




Intestinal protozoan and helminthic infections among hemodialysis and cancer patients

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Abstract

Intestinal parasitic infections (IPIs) can be a severe threat to immunocompromised patients. This is particularly true for those undergoing chemotherapy and hemodialysis. The present research is aimed at identifying intestinal parasites that might be present in immunocompromised patients. In this cross-sectional study 1040 stool samples were collected from March to September 2017. Six hundred and forty-one stool samples from immunocompromised patients (279 samples from hemodialysis patients and 362 samples from chemotherapy patients) and 399 samples from the control group were collected in Guilan province, Iran. The samples were tested by direct, formalin-ether methods for protozoa and ova of intestinal parasites and Ziehl-Neelsen staining methods for coccidian parasites such as *Cryptosporidium* species. The overall parasitic infection rate was highest (15%) in hemodialysis patients and 11.3% in chemotherapy patients, whereas the lowest rate was observed (7.3%) in the control group. The infectivity rates were statistically significant ($P = 0.008$) when compared with the control group. The parasites found were *Blastocystis hominis* (8.9% of the cases), *Entamoeba coli* (1.6%), *Iodamoeba butschlii* (0.8%), *Endolimax nana* (0.6%), *Chilomastix mesnili* (0.5%), *Strongyloides stercoralis* (0.5%), and *Taenia* species (0.15%), whereas *Giardia lamblia* was detected only in the control group. There was not a correlation between prevalence of parasites with age or education levels of the infected individuals. Results of the present study suggest that periodic stool examinations in special parasitological laboratories should be included as part of routine and general medical care.

Keywords Parasites · Dialysis · Chemotherapy · Prevalence · Iran

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Introduction

Intestinal parasitic infestations cause a variety of clinical conditions, the majority of which are related to the gastrointestinal (GI) tract.

There is evidence that some parasitic infections are associated with cancer development. The level of evidence of this association varies from high to low. A long time interval is usually involved in the development of cancer. For example, *Opisthorchis viverrini* and *Clonorchis sinensis* are associated with cholangiocarcinoma and *Schistosoma haematobium* with bladder cancer (Samaras et al. 2010). According to the World Health Organisation (2020), certain viruses, bacteria, and parasites can act as biological carcinogens. It is suggested that in low- and middle-income countries as many as 15% of cancers diagnosed in 2012 could be attributed to carcinogenic infections; de Martel et al. (2020) estimated that in 2018, around 2.2 million infection-attributable cancers were diagnosed worldwide. According to Cancer Research UK (2019), bile

duct cancer and bladder cancer can be directly attributed to parasites.

Changes in social and cultural context have influenced the human-parasite relations during the past century. Currently, parasitic diseases are more prevalent in underdeveloped countries (Bloom and Murray 1992). Patients undergoing chemotherapy or hemodialysis are considered to be immunocompromised and specific steps in healthcare are followed to reduce the risk of infection (Ferreira and Borges 2002). Healthy individuals could also be infected with intestinal parasites; and in these patients, symptoms are more commonly self-limiting, but infections in these hosts can increase the risk of infection for immunocompromised individuals (Bora et al. 2016).

There is also evidence that there is a high rate of parasitic infections in cancer patients, especially those that undergo chemotherapy (Zabolinejad et al. 2013; Barazesh et al. 2015). Directing the awareness of physicians towards the early diagnosis, and rapid and proper treatment of such pathogens could reduce the morbidity and mortality rate as well as reducing the medical costs.

The present study was conducted to investigate the prevalence of IPIs in two groups of immunocompromised patients (hemodialysis patients and chemotherapy patients), compared with the control (individuals not diagnosed with those conditions) group.

Material and methods

Ethics statement

The cross-sectional study was approved by the Ethics Committee of Guilan University of Medical Sciences with ethics number of IR.GUMS.REC.1396.141.

