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ORIGINAL ARTICLE

The hidden impact of different *Blastocystis* genotypes on C-3 and IgE serum levels: a matter of debate in asthmatic Egyptian children

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Abstract *Blastocystis hominis* is highly prevalent with respiratory allergies among Egyptian children. Yet, little is known about the possible immunological relationship. Aims of this study were to measure complement-3 (C-3), total and specific IgE to intestinal allergens in patients' serum regarding the identified *B. hominis* genotypes. In a cross-sectional study, three hundred children (150 asthmatics and 150 non asthmatics) participated in the study from both sexes, mean age 7.5 ± SD (3–4) years after a questionnaire administration. PCR-based genotyping of *B. hominis* selective in vitro cultivation was performed. C-3, total and specific IgE were all measured in patients' serum utilizing ELISA. Blastocystosis was detected in 100 out of 300 children, 65 (43.3%) out of 150 asthmatics and 35 (23.3%) out of 150 non-asthmatics. Vacuolar forms were the most prevalent in both direct wet mount and stool cultures. Forty (61.5%) out 65 asthmatics and 5 (14.2%) out of 35 non-asthmatics were ≥ 5 organisms/HPF. Sex and irritable bowel disease were statistically insignificant (p value < 0.05). Urticaria was coincided in 15.4% of asthmatics and 8.6% of non-asthmatics. Of 100 cases of blastocystosis, eighty-four were genotype-3 and sixteen were genotype-4. Out of these, 55 cases of genotype-3 and 6 cases of genotype-4 were asthmatics. Positive C-3 serum levels were in 46 (54.81%) of genotype-3 and 2 (12.5%) of genotype-4. High total IgE levels in 30 (35.7%) out of 84 cases of genotype-3 and 4 (25%) out of 16 cases of genotype-4. Positive specific IgE was in 25 (29.8%) of genotype-3 and 3 (18.75%) of genotype-4. Genotype-3 was of higher infection intensity (p value = 0.0001). In conclusion, *B. hominis* possess a hidden allergy triggering impact that can be obscured by simultaneous high (total and specific) IgE levels towards specific common intestinal allergens. Blastocystosis induces allergy by increasing C-3 serum levels in a genotype-dependent manner being higher in genotype-3. Virulence of genotype-3 seems to stand beyond increased parasite intensity and wide absorption of intestinal allergens that indirectly elevate IgE serum levels.

Keywords *Blastocystis hominis* · Allergies · Genotype-3 · Genotype-4 · C-3 · IgE

Introduction

Blastocystis hominis is an enteric protozoan infection which affects both humans and animals, that has been listed by the World Health Organization as one of the neglected diseases (Khademvatan et al. 2017). The incidence of this infection has been assessed to reach up to 280 million infections per year, 3–6% in developed countries and 20–50% in developing nations especially those in tropical and subtropical regions (Menounos et al. 2008). Higher disease percentages had been detected in communities with more animal contact, contamination of food and water, and lack of sanitation being related to fecal–oral transmission (Di Prisco et al. 1998).

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The genetic subtype is a deep influencing factor in the virulence of *Blastocystis* spp. (Hussein et al. 2008). Previous studies on the SSU rRNA gene revealed the presence of 17 genotypes of *Blastocystis* spp. (Stark et al. 2007; Tan 2008; Robertson et al. 2010), which display different host specificity and the wide range of clinical manifestations ranging from the asymptomatic carrier, acute gastrointestinal disturbance or chronic gastroenteritis with a period more than 2 weeks (Mohamed et al. 2017; Gonzalez-Arenas et al. 2018). Allergic asthma constitutes 60% among other types of asthma (Geiger et al. 2002). Pathogenesis of asthma encompasses mucosal edema of the small bronchioles; thick mucus secretions, spasm of smooth bronchiolar muscles, increased airway resistance, which is much higher during expiration and would increase intrapulmonary pressure (Van Bever et al. 2004). Continuous hypoxia and air hunger in young age group would affect ATP production by distressing normal Krebs cycle with subsequent cellular death, depressed mental activity and reduced muscular capacity (Ahmad Al Obaidi et al. 2008).

