

Blastocystis sp. and Other Intestinal Parasites in Hemodialysis Patients

Rose Anne Kulik¹, Dina Lúcia Morais Falavigna², Leticia Nishi³ and Silvana Marques Araujo^{2,3}

¹Faculdade Integrada de Campo Mourão; ²Post Graduation Program in Clinical Analysis; ³Post Graduation Program in Sciences of Health; State University of Maringá; Maringá, PR, Brazil

Chronic renal insufficiency disease (CRI) leads to uremia in hemodialysis patients and induces a state of immunodepression that results in higher frequencies of infections and diarrhea. Hemodialysis patients resident in the city of Campo Mourão, Paraná, Brazil were analyzed from April 2006 through September 2007 for *Blastocystis* sp. and other intestinal parasites and for associated diarrhea. Fecal samples from 86 hemodialysis patients and 146 healthy (reference) persons were examined by standard methods for detecting ova, larvae and cysts, which included preservation in 10% formalin and the Kinyoun method. Thirty-three hemodialysis patients (45.1%) and 36 reference individuals (25.7%) were found to be parasitized. The differences in the percentages of parasitism and polyparasitism between the reference group and the chronic renal patients was significant ($p=0.0318$ and 0.0019 , respectively). *Blastocystis* sp. (18%-20.1%), *Endolimax nana* (14%-16.3%), *Cryptosporidium* sp. (4%-4.7%) and *Entamoeba coli* (4%-4.7%) were the most frequent protozoa found in the hemodialysis patients. Parasitism was not significantly associated with diarrhea ($p=0.9947$) or with decreased white blood cell counts ($p=0.7046$) in these individuals. Because parasitic infections may be an important comorbidity factor in hemodialysis patients, we suggest that parasitological stool examinations, especially for *Blastocystis* sp. and *Cryptosporidium* sp., be included in routine medical follow-up examinations of these patients.

Key-Words: *Blastocystis* sp., hemodialysis patients, parasites.

Increased life expectancies and the resulting aging of many human populations have increased the incidence of chronic degenerative diseases, such as chronic renal insufficiency (CRI) [1]. CRI causes progressive and irreversible loss of renal function, in which the human body does not maintain metabolic and hydroelectrolytic equilibrium. The accumulation of non-excreted metabolites by the kidney, such as urea [2], which leads to uremia [3], is one of the complications of CRI. Uremia induces a natural state of immunodepression, most commonly expressed as negativity to delayed hypersensitivity skin tests and an increase in the incidence of infections [4,5], which are responsible for 48% of deaths in patients with CRI [3,6].

Although disease manifestations do not exempt any organic system, diarrhea is one of the most important clinical signs of CRI [2]. In immunocompromised individuals, parasitic infections can cause profuse diarrhea, usually accompanied by weight loss, anorexia, malabsorption syndrome, and in some cases, fever and abdominal pain [5]. *Blastocystis* sp. [7,8], *Cryptosporidium* sp., *Entamoeba histolytica* and *Giardia duodenalis* are protozoan parasites that can cause diarrhea in humans [9-11], although *Blastocystis* sp. is often undiagnosed [12].

Blastocystis sp. is a pathogenic agent in immunodepressed or immunosuppressed individuals, causing symptomatic or asymptomatic infections [13,14]. Water, pets, and raw vegetables can be sources of infection [15]. The prevalence of this parasite ranges from 1.5 to 15% in developed countries, and from 30 to 50%

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Address for correspondence: Dr. Dina Lúcia Morais Falavigna. Bloco I-90, sala 11 – Universidade Estadual de Maringá. Zip code: 47020-900. Maringá – PR, Brazil. Phone: (44) 3224 5688/ 9925 3508. Fax number: (44) 3261 4860. E-mail: falavigna@wnet.com.br.

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in developing countries [15,16]. Several therapeutic agents available for the etiological treatment of intestinal protozoa can improve the clinical condition and quality of life of patients with CRI [13,14].

We assessed the prevalence of *Blastocystis* sp. and other intestinal parasites, and investigated whether these infections are associated with diarrhea in patients undergoing hemodialysis in the city of Campo Mourão, state of Paraná, Brazil.

Material and Methods

This project received approval by the Permanent Committee for Ethics in Research with Human Beings of our institution – Copep/UEM (Protocol CAAE 01210093000-05; Assessment No. 220/2005).

All 86 hemodialysis patients attended at the *Instituto do Rim* (Kidney Institute) in Campo Mourão from April 2006 through September 2007 were included in our study. The patients ranged from 21 to 82 years old, with a mean of 47.9 ± 16.8 years for females (40.2%) and 50.4 ± 13.7 years for males (59.8%).

Fecal exam results for 146 reference individuals, who lived in the same district, did not have kidney problems, were in the same age range and proportion of sexes, and were attended at local public health centers, were used for comparison. Neither the occurrence of diarrhea nor changes in white blood cell counts were investigated in the reference group.

