

Study of *Blastocystis hominis* isolates in urticaria: a case–control study

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Summary

Blastocystis hominis is a common intestinal parasite, with a prevalence in developing countries of up to 50%. The aim of this study was to investigate the association of this parasite with urticaria by determining the genotypic isotypes in the Egyptian population. In total, 54 patients with urticaria and 50 controls were enrolled in the study. Stool samples were examined and assessed by PCR. The parasite was detected in a significantly higher number ($P < 0.001$) of the patient group than the control group. There was no significant difference between the patients with acute and those with chronic urticaria ($P = 0.2$). The amoeboid form was found in 60.6% of *Blastocystis*-positive patients with urticaria, but in none of the healthy controls. Subtype 3 was the only isolate found in both the patient and control groups. We recommend treatment for *Blastocystis*-positive patients with urticaria in developing countries. The prevalence is much lower (around 10%) in developed countries, where treatment should only be considered in the absence of other possible causes of urticaria.

Urticaria is a relatively common skin disorder that has been attributed to immunological, non-immunological and idiopathic causes.¹ Only a few case reports have been published about the association of *Blastocystis hominis* infection and urticaria, but there seems to be an indication that pathogenicity depends on the subtype.^{2,3} The prevalence of this parasite is up to 10% in developed countries and up to 50% in developing countries.⁴ Because a clear correlation between *B. hominis* and urticaria is still lacking, and its prevalence is reported to be relatively high in Egypt, at about 33.3%.⁵ we aimed to study the association between the parasite and urticaria in the Egyptian population with and without gastrointestinal symptoms, and to identify the prevalent genetic subtype.

Report

The study was approved by the local study ethics committee, and written informed consent was obtained from all participants.

In total, 54 patients with urticaria (31 female, 23 male; mean $\pm$ SD age 33.1 ± 15.6 , range 3–65), attending the dermatology outpatient clinic at Ain Shams University Hospital, were enrolled in the study. Patients with a personal or family history of asthma, nasal allergy or hypersensitivity to particular foods or drugs; with a history of drug intake within 2 weeks before appearance of the skin lesions; with fever, arthralgia, insect bites, or history of recent travel; and/or who kept pets were excluded.

The control group consisted of 50 people attending the same clinic for different reasons, who were matched for age, gender and socioeconomic group.

The urticaria group was classified into two subgroups: subgroup A comprised 18 patients with acute urticaria (weals present for < 6 weeks) and subgroup B comprised 36 patients with chronic idiopathic urticaria (weals usually present for > 6 weeks).¹

A complete history was taken from all subjects, who then underwent general and dermatological

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examination, routine laboratory tests, and radioallergo-sorbent test (RAST) to identify any underlying cause for the urticaria. Investigations for a possible underlying internal disorder (hepatic, renal, autoimmune or endocrinological) were also performed.

B. hominis isolates obtained from the stools were grown in culture using a biphasic egg-slant medium overlaid with Locke solution and inactivated horse serum. The cultures were then subcultured for an additional 2–3 days. The parasite was purified by density-gradient centrifugation, and then examined under microscopy at $\times 1000$ magnification. The pellet was stored at -70°C .

DNA was purified from cultured *B. hominis* (QIA-amp® Stool Mini Kit; Qiagen Co. GmbH, D-40724, Hilden, Germany). Amplification of DNA was carried out with the primers shown in Table 1. The amplification products were digested with three restriction enzymes (*AluI*, *HinfI* and *RsaI*; Promega Corp., Madison, WI, USA), separated by electrophoresis in 2% agarose gels, and analysed.

Statistical analysis was performed using SPSS for Windows (version 10; SPSS Inc., Chicago, IL, USA). The Student *t*-test was used for quantitative variables, and the χ^2 test for categorical variables. Data are expressed as mean $\pm$ standard deviation or percentage. $P \leq 0.05$ was considered significant.

Of the 54 patients, 33 (61.1%) were positive for *B. hominis*, whereas only 4 of the 50 controls (8%) were positive, which was a significant difference ($P < 0.001$). The amoeboid form was found in 60.6% of *B. hominis*-

positive patients with urticaria, but in none of the controls, which was also significant (Table 2). All the *B. hominis* isolates, in both patients and controls, were subtype 3 (Fig. 1). There was no significant difference between the two subgroups, A and B ($P > 0.05$) for any of the tested parameters (Table 3).

The significantly higher incidence ($P < 0.001$) of the parasite in the patient group compared with the control group supports previous reports concerning the possible role of this parasite in the pathogenesis of urticaria.^{2,3} The insignificant difference between subgroups A and B regarding the presence of the amoeboid form reflects its association with both types of urticaria, in contrast to the report of Katsarou-Katsari *et al.*³

In this study, subtype 3 was the only isolate in both patients and controls, suggesting the presence of other factors influencing the pathogenicity of the parasite, such as the immune status of the host, the parasite load, the presence of local intestinal disease, or factors affecting parasite behaviour. The subtypes isolated may vary between different areas, and may also be subject to climatic and seasonal variations, and differences in the source of infection.

