Comparing the anti-parasitic effect of Ivermectin versus Albendazole against intestinal nematode worms

Original Article

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ABSTRACT

Background: Albendazole (ABZ) is the drug of choice in treatment of intestinal nematodes. However, development of drug resistance necessitates developing novel drugs or drug repurposing.

Objective: This study aimed to investigate the efficacy of single doses of Ivermectin (IVM) versus ABZ in children infected with intestinal nematodes.

Subjects and Methods: A total of 136 schoolchildren (6-15 years old) infected with *T. trichiura*, *A. lumbricoides*, and *A. duodenale* were enrolled in the study. They were divided into two treatment groups, 68 in each, receiving either ABZ or IVM. At days 0 and 14 post-treatment, one stool Kato-Katz thick smear was performed for cure assessment in ABZ and IVM treated groups. Blood samples were collected at the same time points to determine absolute eosinophil count and IL-5 serum level as additional parameters to assess their prognostic role in drug efficacy.

Results: Compared to the ABZ cure rate (CR), IVM showed equivalent efficacy in treating *A. lumbricoides*. However, it was unsuccessful in treating both *T. trichiura* and *A. duodenale* infections. High eosinophil counts were recorded in the infected rather than non-infected children and their levels dropped significantly after treatment with ABZ and IVM. High levels of IL-5 were observed in the infected children compared to non-infected, with significant reduction after treatment with ABZ and IVM.

Conclusion: A single dose of IVM is a promising therapy for *A. lumbricoides* but was inefficient for treating *T. trichiura* and hookworm infections. Although eosinophil counts and serum interleukin-5 (IL5) levels decreased after treatment with ABZ and IVM, their function as effective prognostic parameters requires more research to establish their relevance.

Keywords: albendazole; *A. duodenale*; *A. lumbricoides*; eosinophilia; IL5; ivermectin; Kato-Katz; *T. trichiura*.

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INTRODUCTION

The three major intestinal helminthes, *A. duodenale* (hookworm), *A. lumbricoides* (roundworm), and *T. trichiura* (whipworm) are common widespread parasites in developing countries, especially in Africa. These infections represent significant health issues in endemic areas accounting for the major burden of soil-transmitted helminths (STH)[1]. Different forms of these endo-parasites (eggs, larvae) contaminate soil and can be easily transmitted between different age groups and sexes through ingestion of the infective stage in a variety of food items or by penetration of skin with the infective filariform larvae in case of hookworms[2]. These worms primarily affect school-aged children due to underdeveloped immunity and bad sanitary health measurements. Most of these infections are asymptomatic in mild infections, whereas frank symptoms such as abdominal discomfort, nausea, vomiting, and diarrhea occur in association with significant worm loads as a result of repeated infection[3]. Intestinal parasites mostly affect young children before they attain a certain level of parasite resistance. Enough protein is necessary during childhood development to sustain an efficient immune response that relies on cell replication and the generation of active protein molecules to overcome the infection. The rate of re-infection decreases as the child host grows older owing to immune system development[3]. These parasites were identified as poverty-causing illnesses that influence the infected child's adult cognitive abilities and effect the future income levels due to loss of ability for work and consequent inability to meet treatment costs[4].

Ascariasis and ancylostomiasis provoke eosinophilia principally during the initial stage of larval migration through the lungs. Eosinophilia was attributed to strong helper T lymphocytes (Th2) that increased IL5 levels[5]. Normally the number of eosinophil cells is relatively low in peripheral blood representing only 5% of all leukocytes. However, its percentage escalates substantially during parasitic disease without a proportional increase in the number of other leukocytes, specifying eosinophilia an immune hallmark of parasitic infections[6].

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Although eosinophilia may become reduced or even disappear with time in many chronic helminthic infections, the release of parasite antigens during anthelmintic treatment may result in a new or aggravated eosinophilic response[7]. Notably, IL-5 regulates and plays a crucial function in all phases of eosinophil development[6]. It was proposed that IL-5 and eosinophils are attractive criteria for evaluation of specific therapy for gastrointestinal worms. This was attributed to their ability to guard against repeated infections by initiating a fast type 2 immune response[8]. Hence, measuring their level in the blood can support the diagnosis of intestinal worms[5].

