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ORIGINAL PAPER

Prevalence of intestinal parasite infections and associated clinical symptoms among patients with end-stage renal disease undergoing hemodialysis

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Abstract
Purpose Intestinal parasitic infections (IPIs) can result in high morbidity and mortality, particularly in immunocompromised patients. Infectious diseases are among the main causes of death in end-stage renal disease (ESRD) patients due to their impaired immune systems. The aim of this study was to determine the prevalence IPIs and their associated symptoms in ESRD patients.
Methods In this case–control study, the fecal samples of 78 ESRD patients undergoing hemodialysis and 140 controls without any kidney problems were analyzed for intestinal parasites using direct-smear, formol-ether and modified Ziehl–Neelsen staining techniques.
Results The difference in the prevalence of IPIs between ESRD patients (30.7 %) and the control group (10.7 %) was significant (OR = 3.7; 95 % CI = 1.8–7.61; $P < 0.001$). *Blastocystis* (14.1 %) and *Cryptosporidium* spp.(11.5 %) were the most common IPIs detected in ESRD patients, and the presence of *Cryptosporidium* spp. was significantly associated with diarrhea in ESRD patients (OR = 16; 95 % CI = 1.54–166.05; $P < 0.05$). Leukocytosis, diarrhea, weight loss, nausea/vomiting and bloating were also significantly higher in the hemodialysis group when compared with the control group.
Conclusion The current study revealed a high prevalence of intestinal parasites and related clinical symptoms in ESRD patients undergoing hemodialysis. Since hemodialysis patients are immunocompromised and intestinal parasites can cause serious clinical complications, we suggest that stool examination for intestinal parasites, with an emphasis on detection of *Cryptosporidium* spp. and *Blastocystis,* should be incorporated into the routine clinical care for these patients. Measures for preventing the acquisition of IPIs are also recommended.

Keywords Intestinal parasitic infections · Hemodialysis · ESRD patients

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Introduction

Failure of renal function is the main cause of death in chronic kidney disease (CKD) patients. The mortality rate increases when CKD develops into end-stage renal disease (ESRD), at which stage it requires the initiation of dialysis or kidney transplantation [1]. The mortality risk in ESRD patients compared to the healthy population is 36-fold and 2-fold greater in patients aged 16–49 years old and those

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over 65 years old, respectively [2]. Infectious diseases are the second most significant factor responsible for death in ESRD patients [3]. Patients with CKD and those in ESRD have weakened immune systems, because of the increased uremia that is connected to a state of immune dysfunction [1, 4]. In other words, the uremic milieu causes polymorphonuclear leukocyte (PMNL) dysfunction, thereby disabling a major component of the immune system and increasing the risk of infection [5].

Intestinal parasitic infections (IPIs) are among the most significant causes of morbidity and mortality worldwide, especially in developing countries. It has been reported that 3.5 billion people are affected, and 450 million are ill due to these parasitic infections [6]. Intestinal parasites (IPs) include *Cryptosporidium* spp., *Isospora belli, Entamoeba histolytica, Giardia lamblia, Blastocystis hominis* and *Strongyloides stercoralis.* These can all cause serious clinical symptoms in immunocompromised patients, such as those with HIV/AIDS [7–9]. Some recent studies have shown a high prevalence of IPIs in ESRD patients undergoing hemodialysis [10–12]. Therefore, early diagnosis and treatment of such infections in ESRD patients can help in improving patients' health and their quality of life.

Despite continual improvement in sanitary conditions over the past three decades, IPIs are still highly prevalent in Iran. It is estimated that the overall prevalence of IPIs in Iran is 38 % [13]. The prevalence rate for some of the more common intestinal parasites in Iran is as follows: *Giardia lamblia* 18.7 %, *Entamoeba histolytica* 1.47 %, *Entamoeba coli* 19 %, *Ascaris lumbricoides* 10.9 %, and *Hymenolepis nana* 2.3 % [13]. The rates for *Blastocystis hominis* and *Strongyloides stercoralis* (in endemic areas) are estimated between 0.008–54.5 % and 4.9–42 %, respectively [14, 15].

The main objective of this study was to determine the prevalence and clinical significance of intestinal parasitic infections in patients with CKD undergoing hemodialysis who were referred to 29-Bahman hospital in Tabriz city, Northwest Iran.

