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Intestinal parasitism in pediatric oncology children receiving chemotherapy: unexpected low prevalence

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ABSTRACT

Background: Children with underlying malignancies and those on chemotherapy are at risk for having intestinal parasitic infections, which can lead to a severe course and death. This cross-sectional study was done to assess the copro-parasitological and copro-molecular prevalence of entero-parasites in children with malignancies and those on chemotherapy.

Procedure: Stool samples were collected from 137 Egyptian hospitalized cancerous children with different malignancies in the National Cancer Institute, and receiving chemotherapy.

Faecal samples were examined microscopically. Genomic copro-DNA was extracted from fecal samples and amplified by 3 separate nPCR assays targeting *Cryptosporidium*, *G. intestinalis* and *Entamoeba histolytica* complex.

Result: The overall prevalence of enteroparasites was 6.6 % (9 cases). Only *Giardia* copro-DNA was encountered in 2 (1.4%) faecal samples of patients. Coproscopy detected parasites in 7 cases: *Blastocystis spp.* in 5 cases (3.6%), *Hymenolepis nana* in 1 case (0.7%) and *Ascaris lumbericoides* in 1 case (0.7%).

Conclusion: Low prevalence may be due to patient's use of prophylactic anti-parasitic and anti-fungal drugs, a standard protocol, basic hygienic practices and good nursing all of which are preventive against enteroparasites transmission. Among studied variables only diarrhoeic individuals who had a solid tumor, and soft/liquid stool with mucus and blood were predictors of intestinal parasitism.

1. Introduction

Malignancies and their treatment weaken the immune system and expose the patients to risk for infectious diseases [1, 2]. More than 20% of neoplasm cases worldwide are accompanied by a viral, bacterial or parasitic infection [3].

Non-opportunistic intestinal parasites particularly; *Giardia intestinalis* (*G. intestinalis*) and *Entamoeba histolytica/dispar/moskoviski* complex (*E. histolytica* complex) are frequently encountered in immunocompromised hosts and are more frequent in children with malignancies, who usually showed manifestations like immunocompetent individuals [4, 5]. In children with malignant tumors, intestinal parasitic infections interfere with disease control leading to reduced quality of life and can lead to a severe course and end fatally and [6, 7, 8]. *Cryptosporidium* is another emerging opportunistic agent among immunosuppressed children particularly with malignancies that can lead to severe illness [9, 10, 11, 12].

Although, coproscopy was regarded as the “gold standard” for traditional diagnosis of intestinal parasites, however, it is of low sensitivity and hindered by the nonspecific clinical presentation [13, 14, 15]. PCR-based methods are used effectively for reliable detection of intestinal protozoa, with high sensitivity and specificity, a fact that has led many authors to consider PCR the “gold standard” method that will take over current conventional techniques [11, 16, 17, 18, 19].

There are very few studies on the prevalence of enteric parasites in immunocompromised patients particularly those receiving chemotherapy [5, 20, 21]. It was recommended that oncologic patients receiving chemotherapy should have a coproparasitoscopic testing to avoid disseminated parasitic infections [22].

This study aimed to determine the copro-parasitological and the

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copro-molecular prevalence of enteroparasites in a cohort study of Egyptian cancerous children.

2. Material and methods

2.1. Study subjects & ethical consideration

Faecal specimens were collected, in this cross-sectional study, from 137 Egyptian children from 1 day to 18 years old (where 14 patients were from 1 day to 2 years old, 58 were from 2 years old to 12 years old and 65 from 12 years old to 18 years old).

Children were suffering from different types of malignancies or receiving treatment by chemotherapy and were hospitalized in the National Cancer Institute (NCI), Cairo University, from May 2013 to January 2015. Their demographic and related clinical data was registered.

The ethical committee of Kasr Al-Ainy Faculty of Medicine ethically approved the study. Informed consent was attained from the patients' relatives, for children it was attained from their parents or guardians, all answered the designed questionnaires.

2.2. Collection of fecal specimens and their processing

Each child in the study provided a single fecal specimen. Collected specimens were screened by the microscope for fecal parasitic stages prior to and after sedimentation concentration according to Washington *et al*, and Amin H.A. and Ali S.A. [23,24] and using acid-fast (AF) stained faecal smears for sporozoa oocysts and microscporidia spores according to Ignatius et al. and Ernest *et al.* [25,26] at the diagnostic and research unit of parasitic diseases (DRUP). For further molecular work, the rest of the fecal sample was kept at -20 °C. All nPCR assays were done at Lab of Molecular Medical Parasitology (LMMP), Department of Medical Parasitology, Faculty of Medicine, Cairo University, Egypt.

