Assessment of *Blastocystis* spp. Infection in hemodialysis patients in two centers of the metropolitan area of rio de janeiro

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Blastocystis spp. is the most common protozoan worldwide. Hemodialysis (HD) patients are immunosuppressed and may be susceptible to infections by opportunistic pathogens. This study aims to evaluate *Blastocystis* spp. infection in HD patients. Fecal samples were collected from 97 patients and 42 healthy individuals from the same social and economic environment. The prevalence of *Blastocystis* spp. was 41.2% among patients and 45.2% among controls (P=0.66). Monoparasitism was more frequent than polyparasitism in both groups. Our findings suggest that *Blastocystis* spp. is relevant in HD patients. Although frequently neglected, investigation for enteroparasites may be helpful for clinical management of HD patients.

Key Words: *Blastocystis* spp., hemodialysis, enteroparasites

INTRODUCTION

Blastocystis spp. is a cosmopolitan enteric protozoan that inhabits the gut of humans and many animals. According to the taxonomic consensus, due to its extensive genetic diversity, all species of the genus *Blastocystis* receive the same denomination, independent of the host. Thus, the species previously called *Blastocystis* hominis is currently known as *Blastocystis* spp [1]. Due to its pleomorphism, the parasite has already been considered a fungus, sporozoan and even as a cyst of other organisms [2]. Although it has been described more than a hundred years ago by Alexeieff, little is known about its pathogenic potential, genetic diversity, host interactions and treatment [3]. Only in the last decade advances on its biology have been achieved [4,5]. More recently, molecular studies based on the PCR technique led to the identification of 17 subtypes (ST) and among them, nine have been found infecting humans with a variable prevalence [6,7]. In fact, only four are common (ST1, ST2, ST3 and ST4) representing about 90% of the isolated subtypes [6] with the vast majority of human infections attributable to the ST3 subtype [7].

As a result of its pleomorphic nature and the difficulty in standardizing laboratory techniques, the diagnosis of *Blastocystis* spp. still produces erroneous and false negatives results [4]. *Blastocystis* spp. is the most common parasite in coproparasitological studies involving either humans or animals8. Interestingly, the parasite is frequently found in fecal samples of symptomatic or asymptomatic individuals with similar prevalence rates. However, it is also indicated as the sole causative agent in some gastrointestinal infections, in which patients complain of symptoms such as abdominal pain, diarrhea, nausea, vomiting, bloating and anorexia. In addition, the parasite was already incriminated in dermatological affections in which the most frequent complaints are urticaria and pruritus of the palms and/or plants as well as infectious arthritis [1,8].

It is believed that around the world, nearly one million people are colonized by *Blastocystis* spp. [1]. The prevalence of this protozoan varies between communities in the same country and also between different countries, perhaps reflecting real differences among populations or the use of different diagnostic techniques. In China and Thailand, for instance, the range can vary from 1.9% to 32.6%, and from 0.9% to 45.2%, respectively [9]. The same is true for developed countries in which the frequency varies, reaching 10% in United States 0.5% in Japan1. In developing countries, the prevalence can be as high as 60%10. High prevalence rates are associated with low socioeconomic levels, suggesting that transmission is increased with deficient basic sanitation, proximity to domestic animals and livestock, and water supply from artesian wells and rivers [5,8]. It is a general consensus that transmission of *Blastocystis* spp. is by fecal-oral route [10,11].

As well as when dealing with other parasitic infections, some specific group populations are more susceptible to infection by *Blastocystis* spp. [9]. In immunocompromised patients, such as carriers of the AIDS virus, cancer patients and transplanted patients, it has been identified as an opportunistic pathogen6. In fact, this may be the case for patients undergoing dialysis.

