EUROPEAN ACADEMIC RESEARCH
Vol. VI, Issue 4/ July 2018

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Diagnosis and Following up the treatment of Acute Blastocystosis

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Abstract:

Blastocystis is a unicellular parasite that is the only member of the Stramenopila associated with human pathological changes and it is increasingly recognized as a potential cause of diarrhoea. Aims of this case report were to diagnose, treat and follow up treatment of un acute enteritis. A sixty four old, healthy male ate an uncooked food as lunch meal and several days after he got watery diarrhea, abdominal

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bloating and pain. Thereafter, the parasitological examination diagnosed the case as blastocystosis. The patient was treated with metronidazole for 10 days. The symptoms like diarrhea and abdominal pain disappeared after three days of treatment. In order to investigate effect of the treatment, numbers of Blastocystis cells were counted after 10 days-treatment at different time intervals 1, 10, 20, and 30 days, were found to be 10, 5, 2 and zero cells, respectively. The findings might suggest that pathogenic and apathogenic different subtypes of Blastocystis were found in the patient sample. The enteritis was related to the pathogenic subtypes that responded to metronidazole and the diarrhoea consequently disappeared.

Key words: acute enteritis, diarrhoea, microscopy, Blastocystis, metronidazole

INTRODUCTION

Blastocystis hominis is a microscopic organism that inhabits the intestine and is sometimes found in the stools of healthy people as well as in the stools of those who have diarrhoea, abdominal pain or other gastrointestinal problems and the infection with *Blastocystis* is called blastocystosis[1].

Life cycle of this parasite consists of vacuolar forms giving origin to multi vacuolar and amoeboid forms. The multi vacuolar form develops to thin-walled cyst that is thought to be responsible for autoinfection. The amoeboid form develops to thick-walled cyst that is excreted in stool and it is believed to be responsible for external transmission, possibly by the fecal-oral route through ingestion of contaminated water or food. The cysts infect epithelial cells of the digestive tract and multiply asexually[2].

This unicellular parasite was first described as yeast and named by Alexieff to *Blastocystis enterocola* in 1911[3] and was renamed to *Blastocystis hominis* by Brumpt 1912[4]. Thereafter, Zierdt et al. 1967 gave morphological and physiological evidence considering *B. hominis* as a protozoan and he placed it in the subphylum Sporozoa[5].

Major revisions were made to classification of Blastocystis by analysis of small-subunit rRNA gene sequences in 1996, which placed Blastocystis in Stramenopiles[6, 7, 8]. Thus, Blastocystis is a genus of unicellular parasites belong unranked Superphylum Heterokonta (Stramenopila); Class: Blastocystae; Order: Blastocystida; Family: Blastocystidae; Genus: Blastocystis; Species: *Blastocystis* species. Subtype nn[7, 9]. Blastocystis is the only member of the Stramenopila associated with human pathological changes[10, 11], and it is suggested that the Blastocystis genus is a species complex comprising 01 to 17 subtypes (STs) at least 9 of which are found in humans[12, 13].

Despite reports in the early 1900s of a possible pathogenic role for *B. hominis* were largely ignored, and it was generally considered non-pathogenic intestinal yeast[14] , there have been many reports, supporting a role for *B. hominis* as a potential pathogen in humans[15, 5, 16, 17, 18, 19, 20, 21, 22, 23, 24]. Thereafter, Blastocystis species is increasingly recognized as a potential cause of diarrhoea[14, 25, 26, 27] and a significant high concentration of Blastocystis cells was found in symptomatic patients than asymptomatic ones[28]. In addition, the infection of *B. hominis* was found to be significantly more in summer than in winter over a three year period study[28], and prevalence of this parasite is usually higher in developing countries, ranging from 30% to 50%, and 1.5% to 15% in developed countries[10, 29]. In the other side, it was reviewed that the incidence of *B. hominis* was first and far in excess of more commonly incriminated parasites such as *Dientamoeba fragilis*, *Giardia intestinalis*, *Entamoeba histolytica/dispar* and *Cryptosporidium parvum*[27].

Many articles and reviews confirmed that *B. hominis* might be a cause of intestinal disease[26, 25, 28]. In this context, the most common symptoms with stools positive only for *B. hominis* were diarrhoea, abdominal pain, flatulence and vomiting[26, 25, 28], and patients with *B. hominis* responded to metronidazole and were fully cured[28, 25].

Aims of this case report were to diagnose, treat and follow up treatment of an acute blastocystosis.