Materials and methods

Study area and population

This case–control study was carried out on 78 hemodialysis patients (54 % male, 46 % female), with a mean age of 51.6 ± 16.4 years, referred to the hemodialysis ward of 29-Bahman hospital in Tabriz city between May 2013 and January 2014. Hemodialysis patients who had not been given any anti-parasitic drugs in the month prior to the study and had been receiving hemodialysis treatment for at least 1 year were included in this study. The control group consisted of 140 healthy individuals without any kidney problems. Based on physician examination and documents, the health condition, residence region, age and sex of the control group were highly similar to patient group. All participants in this study signed a consent form and completed a standardized clinical questionnaire that asked for information concerning symptoms related to intestinal parasitosis and demographic characteristics (e.g., gender, age, and residence location). Clinical symptoms such as diarrhea (having at least three loose or liquid stools per day), weight loss (decrease of more than 5 percent of usual body weight within 6 months), feeling weakness (sustained decrease in the strength), stomach pain (cramps or a dull ache in the tummy), abdominal pain (diffuse pain in the abdominal cavity), nausea or vomiting (the sensation or act of forcible emptying of the stomach frequently) and bloating (any abnormal general swelling or feeling full) were collected by physicians.

Fecal sample collection and laboratory analysis

After completing the questionnaire, three stool samples were collected from each individual in pre-labeled, leak-free, plastic specimen containers, on three consecutive days with one-day intervals. The fecal specimens were examined macroscopically. Any diarrheal stool samples were sent immediately to the laboratory for parasitological examination, while formed stool samples were preserved in 10 % formalin for further analysis. For detection of trophozoites, cysts, ova and larvae of intestinal parasites, the stool samples were examined by microscopic observation (Zeiss, Germany, 100× and 400× magnification) of direct smears using normal saline (0.85 % NaCl solution), Lugol's iodine staining, and formalin ether concentration techniques [16]. To identify oocysts of the opportunistic intestinal coccidia *Cryptosporidium* spp., *Isospora belli,* and *Cyclospora cayetanensis,* a permanent slide was prepared for each sample, then stained with the modified Ziehl–Neelsen acid-fast technique, as described previously [17]. To measure the hematological parameters, blood samples were collected in EDTA containers from all participants. The Sysmex Hematological Analyzer Model XS-800i (Japan) was used for white blood cell counts and differentials. Prior to each series of blood sample tests, the quality control was completed and recorded using commercial specimens. The examiners were unaware of the patient's clinical condition. Data analysis was carried out using the SPSS software version 21 (SPSS, Chicago, IL, USA). The Chi square test was used to compare prevalence of parasites among groups. Associations were tested using odds ratios (OR) and 95 % confidence intervals (CI) after adjustments. A P value of <0.05 was considered statistically significant for differences.

Table 1 Demographic characteristics of hemodialysis patients, according to the presence or absence of intestinal parasites

Variable	Parasitized ($n = 24$)		Non-parasitized ($n = 54$)		Odds ratio (95 % CI[2])	*P* value
	N	%	*N*	%		
Sex						
Male	14	58.3	36	46.1	0.7 (0.26–1.88)	0.479
Female	10	41.7	18	23.1	1.42 (0.53–3.84)	
Age						
20–34[a]	4	16.7	10	18.6	1	0.65
35–49	5	20.8	14	25.9	0.89 (0.19–4.19)	
50–64	7	29.1	9	16.6	1.94 (0.42–8.92)	
≥65	8	33.4	21	38.9	0.95 (0.23–3.93)	
Residence						
Urban	11	45.8	42	77.8	0.24 (0.09–0.67)	<0.05
Rural	13	54.2	12	22.2	4.13 (1.48–11.56)	
Hemogram						
Leukocytosis	13	54.1	3	5.5	20.09 (4.88–82.65)	<0.001
Leukopenia	4	16.6	5	9.2	1.96 (0.48–8.06)	0.445
Type of stool sample						
Diarrhea	13	54.1	4	7.4	14.77 (4.03–54.03)	<0.001
Normal	11	45.9	50	92.6	0.07 (0.02–0.25)	

[a] The first subgroup of age is considered as the reference

Results

Across the 218 participants, the overall prevalence of intestinal parasites was 17.8 % (39/218). No helminth infections were observed among either patients or controls. Based on parasitological examination of the stool specimens, the frequency of parasites was 30.7 % (24/78) in hemodialysis patients and 10.7 % (15/140) in the control group. Patients undergoing hemodialysis were significantly more likely to be infected with intestinal parasites than healthy individuals (OR = 3.7; 95 % CI = 1.8–7.61; $P < 0.001$). As shown in Tables 1 and 2, there were no significant differences in the age and sex distributions between parasitized and non-parasitized patients in the hemodialysis group, nor between the hemodialysis group and control group. Based on residence status (Table 1), hemodialysis patients residing in the rural areas had a significantly higher probability of being infected with intestinal parasites compared to urban residents (OR = 4.13; 95 % CI = 1.48–11.56; $P = 0.005$).

