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Association of *Helicobacter pylori* and protozoal parasites in patients with chronic diarrhoea

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ABSTRACT

Introduction: An association of *Helicobacter pylori* and common protozoal parasites in patients with abdominal discomfort and chronic diarrhoea is unclear and may be pathological.

Materials and methods: One hundred and sixty-one patients with diarrhoea were compared to 114 age and sex matched controls. Stool samples were examined by microscopy and DNA extracted for PCR with specific primers for *H. pylori* and protozoal parasites *Blastocystis* sp., *Entamoeba* sp. (*Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii)* and *Giardia duodenalis* (*G. duodenalis*).

Results: There was a marked difference in the presence of parasites between patients and controls: no parasite 42/75%, one parasite 42/15%, two or more parasites 16/10%, respectively ($p < 0.001$). Patients with diarrhoea were more likely to be infected with *Blastocystis* sp ($p < 0.001$), *E. histolytica* ($p = 0.027$) and *E moshkovskii* ($p = 0.003$). There was no difference in the frequency of *H. pylori* ($p = 0.528$), *G duodenalis* ($p = 0.697$) or *E dispar* ($p = 0.425$). Thirty-three patients and 27 controls had *H. pylori* infection. Of these, 22 patients and 6 controls were infected with *Blastocystis* sp ($p = 0.001$), 6 patients and no controls were infected with *E. histolytica* ($p = 0.02$), whilst 7 patents and 9 controls were infected with *E dispar* ($p = 0.292$).

Conclusion: In this population, diarrhoea is linked to infection with *Blastocystis sp, E. histolytica* and *E moshkoviskii*. In *H. pylori* infection, diarrhoea is linked to *Blastocystis* sp and *E. histolytica* infection. These associations may be linked pathogenically

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Introduction

In developing countries, co-infections with different pathogens are common, and are attributable the feco-oral transmission of bacterial and parasite pathogens brought about by the poor quality of the water consumed by the population at large living in unhygienic conditions, i.e. overcrowding, poor toilet facilities, and absence of quality health care [1]. The prevalence of intestinal parasites such as *Blastocystis* sp, *Giardia duodenalis* and *Entamoeba* sp., etc. has been estimated as 53% in children residing in an urban slum of a metropolitan city of Karachi, Pakistan [2]. Symptoms of abdominal discomfort and or pain, nausea and irregular bowel habit such as diarrhoea are associated with *Entamoeba histolytica*, *Blastocystis* sp and *G. duodenalis* infection. Amoebiasis is a faecal-oral route transmitted infection with the amoebas of the *Entamoeba* group. The symptoms of amoebiasis vary in severity from mild to severe and include loose stools, abdominal cramping, and abdominal pain. These are usually associated with infection by *E. histolytica*, but there are also reports of *Entamoeba moshkovskii* infection [3,4]. Microscopically, *E. moshkovskii, Entamoeba dispar* and *E. histolytica* are indistinguishable and require PCR test using specific primers for differentiation.

Several cross-sectional studies have also reported *Blastocystis* sp. associated with diarrhoea, abdominal pain/discomfort and urticaria [5–7]. Seventeen *Blastocystis* subtypes have been identified according to small subunit ribosomal RNA differences [6]. The percentage of patients testing positive for *Blastocystis* sp. has increased in recent years [8]. *G. duodenalis*, a flagellate parasite, colonizes the small intestine and is transmitted by feco-oral route [2]. Trophozoites are the cause of the symptoms of giardiasis. The most common manifestation of *G. duodenalis* is abdominal pain, bloating and diarrhoea. However, less than 10% of infections may be asymptomatic.

Helicobacter pylori is a common Gram negative bacterium associated with infection of the stomach, and which causes gastritis, peptic ulcer, carcinoma and lymphoma [9]. Locally, *H. pylori* seroprevalence in children aged 11–15 years of 54% is linked with age and poor socioeconomic condition [10–12], a figure very close to the prevalence of intestinal parasites [2], leading to the strong likelihood of co-infection. The gastrointestinal tract is populated by commensal bacteria that influence

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Table 1. Primer sequences.