The fecal samples were obtained after the person signed a form acknowledging informed consent. Three fecal samples were collected from each individual, on non-consecutive days. The fresh fecal material was stored in wide-mouth flasks with threaded lids. These flasks were labeled with the name of the person, delivery date and protocol number. The correct way to collect and store the feces was explained to the persons when the flasks were given to them.

The fresh fecal samples were analyzed with the Faust, Lutz and Rugai methods [17]. A portion of each sample was stored

in a 10% formalin solution at a proportion of 1:1 to analyze for *Cryptosporidium* sp. and *Blastocystis* sp. We used direct smears made from these formalin-preserved samples to inspect for cysts of *Blastocystis* sp., and we used the modified Kinyoun acid-fast staining method to detect *Cryptosporidium* sp. [17]. CRI patient blood count (number of leukocytes) data were obtained from medical records based on examinations made during the same period at the *Instituto do Rim*.

Statistical analyses were performed using Statistica version 6.0. The chi-square test was used to compare the prevalence of enteroparasites in hemodialysis patients and the reference group, the characteristics (except for age) of CRI individuals undergoing hemodialysis who had or did not have intestinal parasites, and to test for a possible association between these parasites and diarrhea. Student's t test was used to compare the mean ages of the individuals in these groups. The significance level adopted was 5%.

Results

Among the 86 hemodialysis patients, 33 (45.1%) had parasites (Table 1) and eight (9.2%) had more than one species of parasite. *Blastocystis* sp. (18%-20.1%), *Endolimax nana* (14%-16.3%), *Cryptosporidium* sp. (4%-4.7%) and *Entamoeba coli* (4%-4.7%) were the most frequent protozoa (Table 2).

Both *Blastocystis* sp. and *E. nana* were found in seven patients (21.2%), and *E. nana* and *E. coli* in one (3.0%). Diarrhea was diagnosed in two of the seven patients infected with *Blastocystis* sp. and *E. nana*, and in the patient who had both *E. nana* and *E. coli*.

Among the 146 individuals in the reference group (Table 1), monoparasitism (23 cases-25.7%) predominated over polyparasitism (13 cases- 0.9%). The most frequent parasites were *Iodamoeba bütschlii*, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Hymenolepis nana*, *Trichuris trichiura* and *Taenia* sp.

Differences in the percentages of parasitism and polyparasitism between the reference group and the hemodialysis patients were significant ($p=0.0318$ and 0.0019 , respectively).

Among the 76 hemograms, 16.4% (13) indicated leucopenia, 7.6% (6) leucocytosis and 7.6% (6) neutrophilia. The finding of parasites was not significantly related ($p=0.7046$) to changes in white blood cell counts (Table 3). Among the 33 parasitized hemodialysis patients, 25 had diarrhea (Table 4). There was no significant association between diarrhea and parasitism ($p=0.9947$).

Discussion

There have been several studies on the incidence of parasites in populations of immunodepressed and/or immunosuppressed individuals, mainly those with HIV [18-22]. However, there is little information on kidney patients [9,10,23,24]. We found that parasitism and polyparasitism were more frequent in hemodialysis patients than in the reference group. Parasitism was more closely related to the degree of compromise of the immune system in these patients than to

exposure to pathogens transmitted via the fecal-oral route; the latter is more important in the immunocompetent population [10,23]. This may be a consequence of the careful orientation about diet given to these patients; they are advised to eat well-cooked greens, vegetables and meat [25] in order to avoid parasitosis. These precautions could explain the absence of parasites such as *I. bütschlii*, *E. vermicularis*, *A. lumbricoides*, *H. nana*, *T. trichiura* and *Taenia* sp. in the hemodialysis patients, compared to the reference group.

Blastocystis sp. has a worldwide distribution, appearing more frequently in tropical and subtropical countries [26]. There are no reports of blastocytosis cases in hemodialysis or kidney-transplant patients in the Brazilian literature. In our study, 20.1% of the hemodialysis patients were infected with *Blastocystis* sp. Although infections by this protozoan have been related to poor-quality drinking water [15,27], it is unlikely that these patients acquired the parasites from contaminated water, because treated water is supplied to nearly 100% of the population of Campo Mourão [28].

Individuals who are undergoing dialysis treatments have a high risk of acquiring infections through contact with nursing staff, equipment and materials, on surfaces or from hands [29]. These patients, already immunodepressed because of their condition, become more susceptible after repeated hospitalizations and as a result of surgical interventions that may be required [30].

The incidence of *E. nana* was significantly higher in the dialysis patients compared to the reference group, again probably a result of the immunological fragility of these patients. In immunodepressed and/or immunosuppressed individuals, *E. nana* causes irritation of the crevices of the intestinal mucosa, progressing to fibrosis, with impaired absorption of food, causing gastrointestinal symptoms such as diarrhea [31].

The frequency of *Cryptosporidium* sp. in the hemodialysis patients was relatively lower (4.6%) than that reported by Seyrafiyan et al. (2006) in Iran, and by Turkcapar et al. (2002) in Turkey, who reported 11.5% and 20.27%, respectively, in adult patients. This may be a consequence of the quality of drinking water [9-11,24], because in Brazil, all water used by the population is subject to quality control [2]. Reinforcing this hypothesis, Chieffi et al. (1996) in a study of immunocompromised individuals in Brazil reported a frequency of infection of *Cryptosporidium* sp. similar to what we found.