Leukocytosis (OR = 20.09; 95 % CI = 4.88–82.65; $P < 0.001$) and diarrhea (OR = 14.77; 95 % CI = 4.03–54.03; $P < 0.001$) were significantly higher in parasite-infected hemodialysis patients than non-parasitized patients; however, no statistically significant difference was observed for leukopenia (OR = 1.96; 95 % CI = 0.48–8.06; $P = 0.445$) (Table 1). Among the hemodialysis patients, 65 (82 %) were symptomatic (Table 2). The most frequent clinical findings were: feeling of weakness 47 (60.2 %), stomach pain 32 (41.0 %), weight loss 26 (33.0 %), abdominal pain 25 (32.0 %), bloating 25 (32.0 %), nausea or vomiting 19 (24.3 %), and diarrhea 17 (21.8 %). Six clinical variables were found to be significantly different between the hemodialysis group and the control group (Table 2), including leukocytosis (OR = 4.90; 95 % CI = 1.92–12.53; $P < 0.001$), presence of diarrhea (OR = 5.29; 95 % CI = 2.08–13.43; $P < 0.001$), weight loss (OR = 3; 95 % CI = 1.54–5.85; $P < 0.001$), nausea or vomiting (OR = 2.68; 95 % CI = 1.27–5.65; $P < 0.05$) and bloating (OR = 16.04; 95 % CI = 5.33–48.28; $P < 0.001$).

The most parasites detected in hemodialysis patients were *Blastocystis hominis* (14.1 %), *Cryptosporidium* spp. (11.5 %), *Entamoeba histolytica/dispar* (2.5 %), *Endolimax nana* (1.3 %) and *Giardia lamblia* (1.3 %). *Blastocystis hominis* (OR = 5.58; 95 % CI = 1.71–18.18; $P < 0.05$) and *Cryptosporidium* spp. (OR = 9; 95 % CI = 1.49–42.79; $P < 0.05$) parasites were significantly more common in the hemodialysis group compared to the control group. No significant difference for *Entamoeba histolytica/dispar* (OR = 3.66; 95 % CI = 0.33-41.00; $P = 0.291$), *Endolimax nana* (OR = 0.89; 95 % CI = 0.08–10.04; $P = 1.00$) or *Giardia lamblia* (OR = 0.44; 95 % CI = 0.05–4.02; $P = 0.657$) was observed (Fig. 1). In the hemodialysis group, infection with *Cryptosporidium* spp. was significantly associated with diarrhea (OR = 16; 95 % CI = 1.54–166.05; $P < 0.05$), whereas no statistical association was found between presence of *Blastocystis hominis* and

Table 2 Comparison of prevalence and identified symptoms of intestinal parasites between hemodialysis patients and control group

Variable	Hemodialysis patients ($n = 78$)		Control group ($n = 140$)		Odds ratio (95 % CI[2])	*P* value
	N	%	*N*	%		
Sex						
Male	14	18.0	9	6.4	0.93 (0.25–3.47)	0.918
Female	10	12.8	6	4.2	1.07 (0.28–3.98)	
Age						
20–34[a]	4	5.1	2	1.4	1	0.853
35–49	5	6.4	5	3.6	0.50 (0.06–4.09)	
50–64	8	10.2	4	2.8	1 (0.12–7.99)	
≥65	7	9.0	4	2.8	0.87 (0.11–7.11)	
Hemogram						
Leukocytosis	16	20.5	7	5	4.90 (1.92–12.53)	<0.001
Leukopenia	9	11.5	7	5	2.48 (0.88–6.94)	0.076
Type of stool sample						
Diarrhea	17	21.8	7	5	5.29 (2.08–13.43)	<0.001
Normal	61	78.2	133	5	0.18 (0.07–0.47)	
Symptoms						
Weight loss	26	33.3	20	14.2	3 (1.54–5.85)	<0.001
Feeling of weakness	47	60.2	10	7.1	19.71 (8.97–43.30)	<0.001
Stomach pain	32	41	55	39.2	1.07 (0.61–1.89)	0.801
Abdominal pain	25	32	38	27	1.27 (0.69–2.31)	0.443
Nausea or vomiting	19	24.3	15	10.7	2.68 (1.27–5.65)	<0.05
Bloating	25	32	4	2.8	16.04 (5.33–48.28)	<0.001