	Sequence	PCR product Size (bps)
Helicobacter pylori		
Helicobacter genus-specific 16S rRNA		400
Forward (C97)	5′-GCT ATG ACG GGT ATC C-3′	
Reverse (C98)	5′-GAT TTT ACC CCT ACA CCA-3′	
UreaseC (glmM) gene		296
Forward	GGATAAGCTTTTAGGGGTGTTAGGGG	
Reverse	GCTTACTTTCTAACACTAACGCGC	
Entamoeba genus specific		
Forward	5′ TAAGATGCACGAGAGCGAAA 3′	
Reverse	5′ GTACAAAGGGCAGGGACGTA 3′	
E. histolytica		
Forward	5′ AAGCATTGTTTCTAGATCTGAG 3′	439
Reverse	5′ AAGAGGTCTAACCGAAATTAG 3′	
E. moshkovskii		
Forward	5′ GAAACCAAGAGTTTCACAAC 3′	553
Reverse	5′ CAATATAAGGCTTGGATGAT 3′	
E. dispar		
Forward	5′ TCTAATTTCGATTAGAACTCT 3′	174
Reverse	5′ TCCCTACCTATTAGACATAGC 3′	
Blastocystis spp sequence-tagged site		
SB83		
Forward	5′ GAAGGACTCTCTGACGATGA 3′	351
Reverse	5′ GTCCAAATGAAAGGCAGC 3′	
SB 155		
Forward	5′ ATCAGCCTACAATCTCCTC 3′	650
Reverse	5′ ATCGCCACTTCTCCAAT 3′	
SB227		
Forward	5′ TAGGATTTGGTGTTTGGAGA 3′	526
Reverse	5′ TTAGAAGTGAAGGAGATGGAAG 3′	
SB332		
Forward	5′ GCATCCAGACTACTATCAACATT 3′	338
Reverse	5′ CCATTTTCAGACAACCACTTA 3′	
SB340		
Forward	5′ TGTTCTTGTGTCTTCTCAGCTC 3′	704
Reverse	5′ TTCTTTCACACTCCCGTCAT 3′	
SB336		
Forward	5′ GTGGGTAGAGGAAGGAAAACA 3′	317
Reverse	5′ AGAACAAGTCGATGAAGTGAGAT 3′	
SB337		
Forward	5′ GTCTTTCCCTGTCTATTCTGCA 3′	487
Reverse	5′ AATTCGGTCTGCTTCTTCTG 3′	
Giardia duodenalis		
Forward	5′ TCA ACG TCA ACC GCG GCT TCC GT 3′	485
Reverse	5′ GTT GTC CTT GCA CAT CTCC 3′	

the behaviour of the protozoan parasites with which they directly interact. *H. pylori* may influence the presence, virulence and pathophysiology of parasitic protozoan infections.

The aim of this study was to determine any associations of *H. pylori* and protozoal parasites in patients with abdominal discomfort and chronic diarrhoea.

Materials and methods

This study was conducted at the Aga Khan University in Karachi, Pakistan: 161 patients with history of intermittent diarrhoea for 6 months were selected as the study group of patients with diarrhoea. These were controlled by 114 local residents who responded to an advertisement posted to fill out a questionnaire and provide a random stool specimen, and whom were without any symptoms of chronic disease or diarrhoea in the previous six months. A medical history and examination was obtained from each participant. Exclusion criteria were age <18 or >65 years, coeliac disease, inflammatory bowel disease, pregnancy, lactation, small bowel bacterial overgrowth, lactose intolerance, use of laxatives, anti-diarrhoeal drugs, bismuth, oral antibiotics, usage of acid reducing drugs e.g. histamine-2-receptor antagonists, proton pump inhibitors in the last one month and unwilling to participate. Both patients and controls provided a stool specimen at the laboratory. The study was reviewed by the university ethics committee. Written informed consent was obtained.

Microscopy of the stool smear was performed with approximately 2 mg of solid stool on the tip of a wooden stick mixed in physiologic saline and Lugol's iodine separately and examined under a light microscope with a

Table 2. Details of the enrolled patients.