Study area and sample collection

The patients, who were eligible for the study, were informed with a written document about the research plan and their consent was taken before recruitment. Participants were referred from Razi Hospital, Guilan Oncology Center, Kianmehr Hemodialysis Center, and Caspian Hemodialysis Center in Rasht, Guilan province, Iran. During March to September 2017, fresh stool samples were collected from 362 cancer patients, 279 hemodialysis patients, and 399 control group individuals. One fecal sample was collected in specimen containers from each individual and the samples were transferred to the Department of Parasitology, School of Medicine, Guilan University of Medical Sciences. During the first stage, demographic data were collected (age, gender, residence, and education). Further self-reported information about their health was obtained. For the participants with cancer, number of completed chemotherapy cycles undergone

and the date of the first chemotherapy session was recorded. For the hemodialysis patients, the following parameters were self-reported: the duration of hemodialysis and the date of their first dialysis were recorded. Participants that took anti-parasitic drugs, antibiotics, mineral oils, barium, bismuth, and non-absorbable anti-diarrhea drugs 2 weeks prior were excluded from the study. The inclusion criteria for the three groups were as follows:

1. Control group: individuals without any symptoms or diagnosis of malignancy.
2. Cancer patients receiving at least one course of chemotherapy.
3. Hemodialysis patients at least 3 months post their first dialysis were included in the study.

Stool sample examination

Macroscopic investigation of stool samples was performed to detect mucus and blood. The microscopic screening included 3 direct wet smear (saline preparation and iodine preparation) and formalin-ether concentration to detect intestinal protozoa (amoeba and flagellate) and ova of intestinal parasites (Bora et al. 2016; Garcia 2007). The oocysts of coccidian parasites (*Cryptosporidium* spp., *Isospora belli*), were examined with a modified acid-fast technique using light microscopy with a magnification of $\times 100$ according to Garcia et al. (1983).

Statistical analysis

The data were statistically analyzed using SPSS software (SPSS version 16). Differences between variable groups were analyzed by Chi-squared test, and test significance level was $p < 0.05$.

Results

This study compares the prevalence of intestinal parasites between 641 immunocompromised patients (including 362 chemotherapy and 279 hemodialysis patients), and 399 control group as defined above. Of the participants, 49.9% were female and 50.1% were male (Table 1). Total infection rates in immunocompromised patients were 13% (Table 2). The IPI rates were 11.3%, 15%, and 7.3% for chemotherapy patients, hemodialysis patients, and the control group, respectively. The IPI rates were significantly different between the three groups ($P = 0.008$). *Blastocystis hominis* was the most prevalent (8.9%) parasite observed in immunocompromised patients followed by *Entamoeba coli* (1.6%), *Iodamoeba butschlii* (0.8%), *Endolimax nana* (0.6%), *Chilomastix*

Table 1 Demographic characteristics of immunocompromised patients and control group

Variable	Immunocompromised patients		Control <i>N</i> = 399 <i>N</i> (%)	Total <i>N</i> = 1040 <i>N</i> (%)
	Hemodialysis <i>N</i> = 279 <i>N</i> (%)	Cancer <i>N</i> = 362 <i>N</i> (%)		
Age group				
≥ 30	12 (4.3%)	17 (4.7%)	19 (4.8%)	48 (4.6%)
31–59	53 (19%)	108 (29.8%)	110 (27.6%)	271 (26.05%)
60–79	164 (58.8%)	188 (51.9%)	202 (50.6%)	554 (53.3%)
≤ 80	50 (17.9%)	49 (13.5%)	68 (17%)	167 (16.05%)
Gender				
Male	160 (57.3%)	171 (47.2%)	190 (47.6%)	521 (50.1%)
Female	119 (42.7%)	191 (52.8%)	209 (52.4%)	519 (49.9%)
Educational level				
Primary school	214 (76.7%)	277 (76.5%)	235 (58.9%)	726 (69.8%)
High school	48 (17.2%)	61 (16.9%)	129 (32.33%)	238 (22.9%)
University	17 (6.1%)	24 (6.6%)	35 (8.77%)	76 (3.36%)
Residing in are				
Urban	207 (74.2%)	131 (36.2%)	248 (62.1%)	586 (56.3%)
Rural	72 (25.8%)	231 (63.8%)	151 (37.8%)	454 (43.6%)

mesnili (0.5%), *Strongyloides stercoralis* (0.5%), and *Taenia* species (0.15%) (Table 2).