The abnormal environment generated by the incomplete correction of homeostasis by hemodialysis may have negative impacts on neutrophil chemotaxis, phagocytosis, bactericidal actions, and on the maturation of T lymphocytes, which may increase the susceptibility to infections [12]. It is known that cellular immunity has an important role in defense against parasitic infections, including that caused by *Blastocystis* spp. [13]. Studies carried out in different countries on HD patients reported a high prevalence rate of *Blastocystis* spp. infection. Of note, some studies fail to prove that prevalence of the parasite is higher in HD patients than in control group [13-16].

Patients undergoing hemodialysis have a broad spectrum of gastrointestinal symptoms, similar to those caused by some enteroparasites, which may act as a confounding factor. Therefore, it is recommended to evaluate them for parasitic infections before therapeutic interventions [14].

In the present study, we provide some recent data on the prevalence of *Blastocystis* spp. in hemodialysis patients compared with a control group, without chronic renal disease, in two municipalities in the metropolitan region of the state of Rio de Janeiro, Brazil.

MATERIAL AND METHODS

Study design and volunteers

This is a cross-sectional observational study in a convenience sample. Patients with end-stage renal disease undergoing hemodialysis for more than 180 days, without gender or age restriction from two dialysis centers located in the cities of Niteroi (Center 1) and Itaborai (Center 2), respectively, were included. The control group was composed by patients' relatives, without declared CKD, who lived in the same residence and therefore subjected to the same possible risk factors for enteroparasite

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infection. This study was approved by the ethics committee of the Medical School of Universidade Federal Fluminense (Protocol number 1.147.848).

Data and sample collection

All patients in this study signed a consent form and completed a standardized clinical and epidemiological questionnaire that asked for information concerning symptoms related to intestinal parasite infection and demographic characteristics. Afterwards, the volunteers were instructed to collect two fecal samples, using universal collectors.

Coproparasitological tests

A fresh sample was employed to perform the Rugai technique. The volunteers were also instructed to collect 3 samples on alternate days and to pack them in the 10% formalin flask for the Hoffman, Pons & Janer technique. The samples were transported in refrigerated boxes to the laboratory of parasitology of the university hospital. All samples were collected from March 2016 to November 2016. For detection of *Blastocystis* spp., the sediments were observed under magnification of 100 and 400x under an optical microscope.

Statistical analysis

Data are presented as mean ± S.D. or frequencies. Continuous variables were compared using t tests; frequencies, by chi-square or proportion test. P values were considered significant when <0.05. Graphpad Prism 5.0 (GraphPad Software, Inc.) was employed for the analysis.

RESULTS

Fecal samples were collected from 97 HD patients aged 57.1 ± 11.8 years, 55.6% male, with a dialysis time of 8.3 ± 9.7 years. The control group consisted of 42 volunteers aged 49.8 ± 17.4 years, from which 69% were women.

The general characteristics of participants are in Table 1. Male gender was more prevalent in patients (55.6% *vs*. 33.3%, P=0.02). Control volunteers were younger (50.1 ± 16.5 years *vs*. 57.1 ± 11.7, P=0.01). In both groups, most of the participants were in the age range of 42-62 years. The whole prevalence rate of enteroparasites in the sample was 56.1%. *Blastocystis* spp. was isolated in 59 cases (42.4%) and was the most frequent parasite representing 75.6% of the identified enteroparasites. The prevalence of the organism was not different between HD patients and controls (41.2% *vs*. 45.2%, respectively, P=0.66).

	All	Patients	Controls	P valuea
N	139	97	42	-
Male gender	68 (48.9)	54 (55.6)	14 (33.3)*	0.02
Age, years	55.0 ± 13.6b	57.1 ± 11.7	50.1 ± 16.5*	0.01
Age range				
21-41 years	18 (13.0)	9 (9.3)	9 (21.4)	
42-62 years	79 (56.8)	55 (56.7)	24 (57.2)	0.08
≥ 63 years	42 (30.2)	33 (34.0)	9 (21.4)	
Incomplete elementary education	n.a.	49 (50.5)	n.a.	
Enteroparasite infection	78 (56.1)	50 (51.5)	28 (66.7)	0.1
Blastocystis spp. infection	59 (42.4)	40 (41.2)	19 (45.2)	0.66

Data is shown as n (%) unless specified

a Controls vs. Patients; b Mean ± S.D.