	Patients with diarrhoea (n = 161)	Control (n = 114)	P value
Age (years) (mean/SD)	41 (15)	42 (14)	0.408
Sex (male/female) *n* (%)	112/49 (70%/30%)	75/39 (66%/34%)	0.508
Parasites detected on PCR (n, %)			
None	68 (42)	85 (75)	<0.001
One species	67(42)	18(16)	
Two or more species	26(16)	11(10)	
Specific parasites present			
Helicobacter pylori yes/no (*n*, %)	33/128 (20/80)	27/87(24/76)	0.528
Blastocystis sp yes/no (*n*, %)	81/80 (50/50)	26/88 (23/77)	<0.001
G. duodenalis yes/no (*n*, %)	16/145 (10/90)	13/101 (11/89)	0.697
E. histolytica yes/no (*n*, %)	16/145 (10/90)	3/111 (3/97)	0.027
E. dispar yes/no (*n*, %)	37/124 (23/77)	31/83 (27/73)	0.425
E. moshkovskii yes/no (*n*, %)	32/129 (20/80)	8/106 (7/93)	0.003

Note: *n* (%) = number and percentage.

cover slip [13]. Specimens were examined microscopically at 100X and 400X.

Stool DNA Extraction kit (Qiagen) was used to extract DNA as described in the user protocol and stored at −20 °C until used PCRs were as follows. Stool DNA was used to amplify *H. pylori* target genes using 16S rRNA and UreaseC (*glmM*) gene of *H. pylori* using primers described previously (Table 1) [14,15]. PCR was performed in an Eppendorf- Mastercycler, Germany. Both controls positive and negative were used with each batch of PCR. DNA from *H. pylori* strains ATCC 43504 was used as a control. The different subtype-specific sequence-tagged-site primers used for typing the *Blastocystis* sp. were previously described [16–26]. PCR for *Blastocystis* sp. was performed as previously described [21] (Table 1). ATCC 50177 (type 1), ATCC 50587(type 3), ATCC 50608 (type 4), and ATCC 50588 (mix type) and ATCC 50613 (mix type) were used as control for PCRs. A PCR for *Entamoeba* sp. was performed as previously described [27] (Table 1). These primers detected sequences of 16S-like ribosomal RNA gene (16S rRNA) of *E. histolytica*, *E. dispar* and *E. moshkovskii*. ATCC *E. dispar* strain SAW760, *E. histolytica* 30459 and *E. moshkovskii* 30041 were used as control for PCR experiments. PCR for *G. duodenalis* was performed using primers as previously described [28] (Table 1). All chemical reagents used were manufactured by Promega (Fitchburg, WI, USA). PCR primer pair amplified a 485-bp *G. duodenalis* glutamate dehydrogenase locus (Table 1). ATCC *G. duodenalis* 30888 was used as a control for PCR work.

Results are expressed as mean with standard deviation for age (analysed by t test) and as number (percentage) (analysed by the chi-square test) for categorical data. $p < 0.05$ was considered statistically significant. Analysis of data was performed by SPSS version 16.0.

Results

The patients and controls were age and sex matched (Table 2). Patients had a considerably greater overall burden of parasites. Specifically, they were more likely to be infected with *Blastocystis* sp, *E. histolytica* and *E moshkovskii,* but not *H. pylori*, *G duodenalis* or *E dispar*.

Thirty-three patients and 27 controls were infected with *H. pylori*. Of the 33 patients, six had no parasites, 14 had one parasite and 13 had two or more. Of the 27 controls, nine had no parasites, 17 had one and one patient has multiple parasites (p = 0.005). Twenty-two patients and 6 controls were infected with *Blastocystis* sp (p = 0.001). Six patients and none of the controls were infected with *E. histolytica* (p = 0.02). Seven patients and 9 controls were infected with *E dispar* (p = 0.02). Two patients and one patient were infected with *G duodenalis*, whilst 4 patients and 1 control were infected with *E moshkovskii* (data not analysed).