One of the WHO's ambitious disease combating aims for 2030 is to interrupt the transmission of intestinal parasitic diseases by implementing annual or semiannual mass chemotherapy strategies[9]. Four drugs, ABZ, Mebendazole, Levamisole, and Pyrantel were assigned for the global control of intestinal nematodes[10]. The benzimidazole drug, ABZ, is the first-line option against ascariasis, trichiuriasis, and hookworm parasitic diseases[2]. A single oral dose of 400 mg ABZ is a low-cost drug for combating STHs in school children[11]. Notably, ABZ was donated by pharmaceutical corporations in response to a great demand for it globally, and hundreds of millions of doses are provided each year to endemic areas for intestinal parasite control[12]. While it showed satisfactory results in controlling the infection rate of ascariasis and ancylostomiasis, poor efficacies were recorded against *T. trichiura*[13]. Although anthelminthic drugs were successful at the individual level in improving physical growth and mental performance, that was reflected in economic progress in targeted nations to end the poverty cycle, their widespread use resulted in anthelmintic resistance documented in livestock nematode populations[14] with possible drug resistance in human populations[15]. Such concerns may endanger the promising outcomes of mass medication deworming initiatives. The WHO highlights the significance of regularly evaluating the efficacy of anthelmintic drugs to guarantee continuous usage of chemotherapy in the management of intestinal nematode infections[16]. As a result, new applications of previously recognized drugs that proved effective for certain therapies were encouraged[17].

In this context, IVM which exhibits diverse actions that have been applied to treat different infections, was considered an efficient target and was included in the WHO essential medications list for clinical testing and for the treatment of intestinal nematodes[18]. Its application to control parasitic diseases in humans (particularly nematodes) was found to greatly influence the global reinfection rates[19]. Originally this drug showed excellent results in treating filariasis leading to a dramatic decrease in infected cases worldwide[20]. It was shown that this synthetic version of naturally occurring avermectin B1 negatively affects nematode worm movement and feeding capacity *via* glutamate-gated chloride channels at nanomolar concentrations. These channels are not found in vertebrates and are assumed to be responsible for IVM's vast safety margin in most mammals[21]. It interferes also with nematode fertility; as evidenced by decreased microfilariae production by filarial worms[21]. A major turning point in global interest in this drug was the COVID-19 pandemic of 2020[22] that disrupted programs of parasitic mass drug administration efforts against intestinal nematodes and other neglected disease control measures[23].

The purpose of the present study is to compare the curative rate of a single dosage of IVM 200 µg/kg with the standard dose of ABZ in children infected by *T. trichiura*, *A. lumbericoides*, and *A. duodenale*. Successful implementation may expand its usage as an alternative to traditional anti-helminthic medications that acquire resistance over time. The secondary objective was to evaluate the diagnostic role of eosinophilia and serum IL5 level as monitoring parameters of drug efficacy in mass drug administration campaign against intestinal nematode worms.

SUBJECTS AND METHODS

This randomized controlled clinical trial was conducted at the Medical Parasitology Department, Faculty of Medicine; Zagazig University, during the period from December 2019 to November 2020.

Study design: In preparation for the study, a parasitological screening for intestinal nematodes was applied for schoolchildren who visited outpatient Pediatric Clinics at Zagazig University Hospitals. From each stool sample, a Kato-Katz thick smear was performed, and examined for identification of *T. trichiura*, *A. lumbericoides*, and *A. duodenale* eggs. At day zero for each infected child, two samples were provided: a single stool sample examined by Kato-Katz thick smear to confirm the infection, and a blood sample to determine absolute eosinophilia and measure IL5 blood level. Each infected child was assigned to one of two therapy groups receiving either IVM or ABZ until we reached an equal number in both groups. At the 14th day after drug administration, an additional single Kato-Katz thick smear, and blood sample were collected and analyzed for another microscopic examination and assessment of CR, as well as determination of eosinophil count and IL5 estimation. Any children who remained positive for any intestinal parasitic infection based on microscopy at the end of the study were administered ABZ.