* P <0.05 vs. Patients; n.a. = not available

Families with access to treated water were more numerous in Center 1 (78.5% *vs*. 47.2%, P<0.001). Accordingly, families with an income of a minimum salary or less were more common in Center 2 (92.7% *vs*. 57.1%, P<0,001). Features of participants stratified by center are in Table 2. Those derived from center 2 tended to be younger but statistical significance was not found. Regarding education, no difference was observed between centers. Forty-two samples of hemodialysis patients were derived from the Center 1: twenty-three were positive for enteroparasites (54.7%) and in 18 of them (78.2%) *Blastocystis* spp. was identified. Sixteen samples of the control volunteers belonged to this center: nine were positive for enteroparasites (56.2%) and *Blastocystis* spp. was observed in 7 of them (77.8%). In Center 2, hemodialysis patients provided 55 samples: twenty-seven (49.1%) had positive results for enteroparasites with *Blastocystis* spp. being found in 22 of them (81.5%). In the 26 samples from the control volunteers of this center, 19 (73.1%) were positive and *Blastocystis* spp. was found in 12 of them (63.1%).

	Center 1	Center 2	P value
Patients N	42	55	-
Male gender	20 (47.6)	34 (82.9)	0.16
Age in years	59.5 ±12.4 a	55.3 ± 10.9	0.08
Incomplete elementary education	28 (66.7)	21 (51.2)	0.15
Enteroparasite infection	23 (54.7)	27 (49.1)	0.57
Blastocystis spp. infection	18 (42.9)	22 (29.3)	0.77
Controls N	16	26	-
Male gender	6 (37.5)	8 (30.7)	0.65
Age in years	52.6 ± 17.2	48.2 ± 17.0	0.4
Enteroparasite infection	9 (56.2)	19 (73.1)	0.26
Blastocystis spp. infection	7 (43.8)	12 (46.2)	0.43

Selected features of participants are again presented in Table 3, this time factored by positivity for *Blastocystis* spp. The frequency of positivity was similar between genders either in patients (38.9% *vs*. 44.1%, P=0.75) or controls (42.9% *vs*. 46.4%, P=0.91). Likewise, the rate of positivity did not differ between the age ranges either in patients (P=0.66) or controls (P=0.77).

Blastocystis spp. was found as mono and polyparasitism. In both groups, the frequency of monoparasitism was statistically higher than polyparasitism (62.5% *vs*. 32.5%, P<0.01, and 68.4% and 31.6%, P=0.025, for hemodialysis patients and controls, respectively).

	Patients		Controls	
	Blast-(n=57)	Blast+(n=40)	Blast-(n = 23)	Blast+(n=19)
Male gender	33 (61.1)	21 (38.9)	8 (57.1)	6 (42.9)
Female gender	24 (55.9)	19 (44.1)	15 (53.6)	13 (46.4)
Age, years	57.1 ± 11.7a	56.9 ± 11.9	50.1 ± 16.8*	49.8 ± 16.6*
Age range				
21-41	4 (44.4)	5 (55.6)	4 (44.4)	5 (55.6)
42-62	33 (60.0)	22 (40.0)	14 (58.3)	10 (41.7)

≥ 63	20 (60.7)	13 (39.3)	5 (55.6)	4 (44.4)

Data is shown as n (%) unless indicated

a Mean ± S.D.

* P<0.01 *vs*. Patients

DISCUSSION

In the recent years, there has been an increase in the number of studies addressing enteroparasitism in end-stage renal disease patients on dialysis. Studies that determine the prevalence of enteroparasites in patients with ESRD show significant infection rates, mainly by protozoa [14,15].