[a] The first subgroup of age is considered as the reference

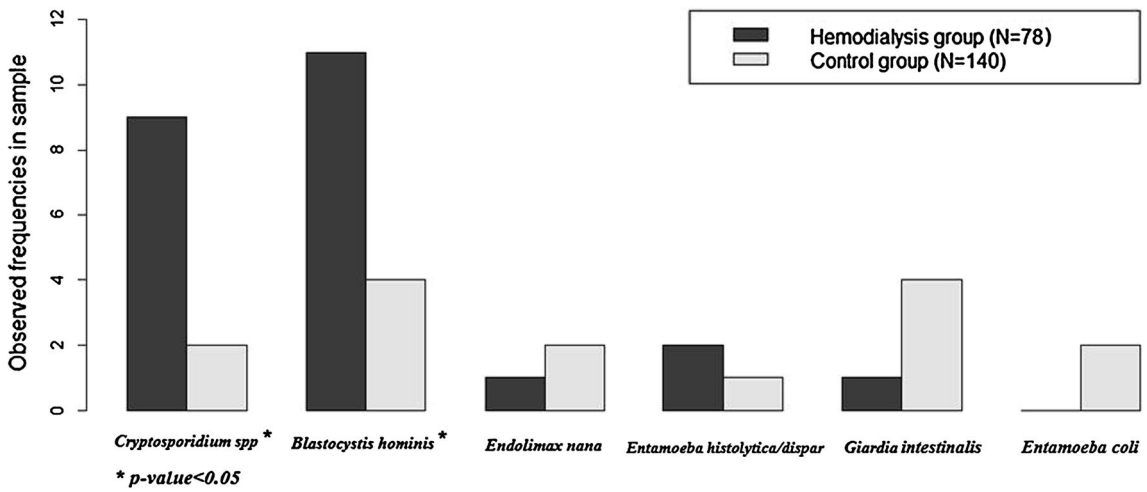

Fig. 1 Prevalence of intestinal parasites in hemodialysis patients and in the control group

diarrhea (OR = 0.25; 95 % CI = 0.05–1.39; $P = 0.217$) (Fig. 2).

The result of this study suggests that there is a significant association between the presence of IPs and diarrhea in hemodialysis patients, whereas this relationship is not observed in control group.

Discussion

A rise in blood urea could cause the death of patients with chronic kidney disease (CKD) if they are not treated with dialysis, as uremia impairs the function of lymphocytes, leukocytes such as neutrophils and monocytes, nitric oxide

