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Blastocystis sp. infection in patients with gastrointestinal complaints: a Cuban study

Gastrointestinal şikayeti olan hastalarda *Blastocystis* sp. infeksiyonu: bir Küba araştırması

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ÖZET

Amaç: Bu çalışmada; yetişkinlerdeki gastrointestinal sistem şikayetleri ile *Blastocystis* varlığı arasındaki ilişkinin tanımlanması amaçlanmıştır.

Yöntem: Ocak-Aralık 2011 tarihleri arasında Küba, Matanzas Şehri'nde bulunan Hijyen, Epidemiyoloji ve Mikrobiyoloji Merkezi'nde tanımlayıcı bir çalışma yürütülmüştür. Toplam 749 erişkin (20-69 yaşları arasında, ortalama yaş 38) bu çalışmaya dahil edilmiştir. Patojenik bakteri, parazit açısından pozitif dışkı sonucu olanlar ve rotavirus sonucu pozitif olanlar bu çalışma dışında bırakılmıştır. Sonuç olarak 240 erişkin çalışma koşullarını karşılamıştır: 128 (%53,3) erkek, 112 (%46,7) kadın ve olguların yaş ortalaması 40 idi. Karşılaştırmayı kolaylaştırmak için grup ikiye bölünmüştür: Gastrointestinal şikayetleri olan 110 erişkinden oluşan birinci grup ve şikayetleri olmayan 130 erişkinden oluşan ikinci grup. Her iki grupta da cinsiyet dağılımı homojen olmuştur. *Blastocystis* sp. varlığını tanımlamak için taze dışkı örnekleri nativ yöntemiyle direkt olarak incelenmiştir. Formalin-eter (Ritchie) yöntemi intestinal parazitlerin yanlış tanısından kaçınmak amacıyla kullanılmıştır. Her iki yöntemde de taze dışkı örnekleri ışık mikroskobu ile incelenmiştir.

Bulgular: Semptomatik grupta yer alan 49 hastada (%44,5) ve asemptomatik grupta yer alan 28 (%22,4)

ABSTRACT

Objective: This study is aimed to determine the association between the presence of *Blastocystis* sp. and gastrointestinal complaints in adults.

Method: A descriptive study was carried out from January to December 2011 at the Centre of Hygiene, Epidemiology and Microbiology in Matanzas City, Cuba. A total of 749 adults (aged from 20 to 69 years old, mean age 38) had the opportunity to be included. Patients who had positive stool results for pathogenic bacteria, parasites and those with positive result to rotavirus were excluded from the study. A total of 240 adults were finally included, 128 (53.3%) male and 112 (46.67%) female with a mean age of 40 years old. To facilitate the comparison patients were divided into two groups: 110 adults with gastrointestinal complains and a second group of 130 adults without gastrointestinal disturbances. Sex distribution was homogenous in both groups. The fresh fecal samples were examined to determine the presence of *Blastocystis* sp. using the direct wet mount. Formalin-ether (Ritchie) technique was used to avoid the misdiagnosis of other intestinal parasites. In both techniques, the fresh samples were examined under light microscope.

Results: *Blastocystis* sp. was detected in 49 patients (44.5%) in the symptomatic group and 28 (22.4%) in the

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hastada *Blastocystis* sp. saptanmıştır. Karın ağrısı (%52,7) veya şişkinlik (%38,2), akut ishal (%26,4), iştah kaybı (%18,2), ve dispepsi (%16,4) septomatik grupta yer alan hastalarda tespit edilen gastrointestinal şikayeler olarak belirlenmiştir. Gastrointestinal şikayeti olan hastalarda *Blastocystis* sp. tanımlanma olasılığının, asemptomatik hastalardan 2,9 kez daha yüksek olduğu bulunmuştur.

Sonuç: Mevcut çalışmamız *Blastocystis* sp.'yi patojen olarak tanımlayan yazarları desteklemektedir.

