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Prevalence of *Blastocystis hominis* in Patients with Gastrointestinal Trouble

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Abstract

Blastocystis hominis has a worldwide distribution with a marked prevalence in many countries, it can be confused with other microorganism, the most common approaches for the detection of *Blastocystis sp.* consist of direct smear examination by light microscopic or in vitro culture and molecular diagnosis. So the objective of this study was molecular identification of *Blastocystis hominis* to estimate its prevalence. Cross section study was conducted during the period From January till December 2019 on 100 patients attending King Faisal Medical Complex in Taif, presented with symptoms of gastrointestinal trouble (i.e., nausea, vomiting, loss of appetite, passage of loose stool, passage of mucoid stool, and abdominal pain). Stool samples were collected one sample from each patients and every sample was divided into two parts one immediately deep frozen for PCR and other part was examined directly by wet mount preparation using saline and iodine methods, and then reexamined after concentrating them in formalin-ether. This study showed that the prevalence rate of *Blastocystis hominis* detected by molecular diagnosis was 10% and there was a statistical significant in *Blastocystis hominis* diagnosed in cases with irritable bowel diseases, Chron's disease and Eczema dermatitis.

Keywords: Blastocystis hominis; Prevalence; Molecular diagnosis

Introduction

Blastocystis is a common intestinal parasite infecting and responsible for diarrhea in humans. Recent studies "in vitro" and "in vivo" have shed new light on the pathogenic power of this parasite. Blastocystis infection is associated with a variety of gastrointestinal disorders, irritable bowel syndrome (IBS), and cutaneous lesions [1]. Moreover, the patients with Blastocystis as the only detected possible pathogen were observed to relieve from gastrointestinal symptoms after successful treatment of Blastocystis. Molecular data reveal extensive genetic diversity within Blastocystis genus in the small subunit (SSU) rRNA gene. To date, 17 subtypes (STs) have been reported based on sequence analysis of the SSU rRNA gene, and nine of them have been identified in humans, with eight being zoonotic [2].

Diarrhea is simply an altered movement of ions and water that follows an osmotic gradient. Under normal conditions, the gastro-intestinal tract has tremendous capacity to absorb fluid and electrolytes, where 8–9 liters of fluid are presented to the intestine daily and only 100-200ml are excreted in the stool. Enteric pathogens, however, can alter this balance towards net secretion, leading to diarrheal disease [3]. Diarrhea is described as three or more loose or watery stools a day. Infection commonly causes acute diarrhea [4]. Infectious Diarrhea is caused by a virus, parasite, or bacterium. It can spread quickly from person-to-person. Some of the causes of infectious diarrhea, such as Campylobacteriosis, shiga-toxin producing E. coli, giardiasis, salmonellosis and shigellosis, parasites as cryptosporidiosis, amoebiasis, Giardiasis, Blastocystiasis and viruses such as Rotavirus [3].

Parasites can enter the body through food or water and settle in the digestive system. Parasites that cause diarrhea include *Giardia lamblia*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Cryptosporidium* and *Blastocystis hominis* [5]. The most common approaches for the detection of *Blastocystis* sp. consist of direct smear examination by light microscopic or xenic in vitro culture. However, given the occurrence of different forms of *Blastocystis* sp. (especially the hardly recognizable cystic form), deterioration caused by environmental conditions or drug treatment and the fact that *Blastocystis* sp. can be confused with other microorganisms, this method seems to have largely underestimated this parasite in the context of enteric parasite diagnosis. Moreover, culturing this parasite is time consuming and can bias subsequent genotyping due to the different ability of isolates to grow in selective medium. Therefore, to overcome these limitations, several molecular polymerase chain reaction (PCR)-based diagnostic approaches using faeces directly or after culture of faecal specimens have been described [1].