Fresh frozen stool specimens were subjected for extraction of genomic fecal DNA by commercial Favor Prep stool DNA isolation Mini Kit (Favorgen Biotech corporation ping-Tung, Taiwan) following kit's instructions preceded by thermal shocking of fecal specimens in the form of 5 cycles of boiling in water bath and deep freezing each for 5 minutes and incubating samples at 95 °C for one hour after 10 minutes at 56 °C.

Three separate nested-PCR (nPCR) reactions were used to amplify extracted copro-DNA. First for *Cryptosporidium* by nPCR designed to target COWP gene, using two primer pairs; outer primer pair amplifying a 796 bp fragment and inner primer pair which amplify a 553 bp fragment [27, 28]. The reaction condition and mixture were performed following Spano *et al.* [27] in a volume of 25μl. The nPCR products were electrophoresed by 1.5% agarose gel electrophoresis containing ethidium bromide. To determine *Cryptosporidium* genotype, nPCR products were cut by *Rsa*I enzyme (Fermentas UAB), the obtained fragments where stained by ethidium bromide and visualized by UV after being electrophoresed in 3.2% agarose gels. Second for *G. intestinalis* by nPCR designed to target β giardin gene, using two primer pairs; outer primer pair amplifying a 753 bp fragment and inner primer pair which amplify a 511 bp fragment [29].

The reaction condition and mixture were performed following Caccio *et al.* [29] and Lallea *et al.* [30] in a volume of 25μl. The nPCR products were electrophoresed by 1.5% agarose gel electrophoresis containing ethidium bromide. To determine *Giardia* assemblage, the obtained products by nPCR were cut by *Hae*III enzyme (Fermentas UAB), the obtained fragments where stained by ethidium bromide and visualized by UV after being electrophoresed in 3.2% agarose gels. Third for *E. histolytica* complex by nPCR targeting 16S-like gene, using two primer pairs; outer primer pair amplifying a ~800 bp fragment and inner primer pair amplifying one or more of the following fragments; 174 bp in presence of *E.dispar*, 439 bp in presence of *E.histolytica* and 553 bp in presence of *E. moshkovskii*. The reaction condition and mixture were performed according to Ngui *et al.* [31] in a volume of 25μl. The obtained PCR fragments were stained by ethidium bromide and visualized by UV after electrophoresis in 1.5% agarose gels.

2.3. Statistical analyses

All obtained data were collected, displayed in tables and analyzed statistically by SPSS software version 20. Percentages were used to express the positive rates. Chi square test was performed to compare the difference in rates of prevalence among different groups of the studied variables. *P*-value at < 0.05 was of statistical significance.

3. Results

Among the 137 fecal specimens assayed by nPCR targeting *Giardia*, *Cryptosporidium* and *E.histolytica* complex copro-DNA, only *Giardia* Copro-DNA was detected in 2 (1.4%) stool samples of patients. Using microscopy with and without AF staining detected 7 cases of intestinal parasitism, *Blastocystis spp.* was the most prevailing parasite (5 cases, 3.6%) followed by *Hymenolepis nana* (*H.nana*) (one case, 0.7%) and *A.lumbericoides* (one case, 0.7%) (Table 1).

Among studied variables, there was a statistically significant association between detection of intestinal parasites and clinical intestinal manifestations (diarrhoea), stool contents (pus and mucus) and stool consistency (soft and liquid stool) (Tables 2, 3, 4, 5, and 6).

4. Discussion

Children with underlying malignancies and those receiving chemotherapy have an increased incidence of acquiring parasitic infections, with higher severity than immunocompetent subjects [32].

High prevalence of intestinal parasites among immunocompromised patients including malagnancies was reported in many studies using microscopy [20, 33, 34, 35, 36]. However, we obtained a relatively low level of intestinal parasites (6.3%) among pediatric oncology children receiving chemotherapy using coproscopy and more sensitive copro-nPCRs. *Blastocystis* spp. was the most common type followed by *G. intestinalis*.