Target population: The study included school-age children of both sexes ranging in age from 6-15 years

who had symptoms suggestive of intestinal parasitism such as diarrhea, impaired appetite, and abdominal discomfort. A questionnaire form was designed for each child including date, gender, age, residence, infecting nematode, and medicine administered. Anthropometric measures were done for all children included in the study. Each child infected with any of the researched parasites was considered eligible to participate in our study and was randomly assigned to one of two therapy groups. Each candidate had an equal probability of being accepted into either category unless he or she refused to sign the consent form, had received any anthelminthic therapy during the preceding three months, or had a history of IVM or ABZ hypersensitivity.

Children who were unable to produce a stool sample at the follow-up visit, or who had a serious concomitant medical condition were excluded from the study. The study-group allocation was known to study-site investigators, but participants and laboratory personnel were not. Any child who vomited within 2 h of receiving the anthelminthic medicine was omitted because the specific remaining amount of anthelminthic drug taken would be unknown.

Drugs and administration dose: Children were administered a single dose of either 400 mg/kg ABZ (purchased as Alzental from EIPICO, Egypt)[11], or 200 µg/kg IVM (purchased as Iverzine from UNIPHARMA, Egypt)[18]. All 136 infected children were deemed eligible for the trial and were randomly assigned to one of two therapy groups. Each candidate had an equal probability of being accepted into either category. To guarantee compliance, all participant candidates were required to take medication at the hospital.

Collected samples

- **Stool samples:** All eligible children were trained on how to take the sample in order to avoid contamination with urine. About 30 g of fresh stool were collected in code-labeled, clean, dry, wide-open plastic containers with a sealed lid, allowing the stool sample to retain moisture while avoiding dryness and bacterial contamination.

- **Blood samples:** Five ml blood were withdrawn, 3 ml for serum preparation, and 2 ml for eosinophil count. Serum samples were kept at -20°C for later use in IL-5 measurement.

Stool analysis: Kato-Katz thick smear was prepared from each individual sample and all slides were examined microscopically using a X10 magnification within 30-60 min to minimize over clearing of *A. duodenale* eggs that are sensitive to the time interval between slide preparation and reading[24,25]. No preservative was added. Before a slide was considered negative, ten X40 magnification fields of the stool smears were inspected.

Eosinophils count: Absolute values in one ml^3 of blood were calculated as a component of the complete blood count of peripheral blood smears from infected children before and after treatment with IVM and ABZ using an automated blood analyzer. The presence of eosinophilia was defined as an absolute eosinophil count of values greater than 500 eosinophil cells/ ml^3[26].

Serum IL-5 measurement: Concentrations in the serum samples were evaluated using the double sandwich ELISA immunoassay (SunLong Biotech Co., LTD, China, Cat NO. SL0998Hu) according to the manufacturer's guidelines[27]. The optical density was determined spectrophotometrically at a wave-length of 450 nm, and sample IL-5 concentrations were recorded according a plotted standard curve. The assay range was 1.6-100 pg/ml.

Parameters used for CR and treatment failure: The CR is defined as the clearance of stool samples from STH eggs on day 14 of treatment. Failure of treatment is defined as the existence of eggs two weeks after initiation of therapy[28]. Response to therapy was expressed by CR calculated for each individual parasite as the percentage of egg-positive children at day zero who became egg-negative following therapy at a 14th day follow-up. The following equation was used: CR = (number of study children who were negative after treatment on day 14/total number of positive participants in the same group) X 100. In addition, a drop of at least half of the original eosinophil count and/or a decrease of at least half of the initial IL5 level were considered prognostic of the drug efficiency[29].

Statistical analysis: The collected data were coded and recorded into the Statistical Package for Social Sciences (SPSS) version 22.0. The data were expressed as means ± standard deviation (SD) and percentages. The t-test was used to compare the means of each two groups. The association between each two variables was analyzed and assessed using the chi-square test. Statistical significance was considered when P values were ≤ 0.05.