Blastocystis sp. and *E. nana* were found together in 21% of the hemodialysis patients studied. In mixed or single infections, they may be related to diarrhea, especially when they are abundant in patients [31,32]. The presence of these parasites in the gastrointestinal tract modulates the immune response, predisposing to infection with other enteropathogens and favoring secondary infections and multi-parasitism [31].

Cases of leucocytosis and/or leucopenia, such as those we found in our study, may occur in simple infectious processes, such as pharyngoamygdalitis, not necessarily associated with parasitism [34]. However, the white blood cell counts and neutrophilia found in some of the hemodialysis

Table 1. Prevalence of enteroparasites in hemodialysis patients and in the reference group.

Parasites	Hemodialysis patients		Reference group	
	N	%	N	%
Positive	33*	45.1	36*	26.7
Negative	53	55.0	110	73.3
Total	86	100.0	146	100.0

*p=0.0318 (chi-square test; significance level of 5%).

Table 2. Prevalence of enteroparasites by species, in hemodialysis patients and in the reference population.

Parasite species	Hemodialysis patients		Reference group		p**
	N	%	N	%	
<i>Blastocystis</i> sp.	18	20.9	Nd	-	-
<i>Endolimax nana</i>	14	16.3	2	1.1	0.0001
<i>Cryptosporidium</i> sp.	2	4.7	Nd	-	-
<i>Entamoeba coli</i>	4	4.7	3	1.7	0.2873
<i>Strongyloides stercoralis</i>	2	2.3	1	0.6	0.2977
<i>Giardia duodenalis</i>	1	1.2	2	1.1	0.8866
Other parasites *	0	0	28	21.2	0.0001

Nd=not determined. **Iodamoeba bütschlii*, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Hymenolepis nana*, *Trichuris trichiura*, *Taenia* sp.

Table 3. Characteristics of chronic renal insufficiency patients undergoing hemodialysis, according to the presence or absence of intestinal parasites.

Characteristics	Parasitized		Non-parasitized		p
	N	%	N	%	
Age (mean)	45.9 ± 13.8		51.5 ± 15.5		0.046538*
Gender					
Male	17	51.5	33	62.3	0.6454**
Female	16	48.5	20	37.7	0.5020**
Hemogram					
Leucopenia	4	12.1	9	17.0	0.5506**
Leucocytosis	3	9.1	3	5.7	0.6127**
Neutrophilia	0	0%	6	11.3	0.0533**
Diarrhea					
Present	25	75.8	40	75.5	0.9911**
Absent	8	24.2	13	24.5	0.9813**
Examined patients (N)	33		53		

Statistical comparisons were carried out by Student's t* or chi-square test**; significance level of 5%.

Table 4. Association between the presence of parasites and diarrhea in hemodialysis patients.

Parasite	Diarrhea				p*
	Present		Absent		
	N	%	N	%	
<i>Blastocystis</i> sp.	11	61.1	7	38.9	0.2726
<i>Endolimax nana</i>	8	57.1	6	42.9	0.2019
<i>Cryptosporidium</i> sp.	2	100.0	0	0.0	0.4279
<i>Entamoeba coli</i>	4	100.0	0	0.0	0.2660
<i>Strongyloides stercoralis</i>	2	100.0	0	0.0	0.4279
<i>Giardia duodenalis</i>	1	100.0	0	0.0	0.5734
Total	25		8		0.9947

*Chi-square test; significance level of 5%.

patients in our study are indicative of immunodepression, which can increase susceptibility to parasitosis [2,27]. Infections may cause or worsen anemia in hemodialysis patients, aggravating immunodepression. This underscores the need for early diagnosis of parasitosis and treatment to minimize its impact on the patient [35].

The frequency of diarrhea in our hemodialysis patients was not significantly different from that of the patients without parasites. However, *Blastocystis* sp. and *E. nana* may be considered pathogenic, especially when they provoke diarrhea and other diarrheagenic agents are excluded [31-33]. Thus, when diagnosed, these patients must be treated [14], since subclinical infections may appear in about two-thirds of individuals who undergo kidney transplants [13]. The combination of drugs used to prevent organ rejection, markedly favors reactivation of the protozoon, leading to persistent diarrhea and dysentery [13,33].

The effects of currently-available treatments and procedures for handling chronic diseases on the epidemiology of infectious agents, such as *Blastocystis* sp., which has been ignored for many decades by health professionals, deserves consideration. We suggest that parasitological stool examinations with emphasis on *Blastocystis* sp. and *Cryptosporidium* sp. be included in routine follow-up exams of individuals undergoing hemodialysis. In addition, repetition of parasitological tests is necessary to establish the etiology of the diarrheal crisis, or to detect possible asymptomatic hosts, especially in hemodialysis patients, since the drugs used to treat protozoonosis are not always effective against blastocystosis and cryptosporidiosis.

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