Blastocystis spp. is a protozoan with worldwide distribution with a higher prevalence rate in tropical and subtropical regions. If this parasite has a higher prevalence rate in hemodialysis patients than in the general population is still a matter of controversy. In the present study we resorted to a control group composed of individuals sharing the same residence of the HD patients, aiming a more appropriate comparison between groups.

Male gender was more preponderant in HD patients. It is our view, however, that this difference did not affect our findings since the majority of studies [17-19] did not assign gender as a risk factor for enteroparasitism. The age in the control group was slightly lower than in HD patients but in both groups the majority of participants was in the age range of 42-62 years.

In the present study, *Blastocystis* spp. was the intestinal parasite most commonly observed in HD patients (75.6%) and controls (67.8%) without statistically significant difference between groups. Our findings are in agreement with those of Karadag et al. [13] and Omrani et al. [12] but not with the ones from Gil et al. 16 that observed higher frequency of *Blastocystis* spp. in the control group compared to the HD group.

The prevalence rate of 41% of *Blastocystis* spp. among HD patients was higher than in previous Brazilian reports, such as those described by Gil et al. [16] (24.5%) and Kulik et al. [15] (20.9%). When compared to international studies, the difference can become even more marked. In Iran, for instance, studies by Omrani et al. [12] and Barazesh et al. [14], both addressing HD patients, reported the parasite in 14.1% and 13.6%, respectively. In Turkey, Karadag et al. [13] reported a prevalence rate of 23.9%, a number still lower than ours but comparable to other Brazilian studies.

Patients from Center 1 had more access to treated water and a higher income per capita. In spite of the differences in some socioeconomic indicators, the prevalence rates of enteroparasites as a whole and *Blastocystis* spp. were not different between centers. These results go against our expectation but we could not find a definite explanation for that. We wonder if the differences between their basic sanitary conditions were not substantial enough to impact in the prevalence of the infections.

Consistent with the studies by Omrani et al. [12] and Barazesh et al. [14], positivity for by *Blastocystis* spp. in the present study did not seem not be influenced by neither gender nor age either in patients or controls.

Blastocystis spp. was found as a sole infection or in association with other parasites. *Blastocystis* spp. as monoparasitism was approximately twice more frequent than as polyparasitism in both groups.

Karadag et al. [13] also reported infection by *Blastocystis* spp. as monoparasitism to be more frequent than as polyparasitism in dialysis patients with the difference reported exhibiting even a higher magnitude than in present study (82% *vs*. 18% and 62.5% *vs*. 32.5%, respectively). Monoparasitism seems to be the main form of presentation of enteroparasitism irrespective of the infectious agent. Kulik et al. [15], for instance, found statistically significant differences between the frequency of mono - and polyparasitism in favor of the first in hemodialysis patients as well as in the control group. Barazesh et al. [14] reported enteroparasitism in a frequency of 28% with monoparasitism representing 84%.

The pathogenicity of *Blastocystis* spp. is still a subject of controversy. However, it is now known that this parasite can damage the intestinal epithelium and release toxins, favoring the occurrence of intestinal and extra intestinal injury, especially in immunosuppressed patients, such it seems to be the case for ESRD patients [1]. The Center for Disease Control and Prevention (CDC) advises that symptomatic patients in whom no other causes are found, once this parasite is identified, treatment becomes necessary [12].

CONCLUSION

The present report is mainly focused on the occurrence of *Blastocystis* spp. in HD patients compared to a control group. Our results indicate that HD patients did not have higher prevalence rates of *Blastocystis* spp. infection. Of note, the parasite was a frequently finding in the coproparasitological tests from participants from both counties either in patients or controls, reinforcing the need for its diagnosis and treatment in symptomatic patients in whom no other causes are identified. The sample of our study is relatively small limiting the generalizability of the finding. Further studies are required to better assess the clinical and epidemiological data of this protozoan, especially in immunosuppressed patients.

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