There has been an escalating number of studies inspecting the causal associations between blastocystosis and allergic manifestations (Clark 1997) owing to type 1 hypersensitivity reactions in children (Hennino et al. 2006) in comparison to studying groups liberated from this protozoan infection (Bakiri and Mingomataj 2010; Hameed et al. 2011). Moreover, *B. hominis* infection was concluded as an etiology for resistance against ordinary therapeutic regimens in allergic reactions of unknown etiology that would be justified by prescribing anti-protozoan medication (Rossignol 2010).

The aim of the present study was to observe the relationship between different *Blastocystis hominis* genotypes and asthma. In this context, IgE responses (total and specific) towards common intestinal allergens versus C-3 were measured in patients' serum regarding different genotypes in an attempt to study the possible immunological impact of the parasite.

Materials and methods

Patient population

The study was done during the period (December 2017–July 2018). Microscopic examination and *Blastocystis* culture were performed in the Parasitology Department; Cairo University Hospitals, Egypt, which includes a large outpatient laboratory unite and processes more than 27,000 stool samples per year. Throughout the study period, a total of 300 children (150 having bronchial asthma and 150 were non-asthmatic controls) were referred from the outpatient clinics of Children's Hospital, Cairo University. Asthma was diagnosed by a specialized pediatrician according to the workshop on the Global Strategy for Asthma "The National Heart Blood and Lung Institute/World Health Organization (NHLBI/WHO)". Asthmatic children were under control of their anti-asthmatic drugs during the study. Informed consent was obtained from the patients or their parents to participate in the study and respond to the study questionnaire as shown in Fig. 1.

The questionnaire was inquired for the clinical data and a follow-up stool examination. Analysis of the collected data was then performed. Patients who were included in the study didn't perceive any anti-parasitic medication in the previous 12 months and agreed to provide the research team with three fecal samples on alternate days. Patients were solely infected with *B. hominis*. Patients with current respiratory tract infections e.g. common cold, influenza, tonsillitis, ear, or sinus infections, bronchitis, and pneumonia were excluded from the study.

Stool collection

Stool specimens from each patient were collected in a clean stool cup. The stool samples were then transported to the Medical Parasitology lab.

Parasitological examination of stool analysis

All specimens were screened for *B. hominis* by direct and formalin-ether concentration method. The microscopic data from the iodine stain comprised recording parasitic forms and the number of parasites per high-power field (400 ×) to determine the infection intensity (Hoffman et al. 1934).

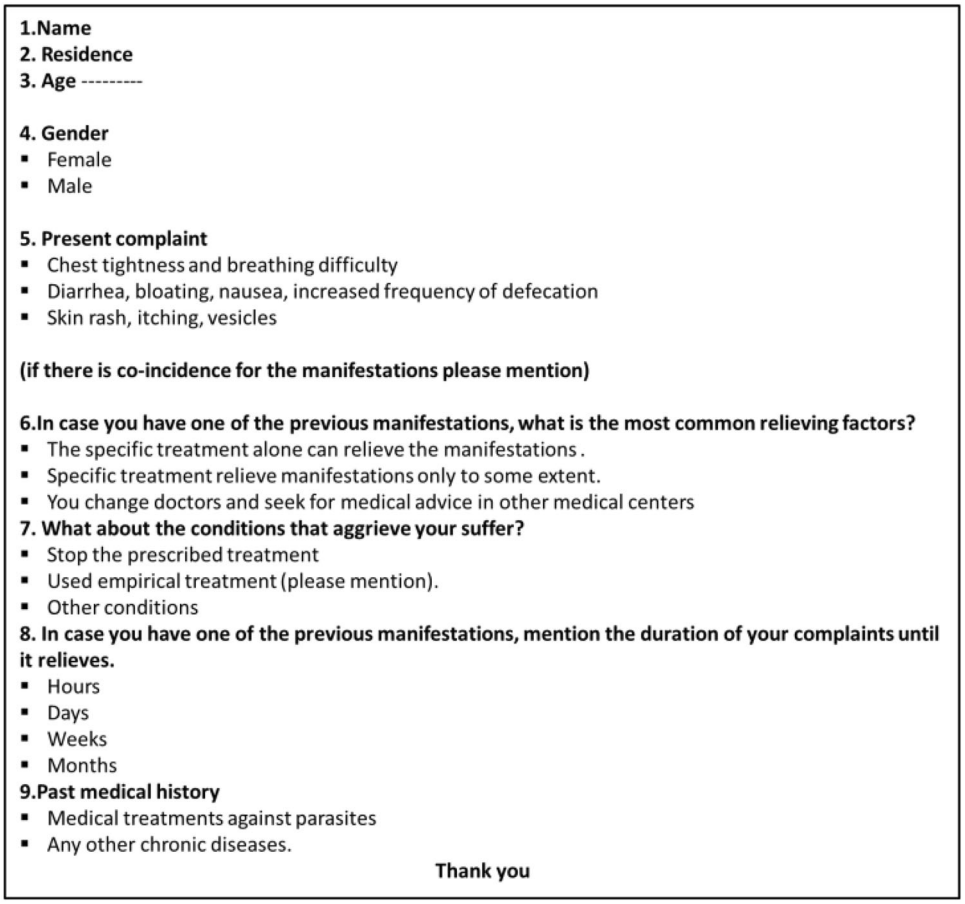
Microbiological examination of stool samples