In Egypt, Hammouda et al. [33] microscopically detected *G.lamblia* and *Cryptosporidium* oocysts in 17.7% and 13.3%, respectively in patients receiving chemotherapy. Joshi et al. [34] microscopically detected *E. histolytica*, *Cryptosporidium* and *G. lamblia* infections in 14.9%, 8.5% and 4.3%, respectively in 94 immunocompromised patients with acute or chronic diarrhea. Botero et al. [20] reported intestinal protozoa using microscopy: *E. histolytica/dispar* (10.0%), *G. lamblia* (7.2%) and *Cryptosporidium* (3.6%) of 111 immunocompromised individuals. Authors explained that the prevalence was affected by the prophylactic treatment with albendazole to all patients except HIV patients. Al-Megrin, [35] found prevalence of 8.1%, 6.6% and 5.2% for *Cryptosporidium*, *G. lamblia E. histolytica,* respectively in immunocompromised patients using microscopy. Jiménez-Cardoso et al., [36] detected microscopic prevalence

Table 1
Diagnostic yield of coproscopy with and without AF staining and copro-PCR assay for detection of intestinal parasites among study group.

		Frequency	%
Copro-nPCR	*G. intestinalis*	2	1.4
	E.complex	0	0
	Cryptosporidium spp.	0	0
Coproscopy*	*Blastocystis spp.*	5	3.6
	A.lumbericoides	1	0.7
	H.nana	1	0.7
Parasites detected		9	6.4
No parasites		128	93.6
Total		137	100%

* Using AF staining microscopy for *Cryptosporidium* sp, *Cyclospora*, *Cystoisospora* & *Microsporidia* revealed no parasites.

Table 2
Demographic and environmental data of positive cases intestinal parasitism.

		Frequency (n = 137)		%		*P* value*
		Positive (n = 9)	Negative (n = 128)	within Parasitism (n = 9)	Of total (n = 137)	
Age Group	Infant (1d-<2y)	0	14	0.0	0.0	0.15
	Early childhood (2y-<12y)	2	56	22.2	3.4	
	Late childhood (12y-<18y)	7	58	77.8	10.8	
Gender	Male	4	72	44.4	5.3	0. 49
	Female	5	56	55.6	8.2	
Water Type	Tape	9	109	100	**6.6**	0.46
	filter	0	3	0.0	0.0	
	mineral	0	16	0.0	0.0	
Animal Contact	Yes	1	37	11.1	0.7	0.25
	No	8	91	88.9	5.8	

Data presented as n, with (*) *P* value < 0.05 is significant.

Table 3
Mean age of positive cases for intestinal parasitism in the study group.

	Minimum	Maximum	Mean	SD*	*P* value*
Age (Years)	7	8	2.8	4.47	0.14

Data presented as mean, ± SD = Standard deviation, with (*) *P* value < 0.05 is significant.

Table 4
Associated clinical manifestations among intestinal parasitism positive cases.

	Frequency (n = 137)		%		*P* value*
	Positive (n = 9)	Negative (n = 128)	Within Parasitism (n = 9)	Of total (n = 137)	
Diarrhoea	9	45	100	**6.6**	0.005*
Diarrhoea & vomiting	0	9	0.0	0.0	
Diarrhoea & fever	0	12	0.0	0.0	
Diarrhea & abdominal pain	0	2	0.0	0.0	
Asymptomatic	0	60	0.0	0.0	

Data presented as n & %, with (*) *P* value < 0.05 is significant.

of 9.1% and 2.6% for *Cryptosporidium spp*. and *Giardia intestinalis,* respectively of 173 individuals infected with HIV or having acute lymphoblastic leukemia with or without diarrhea.

This variation in the prevalence of parasitic infection in immune-compromised children could be due to the state and severity of malnutrition [37], time and duration of chemotherapy [38], other accompanying infections and possibly animal contact [39].

Our unexpected low prevalence of enteroparasites might be attributed to prophylactic treatment with anti-parasitic (albendazole) and anti-fungal drugs, a standard protocol. Also, patients received metronidazole for any case of diarrhea in cancer patients. Furthermore, chemotherapy might play a role in killing protozoa. Also, hygienic procedures e.g. washing hands prior to meals and other precautions were done for those children in addition to having healthy and clean foods and drinks and good nursing, as those children were frequently hospitalized over long periods to receive their treatment. Reducing risk of exposure of immune-compromised patients to social and environmental sources including drinking of contaminated water, presence of animals at home, over-crowded areas are important factors in the reduction of the transmission of enteric infections [40, 41]. All these factors may be preventive against faeco-orally transmitted intestinal parasitic infections in our study.