Ethical considerations: This study was conducted in accordance with the clinical standards suggested by the Ethics Committee of the Faculty of Medicine at Zagazig University that reviewed and approved the study protocol. After outlining the study's goal and objectives, the parents/guardians signed an Arabic copy of written consent. Participants' test findings were kept secret. Anthelminthic drugs were prescribed for children that had parasitic infection other than STHs.

RESULTS

Table (1) represents the initial baseline characteristics of our study population. Using the

Table 1. Baseline anthropometric measures, demographic criteria, and predisposing risk factors for intestinal parasitic infections among both infected and non-infected groups.

	Infected group	Non-infected group	Total	Statistical analysis	
	(N=136)	(N=136)	(N=272)	*T* test	*P* value
		Mean ± SD			
Age (year)	9.3±1.2	11.6±1.8	10.45±1.5	2.3	0.07
Weight (Kg)	25.4±2.9	31.8±3.7	28.6±3.3	4.2	**0.02***
Height (cm)	126.3±13.4	139.3±16.3	132.8±14.9	6.9	**0.000***
		No. (%)			
Gender					
Male	87 (64)	69 (51)	156 (57)	4.87	**0.03***
Female	49 (36)	67 (49)	116 (43)		
Resisdence					
Rural	76 (56)	86 (63)	162 (60)	1.52	0.22
Urban	60 (44)	50 (37)	110 (40)		
Contact with soil					
Yes	65 (48)	33 (24)	98 (36)	16.3	**0.000***
No	71 (52)	103 (76)	174 (64)		
Raw vegetable and fruits					
Yes	73 (54)	46 (34)	119 (44)	10.89	**0.000***
No	63 (46)	90 (66)	153 (56)		

N: Number of cases, **SD:** Standard deviation, ***:** Significant difference ($P < 0.05$).

student's *t*-test there was no statistically significant difference in age. Analysis of the anthropometric measures (weight and height) reported a significant difference between both infected and non-infected groups (P=0.02 and <0.000, respectively). Infection with intestinal nematodes proved directly proportional to some predisposing factors like gender (more in boys than girls) (P=0.03), contact with soil, and consuming raw vegetables and fruits (P<0.000, respectively). Areas of residence whether rural or urban had no effect on the rate of infection with intestinal nematodes, i.e., non-significant. There were 76 children (56%) infected with *A. lumbericoides*, and *A. duodenale* ova were detected in stools of 38 children (28%), while *T. trichiura* positive stool results were recorded in 22 children (16%).

Table (2) illustrates the parasitological CR of the infected group 14 d after ABZ and IVM therapy for *A. lumbricoides*, *A. duodenale* and *T. trichiura*. It was observed that CR of IVM (97%) was close to ABZ against *A. lumbricoides* (95%) with no statistically significant difference. However, ABZ (74%) was more effective against *A. duodenale* than IVM (37%) with statistically significant difference (P=0.02). The CR of IVM (55%) recorded no statistically significant difference for treatment against *T. trichiura* when compared with ABZ (73%). Overall, IVM efficacy was 74% against the studied intestinal nematodes with statistically insignificant difference when compared with CR of ABZ that recorded 85% efficacy.

Children infected with intestinal nematodes showed significant high levels of blood eosinophilia and IL-5 (P<0.000, respectively) compared to non-infected children. When measured after treatment, a significant difference of values was recorded between both ABZ (P=0.02 and=0.003, for eosinophilia and IL-5 respectively) and IVM treatment groups (Tables 3 and 4).

Table 2. Comparison of the CR of both ABZ and IVM 14 days post-treatment.

Parasite	Pre-treatment	Albendazole		Ivermectin		Statistical analysis	
		Successful treatment	CR	Successful treatment	CR	X^2 test	*P* value
A. lumbricoides	38	36	95%	37	97%	0.35	0.56
A. duodenale	19	14	74%	7	37%	5.2	**0..02***
T. trichiura	11	8	73%	6	55%	0.8	0.38
Total	**68**	**58**	**85%**	**50**	**74%**	**2.88**	**0.09**

Pre treatment: Number of positive children before treatment; **Successful treatment:** Number of negative children after treatment; **CR:** Cure rate; ***:** Significant difference ($P < 0.05$).