MATERIAL AND METHOD

Case presentation

A sixty four old, healthy male ate an uncooked food as lunch meal in July 2015. The meal consisted of a cold sandwich (cheese and raw vegetables enriched with sauce). Several days after, the patient got watery diarrhea, abdominal bloating and pain. The patient examined his own parasitological stool preparations.

Parasites identification and methods of examination

The stool samples were prepared by the formalin-ethyl acetate concentration method. Iodine wet mounts of concentrated stool samples were examined microscopically for demonstrating trophozoites and cysts of protozoa and ova or larvae of helminths[30].

Identification of the detected parasites was based on microscopic morphology that was compared with those in standard texts, literature and micrographs according to Centers of Disease Control and Prevention, standard methods for diagnosis of intestinal parasites[30].

RESULTS

Identification, treatment and following up the treatment

Laboratory investigation by utilizing the formalin-ethyl acetate concentration method, light microscopy and depending on the morphology of found parasites, uncovered that the prepared stool samples from the patient before treatment were overwhelmed by Blastocystis cells. These eukaryotic cells have round, oval, or ellipsoidal shapes measuring 5-30 μm in diameter with usual range of 8-10 μm. The cell contains large central body surrounded by a thin rim of cytoplasm containing up to six nuclei as described by reference[30].

The detected Blastocystis cells found in different seizes (anisocytosis) and the small seize cells were the dominant. Before treatment, the mean number of Blastocystis cells per high power filed (HPF) calculated from 10 fields was 70 ± 7, the minimal and maximal numbers were 60 and 80 cells/HPF.

The patient was treated with metronidazole for 10 days. The symptoms like diarrhea and abdominal pain disappeared after three days of treatment.

In order to investigate effect of the treatment, numbers of Blastocystis cells were counted after 10 days-treatment at different time intervals 1, 10, 20, 30 days, were found to be 10, 5, 2 and zero cells, respectively (Figures 1, 2, 3).

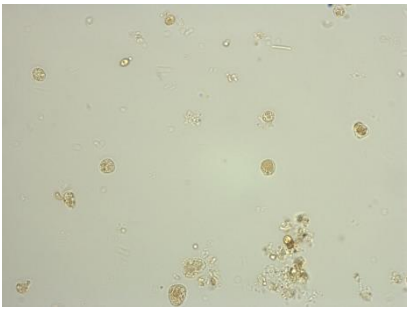

Figure1: One day after 10- days' treatment showing 10 Blastocystis cells per HPF

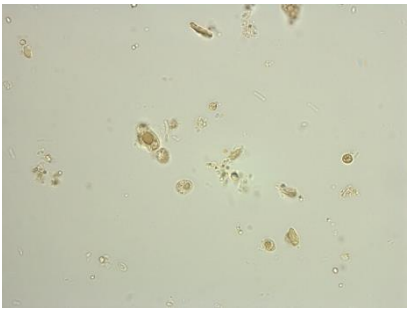

Figure 2: Ten days after 10- days' treatment showing 5 Blastocystis cells per HPF

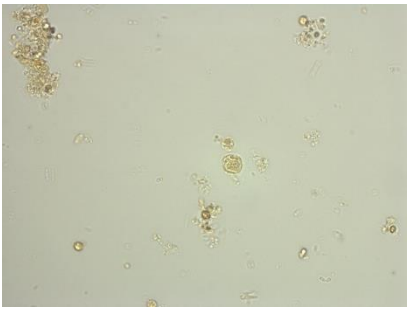

Figure 3: Twenty days after 10- days' treatment showing 2 Blastocystis cells per HPF

The statistical analysis showed that the number of Blastocystis cells was negatively correlated with the treatment and with the 20 days-post treatment time as well, according to the regression equation (y = -14,8x + 61,8) and coefficient factor (R^2 = 0,6231) (Figure 4).

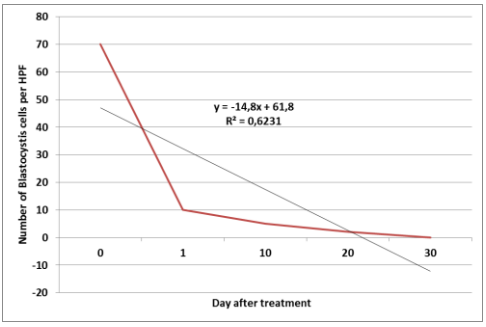

Figure 4: relationship between numbers of Blastocystis, metronidazole's affect and post treatment time

DISCUSSION

Enteritis, which is an inflammation of the small intestine, is most commonly caused by food or drink contaminated with pathogenic microbes[31]. Blastocystis species is a microscopic organism that inhabits the intestine and is sometimes found in the stools of healthy people as well as in the stools of those who have diarrhea and abdominal pain[1].

