girls 46.2%.

Correlation of mean weights of 24±1.8 kg in infected group with 27±2.3 kg in non-infected group was statistically insignificant. Also, the difference between mean heights of infected and non-infected groups (129±12.7 cm and 131±10.2 cm, respectively) was insignificant. Similarly Soukhathammavong *et al.*,[23] found that the mean weight of infected group taking both ADZ and mebendazole for treating *A. duodenale* and *A. lumbricoides* was 24.0±6.0 and 25.2± 6.0 respectively, and mean height was 124.1±11.0 and 127.0±11.0, respectively.

As expected results of prevalence of parasitic infections according to residence, was statistically greater among cases living in rural areas (65.4%) than those living in urban areas (34.6%). Likewise Dyab *et al.*,[34] and Abd Ella *et al.*,[27] reported higher rates of parasitic infection in rural than urban areas (60% and 40%) and (50.95% and 38.35%), respectively. Esrey *et al.*,[35] suggested that poor sanitation and usage of feces as fertilizers are the main contributing factors for elevated risk of parasitic infection in rural communities.

Consequently, children living in rural areas of developing countries have increased risk of anemia, poor growth and STHs infections. These are usually strongly associated with long-term nutritional stress manifested clinically as anemia, retarded growth, and cognitive impairment[36]. Regarding anemia, in our work, the recorded Hb levels were significantly lower (35.9%) in infected cases than in non-infected ones (9.7%). Crompton and Nesheim[37] and WHO[38] established that parasitic infections as ascariasis are usually associated with anemia as a sequel of inflammation and malnutrition.

Because of these worm infections gastrointestinal manifestations as diarrhea and colic were the main symptoms. Diarrhea and colic were respectively more significantly prevalent in infected patients (39.7% and 62.8%) than non-infected ones (5.5% and 8.9%). These results coincide with the report by Dyab *et al.*,[34] who found significant increase of diarrhea, recurrent abdominal pain and pallor in infected students when compared to those who were non-infected. A similar result was also reported by Khadka *et al.*,[39] who detected increased prevalence of intestinal parasitosis among school children with abdominal discomfort, pain and diarrhea.

ADZ is widely used drug in preventive chemotherapy programs targeting STHs infections worldwide[40]. Its wormicidal activity is mainly through binding to intracellular microtubules and preventing their elongation. This leads to inhibition of absorption of molecules that are critical for parasite growth. This action affects the parasites rather than the host[41].

In our study, the cure rate by ADZ was substantial for *A. lumbricoides* recording 93.8% (95% CI: 91.14 - 96.46 %), while for *A. duodenale* it was 88.2% (95% CI: 84.64 - 91.76%). Comparatively, the ERR for *A. lumbricoides* was 96.1% (95% CI: 93.96 - 98.24%) while for *A. duodenale* it was 91.2% (95% CI: 88.07 - 94.33%). In another report, Keiser and Utzinger[40] estimated ADZ post-treatment cure rates of 88% (95% CI, 79%-93%) for *A. lumbricoides* and 72% (95% CI, 59%-81%) for hookworm. Also, in agreement with our results Adugna *et al.*,[42] noted that ADZ had cure and ERR rates of 83.9% and 96.3% for *A. lumbricoides* and 84.2% and 95% for hookworm infection respectively. Vaz Nery *et al.*,[24] reported that ADZ was highly efficacious against *Ascaris* spp., with a cure rate of 91.4% (95% CI: 85.9–95.2%) and infection intensity reduction rate of 95.6% (95% CI: 88.3–100%), but it was less effectual against hook worms with a cure rate of 58.3% (95% CI: 51.4–64.9%) and infection intensity reduction rate of 88.9% (95% CI: 84.0–97.0%). Also, Soukhathammavong *et al.*,[23] noted that the single dose of ADZ has low therapeutic efficacy on hook worms with a cure rate of 36.0% and ERRs of 86.7%, while in *A. lumbricoides* it produces high efficacy with a cure rate of 92.9% and ERRs of 100%. The author suggested that the apparent lower effect of ADZ on hook worm may be attributed to failure of some children to swallow the tablet correctly, other host factors, co-infections with other helminthes, differences in strains and species susceptibilities, or development of ADZ resistance.

Until now, the standard method for diagnosing STHs infections is microscopic examination for detection of helminth eggs or larva in stool samples. Although this method is easy, simple and of low cost, it suffers from low sensitivity, especially in cases of light infection. The other alternative is PCR assay, which is a highly sensitive technique that can be used for diagnosis of STHs[43]. In our study we used conventional multiplex PCR assay that can simultaneously detect *A. lumbricoides* and *A. duodenale* infections with low cost and requiring only a standard thermal cycler. In our study multiplex PCR sensitivity was 100% for detection of both worms. While, PCR specificity was 96.8% for *A. lumbricoides* detection, it was 93.8% for *A. duodenale*. Phuphisut *et al.*,[11] who compared multiplex PCR with microscopic examination of fecal samples detected a sensitivity and specificity of 87% and 83%, respectively. They accordingly suggested that multiplex PCR assay provides an alternative method for routine diagnosis of STHs infection. Also, Llewellyn *et al.*,[44] reported that multiplex PCR has superior sensitivity for determination of infection with Ascaris and hook worms compared to microscopy, especially in samples exhibiting polyparasitism.

In conclusion, the estimated cure rates and ERR for *A. lumbricoides* and *A. duodenale* demonstrate the high efficacy of ADZ against these STHs and its usefulness as a mass chemotherapy agent in Egypt. Furthermore, this

study demonstrates the practicality of conventional multiplex PCR as a method to measure the therapeutic efficacy of anthelminthic drugs.

Author Contribution: SH Yahia planned the study design, HSF Moawad collected the stool samples and both shared TI Farag and SM Mohammad in parasitological and molecular examination and writing and reviewing the manuscript.

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