Table 3. The percentage of eosinophil cells count in blood among infected and non-infected groups.

		Eosinophil (%)	Statistical analysis	
		Mean ±SD	*T* test	*P* value
Infection	Infected	6.7 ± 1.8	6.4	0.000*
	Non-infected	2.4 ± 0.6		
Post-ttt by	Albendazole	4.3 ± 1.6	2.86	0.02*
	Ivermectin	5.1 ± 1.9		

SD: Standard deviation, **ttt:** Treatment; *: Significant ($P < 0.05$)

Table 4. Serum levels of IL-5 among infected and non-infected children..

		IL-5 (pg/ml)	Statistical analysis	
		Mean ±SD	*T* test	*P* value
Infection	Infected	19.4 ± 6.2	12.16	0.000*
	Non-infected	12.8 ± 4.7		
Post-ttt by	Albendazole	14.2 ± 4.3	3.24	0.003*
	Ivermectin	16.7 ± 5.5		

SD: Standard deviation, **ttt:** Treatment; *: Significant ($P < 0.05$)

DISCUSSION

An important category of parasitic infections that cause significant morbidity, particularly in impoverished populations living in tropical and sub-tropical regions in developing countries is that of STHs, i.e. *A. lumbricoides*, *T. trichiura*, *A. duodenale*[30]. These infections markedly affect children's health causing poor nutrition and impaired somatic growth. The use of accurate, quick, and low-cost diagnostic approaches; the application of safe and effective drugs; and the implementation of well-structured mass preventative and control measures are all prerequisites to achieve significant progress in the control or elimination of such infections[31]. Periodic therapy with ABZ demonstrated a convincing reduction in worm burden to the low level at which helminthic-associated morbidity completely disappeared[32]. There are worries regarding efficacy and development of drug resistance as a result of continued usage of therapies in endemic areas[33]. Efficacy monitoring should be carried out during human mass drug application programs to ensure sustained effectiveness and guide best practices to maximize treatment results[34]. In this context, IVM proved to be an effective and cost comparable alternative to drugs in common use against intestinal parasites in different sex and age groups. However, in absence of standardized guidelines for drug efficacy studies, these results could not be generalized globally as each geographical area or population group has its unique characteristics that largely affect the efficacy of the investigated drug[34]. Because of IVM broad anti-parasitic spectrum, it may be considered an appealing drug in integrated therapy regimens in countries where multi-parasitism is known[35]. Oral IVM treatment is generally acknowledged for its high compliance and ease of administration. The majority of IVM safety and pharmacokinetic research studies was limited to a dosage range of 0.15–0.20 mg/kg, with 0.15 mg/kg being the standard anthelmintic recommended dose[36,37].

Our main aim was to evaluate the effectiveness of 200 µg/kg international dose of IVM versus the standard 400 mg single oral dose of ABZ used commonly to treat children aged 6 to 15 years old infected with one of the STHs. We used Kato-Katz as a standard method of diagnosis and parasitic CR as a parameter of effectiveness. The secondary objective was to evaluate diagnostic role of eosinophilia and serum level of IL5 as measuring parameters of drug efficacy in mass drug administration campaign against STHs.

Accordingly, the greatest efficacy of IVM (97%: 37/38) was found against *A. lumbericoides* when compared with that of ABZ (95%: 36/38) but with no significant difference. When we compared 14th day stool analysis results with baseline values, ABZ had a parasitological cure rate of 74% (14/19) for *A. duodenale*, which was significantly greater than the 37% (7/19) of IVM (P=0.02). No significant difference was recorded between ABZ (73%: 8/11) and IVM (55%: 6/11) when tested for *T. trichiura*.