Stool specimens were transported to the Bacteriology Unite, Clinical Pathology Department, Kasralainy School of Medicine via Remel Cary Blair transport medium. Bacterial analyses were accomplished using standard culture techniques to exclude *Shigella* spp., *Salmonella* spp., *Campylobacter jejuni*, *Aeromonas* spp. and *Yersinia enterocolitica* (Old and Duguid 1970).

Endemic viral infections, *Adenovirus* and *Rotavirus*, were ruled out using (CORIS-BioConcept-Adeno-Strip-Catalogue no. # C1002) and (CORIS-BioConcept-Rota-Strip-Catalogue no. # C1001) kits respectively. The test was performed by rapid dipstick test to exclude endemic viral infections in watery and loose stool.

Selective culture for *Blastocystis hominis*

Establishment of *Blastocystis* growth in vitro was done to enhance the efficacy of the subsequent molecular amplification and genotyping of the organism (Vennila et al.



1.Name
2. Residence
3. Age ---------

4. Gender
- Female
- Male

5. Present complaint
- Chest tightness and breathing difficulty
- Diarrhea, bloating, nausea, increased frequency of defecation
- Skin rash, itching, vesicles

(if there is co-incidence for the manifestations please mention)

6.In case you have one of the previous manifestations, what is the most common relieving factors?
- The specific treatment alone can relieve the manifestations .
- Specific treatment relieve manifestations only to some extent.
- You change doctors and seek for medical advice in other medical centers

7. What about the conditions that aggrieve your suffer?
- Stop the prescribed treatment
- Used empirical treatment (please mention).
- Other conditions

8. In case you have one of the previous manifestations, mention the duration of your complaints until it relieves.
- Hours
- Days
- Weeks
- Months

9.Past medical history
- Medical treatments against parasites
- Any other chronic diseases.

Thank you

Fig. 1 Questionnaire. Data collection was performed via a tailored questionnaire for subjective evaluation of the current clinical condition

1999). 10 g of positive stool samples were seeded in Jones' medium supplemented by 10% horse serum. These axenic cultures were incubated under anaerobic conditions at 37 °C for 48 h. The sediment between the basal residue and the liquid interface of the culture was examined on a 40 × objective to check for the growth of the parasite (Irikov et al. 2009). The determined inoculum was obtained and kept at − 20 °C for further molecular amplification (Suresh and Smith 2004).

Molecular characterization of *Blastocystis* isolates

The products of *Blastocystis* selective culture were processed for PCR assay according to Stensvold (2013) using Blas-F: (GGA GGT AGT GAC AAT AAA TC) and Blas-R: (ACT AGG AAT TCC TCG TTC ATG) primers. The target of SSU rRNA gene after genomic DNA extraction was performed using Favor Prep-stool DNA isolation Mini Kit (Favorgen Biotech corporation Ping-Tung 908, Taiwan, Cat. No. FASTI001).