Our results as well as most of the results of other studies could be limited by involving a small number of patients and short time. T cell population count was not done, which is a significant risk factor for parasitic infection susceptibility. In addition, in the current study, a single stool sample from each individual was examined with less diagnostic yield. Some studies recommended examining more than one sample [42, 43], while, others did not recommend, especially when using AF staining [44]. We used the copro-nPCR, a highly sensitive method, to overcome any defective diagnostic yield.

Among studied variables; gender, age, symptoms, type of drinking water, contact with animals and stool consistency as an association with detection of parasites in faeces, only symptomatic (diarrhoeic)

Table 6
Type of tumor among intestinal parasitism positive cases.

	Frequency (n = 137)		%		*P* value*
	Positive (n = 9)	Negative (n = 128)	n Parasitism (n = 9)	Of total (n = 137)	
Solid tumor	5	28	0.6	3.6	0.02*
Haematological tumor	4	100	44.4	2.9	

Data presented as n & %, with (*) *P* value < 0.05 is significant.

Table 5
Yield of macroscopic examination of stool collected from parasite positive cases.

			Frequency (n = 137)		%		*P* value*
			Positive (n = 9)	Negative (n = 128)	Within Parasitism (n = 9)	Of total (n = 137)	
Stool Contents	Mucous	Yes	5	31	55.6	3.6	0.04*
		No	4	97	44.4	2.9	
	Pus	>5	7	93	77.8	1.5	0.01*
		0–5	2	35	22.2	5.1	
	RBCs	Yes	2	35	22.2	1.5	0.74
		No	7	93	77.8	5.1	
Stool consistency	Liquid		6	11	66.7	4.4	0.0001*
	Soft		3	79	33.3	2.2	
	Formed		0	38	0.0	0.0	

Data presented as n & %, with (*) *P* value < 0.05 is significant.

individuals, who had solid tumor and soft/liquid stool with mucus and blood showed association with intestinal parasitism of statistical significance (P value >0.05).

Similar to our findings Steer, [45] found that the incidence of *Blastocystis* is higher in patients with solid tumor (colorectal carcinoma, 53%) which may enforce the theory that *Blastocystis* spp. may implicated in the development of colorectal carcinoma. Contrary to our results, for children with leukemia and lymphoma, cryptosporidiosis was detected in 14.8% of diarrhoeic children with a prevalence of 8.3% in patients with solid tumours [46].

No cryptosporidiosis was detected in non-diarrhoeic children. However, in all the aforementioned studies no statistical results were provided and therefore interpretation of the results could not be performed.

In accordance with our results, Perch et al., [47] and Gatei et al., [48] found that infection rates did not differ with gender distribution. However, some studies [49, 50, 51] showed variation with gender of statistical significance. Al Hindi et al., [49] found that the number of infected females was significantly higher than males, while, Park et al., [50] and Mumtaz et al., [51] declared higher prevalence rates in males.

The mean age of positive cases for intestinal parasitism in the study group was 12.8 years. Infection was not found in infants, and though it was found in children, it was of no statistical significance (P value = 0.15).

Several studies in Egypt identified drinking water as an important source of human infection [52, 53]. In this study, tap water was the main source in all positive cases of the study group (100%) with no statistical significance (P value = 0.46).

In this study, most of children (88.9%) had no history of animal contact of no statistical significance (P value = 0.25). Many studies failed to find an association with animals, confirming the importance of other modes of transmission [54].

5. Conclusion

Prophylactic anti-parasitic and anti-fungal drugs as standard protocol in pediatric malignancies patients receiving chemotherapy with basic hygienic practices and good nursing have the preventive benefits against enetro-parasites and reduction in their prevalence. Patients who have solid tumor with diarrhea, and who have mucoid, bloody, soft/liquid stool are predictors for detection of intestinal parasitism.

Declarations

Author contribution statement

Abeer Al-Antably, Ayman Elbadry, Samar El Sayed, Rafiaa Hussein, Youssef Said, Marwa Hassan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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