Interest in Blastocystis species has significantly grown in recent years, due to its potential role as a human pathogen[32].The prevalence of Blastocystosis varies depending on the study setting, reaching the highest percentages (68–100 %) among children from developing countries[33, 34]. Moreover, a case report about a colonic ulcer caused by *B. hominis* has published and mentioned that *B. hominis* is a pathogen to bear in mind when large colonic ulcers are diagnosed, especially in patients with a travel history and diarrhoea[35].

The present case study detected Blastocystis cells in stool sample from a patient with diarrhea by microscopy and found that symptoms and number of Blastocystis cells as well, were negatively correlated with the treatment by metronidazole.

There are many reports about number of *B. hominis* seen per high-power field (x400 magnification) and their correlation with

symptomatic disease. An empirical figure, five or more per high-power field, correlates with the presence of symptoms associated with blastocystosis[3, 19]. Kain et al., 1987, however, presented convincing data that patients with ≤ 5 *B. hominis* per oil immersion field (OIF; x1000) expressed symptoms as often as those with ≥ 5 *B. hominis* per OIF[19]. This classic well-controlled work is the definitive modern study of *B. hominis* infections[36].

Despite being discovered more than 100 years ago[37, 38, 39], it is difficult to argue the clinical significance and pathogenic potential of Blastocystis[39], since infections do not consistently lead to intestinal symptoms[40, 41, 42]. While a large number of infected individuals presents with clinical symptoms[40, 43], asymptomatic carriage of the parasite is also common[44]. Moreover, in symptomatic patients, the duration and severity of symptoms vary from acute enteritis to chronic and mild diarrhea[42, 45].There is no consensus on the reasons for the observed diverse intestinal symptoms. A number of reports have suggested a strain- or subtype-dependent variation in parasite pathogenicity[36, 42, 45, 46, 47, 48, 49].

Studies associate Blastocystis subtypes 1, 4 and 7 with pathological alterations in humans, whereas subtypes 2 and 3 are considered apathogenic[40, 42, 46]. Also, the presence of both pathogenic and apathogenic strains within one subtype has been reported[50, 51]. Another issue complicating the pathogenic potential of Blastocystis is reports of treatment failure[43, 45, 52, 53, 54, 55]. Although metronidazole is the treatment of choice, physicians are often skeptical about prescribing antibiotics for Blastocystis infections due to frequent reports of non-responsiveness to chemotherapy[42]. Strain-to-strain variation within Blastocystis in susceptibility to the metronidazole and other antiparasitic agents among Blastocystis strains is commonly reported[56, 57], and has been proposed to be the reason for frequent treatment failures in parasite infections[43, 56].

Recently, Blastocystis research Foundation reviewed 174 studies positive for Blastocystis species and found that these parasites were pathogenic in 157 studies and non-pathogenic in 17 studies[58].We utilised $\chi 2$-statistical analysis and found that number of studies reviling Blastocystis as pathogenic parasite is very highly significant than that number of non-pathogenic ($p < 0.0001$).

Finally, we observed in our case that the diarrhea and abdominal pain disappeared after three days of treatment. It was found also that 60 out of 70 Blastocystis cells (86%) were disappeared under 10 days treatment. These finding might suggest that pathogenic and nonpathogenic different subtypes in the population of Blastocystis were found in the patient sample. The pathogenic subtypes (86%) of the population were eliminated by metronidazole under treatment time compared to the nonpathogenic subtypes that were eliminated within 20 days after treatment. In this context, our statistical analysis was negatively correlated the number of Blastocystis cells with the treatment and with the 20 days-post treatment time as well according to the regression equation and coefficient factor, as shown in result figures 1-4. These findings might suggest that different subtypes will respond differently to the treatment.

CONCLUSIONS

Our findings confirmed that Blastocystis microorganism was the causative agent of this enteritis case. The findings might suggest that pathogenic and apathogenic different subtypes of Blastocystis were found in the patient sample. The blastocystosis was related to the pathogenic subtypes that responded to metronidazole and the diarrhoea consequently disappeared.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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