The A260/A280 ratio was used to test for DNA purification and concentration. In an attempt to improve PCR, primers were titrated using primer concentration 50, 100, 200 and 400 nM. Each titer has its annealing gradient temperature ranging from (45 to 60 °C) and its DNA template concentration (1, 3, 5 and 7 µl) according to Khademvatan et al. (2017).

Regarding the optimized PCR conditions the following components were used: PCR master mix 12.5 µl, reverse primer 1 µl, forward primer 1 µl, template DNA 2.5 µl, Taq polymerase 0.5 µl and sterile distilled water to get the final volume to 25 µl according to the manufacturer's instructions. Genotyping of *Blastocystis* was performed using RFLP technique to obtain SSU rRNA gene product 1.1 kbp by *HinfI* enzyme (New England Bio Labs Inc., MA, USA) according to the manufacturer's instructions (Yoshikawa et al. 2009). Digested DNA samples were loaded into wells of a 0.8% agarose gel impregnated with Ethidium Bromide, and an electric field was applied. DNA Fragments were visualized by UV light and measured using a 100-bp. ladder marker.

Serology tests

IgE test

Total IgE was tested by ELISA kit (Cat. # AB108650). Specific IgE was detected serologically as an alternative to

the skin prick test and the intradermal test using ELISA Kit (ABNOVA-Catalog # KA4000). Specific IgE included antigens of some food types; cow's milk, egg whites, peanuts, wheat, and sesame seeds.

C-3 test

C-3 antigens were detected qualitatively by double antibody sandwich ELISA (Abcam, Human Complement C-3 ELISA Kits, Catalog# ab108823).

Infection intensity

The count of *Blastocystis hominis* per 1 g stool (Robertson et al. 2010) was calculated to determine the infection intensity regarding the identified genotypes.

Statistical analysis

The mean value including the standard deviation and Chi square test were used to examine the relationships between every two variables. Results with p values less than or equal to 0.05 were considered statistically significant.

Results

Microscopic examination revealed that the overall detection rate of blastocystosis was 100 (33%) out of 300 children, 65 cases of 150 asthmatic children and 35 cases of 150 non-asthmatic group. In this study, mean age value of the participating children was 7.5 ± SD (3–4) years, 5.5 in asthmatic children and 8.5 in non-asthmatics. Further demographic and clinical data were shown in Table 1.

In spite of the endemicity of geohelminthic infections, there was no interloping from intestinal worm co-infections in the samples collected during this study. In addition, during a comprehensive deworming campaign held by the ministry of health in Egyptian schools, 70 children received a single dose of anti-helminthic treatment. Cases with positive results for bacterial, fungal or viral co-infections were all excluded during collection in the present study.

The vacuolar form was mainly detected in the microscopic examination and Jones' medium. It was characterized by its large central vacuole, a thin peripheral rim of cytoplasm and cell organelles, Fig. 2.

The number of parasites ≥ 5 organisms/HPF was considered statistically significant between both asthmatic and non-asthmatic groups with a p value (0.0016), nevertheless, the number of parasites less than 5 organisms/HPF was statistically insignificant (p value = 0.1336), Table 2.

Results regarding sex and manifestations of irritable bowel disease were statistically insignificant (p value < 0.05) on the other hand co-incidence of urticarial skin disease was obvious in 15.4% of the asthmatic group and 8.6% of non-asthmatic group (p value = 0.0044) (Table 3).

Products of PCR were digested using the restriction enzyme *HinfI* (RFLP analysis) to target gene SSU rRNA 1.1 Kbps. Two *Blastocystis hominis* genetic subtypes were reported in the present study groups, genotype-3 (1100 and 280 bp) and genotype-4 (680, 500, 350 and 270 bp) as shown in Fig. 3.

Infection intensity in genotype-3 ranged from 5.2 × 10 (5) and 8.1 × 10 (5) organisms/g of feces. There was a significant difference (p value = 0.0001) when compared with the genotype-4 with infection intensity ranged from 20 and 100 organisms/g.