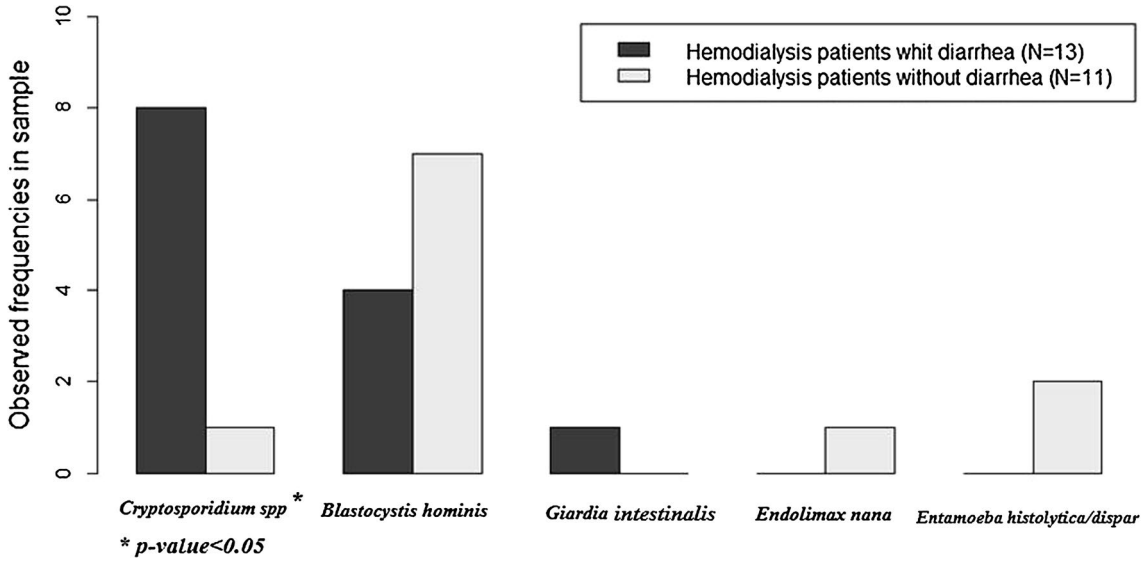

Fig. 2 Prevalence of intestinal parasites in hemodialysis patients with and without diarrhea

(NO) and platelets [1, 4, 5]. In some cases, impairment of phagocytosis, chemotaxis and regulation of chemokines and cytokines has also been observed as a result of uremia [1, 18, 19]. This impairment increases the risk of infection, which is the cause of 40 % of deaths in patients with CKD [20, 21]. Dialysis removes waste, excess water and urea from the blood and, therefore, plays an important role in survival and improving the quality of life of these patients [22].

Studies determining the prevalence and related symptomatology of IPIs in ESRD patients undergoing dialysis are rare but have shown high infection rates, mainly by protozoan parasites [10–12, 23–25]. The reported prevalence of IPs in patients with CKD has been from 11 % to 51 % in different studies. *Blastocystis hominis*, *Cryptosporidium* spp. and *Endolimax nana* have been reported as the most common IPs in such patients [10–12, 23–25].Our findings are in agreement with the results of those studies. Among the IPs detected, *Cryptosporidium* spp. was encountered at a frequency of 11.5 % in ESRD patients. This is consistent with Seyrafian et al. [24] who in 2006 reported that *Cryptosporidium* spp. has a prevalence of 11.5 % in Iran. This prevalence is higher than that reported by Kulik et al. [10] in Brazil (4.6 %), and lower than those reported by Turecapar et al. [23] in Turkey (20.2 %) and Gil et al. in Brazil (26.4 %) [11]. Cryptosporidiosis in immunosuppressed patients can cause severe chronic diarrhea leading to electrolyte imbalance, malabsorption, and profound weight loss [26].

In our study, *Blastocystis hominis* was the most common intestinal parasite in both patient and control groups. The prevalence of *Blastocystis hominis* in our hemodialysis patients was lower than those reported by Kulik et al. (20.9 %) and Gil et al. (24.5 %) in Brazil and Karadag et al. (23.9 %) in Turkey but was higher than that reported previously by Seyrafian et al. (8 %) in Iran [10–12, 27]. There is still much debate over whether *Blastocystis hominis* is actually pathogenic for humans or just part of normal flora [10]. However, in *Blastocystis* infection, lysis of intestinal epithelium and release of diarrheagenic toxins apparently occurs, particularly in immunosuppressed patients [28]. The Centers for Disease Control and Prevention (CDC) recommends that when there are no other identified causes of diarrhea, *Blastocystis hominis* should be considered pathogenic, and so when it is diagnosed in such cases, treatment becomes necessary [29].

There were significant differences in symptoms, specifically weight loss, nausea or vomiting, feeling of weakness, bloating and leukocytosis, between ESRD patients and the control group. Gil et al. in Brazil found that abdominal pain, bloating and postprandial fullness in hemodialysis patients were higher than the control group [11]. Leukocytes, including neutrophils and monocytes, are the main cells of the innate immune system that act against infectious agents, and are especially increased in number of parasitic infections. It is possible that leukocytes also increase during hemodialysis, but there is a functional abnormality for leukocytes in ESRD patients that is directly linked to the elevated infection risk in these patients. Studies have shown that end-stage renal disease is associated with hyporeactivity of monocytes [1]. In addition, the killing capability of neutrophils, the main cells in host defense against infection agents, decreases although their number remains unchanged [1, 30]. Although the exact mechanism

responsible for the reduction of neutrophil function is not well known, some factors that are involved have been proposed. They include iron overload, zinc deficiency, increased intracellular calcium, anemia of renal disease, malnutrition, uremic toxins, time since the beginning of dialysis, and also dialysis therapy per se [30].