Studies comparing the relative performances of these various diagnostic methods showed that the PCR approach was as sensitive as the culture approach, real-time quantitative PCR (qPCR) assay developed to detect *Blastocystis* sp. in stool samples. This assay targets a region of the small subunit rRNA gene (SSU-rDNA) and allowed subtyping of isolates by direct sequencing of qPCR products. Moreover, Stensvold and colleagues developed a qPCR on stool samples using the SSU-rDNA marker, including an internal process control enabling the evaluation of potential PCR inhibitors. This approach had the advantage of increasing the specificity and avoiding the amplification of false positives. Therefore, currently, SSU-rDNA genotyping is the method of choice for diagnosis [1]. So the aim of this study is molecular identification of *Blastocystis hominis* to estimate its prevalence.

Subjects and Methods

Cross section study was conducted during the period From January till December 2019 on 100 patients attending King Faisal Medical Complex in Taif, presented with symptoms of gastrointestinal trouble (i.e., nausea, vomiting, loss of appetite, passage of loose stool, passage of mucoid stool, and abdominal pain). Patients had recently received anti-parasitic medications were excluded from this study.

Ethical Considerations

The study was ethically approved by the ethical committee of Taif University. Participants were provided with detailed information regarding the study and asked to sign out a written consent form.

History of the Diseases

Data about the basic sociodemographic variables (age, gender and residence) and the associated gastrointestinal symptoms (diarrhoea, abdominal pain, fever, nausea, vomiting, watery stool or mucous in stool) and other diseases complains were collected from patients sheets.

Samples Collection

Microscopic examination

Stool samples were collected one sample from each patients and every sample was divided into two parts one immediately deep freeze for PCR and other part was examined directly by wet mount preparation using saline and iodine methods, and then reexamined after concentrating them in formalin-ether .

PCR-based molecular assay

Aliquots of frozen faecal specimens were subjected for DNA extraction and subsequent PCR, DNA was isolated from stool samples using the genomic DNA purification Kit QIA amp® Fast DNASTool Mini Kit (cat. No. 51604 Qiagen-Germany) which has been validated for extraction from stool samples. The PCR procedure included specific primers: forward, RD 5'-ATCGCCACTTCTCCAAT-3' and reverse: RD 5'-GAGCTTTTAACTGCAACAACG-3', amplifies the 600 bp product of 18S SSU rDNA of *Blastocystis*. 50 µl of Go Tag® Green master mix (Promega, USA), were added to 10 µl of DNA extract and 0.8 µg/µl of each primer were added. The amplification cycles consist of 30 cycles. Each cycle consisted of 1 minute of denaturation at 95°C, 2 minutes of annealing at 56°C, and 1 minute of primer extension at 72°C with an additional extension at 72°C for 7 minutes after the last cycle. Positive and negative controls were used for each run. Positive control was DNA extracted from pooled known positive stool samples, by stool examination; while negative control was a blank containing all PCR reagents but no DNA. For detection of PCR products, 10µl of each PCR mixture and DNA molecular size marker were electrophoresed in a 1.4% agarose gel for 1hour and was stained with a 0.5µg/ml ethidium bromide, visualized in a UV transilluminator. The positive control lane showed a specific band at 600 bp, negative control lane was free from any band and samples showing a band opposite to the positive control band were considered as positive.

Analysis of Data

Data collected were tabulated and SPSS version 22.0 was used to analyze them statistically using the frequency test.

Table 1: The demographic features and the clinical symptoms of study's participants.

Character	Cases Total = 100	<i>Blastocystis hominis</i> No.	P-value
Age			
20-40 years	70	6	>0.05
>40 years	30	4	
Gender			
Male	60	7	>0.05
Female	40	3	
Residence			
Urban	65	6	>0.05
Rural	35	4	
Abdominal pain			
Yes	20	7	>0.05
No	80	3	
Diarrhea			
Watery	25	2	>0.05
Mucous	75	8	
Nausea or vomiting			
Yes			
No	55	4	>0.05
	45	6	
Fever			
Yes	39	3	>0.05
No	61	7	

Results

During the study period, 100 patients with gastrointestinal complaints their age range between 20-59 years. The basic demographic characteristics and the clinical features were described in Table 1.