Discussion

This study was based on fecal samples positive for *Blastocystis hominis* from Egyptian children in which the prevalence of infections was 100 (33%) out of 300 participants. This was similar to the prevalence percentage (33.3%) reported by Zaglool et al. (2012) and Mohamed et al. (2017).

The study findings could be attributed to a range of various factors, including endemicity of blastocystosis, fecal–oral transmission in this young age group and

Table 1 Distributive patterns of gender (male/female), IBS, and other allergic manifestations (urticaria) in both asthmatic and non-asthmatic groups were evaluated using Chi square test (χ^2) and p value statistics

Distributive criteria	Asthmatic n = 65	Non-asthmatic n = 35	Total 100	χ^2	p value	Significance
Male	n = 36 (55.4%)	n = 13 (37.1%)	49	1.13	0.2878	Non-significant
Female	n = 29 (44.6%)	n = 22 (62.9%)	51	0.32	0.57	Non-significant
Irritable bowel syndrome (IBS)	n = 55 (84.6%)	n = 25 (71.4%)	80	1.67	0.1963	Non-significant
Urticaria	n = 10 (15.4%)	n = 3 (8.6%)	13	8.125	0.0044	Significant

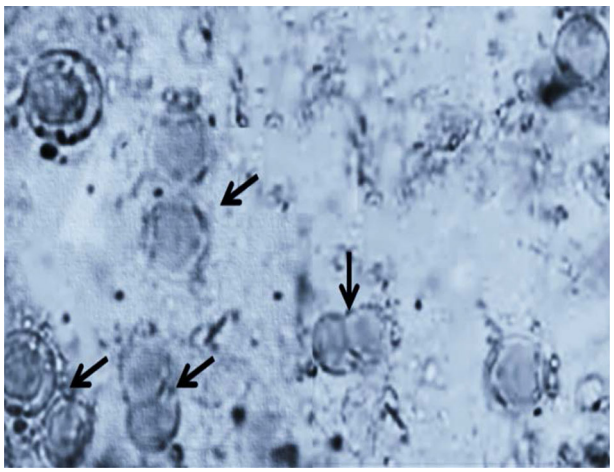


Fig. 2 Photomicrograph. Vacuolar forms of *Blastocystis hominis* during binary fission (arrows) in stool cultures (magnification power, 40 ×)

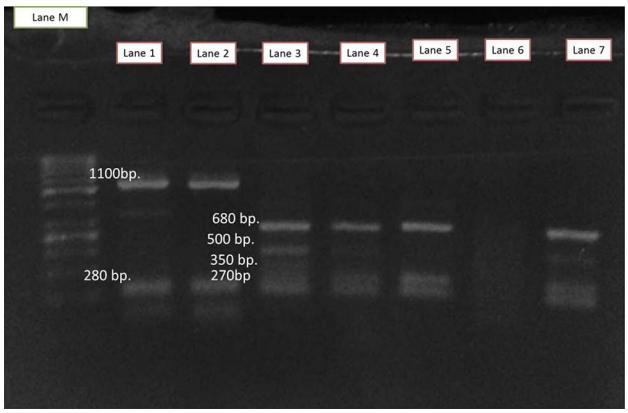


Fig. 3 An agarose gel electrophoresis showing RFLP products after digestion with *Hin*fI. Lane M shows the DNA marker ladder. Lane 1 and 2 show subtype-3 riboprint patterns of *Blastocystis* isolates digestion products at 1100 and 280 bp. Lane 3, 4, 5, and 7 show subtype 4 digestion products at 680, 500, 350 and 270 bp

random use of antimicrobial drugs that may suppress related gastrointestinal manifestations without genuine case treatment (Di Prisco et al. 1998; Northrop-Clewes et al. 2001).

In the present study, there were no statistical significant gender-differences assuming both the asthmatic and non-asthmatic groups. However, previous studies evidenced the overwhelming influence of sex steroids on immune response associating parasitic diseases (Stemeseder et al. 2017).

The incidence of *B. hominis* infections was statistically significant in asthmatic patients (n = 65) and non-asthmatics (n = 35). This finding implicated the further impressive variations in the virulence of various genotypes of the parasite (Puthia et al. 2008). However, previous authors who reported the association of *Blastocystis* infection with allergic diseases (Geiger et al. 2002; Gupta and Parsi 2006; Hameed et al. 2011) conducted their observations only on urticarial diseases (Menounos et al.