Discussion

The high rates of co-infection with *H. pylori* and intestinal parasites leads to the hypothesis that there may be a mechanistic or pathological link. Unsurprisingly, we found that patients with chronic diarrhoea are more likely to carry a greater burden of intestinal parasites, notably *Blastocystis* sp, and *Entamoeba* species. Overall, there was no difference in the rate of *H. pylori* infection. This is consistent with the high prevalence of the *Blastocystis* sp. in local paediatric population [2], and is similar to that published over 20 years [29]. However, the infection rate for *H. pylori* of 20–24% in our adults is far lower than that of 72.3% of apparently healthy children were found harbouring *H. pylori* [30], possibly because of antimicrobial therapy.

Patients with *H. pylori* infection were more likely to be co-infected with *Blastocystis* sp and *E. histolytica.* This is consistent with a study describing *H. pylori* and *Blastocystis hominis* co-infection in Taiwanese healthy adults [31]. Amongst *Entamoeba* species, the prevalence of *E. dispar* exceeded that of *E. histolytica* and *E. moshkovskii*. However, no association of *H. pylori* with *E. dispar* was found. The low rate of *G duodenalis* infection (ca. 10%) is slightly higher than that for *G. lamblia* (ca. 7%) [32]. A study of patients with duodenal biopsies who also had a gastric biopsy found that those with giardiasis were more likely to be colonized with *H. pylori* [33].

Blastocystis sp. appears to be a stable component of the intestinal microbiota once established and appears

to be more common than other protozoal pathogens such *E. histolytica*, *G. duodenalis*, etc. The association of *Blastocystis* sp. with *H. pylori* may be attributed to their common high prevalence as compared to *E. histolytica* and *G. duodenalis*. The association between *H. pylori* and *Blastocystis* sp. infections may also be associated with tolerogenic host immune response besides sharing the route of spread of infection and prevalence in the population. Mucosal dendritic cells (DCs) are involved in creating an overall tolerant state towards intestinal antigens. Infection is responsible for increasing the rate of entry of DC precursors from blood circulation into the tissues to become DCs. The activation of DCs by infiltrating pathogens impairs their ability to secrete IL-12 and stimulate Th1-cellular responses following exposure to *H. pylori* unless conditioned by intraepithelial cell-derived factors [34]. The uptake of pathogenic species by DCs residing in the mesenteric lymph nodes also contributes to the tolerant state. *H. pylori* is generally a non-invasive bacterium living within the stomach mucosa, the cluster of differentiation (CD)4^+ T cells responses directed against *H. pylori*, initiated within Peyer's patches, mesenteric lymph nodes and stomach draining lymph node are more tolerogenic than pro-inflammatory [35]. *Blastocystis* sp transmission occurs through water or by faecal-oral contamination. Infection follows through ingestion of contaminated food and water with cysts from humans and domestic animals. These are zoonotic parasite mainly found in wildlife as a result of environmental contamination with parasites from humans or pets. Higher parasite prevalence in patients is associated with significantly lower levels of digestible energy and a higher level of non-starch polysaccharides (NSPs) in their diets [36]. Dietary NSPs and environmental hygiene appear to be important factors controlling prevalence of *H. pylori* and parasites infection. Food and substances including refined and processed foods such as refined carbohydrates, antibiotics, etc. are known to contribute to bacterial imbalance. Indigenous microbes affect the host responses to non-indigenous parasites i.e. colonization resistance, host immunity and modulation of body function. Many bacteria produce factors that change the motility and surface of host cells so that the parasite is actively taken up by the host cell [37]. It follows that we speculate that infection with *H. pylori* may predispose to infection with *Blastocystis* sp. and *E. histolytica* or vica versa.

Our observational study is limited by small numbers and the possibility that some infections will be asymptomatic, leading to the need for our data to be confirmed in larger numbers.

This work is an advance in biomedical science because it shows a significant association of *H. pylori* with *Blastocystis* sp and *E. histolytica* in patients with diarrhoea.

Summary table

What is known about this subject
- *H. pylori* is a common gastric pathogen
- Numerous intestinal parasites cause diarrhoea

What this study adds
- Blastocystis sp. as the most common protozoal parasite present in adults with diarrhoea.
- A significant association of *H. pylori* with *Blastocystis* sp. and *E. histolytica*.
- No association between *H. pylori* and *E dispar*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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