Yan *et al.*⁶ speculated that subtype 3 might contribute to the pathogenic potential of *Blastocystis* only when the amoeboid form is present. However, we found the amoeboid form in only three-fifths (60.6%) of our *B. hominis*-positive patients with urticaria, indicating the pathogenic potential of the parasite, whether in its amoeboid form or not. It has been suggested that the amoeboid form might adhere better to the epithelial cells lining the gut, affecting gut immune homeostasis⁷ and resulting in an immune response against antigens on the surface of the parasite, with consequent inflammatory-cell recruitment. Histamine-releasing factors could then be released by immigrant cells, activating mast cells and basophils, and leading to short-lived urticaria.³

Table 1 Primers used for PCR.

Primer name	Sequence (5' $\rightarrow$ 3')
R1 (forward)	GGAGGTAGTGACAATAAATC
Reverse	CGTTCATGATGAACAATTAC

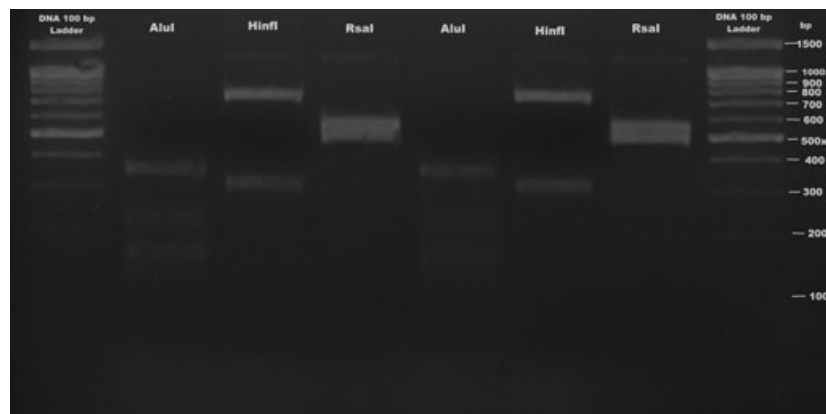


Figure 1 Restriction profiles of part of the 16S-like rDNA of different *B. hominis* isolates on 2% agarose gel. Lanes 1 and 8 are a molecular weight marker (100 bp ladder). DNA in lanes 2 and 5 was digested with *AluI*, DNA in lanes 3 and 6 was digested with *HinfI*, and DNA in lanes 4 and 7 was digested with *RsaI*. Restriction digests in lanes 2–7 stand for subtype 3.

Table 2 Comparison between patients and controls.

Parameter	Patients, n = 54	Controls, n = 50	χ^2	P	Σ
Gender					
Male	23 (42.6)	21 (42)	0.004	0.95	NS
Female	31 (57.4)	29 (58)			
<i>Blastocystis</i> in stool					
Negative	21 (38.9)	46 (92.0)	10.93	0.001	S
Positive	33 (61.1)	4 (8)			
Amoeboid form					
Negative	34 (63)	50 (100)	22.92	0.001	S
Positive	20 (37)	0 (0.0)			

NS, nonsignificant; S, significant. Data are n (%).

Table 3 Comparison between subgroups A and B.

Parameter	Urticaria, n (%)		χ^2	P	Σ
	Acute, n = 18	Chronic, n = 36			
Gender					
Male	9 (50)	14 (38.9)	0.61	0.44	NS
Female	9 (50)	22 (61.1)			
<i>Blastocystis</i> in stool					
Negative	9 (50)	12 (33.3)	1.40	0.24	NS
Positive	9 (50)	24 (66.7)			
Amoeboid form					
Negative	10 (55.6)	24 (66.7)	0.64	0.43	NS
Positive	8 (44.4)	12 (33.3)			
GI symptoms					
Asymptomatic	10 (55.6)	23 (63.9)	0.35	0.55	NS
Symptomatic	8 (44.4)	13 (36.1)			

GI, gastrointestinal; NS, nonsignificant.

Furthermore, the parasite antigen induces the activation of specific clones of T-helper 2 lymphocytes to release cytokines, with consequent IgE production and mast-cell activation, which may be the underlying cause for chronic urticaria.⁸ The significant oxidative stress associated with *B. hominis* infection⁹ is currently considered a predisposing factor in the pathogenesis of chronic urticaria,¹⁰ and the ability of the vacuolar form of *B. hominis* to invade the gut wall⁷ facilitates absorption of other antigens, precipitating urticaria.

Metronidazole is the conventional treatment for this parasite; however, a metronidazole/paromomycin combination² are promising alternative effective treatments.

In conclusion, our study indicates that *B. hominis* might be a causative factor for urticaria of unknown aetiology, whether acute or chronic. We recommend investigation of patients with urticaria in countries with a high prevalence of this parasite; in developed countries, only resistant cases would need to be investigated.

Further studies are needed to detect the pathogenic subtype in each population, factors influencing its pathogenicity and source, and the effect of its treatment on patients with urticaria positive for the parasite.

Learning points

- Urticaria is reported as a symptom of *B. hominis* infections.
- Different parasite subtypes have been isolated, and it has been reported that the pathogenicity of the parasite may depend on its subtype.
- We studied the possible relation of acute and chronic urticaria to the parasite with determination of the isolated subtypes in our cases.

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