In the present study, *S. stercoralis* and *Taenia* species were the only pathogenic helminths detected in immunocompromised patients. Both patients were treated with anti-parasitic drugs. Similar to the immunocompromised patients, *B. hominis* was the most prevalent (4%) parasite observed in the control group, followed by *G. lamblia* (2%), *I. butschlii* (0.5%), *E. coli* (0.25%), *E. nana* (0.25%), and *C. mesnili* (0.25%). The protozoan, *G. lamblia*, was the only pathogenic parasite detected in the control group (Table 2). Multiple infections were detected in five participants (four in the

hemodialysis and one in the cancer group). In all multiple infection cases, *B. hominis* was a constant finding. The association among the prevalence of IPIs and demographics (age, gender, education, residence) is presented in Table 3. The current study demonstrates a significant association between IPIs prevalence and residence ($P = 0.001$) (9.6% among 438 patients living in cities and 18.7% among 203 patients living in rural areas). However, demographic characteristics (age, gender, education) were not associated with the prevalence of IPIs (Table 3).

Hemodialysis patients were put into 3 groups, including with a duration of hemodialysis less than 12 months, between 13 and

Table 2 Prevalence of intestinal parasitic infections and infection rate among immunocompromised and control groups

Parasite	Immunocompromised patients		Total of immunocompromised, <i>N</i> (%)	Control, <i>N</i> (%)	<i>P</i> value
	Hemodialysis, <i>N</i> (%)	Chemotherapy, <i>N</i> (%)			
<i>Blastocystis hominis</i>	28 (10.1%)	29 (8%)	57 (8.9%)	16 (4%)	
<i>Entamoeba coli</i>	7 (2.5%)	3 (0.8%)	10 (1.6%)	1 (0.25%)	
<i>Endolimax nana</i>	2 (0.7%)	2 (0.6%)	4 (0.6%)	1 (0.25%)	
<i>Iodamoeba butschlii</i>	2 (0.7%)	3 (0.8%)	5 (0.8%)	2 (0.5%)	
<i>Chilomastix mesnili</i>	2 (0.7%)	1 (0.3%)	3 (0.5%)	1 (0.25%)	
<i>Giardia lamblia</i>	0 (0%)	0 (0%)	0 (0%)	8 (2%)	
<i>Cryptosporidium</i> spp.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
<i>Strongyloides stercoralis</i>	1 (0.36%)	2 (0.55%)	3 (0.5%)	0 (0%)	
<i>Taenia</i> spp.	0 (0%)	1 (0.28%)	1 (0.15)	0 (0%)	
Infected	42 (15%)	41 (11.3%)	83 (13%)	29 (7.3%)	0.008
Non-infected	237 (85%)	321 (88.7%)	558 (87%)	370 (92.7%)	

Table 3 Association between demographic variables with positive cases for intestinal parasites in immunocompromised patients

Variable	Total	Neg. (%)	Positive patients, <i>N</i> (%)	<i>P</i> value
Age group				
≥ 30	29	25 (86.3%)	4 (13.7%)	0.39
31–59	161	145 (90%)	16 (10%)	
60–79	352	302 (85.8%)	50 (14.2%)	
≤ 80	99	90 (91%)	9 (9%)	
Gender				
Male	331	290 (87.62%)	41 (12.38%)	0.96
Female	310	272 (87.75%)	38 (12.25%)	
Educational level				
Primary school	491	430 (87.6%)	61 (12.4%)	0.87
High school	109	94 (86.2%)	15 (13.8%)	
University	41	37 (90.25%)	4 (9.75%)	
Residing in are				
Urban	438	396 (90.4%)	42 (9.6%)	0.001
Rural	203	165 (81.3%)	38 (18.7%)	

24 months, and more than 24 months. It appears that there is no significant correlation between the prevalence of IPIs and the duration of hemodialysis (Table 4). The effect of the chemotherapy length on the IPI was also investigated. Chemotherapy is not usually a single treatment. Patients have a course of treatment, which includes a number of chemotherapy cycles. Most chemotherapy treatments are given in repeating cycles. During a course of chemotherapy, cancer patients usually have between 4 to 8 cycles of treatment. A cycle is the time between one round of treatment until the start of the next. In present study, the cancer patients were grouped according to the number of chemotherapy cycles, including group one that included participants that completed less than half of the planned cycles, the second group included participants that had completed half of the planned cycles, and the third included participants that had completed more than half of the chemotherapy cycles. The rate of parasitic infection was 9.8%, 22.2%, and 8.2% in the first, second, and third group, respectively. The difference was statistically significant ($p = 0.02$) (Table 5).