Anahtar Kelimeler: *Blastocystis* sp.; patojen; gastrointestinal bulgular

asymptomatic one. Abdominal pain (52.7%) or distension (38.2%), acute diarrhea (26.4%), loss of appetite (18.2%), and dyspepsia (16.4%) were the gastrointestinal complains notified by patients included on the symptomatic group. The probability to identify *Blastocystis* sp. in patients with gastrointestinal complains was 2.9 times higher than in asymptomatic patients.

Conclusion: Current results support those authors considering Blastocystis sp. as pathogen.

Key Words: *Blastocystis* sp.; pathogen; gastrointestinal symptoms

INTRODUCTION

Blastocystis sp. is a unicellular protozoon found in the large intestine in humans. While *Blastocystis* sp. infection is common worldwide, it is observed more frequently in tropical climates and developing countries with a wide host population including mammals, birds, reptiles, and arthropods (1). This parasite spreads through the fecal-oral route particularly under poor hygiene conditions (2, 3). The use of unboiled water has been also considered to have a significant role in the spread of the infection (3,4).

High prevalence of *Blastocystis* sp. has been reported in developing countries (5-8); however, in developed countries, the prevalence could be lesser (2, 9). Despite *Blastocystis* being discovered almost 100 years ago, its clinical significance and many aspects regarding its biology remain unresolved (10). *Blastocystis* can be isolated from individuals with gastrointestinal and extra-intestinal symptoms and asymptomatic individuals with an almost equal prevalence (10). Occasionally, a higher prevalence can be found in asymptomatic than in symptomatic individuals (11). Many researchers classify *Blastocystis* as a commensal or opportunistic pathogen (12, 13), whereas others report finding *Blastocystis* more commonly in stools from symptomatic patients than from asymptomatic individuals (1, 3).

Considering the existing discrepancies related with the pathogenic roll of this common intestinal parasite and the inexistence of other works in Cuba about this topic the aim of the present cross-sectional study was to determine the association between gastrointestinal complaints and the presence of *Blastocystis* sp. in adults.

MATERIAL AND METHOD

Study population:

A total of 749 adults (aged from 20 to 69 years old, mean age 38) with or without gastrointestinal complaints attending to the Centre of Hygiene, Epidemiology and Microbiology, Matanzas City- Cuba from January to December 2011 had the opportunity to be included in this descriptive prevalence study.

Patients who had positive stool results for pathogenic bacteria like *Campylobacter*, *Salmonella* sp., *Shigella* sp. or enteropathogenic *E. coli*), those whose microscopic stool examination revealed pathogenic parasites (*Giardia lamblia, Entamoeba histolytica,* etc.) and those with positive result to

rotavirus were excluded from the study. Patients who rejected to participate were excluded too.

One group was represented by 110 patients with gastrointestinal complaints while the second (n=130) without any accompanying gastrointestinal disturbances, was selected from population attending the centre within similar period for routine control.

Collection of Fecal Samples:

Three fecal samples from each patient were collected in a wide mouth screw capped containers free of preservative at intervals of two days. The samples were collected by patients and immediately sent to the Department of Parasitology of the institution. Diarrheal samples were examined with appropriate techniques searching for rotavirus or pathogenic bacterias. The fresh fecal samples were examined to determine the presence of *Blastocystis* sp. using the direct wet mount. Formalin-ether (Ritchie) technique was used to avoid the misdiagnosis of other intestinal parasites. In both techniques, the fresh samples were examined under light microscope.

Three slides of each sample were prepared and examined by three analysts (1 technician and 2 parasitologist); such that 3 slides per sample were analyzed searching for *Blastocystis* sp. The sample was considered as positive, if it was approved by two of them.

Community Return:

All patients received the results of the laboratory diagnosis. The positive cases were referred to appropriate healthcare units, where they received specific treatment and follow-up.

Data Collection:

A questionnaire was administered by researchers to each parent seeking for gastrointestinal complaints, demography. All patients had no history of medication one month before the study commencement.

