EFFICACY OF FECAL CALPROTECTIN AS A MARKER FOR THE PATHOGENICITY OF BLASTOCYSTIS HOMINIS

By

AMIRA RAAFAT, IMAN R. ABDEL-SHAFI, MOUSA A. M. ISMAIL*,
And NOHA M. AMIN

Department of Medical Parasitology, Faculty of Medicine, Cairo University, Cairo, Egypt (*Correspondence: dr.mousa.abdelgawad@gmail.com)

Abstract

Fecal calprotectin (f-CP) is a marker of inflammation in intestine, calprotectin is released by white blood cells, macrophages, and epithelial cells.

This study used the f-CP level as a factor to determine the pathogenicity of Blastocystis hominis. The f-CP levels were measured in Blastocystis hominis patients as compared to negative B. hominis ones with gastrointestinal symptoms related to healthy controls.

A total of 235 stool samples were divided into three groups. G1: 88 stool samples of patients with gastrointestinal complaints positive only for Blastocystis hominis. G2: 75 stool samples of patients with gastrointestinal complaints and B. hominis free. G3: 72 stool samples of healthy volunteers, with neither gastrointestinal symptoms nor microbiological infection.

The levels of f-CP were determined by specific ELISA showed that the f-CP concentration was highest in G1 & G2 as compared separately with G3, with significant differences (P < 0.001), but without significant difference between G1 & G2 (p =0.453).

Keywords: Blastocystis hominis, Fecal calprotectin, Gastrointestinal symptoms.

Introduction

Blastocystis hominis is a protozoan that infects the human gastrointestinal tract. The prevalence of Blastocystis varied from 3% to 50% in different countries worldwide. Nevertheless, the parasite is a neglected parasite of the gastrointestinal tract (Al and Hökelek, 2007). However, the presence of Blastocystis hominis in patients without other pathogens causing gastrointestinal symptoms should be considered as a pathogen that causes symptoms. Blastocystis hominis patients commonly experience diarrhea and abdominal pain vomiting or fatigue (Ning et al, 2020). Despite findings that support Blastocystis hominis as a possible pathogen, some doubts remain regarding its pathogenicity (Fouad et al, 2011). However, it's associated with a broad gastrointestinal symptoms were reported (Abou El Naga and Negm, 2001). By proper treating of B. hominis infected patients the symptoms disappeared by cure of intestine was inflammation of mucosa (Ning et al, 2020).

Fecal calprotectin (f-CP) binds calcium and zinc was reported in 30 to 60% of neutrophil cytosol (Chen et al, 2012). F-CP concentrations were measured in bacteria, viruses, parasites, colorectal cancer, and inflammatory bowel diseases of the gastrointestinal, which could be detected in patients' feces (Aykur et al, 2019). Besides, gut diseases, neutrophils migrate into lumen, releasing calprotectin damaging mucosal epithelial cells (Bustinduy et al, 2013).

This study aimed to evaluate fecal calprotectin inflammation levels of Blastocystis hominis in patients with gastrointestinal troubles as compared to patients with gastrointestinal troubles but without B. hominis, also, to evaluate f-CP of B. hominis potential pathogenicity.

Materials and Methods

The participants (235) were divided in three groups. G1: 88 patients with gastrointestinal symptoms and only infected with Blastocystis hominis. G2: 75 patients with gastrointestinal symptoms and B. hominis free. G3: 72 health cross-matched volunteers. Medical sheets were filled out on each one.

Patients’ classification was based on the morning stool examined of stained smears (El Saftawy et al, 2019). Enhancement of growth of Blastocystis was done by Jones’ medium using 10% horse serum (Irikov et al, 2009).

Fecal calprotectin was ELISA evaluated (Drg: Hybridx1 Calprotectin Kit) in feces
200 μg/g was positive (Radin et al., 2014).

Statistical analysis: Data were computerized and analyzed by the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Comparisons between quantitative variables were done by using non-parametric Kruskal-Wallis & Mann-Whitney tests (Chan, 2003). Exact test was used instead when expected frequency was < 5 P-values less than 0.05 were considered as statistically significant.

Ethical Statement: Ethical approval was obtained from the ethical committee of Faculty of Medicine, Cairo University and its consecutive adjustments and analogous ethical standards. All procedures related to the human samples were covenant with the ethical standards settled by the National Research Committee and the 2000 Helsinki Declaration.

Results

The participants were <20 years old, >20-40 years old and > 40 years old, with the f-CP level was high in G1 & G22 than control, highest level was among age group 20-40 of G1, but with significant differences (p < 0.01). But, no significant difference between ages in same group (p > 0.05). The gastrointestinal symptoms in G1 were diarrhea, abdominal pain and weight loss (45.5%, 18.2% &18.2%), followed by vomiting and fever (17% & 13.6%) respectively. Symptoms in G2 were diarrhea, abdominal pain and fever (45.3%, 18.7% & 17.3%), followed by vomiting and weight loss (16% & 16%) respectively. The f-CP concentration was highest in G1. F-CP levels of G1 & G2 when compared separately with control showed significant (p < 0.001), but without difference between G1 & G2 (p =0.453). F-CP patients with clinical data were not significant except for abdominal pain and fever.