The reported symptoms in our study are commonly caused by intestinal parasites, but also by bacterial, viral and fungal infection agents. All patients studied were visited by a physician and their medical records were also evaluated to exclude any patients with co-infections or malignancy from the study. Therefore, to the best of our knowledge, ESRD patients enrolled in this study were not co-infected with other agents nor had malignancies that could account for the symptoms reported.

Helminthic infections were not found in our study. This low prevalence is in line with the previous studies in other countries [10–12, 23–25]. Advice given on diet and eating practices, such as using well-cooked vegetables and meat, could explain the low frequency of intestinal helminthes in these patients, whereas intestinal protozoa such as *Cryptosporidium* spp. can be transmitted through contaminated soil and water. Nevertheless, previous studies have shown *Strongyloides stercoralis* to be one of the most common helminthic infections in immunocompromised patients [9, 31, 32], renal transplant recipients [33–35] and CKD patients [36]. *Strongyloides stercoralis* can be the cause of a fulminant fatal illness in patients with compromised immunity and transplant recipients receiving glucocorticoid drugs [37]. It has been reported that the number of cases of hyper-infection with *Strongyloides stercoralis* following renal transplant is reduced by the use of cyclosporine [37]. Microscopic examination of stool samples is the most common diagnostic method [38]. A single stool exam for screening is inadequate and has only 50 % sensitivity for the detection of *Strongyloides stercoralis*, but three stool exams on consecutive days can significantly improve the sensitivity. The formalin-ethyl acetate and Baermann concentration techniques and agar plate culture have been widely used to increase the sensitivity of stool exams [37]. In this study, we performed a formalin-ethyl acetate concentration method on stool samples collected on three consecutive days, but we did not find any larvae of *Strongyloides stercoralis*. This can be explained in at least two ways: (1) Tabriz city is non-endemic area for *Strongyloides stercoralis*. It is well known that provinces in the north (Mazandaran and Guilan) and south (Khuzestan) of Iran are endemic for *Strongyloides stercoralis*. Those areas have a hot-humid climate, whereas Tabriz city has cold-dry climate and *Strongyloides stercoralis* infection in this area is rare. (2) Our study had a relatively small sample size and our detection methods did not include Baermann concentration or agar plate culture.

At the end of the study, the clinical and laboratory data for each patient were sent to the relevant specialist physician. In infected ESRD patients and control individuals, we recommended metronidazole as a broad-spectrum treatment for bacterial and protozoan infections. For ESRD patients who were not infected with intestinal parasites but had associated symptoms, we suggested additional tests for other infectious agents. It is reported that in a hemodialysis session lasting for 4 to 8 h, the extraction ratio of an administered metronidazole dose was 40–65 %, depending on the type of dialyzer membrane used and the duration of the dialysis session [39]. Therefore, if the administration of metronidazole cannot be separated from the dialysis session, it is recommended that a supplementary metronidazole dose following hemodialysis should be considered. However, it is possible that in ESRD patients, excretion of metronidazole and its metabolites in the urine is slow, resulting in a significant accumulation of metronidazole metabolites.

In conclusion, the results of this study revealed that intestinal parasites and related clinical symptoms in ESRD patients undergoing hemodialysis were more frequent than in a matched control group. Considering that patients under hemodialysis are immunocompromised, intestinal parasites could cause them serious clinical complications due to their weakened immune system. Some recommendations for better management and treatment of hemodialysis patients and subsequent studies are as follows:

1. We suggest that stool examination for intestinal parasites, with special attention to *Cryptosporidium* spp., *Blastocystis hominis* and *Strongyloides stercoralis*, should be incorporated into the routine clinical care of these patients.
2. Periodic repetition of the stool examinations with the use of sensitive methods such as Baermann and agar plate culture is strongly suggested for the detection of *Strongyloides stercoralis.*
3. Measures to prevent acquisition of intestinal parasites are also recommended.
4. It is strongly suggested that additional tests should be done, particularly for common bacterial, viral and fungal agents known to be responsible for symptoms reported in this study.
5. More studies are recommended to evaluate the side-effects of metronidazole on ESRD patients.

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Conflict of interest None of the above authors have any conflict of interest.

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