Ethical Considerations:

Ethical clearance was granted by the Institutional review board from Centre of Hygiene, Epidemiology and Microbiology, Matanzas - Cuba under the designation Cód. 2010 - 10 in November 2010. The enrolment also required that the agreement model was signed by patients, after being fully informed about the aim of the study. Instructions on how to collect the stool samples were also provided in writing.

Data management and statistical analysis:

Data regarding the parasitological results was noted on pre-designed record forms. Relative frequencies as percentage were performed using EpiInfo 6.04 software (Public Health Domain Software, CDC, Atlanta, GA, USA).

RESULTS

A total of 240 adults were finally included, 128 (53.33%) male and 112 (46.67%) female with a mean age of 40 years old. Sex distribution was homogenous in both groups.

Abdominal pain (52.7%) or distension (38.2%), acute diarrhea (26.4%), loss of appetite (18.2%), and dyspepsia (16.4%) were the gastrointestinal complaints notified by patients included on the symptomatic group.

Blastocystis sp. was detected in 49 patients (44.5%) in the symptomatic group and 28 (22.4%) in the asymptomatic one. The probability to identify *Blastocystis* sp. in patients with gastrointestinal complaints was 2.9 times higher in symptomatic patients than in asymptomatic. It is important to notice that all *Blastocystis* sp. infected patients had abdominal pain or distension.

DISCUSSION

Parasitic infections of the gastrointestinal tract are very common worldwide. Among these parasites,

Blastocystis sp., represents an interesting and not totally understood topic.

There is still much uncertainty about the pathogenic potential of *Blastocystis* (14). Most authors consider this intestinal parasite frequent with prevalence ranging from 1.5% to 10% in developed countries and from 30% and 50% in developing countries (2, 5-8, 9, 14, 15-17). In Cuba, two different studies carried out in children in different sites identified *Blastocystis* sp. as common as *G. lamblia* (17, 18), the most notified intestinal protozoan parasite in the country.

In vivo endoscopy and biopsy analyses in symptomatic patients indicated that *Blastocystis* sp. do not invade the colonic mucosa, but lead to disturbances on the barrier function and permeability. Suggested mechanisms for *Blastocystis*-induced pathogenesis include: elicitation of toxic-allergic reactions; degradation of human secretory IgA by proteases; changes in epithelial permeability, by inducing apoptosis of host intestinal cells and disruption of the epithelial barrier function; modulation of immune response and cytokine release from colonic epithelial cells (10).

In the Islamic Republic of Iran a case-control study published in 2010 (19), demonstrated no significant relation between the presence of gastrointestinal symptoms and the incidence of *Blastocystis* sp. however; another study from Turkey (20), found a strong correlation between the presence of *Blastocystis* sp. and lower anthropometric indexes in children. Other authors report the association of this intestinal parasite with irritable bowel syndrome or inflammatory colonic conditions (1, 3, 21-24).

The frequency of *Blastocystis* sp. in this study was higher in symptomatic patients than in asymptomatic, so we agree with those authors considering *Blastocystis* sp. as pathogen. Abdominal pain or distension, acute diarrhea, loss of appetite, and dyspepsia were the symptoms notified among the symptomatic group. Abdominal pain or distension was notified by all *Blastocystis* sp. infected patients. This result agrees with other studies about this topic (19, 25, 26).

One weakness of the present work was the limitation to use other diagnostic methods like culture or molecular biology (major sensitivity and specificity) because they are unavailable in Cuba. To increase the accuracy the diagnostic method used three slides of each sample which were prepared and examined by three different and independent analysts.

The diseases caused by intestinal parasites, once considered a rare phenomena confined to the tropics, are now being diagnosed with increased frequency in industrialized countries (27). This trend can be attributed to various factors, including globalization of the food supply, the increased consumption of fresh foods, increased travel to developing countries, and more intensive immigration originated from these areas. *Blastocystis* sp. is a worldwide distributed intestinal parasite not totally understood so other studies are needed to clarify its role over human health.

Considering that *Blastocystis* sp. was more identified among patients with gastrointestinal complaints than in patients without gastrointestinal disturbances, despite methodological limitations, this study supports those authors considering *Blastocystis* sp. as pathogen.

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