The results were shown in tables (1, 2, 3, 4 & 5) as well as figures (1 & 2)

Discussion

Generally, the B. hominis is one of the common zoonotic intestinal parasite (Windsor et al., 2005), of worldwide distribution (Stensvold, 2013). Blastocystis consists of several species, living in the gastrointestinal tracts of species as diverse as humans, farm animals, birds, rodents, reptiles, amphibians, fish, and even cockroaches (Yoshikawa et al., 2007). Fecal-oral transmission is the most accepted pathway, and recent studies have shown that transmission involves only the
cyst form of the parasite (Yoshikawa et al, 2004). The extent to which human-human, human-animal, and animal-human transmission occurred was still unknown, but genomic studies gave evidence for all three routes, though experimental studies have yet to provide conclusive proof for the existence of either (Yoshikawa et al, 2000). Infections with this parasite are spread worldwide and it is frequently the most commonly isolated protozoan in parasitological surveys (Chandramathi et al, 2010). In developing countries, *B. hominis* was more predominant (30 to 50%) as compared to (1.5 to 10%) in developed countries (Li et al, 2007).

In the present study, the main clinical manifestations in a descending order were diarrhea, abdominal pain, weight loss, vomiting and fever among *B. hominis* patients as compared to less severity of diarrhea, abdominal pain, fever, vomiting, and weight loss among *B. hominis*-free patients. Ok et al. (1999) reported that the pathogenicity of *B. hominis* occurred in patients without or with gastrointestinal symptoms. Yakoob et al. (2004) reported that *B. hominis* caused irritable bowel syndrome. Boorum et al. (2008) found that *B. hominis* was the commonest protozoa in patients presenting with gastrointestinal symptoms. Laodim et al. (2012) reported that abdominal pain was the most frequent symptom in *B. hominis* patients followed by diarrhea. Also, Ibrahim et al. (2020) reported that *Bastocystis* patients complained of altered bowel habits with the predominance of diarrhea.

In the present study, there was no significant difference between male and females blastocystosis patients. Dagci et al. (2014) stated that blastocystosis in males and females was more or less in equal percentage. Yunus et al. (2015) reported that blastocystosis was 0.56% in females and 0.53% in males, but without a risk factor. Also, Ibrahim et al. (2020) reported infection of 39% males and 61% females, without significant difference as to ages or sexes. In the present study, among the three different groups, the f-CP level was high in blastocystosis patients and blastocystosis-free patients than control. The highest level was in age group 20-40 in blastocystosis patients. The f-CP levels were significant between blastocystosis patients and blastocystosis-free patients than control in all age groups (p<0.01), without significant difference among ages of same group (p > 0.05). But, f-CP levels were no significant among symptomatic and healthy ones (p < 0.01). This agreed with Aykur et al. (2020) who didn’t find significant difference among age groups of same group (p > 0.05).

In the present study, the f-CP levels were highest among *B. hominis* patients with gastrointestinal symptoms than patients with gastrointestinal symptoms, without significant difference (p =0.453). Yahya et al. (2017) found f-CP was (3.22%) positive in stools of *Blastocystis hominis* patients compared to (41.75%) *Entamoeba histolytica* patients, followed by (21.27%) in *Giardia lamblia* patients (P<0.05). Lam et al. (2014) found a significant positive correlation between gastrointestinal pathogens and f-CP levels (P<0.0001). Also, Rady et al. (2019) reported fecal calprotectin significant association (p<0.05) with *Cryptosporidium parvum* (52.63%), and *G. lamblia* (45.45%). No doubt, Calprotectin a 36-kDa zinc and calcium-binding protein of about 60% as dissolved in cytoplasm of neutrophils, which was measured in several body fluids, such as the urine, plasma, and cerebrospinal fluid, and tissue samples and feces (McMahon and Chhabra, 2018). Also, the non-invasive usage of f-CP was a stool marker to monitor inflammation (Rutgeerts et al, 2012, Caimmi et al, 2018), and for mucosal inflammatory activity in IBD (Wei, 2016). Kostakis et al. (2013) reported that the f-CP levels of patients with gastrointestinal disorders were higher than healthy controls. Aykur et al. (2020) found that the most common gastrointestinal symptoms in groups of patients with gastrointestinal symptoms were diarrhea and abdominal pain. They in contrast found statistically significant difference in f-CP levels in the groups presented with gastrointestinal symptoms such as diarrhea, abdominal pain and weight loss,
when compared with healthy controls (p < 0.0001).

In the present study, *B. hominis* infected patients showed the highest value of f-CP indicating an inflammation of the mucosa of the gastrointestinal tract, supporting the potential pathogenicity of *Blastocystis hominis*. Lam *et al.* (2014) reported a substantial correlation between the occurrence of intestinal pathogens and f-CP levels (P= 0.0001), and that f-CP levels were higher in patients with positive intestinal pathogens than those without. Also, Abu El-Fetouh *et al.* (2021) found that colonic mucosa of experimentally infected Albino rats a week after *B. hominis* infection showed focal atrophy with unusual inflammatory cells, with aggregates of eosinophils and parasites present in the colon.

**Conclusion**

Using fecal calprotectin clarified the pathogenicity of *Blastocystis hominis* as a reliable indicator of inflammation in patients’ intestines. The f-CP can be a good marker for pathogenicity of *Blastocystis hominis*.

**Authors’ contributions:** All authors equally contributed in this work.

**Conflicts of interest:** Authors declared that they have neither conflict of interests nor received fund.

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**References**


Explanation of figures
Fig. 1: Evaluation of fecal calprotectin levels by age groups.
Fig. 2: Relationship of fecal calprotectin values among groups.
Fig. 3: B. hominis life cycle after Tan KS, 2004: Blastocystis in humans and animals: new insights using modern methodologies. Vet. Parasitol. 126, 1/2:121-44

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