Table 2 Number of organisms per HPF in asthmatic and non-asthmatic groups was statistically studied using Chi square test (χ^2), *p* value, and significance determination

Distributive criteria	Asthmatic group	Non-asthmatic group	χ^2	*p* value	Significance
	n = 65	n = 35	8.25	0.0047	Significant
≥ 5 organisms/HPF	n = 40 (61.5%)	n = 5 (14.2%)	10	0.0016	Significant
< 5 organisms/HPF	n = 25 (38.4%)	n = 30 (85.7%)	2.25	0.1336	Non-significant

Table 3 Prevalence patterns of asthma and interactions of C-3, total and specific IgE serum levels in genotype-3 and genotype-4 were statistically studied using Chi square test and *p* value

Distributive items	Subgroups	Genotype-3	Genotype-4	χ^2	*p* value	Significance
		n = 84 (84%)	n = 16 (16%)	5.19	0.0227	Significant
Clinical presentation	Asthmatic	55 (65.47%)	6 (37.5%)	6	0.0143	Significant
	Non-asthmatic	29 (34.5%)	10 (62.5%)	11.31	0.0008	Significant
C-3	Positive	46 (54.8%)	2 (12.5%)	5.4	0.0201	Significant
	Negative	38 (45.2%)	14 (87.5%)	1.98	0.1594	Non-significant
Total IgE (IU/ml)	< 400	30 (35.7%)	4 (25%)	4.101	0.0429	Significant
	>400	54 (64.2%)	12 (75%)	0.471	0.4925	Non-Significant
Specific IgE (IU/ml)	Positive	25 (29.8%)	3 (18.75%)	14.35	0.0002	Significant
	Negative	59 (70.2%)	13 (81.2%)	5.4	0.0115	Significant

2008; Javaherizadeh et al. 2014; Macdougall and Vernon 2017). Yet, in the context of respiratory allergies, the associative role of blastocystosis remains negligible. Moreover, prevalence of irritable bowel disease in both asthmatic and non-asthmatic children infected with *Blastocystis hominis* was found to be statistically insignificant. Regarding this context, there had been wide argument during the past era concerning the pathogenic potential of *Blastocystis* spp. as the organism was identified in both asymptomatic and symptomatic patients (Udkow and Markell 1993). Nevertheless, several recent in vivo and in vitro studies had demonstrated strong suggestions regarding pathogenicity of this protist (Stark et al. 2007; Tan 2008).

Out of 100 patients with *B. hominis* infections included in the present study, the coincidence of urticaria was detected in 10 (15.4%) of the asthmatic group and 3 (8.6%) of the non-asthmatic group. Dermatologists published the statistically significant prevalence rate of urticarial with the protozoa infections, *Giardia intestinalis* and *B. hominis* (Menounos et al. 2008; Rayan et al. 2007). Urticaria could be a consequence of a diversity of pathophysiological mechanisms (Hennino et al. 2006). Various environmental antigens might be allergenic however concomitant exposure to the parasite would augment these allergic reactions by cumulative absorption of these antigens or by modifying mediators' issue (Gupta and Parsi 2006). However, full etiology beyond pathogenesis of urticaria in blastocystosis remained unidentified (Menounos et al. 2008; Javaherizadeh et al. 2014; Macdougall and Vernon 2017).

We observed that seventy children out of 100 who previously received a single dose of anti-helminthic treatment suffered from blastocystosis as a sole enteric infection. Similarly, Northrop-Clewes et al. (2001) noticed that empirical anti-helminthic treatment despite the lower rate of soil-transmitted helminthic infections e.g. *Trichuriasis* and *Ascariasis*, the incidence of intestinal protozoa infections increased. However, there is no obvious description for this observation. It is noteworthy to suggest that these results reflected a successful image for the wide school-based deworming campaign held by the Egyptian ministry since 2016, that targeted more than 2 million children for better nutritional and growth status.