Discussion

Blastocystis hominis is protozoan parasite with a worldwide distribution frequency reaching 100% in developing countries and exceeding 56% in developed countries. Blastocystis can cause diarrhea, flatulence, bloating, urticaria and irritable bowel syndrome (IBS) (Sánchez et al., 2017). The incidence and prevalence of Blastocystis is most likely due to lack of natural or acquired resistance and differences in behavior and habits closely related to environmental and socioeconomic determinants, food, inadequate drinking water, wastewater use, lack of sanitation and vulnerable socioeconomic conditions. Thus, the prevalence of intestinal parasitic infections in populations with these sociodemographic characteristics is maintained, creating a risk for transmission dynamics among its inhabitants and a public health problem in the country [6].

Blastocystis has a marked prevalence in many countries. According to most epidemiological studies, nearly all countries of the world have been classified into well-developed, with a moderate prevalence (10-15%), or under-developed, with a high prevalence (55–70%), attributed to the levels of hygiene and the presence or absence of contact with animals and/or contaminated water and food. The information on Blastocystis sp. epidemiology in Makkah city, revealing a relatively moderate prevalence (10.5%), as well as the presence of three subtypes: ST3, the most predominant particularly among symptomatic patients; ST1; and ST2 [7]. This study in agreement of our which detected 10% infected with *Blastocystis hominis* detected by PCR.

Table 2: Diagnostic performance of microscopy versus PCR.

Method	Positives	Negative	SE	SP	PPV	NPV	DA
	Blastocystis	Blastocystis	%	%	%	%	%
	No	No					
Microscopy	12	88	86	100	100	88	98
PCR	10	90	100	100	100	100	100

SE: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; DA: Diagnostic Accuracy

In Jeddah, Western Saudi Arabia the Blastocystis hominis infection was associated with anorexia in one student and diarrhea in another. Thus, most of Blastocystis hominis infections were asymptomatic. This may be explained by the fact that there is still controversy about the pathogenicity of B. hominis because this parasite is common in the healthy population without causing any symptoms [8]. This in contrast to our study which showed that most of the patients complain of mucoid diarrhea and abdominal pain the cause for this contrast may be due to selection of cases with gastrointestinal troubles while in study of Mohammed et al have symptoms consisted of abdominal pain in approximately 76%, diarrhea in approximately 27% and other gastrointestinal complaints in approximately 16% of the symptomatic patients. Blastocystis detection differed in the study patients as regard age, gender and residence. Blastocystis more detected in the age group between 20-40 years. Male had a higher infection rate than

Female for Blastocystis (Table 1). In contrast to the study done by Zhang et al. [2] that showed females had a higher infection rate than males for Blastocystis (9.3 and 5.5% respectively). Table 1 this may be due to the difference in the geographical regions.

The number of positive cases were detected by diagnostic PCR is 10 were but positive cases by direct microscopy were 12 cases. The sensitivity of direct microscopy compared with that of PCR shows in Table 2. while in study of Zhang et al, 2017 showed that 133 positive cases were detected by PCR, of which 122 were also positive by the culture method and 83 by direct microscopy and the sensitivity of direct microscopy is 62 % compared with that of PCR. the discrepancy may be due to the morphological diversity of *Blastocystis hominis*

Giardia lamblia and Entamoebae histolytica were diagnosed in 8% and 4% of cases respectively Table 3. There was a statistical significant in *Blastocystis hominis* diagnosed in cases with irritable bowel diseases, Chron's disease and Eczema dermatitis Table 4 this finding in agreement with the study done by Sánchez [6].

Table 3: Other enteric parasites identified in the study's participants.

The other Parasites	No of cases	Frequency (%)
<i>Giardia lamblia</i>	8	8%
<i>Entamoebae histolytica</i>	4	4%

Table 4: Frequency of *Blastocystis hominis* with other diseases.

P-value	<i>Blastocystis hominis</i> No (%)	No. of cases	Other diseases
<0.001**	4(40%)	10	Irritable bowel Syndrome
<0.05*	2(50%)	4	Chron's disease
<0.001**	2(67%)	3	Eczema dermatitis

**High statistically significant

*Statistically significant

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