Discussion

The prevalence of intestinal parasites in chemotherapy patients, hemodialysis patients, and the control group were

Table 4 Relationship between prevalence rate of IPIs and the duration of hemodialysis

Duration of dialysis	Positive	Negative	Total	<i>P</i> value
1–12 month	16 (17.4%)	76 (82.6%)	92 (100%)	0.39
13–24 month	4 (8.7%)	42 (91.3%)	46 (100%)	
≤ 25	22 (15.6%)	119 (84.4%)	141 (100%)	

11.3%, 15%, and 7.3%, respectively. Rasti et al. (2017) reported IPIs of 7.6% in cancer patients and 11.9% of IPIs in hemodialysis patients of Kashan, Iran. The main reason for the lower incidence in cancer patients compared with its prevalence in the hemodialysis patients may be because of the short-term effects of the drugs used in chemotherapy (Barazesh et al. 2015). Hemodialysis patients sometimes spend up to 20 years undergoing hemodialysis. Such long-term care increases the risk of contamination from parasitic infections including from the health care services (Karadag et al. 2013; Alter et al. 2001). Individuals, who are undergoing dialysis treatment, have a high risk of acquiring infections through contact with nursing staff, equipment and materials, on surfaces or from hands (Kulik et al. 2008). The prevalence of intestinal parasites in the present study is lower than the prevalence that has been reported among cancer patients in India (80%) and Egypt (18%) (Baiomy et al. 2010; Bora et al. 2016), and hemodialysis patients in Brazil (45.1%) (Kulik et al. 2008). The prevalence rate of IPIs was 10–63% among the patients with different immunosuppressed status compared with the control group (Bora et al. 2016; Kulik et al. 2008; Al-Qobati et al. 2012; Togeh et al. 2000; Al-Megrin 2010; Ashrafi et al. 2010). The wide variation of prevalence may be attributed to the differences in geographical distribution of parasites, sanitary practices, and different selection criteria of cases (Bora et al. 2016). In the present study, the non-pathogenic intestinal protozoan, *B. hominis*, was the most prevalent (8.9%) parasite observed in immunocompromised patients followed by *E. coli* (1.6%), *I. butschlii* (0.8%), *E. nana* (0.6%), and *C. mesnili* (0.5%) (Table 2). *B. hominis* was found to be the most common intestinal parasite in all studied groups; *G. lamblia* was found only in the control group (Table 2). Similar findings have been reported in other studies (Bora et al. 2016; Kulik et al. 2008; Al-Qobati et al.

Table 5 Frequency of IPIs regarding the number of chemotherapy cycles to total number of scheduled cycles within course of treatment in cancer patients

The number of done treatment cycles to total scheduled cycles	Positive	Negative	Total	<i>P</i> value
Less than half of total treatment cycles	13 (9.8%)	120 (90.2%)	133 (100%)	0.02
Equivalent	10 (22.2%)	35 (77.8%)	45 (100%)	
More than half of total treatment cycles	15 (8.2%)	169 (91.8%)	184 (100%)	

2012; Togeh et al. 2000; Al-Megrin 2010; Ashrafi et al. 2010; Kazemi et al. 2014). *B. hominis* may act as a pathogen in immunocompromised subjects and lead to various types of infections (with or without symptoms). Its infection rate in Iran, vary from 4.6 to 51.4% (Azizian et al. 2016). Water, pets, and vegetables are probably the main sources of infections (Rao et al. 2003; Motta and Silva 2002; Stensvold et al. 2007; Abdel-Hameed and Hassanin 2011; Iguchi et al. 2009). *B. hominis* and four non-pathogenic protozoans, namely *E. coli*, *I. butschlii*, *E. nana*, and *C. mesnili*, had the highest prevalence among the patients and control groups (Table 2).