Vacuolar forms were the most dominant in direct and stool cultures. Prevalence of ≥ 5 organisms/HPF during direct microscopic examination of stool samples was statistically significant in 40 (61.5%) out of 65 in the asthmatic group and 5 (14.2%) out of 35 in the non-asthmatic group. Previous studies revealed the major role of central vacuole in the pathogenesis of *Blastocystis* spp. Puthia et al. (2008) stated that it might act as a storage organelle for cysteine proteases. Besides, other authors suggested that the existence of apoptotic bodies in the parasite's vacuole and stated its probable role in the programmed cell death of the parasite. Other reports mentioned the role of the central vacuole in the parasite's reproduction in a schizogony-like process (Udkow and Markell 1993; Nagel et al. 2015). Contents of protease enzymes are unique virulence factors for *Blastocystis* spp. that facilitate protein degradation, human tissue invasion, cleavage of human secretory IgA and immune-evasion of the parasite (Gonzalez-Arenas et al. 2018).

Khademvatan et al. (2017) suggested the same findings, however, they stated the poor correlation between various parasite's forms and patients' symptoms. On the contrary, previous authors deduced amoeboid forms to be the only form that adheres to the intestinal epithelial lining and contribute to the pathogenicity of *Blastocystis* spp. (Katsarou-Katsari et al. 2008; Malheiros et al. 2011). Hameed et al. (2011) demonstrated the amoeboid form in 95.2% of patients with blastocystosis.

Genotype-3 was significantly prevalent in 84 children while genotype-4 was detected in 16 out of 100 *Blastocystis hominis* infections in this study. Similarly, Souppart et al. (2010) reported that the most prevalent genetic subtype in 61.90% in Egyptian patients was genotype-3. Hameed et al. (2011) recovered *B. hominis* genotype-3 as the sole genetic subtype in their Egyptian study groups. Analogs to these results, *Blastocystis* genotype-3 was identified in North (30.5%) and West (56%) of Iran (Sardarian et al. 2012; Badparva et al. 2014). Yet, previous studies in Spain, Nepal, and France recognized genotype-4 as the most dominant genotype. Moreover, in China and Brazil genotype-1 was the chief genetic subtype (Yan et al. 2006). Hussein et al. (2008) concluded that genotype-1 was the most virulent, whereas genotype-3 and 6 was comprised of various strains with a wide range of pathogenicity. Genotype-3 had been identified as the only genetic subtype of human origin; hence, it is supposed to be a genuine *B. hominis* infection (Tan 2008).

In the present study, genotype-3 exhibited asthma in fifty-five children while genotype-4 was prevalent in only 6 cases. This statistically significant result assured the pathogenicity of *Blastocystis* genotype-3. Likewise, previous authors conducted genotype-3 in the pathogenesis of dermatological allergic reactions (Katsarou-Katsari et al. 2008; Malheiros et al. 2011). Nevertheless, Shahar et al. (2006) and Vogelberg et al. (2010) linked *Blastocystis* species genotype-2 with the simultaneous occurrence of allergy and gastrointestinal disturbances. Furthermore, Gonzalez-Arenas et al. (2018) and Lee et al. (2012) deduced the high incidence of genotype-4 among symptomatic allergic patients. Interestingly, some authors wondered if such variations in genotype pathogenicity might afflict response to the same therapy in some patients (Rossignol 2010; Stensvold et al. 2008).

Prevalence of positive C-3 serum levels was significantly reported in our study groups. Previous studies advocated the strong association between C-3 fragments of serum levels and the severity of asthma in childhood. Shen et al. (2013) pointed to proteases as immunogenic molecules that enhance the progressive accumulation of an active network formed mainly of the inflammatory cells: neutrophils, eosinophils, and lymphocytes. Neutrophils produce C-3 that in addition to toxins released namely during infections of *B. hominis* genotype-3 were found to activate complement pathway and generate anaphylatoxins; C-3a and C-5a (Javaherizadeh et al. 2014). Subsequently, these complement fragments interact with their specific receptors on mast cells and basophils to trigger the release of histamine, the slow reacting substance of anaphylaxis (SRS-A), eosinophilic chemotactic factor and bradykinin (Van Bever et al. 2004).