Our results for *A. lumbricoides* confirmed previous research that demonstrated a single dosage of IVM to be superior or equivalent to ABZ in terms of effectiveness, giving strong evidence of IVM potential to be an effective alternative for ABZ in the treatment of ascariasis[38,39]. When compared to ABZ, inadequate IVM efficacy for trichuriasis, is consistent with several studies indicating this drug's inability to be a convincing alternative to ABZ in the treatment of *T. trichiura* infection[38-40]. This conclusion stands in contrast to Wen *et al*.[39] who reported comparable effectiveness of both IVM and ABZ in the treatment of trichuriasis. Geographical variance of strains might explain some of these variances discrepencies. Our results for *A. duodenale* were also consistent with several studies indicating limited efficacy of IVM in treating hookworm infection, which was shown to be more sensitive to ABZ but not IVM[38-41]. Overall, our study validates prior findings that single dose IVM therapy is most successful for ascariasis but is somewhat ineffective for trichuriasis and ancylostomiasis.

The present results could not be compared to other reports with different or similar findings as the great majority of existing effectiveness data were obtained from clinical trials that lack exclusive guidelines to routinely check efficacy[34,42]. Treatment responses to anthelmintic drugs vary among individuals (age, sex, nutritional status) and among different populations due to demographical criteria. Other than developing resistance, these naturally occurring variables can

influence treatment effectiveness over time and space. Genetic diversity of a population that affects the drug metabolism and other factors whether related to the distributed drug (its quality, drug interactions, emerging anthelminthic resistance) or to methodology and study design, greatly alter the apparent treatment response of the investigated drug[42]. Although IVM resistance by animal gastrointestinal nematodes was commonly reported[43], anthelmintic resistance could not be explicitly proved in our study.

The poor response of IVM observed in our work especially against *T. trichiura* and *A. duodenale* could be attributed to low efficacy of single dose regimen that is too small to achieve acceptable therapeutic efficacy. It was suggested that increasing the duration of the drug intake[44] or adopting a combined therapeutic regimen with two drugs may be necessary to obtain a significant control impact[45]. Another explanation of the treatment failures obtained in such studies conducted in endemic areas are the guiding metrics used to measure the efficacy of an anthelminthic drug, e.g. CR parameters were found to be not the best choice in endemic areas with high transmission rates since it is susceptible to variations in the severity of infection prior to treatment[46]. Furthermore, CR assesses drug efficacy at a single moment in time while neglecting the effect of the same treatment when used on a regular basis in preventative programs[47]. So assessing effectiveness is best recommended after a sufficient period of drug application.

Patients' nutritional and immunological status, as well as their gut flora are hypothesized to exert an impact on drug pharmacokinetics, thus controlling successful clearing of parasite infections[48]. In order to function properly, IVM requires binding to plasma proteins, mainly albumin. As a result, any decrease in serum plasma proteins increases the free fraction of this medicine in blood, which significantly impacts its efficacy[49]. This impact might explain IVM poor efficacy in our studied children, as malnutrition and hypo-albuminemia are real possibilities. In addition, a pharmacokinetic investigation showed that IVM-treated children under the age of 12 achieve half the peak concentration, and a dosage increase for young children is recommended[50]. This shows that the typical 200 µg/kg dosage of IVM may not reach efficacious levels against intestinal worms in little children. Notably it was suggested that parasite genetics that largely influence species susceptibility to treatment is a potential explanation of a drug's low efficacy[51].

Notably, in resource-constrained regions, the Kato-Katz technique is a common tool for diagnosing STHs during epidemiological surveys and monitoring treatment effectiveness studies[52]. As no worldwide criteria exist to assure the validity of this technique's results, we aimed to examine anthelminthic efficacy using additional markers associated with intestinal nematode infection, such as blood eosinophilia and serum IL5. It is established that intestinal nematode worms elicit Th2 immune responses characterized by increased production of eosinophil cells with a double role of defense against newly invading larval stages or by contributing to inflammatory pathology caused by the intestinal parasites. In fact, IL-5 is the most significant cytokine in eosinophil transformation and development, acting as an eosinophil activator. Eosinophilia in individuals with helminthic infections might vary depending on the parasite's location, degree of maturation, rate of migration, and parasite burden[5]. A combined diagnostic strategy (hematological test with excellent fecal process) was useful in the diagnosis and follow-up of intestinal parasitic infections because a negative fecal test is not a sufficiently accurate indication of cure due to its low sensitivity[29,53]. Blood cells and their values are crucial indications of a person's illness state as any drop or rise in their values provides information about the patient's response to treatment.