In previous studies, two important waterborne parasites *Cryptosporidium* and *Giardia* were detected in surface water samples (Mahmoudi et al. 2011, 2013, 2015a, b, 2017). Examination of stool specimens with formalin-ether and Ziehl–Neelsen staining revealed no positive *Cryptosporidium* oocysts in any of the three groups (Table 2). Infections by these protozoans have been related to poor-quality drinking water (Mahmoudi et al. 2017; Seyrafi et al. 2006; Botero et al. 2003; Chieffi et al. 1998), but it cannot be discounted that these patients acquired the parasites from contaminated water, despite the fact that in Guilan, water used by nearly 100% of the population is subject to quality control. The frequency of *Cryptosporidium* spp. in adult hemodialysis patients was reported to be 4.6% in Brazil (Kulik et al. 2008), 11.5% in Iran (Seyrafi et al. 2006), and 20.3% in Turkey (Turkcapar et al. 2002). In addition, in the present study, two pathogenic helminths, *S. stercoralis* (0.5%) and *Taenia* species (0.15%) were the only helminths detected in the examined immunocompromised patients. Strongyloidiasis is a soil transmitted endemic disease, and most immunocompromised patients are exposed to the infection and its side effects, such as diarrhea (Ashrafi et al. 2010).

We observed three cases (0.5%) of infection with *S. stercoralis* in one hemodialysis and two cancer patients. The number of cases observed are lower than the number of cases reported in another study in Iran (9.7%) (Sharifdini et al. 2018). Undoubtedly, certain measures, such as not using human feces as fertilizer on farms, and health education for farmers could be recommendations for the reduction of worm infections and protection of public health.

Positive cases for intestinal parasites were similar in males and females in immunocompromised patients; however, there

was no statistically significant difference in the prevalence of intestinal parasites in male versus female immunocompromised patients (Table 3). Several studies have suggested that the prevalence rate of IPIs in males differ from females (Al-Qobati et al. 2012; Kia et al. 2008; Sayyari et al. 2005). The IPIs were significantly different in relation to the age, where the prevalence rates of IPIs (14.2%) in the age group 60–79 years was higher than other age groups (Table 3). In some similar studies, the highest prevalence was reported in the age group above 50 years (Barazesh et al. 2015; Monsef et al. 2008). The present research demonstrated no significant association between education level and prevalence rate of IPIs (Table 3), which is in line with the finding of Brazesh et al. (2015). Nevertheless, several studies observed a significant relationship between education level and IPIs prevalence (Naeini et al. 2012; Choy et al. 2014; Daryani et al. 2012). The current study demonstrates a significant association between IPIs prevalence and residence (9.6% among 438 patients living in cities and 18.7% among 203 patients living in rural areas). Omrai et al. (2015) reported a relationship between IPIs prevalence and place of residence but Naeini et al. (2012) and Barazesh et al. (2015) found no significant difference between these two variables.

The differences may be the result of agriculture activities and other factors that affect the growth, reproduction, and transmission of parasites in rural areas as compared with cities (Barazesh et al. 2015; Naeini et al. 2012; Omrani et al. 2015). In present study, no significant correlation was observed between IPIs prevalence and hemodialysis duration (Table 4). The association between the number of completed chemotherapy cycles to total number of scheduled cycles within course of treatment and IPIs prevalence was assessed by allocating the cancer patients into three categories according to the chemotherapy cycles received (Table 5). Using this classification, we demonstrated that IPIs are more prevalent in cancer patients that had completed half their chemotherapy cycles and the difference is statistically significant (Table 5). Chandramathi et al. (2012) used the same categorization and demonstrated the higher prevalence of *B. hominis* and *Microsporidium* species in cancer patients during the intermediate chemotherapy cycles (Chandramathi et al. 2012). The poisonous effect of chemotherapy medicine on the immune system reduces the immune function (Koivusalo and Hietanen 2004; Solomayer et al. 2003), thus augmenting parasitic

infections. At the end of the therapy course, the anti-oxidant system of patients reacted to the poisonous effects of chemotherapy drugs (Chandramathi et al. 2012), which may boost their immune system and eventually overcome the infection with the parasites.

Conclusions

In the present study, most of the observed protozoans were non-pathogenic. However, as pathogenic helminths (*S. stercoralis* and *Taenia* species) were detected, periodic parasitological examinations are highly recommended for hemodialysis and chemotherapy patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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