This mechanism was explained as "pseudo-allergic reactions" since it was not mediated by IgE (Macdougall and Vernon 2017). Genotype variability of *Blastocystis* spp. affects the extent of the proteolytic activity of these unicellular protists (Geiger et al. 2002; Moosavi et al. 2012; Gonzalez-Arenas et al. 2018). This was assured in the present study where 46 (54.81%) of genotype-3 exhibited positive complement-3 while 2 (12.5%) of genotype-4 recorded positive results.

Even though, high total IgE levels were evidenced in 30 (35.7%) of 84 cases of genotype-3 and 4 (25%) of 16 cases of genotype-4 and these results matched with positive specific IgE in 25 (29.8%) in genotype-3 and 3 (18.75%) in genotype-4. Several epidemiologic findings emphasized the relationship between IgE (total and specific) serum levels and pathogenesis of asthma (Ahmad Al Obaidi et al. 2008). In addition, the amount of parasite per 1 g stool in genotype-3 infections was significantly higher than genotype-4. The previous experimental study revealed the presence of a positive relationship between the intensity of parasite infection and the severity of the inflammatory reactions. The same study reported infiltration of leukocyte cells to injured sites together with lymphocytes hyperplasia to combat the infectious agents (Suresh et al. 2009).

Accordingly, a causal association of allergy in blastocystosis would be attributed to the expedited absorption of various allergens due to the resultant pathological changes in the mucosa of large intestine including hyperemia and edema (Hanevik et al. 2009; Prieto-Lastra et al. 2006). Subsequently, such action would be translated into elevated IgE serum levels (Ahmad Al Obaidi et al. 2008). Besides, previous authors stated that responses of total IgE can be influenced by exposure pattern to various allergens (Fitzsimmons et al. 2007). Allergen-specific IgE was found to be significantly affected by lifestyle, hereditary factors, exposure to smoking, body mass index of the child, and rate of colds recurrence (Gleich et al. 1982; Stemeseder et al. 2017). Previous authors stated that immunotherapy blocks and reduces the total serum IgE level by 36% only (Ahmad Al Obaidi et al. 2008).

Regarding model of luminal parasites, previous studies assured activation of Th2 cytokines, mediated mucosal IgE production, and eosinophil degranulation. Hence, in a process called frustrated phagocytosis, cell membrane permeability of the parasite increases significantly (Yoshikawa et al. 2009; Cooper et al. 2004). Nevertheless, it is worthy to mention that elevations in mucosal IgE due to protozoan infections are much lower than helminthic infections, a hypothesis that liberates blastocystosis from an even effect on the mucosal IgE (Durmaz et al. 1996; Yan et al. 2006). To summarize, our data highlighted the ignored immunological pathway enrolled by *Blastocystis hominis* infections in asthma. *Blastocystosis* was observed to be related dramatically to the upturn of C-3 serum levels. However, this effect is always obscured by the apparent upgraded total and specific IgE serum levels in response to one of the common intestinal allergens. Virulence of genotype-3 was obvious in the increased parasite intensity that seems to affect allergens absorption and thus indirectly elevate their (total and specific) IgE serum levels.

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Author's contribution EAE and NMA contributed together in data collection using questionnaire, stool and serum samples collection, parasitological examination and performance of *Blastocystis hominis* culture, PCR and genotyping of the extracted isolates. EAE performed the ELISA for C3 and total and specific IgE. NMA performed the special bacteriological examinations. DHH and AE supplied the research with the clinical data and referred children to the Medical Parasitology Department. SA was incorporated in the molecular study of the parasite.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights All procedures performed in the study involving human participants were in accordance with the ethical standards of the National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Permission to conduct this study and ethical approval was

received from the Deanship of Higher Education and Scientific Researches, Faculty of Medicine, Cairo University. The present study was conducted only on stool and serum samples of the selected cases.

Informed consent Aims and procedures of the study were explained and parents of all participants assigned informed consent before being enrolled in the study. Patients suffered from cancers were not included. After sample collection, *Blastocystis* infected patients were offered the antiprotozoal drug "metronidazole" with the standard dose of 40 mg/kg body weight.

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