Our findings of elevated eosinophil and serum IL-5 levels during nematode infection, as well as a simultaneous decline following IVM/ABZ anthelminthic therapy, imply that they might be a useful marker of intestinal parasite infection. However, because of insufficient worldwide recorded reference standards and shortage of comparable research efforts, the observed lowered values of eosinophil count and IL5 serum level following therapy in the current study should be cautiously considered as therapeutic success. More intriguing is the observation that without medication some individuals exhibit a spontaneous decline in eosinophil levels, signaling active eosinophilia down-regulation during infections[54]. The same researchers indicated that cases who fail to record reduced eosinophil count after therapy should not be considered as treatment failure because two weeks may be insufficient time to observe a significant drop in blood eosinophilia and serum IL-5 levels; as recorded with our cases with hookworm infections. Notably, persistent peripheral eosinophilia was found to be common in the first two weeks after anthelminthic drug therapy[6], to return to normal values within three months of therapy[54].

Accordingly, a longer observation period would show a better test performance. It should also be noted that, although the detection of eggs in feces showed lower values within a few weeks of an efficient therapy, the serum antibody tests take several months to turn negative[29], i.e., this might occur with blood parameters such as eosinophil count or IL5 values.

From a clinical point of view, eosinophilia may be a marker of a helminthic infection in locations where intestinal infections are uncommon. However, this view is impractical in endemic settings with high daily reinfection rates, evoking a robust eosinophilia

response to tissue-invasive developmental stages in acute-early infections. This may give an elusive response of treatment failures to the tested anthelminthic drug[5-7]. The main criticism of our work is the evaluation of individual immune response to a single parasite at a specific time point while neglecting poly-parasitism, which is common in endemic areas and has a significant impact on the individual immune response. Furthermore, the specificity of the test is influenced by the prevalence of other medical diseases in which eosinophilia is a distinguishing trait. Large-scale definitive randomized controlled trials with uniform sex, age, infestation degree, and having the same socioeconomic features are required to demonstrate the diagnostic value of eosinophilia and interleukin response during nematode infections. Despite these observations, our study highlights the use of other diagnostic procedures beyond stool analysis to evaluate anthelmintic effectiveness.

In conclusion, the current study employed a single dose (200 µg/kg) of locally manufactured IVM in Egypt to treat three intestinal nematode infections namely *A. lumbricoides*, *A. duodenale*, and *T. trichiura*. Our data suggest that IVM is as effective as the current standard single dose of ABZ treatment against *A. lumbricoides*. Suboptimal therapeutic effectiveness of IVM against *A. duodenale* and *T. trichiura* was recorded in our work. Eosinophil counts and serum IL5 levels are not typically sensitive enough to be clinically effective as predictors of drug efficacy against intestinal parasites in endemic areas. However, our findings emphasize the necessity for more studies to examine diagnostic measures other than stool examination to evaluate the treatment efficacy of the tested anthelminthic.

In areas where the efficacy of IVM or any other alternative to ABZ is to be tested, future well-constructed studies using larger sample sizes and more accurate efficacy parameters under unified analytical methods are recommended, if community wide mass drug administration programs against intestinal nematodes are to continue in the future. Due to the limited sensitivity of the Kato technique and CR suboptimal accuracy as a measuring criterion of drug effectiveness trial, the role of blood parameters in the diagnosis of intestinal nematode infections is to be re-evaluated especially in endemic areas.

Author contribution: Yahia SH planned the study design, and shared Moawad HSF in performing the parasitological examination. Ahmed AA performed children' anthropometric measurements. Alshafey MA counted eosinophils and measured IL-5 levels. All authors shared together in writing and revising the manuscript.

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