The Phenotypic Characterization of Symptomatic and Asymptomatic Blastocystis hominis Subtypes Isolated from Irritable Bowel Syndrome Patients in Diwanyiah City of Iraq

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Abstract

The phenotypic characterization of protein profiles differences between the pathogenic and non-pathogenic Blastocystis hominis and this differences explained the phenomenon of intra subtype variation in pathogenicity. The present study was aimed to investigate phenotypic characteristics of Blastocystis hominis based protein profiles by SDS PAGE analysis was used to discriminate between pathogenic potential symptomatic and asymptomatic Blastocystis hominis subtypes isolates. The SDS-PAGE protein profiles analysis results of symptomatic and asymptomatic Blastocystis hominis positive subtypes isolates were showed the presence high variable numbers of both high and low weight (KDa) protein bands in Blastocystis hominis positive ST3, ST7, and ST1 subtypes. The Blastocystis hominis positive subtype ST3 isolates were showed 19 protein bands in symptomatic patients isolates which ranged from (200KDa-17KDa) and 17 protein bands in asymptomatic patients isolates which ranged from (135KDa-17KDa). The Blastocystis hominis positive subtype ST7 isolates were showed 13 protein bands in symptomatic patients isolates which ranged from (80KDa-30KDa) and 8 protein bands in asymptomatic patients isolates which ranged from (80KDa-30KDa). The Blastocystis hominis positive subtype ST1 isolates were showed 16 protein bands in symptomatic patients isolates which ranged from (180KDa-30KDa) and 13 protein bands in asymptomatic patients isolates which ranged from (80KDa-30KDa). Statistical analysis of protein profiles revealed significant differences in symptomatic and asymptomatic Blastocystis hominis positive subtypes isolates. In Blastocystis hominis subtype 3 was showed high significant differences at (P value 0.0034) and found (76.42%) in symptomatic isolates and (45.37%) in asymptomatic Blastocystis hominis subtype 3 isolates The Blastocystis hominis subtype 7 was showed non-significant differences at (P value 0.1092) and found (61.54%) in symptomatic isolates and (38.46%) in asymptomatic Blastocystis hominis subtype 7 isolates. The Blastocystis hominis subtype 1 was showed significant differences at (P value 0.0193) and found (66.12%) in symptomatic isolates and (39.06%)in asymptomatic Blastocystis hominis subtype 1 isolates. In conclusion, this work is demonstrating the presence of a significant difference in protein profiles between symptomatic and asymptomatic

Blastocystis hominis isolates and The pathogenic potential was related especially into asymptomatic Blastocystis hominis subtype 3 isolates.

Keywords: *Blastocystis hominis, SDS PAGE, Subtypes, Protein profiles.*

1. INTRODUCTION

Blastocystis hominis is a type of intestinal parasite that infects humans and other animals. It was discovered by a Russian physician in 1870, and its pathological significance has remained unknown since then [1]. Cystic donors can infect both toddlers and adults, and their geographical distribution appears to be global; in parasitic surveys, they are often the most isolated primary organisms [2]. Blastocystis hominis was previously overlooked as a disease due to its relationship with minor gastrointestinal symptoms and several asymptomatic illnesses [3]. The cystic donor is common in various nations across the world. Almost all of the world's countries were classed as either industrialized countries, with a moderate prevalence (10-15%), or poor countries, with a high prevalence (55-70%), based on hygiene levels and contact with contaminated animals, water, and food, according to most epidemiological research [4-5].

Blastocystis hominis is a parasite that is regularly discovered in stool samples and is one of the most common parasites identified in the human colon. It has four stages: vascular, granular, amoebic, and cystic. The next phase is the most common in the environment (soil and water), hence it serves as a route of parasite transmission to the host [6]. To prevent the spread of parasites in underdeveloped countries, health care standards, rubbish management, and food and water contamination must all be improved [5]. Blastocystis hominis is a zoonotic parasite that has no known hosts. [7-8]. Cystic donors are transmitted mostly by feces, which is aggravated in unsanitary environments [9]. Nausea, anorexia, abdominal pain, bloating, and severe or chronic diarrhea are some of the symptoms associated with Blastocystis hominis infections [10-11].

Irritable bowel syndrome (IBS) is a digestive illness marked by recurrent stomach pain, constipation, diarrhea, or bloating [12]. Irritable bowel syndrome is caused by bacteria, parasites, fungus, and perhaps viruses, according to new investigations. Microbial damage (particularly parasite infections) is one of the strongest risk factors for the development of IBS, according to research [13-14]. The phenotypic variations between pathogenic and non-pathogenic Blastocystis hominis can be attributed to the phenomena of intra-subtype variation in pathogenicity. This study was aimed to discriminate pathogenic potential symptomatic and asymptomatic Blastocystis hominis subtypes isolates by using SDS PAGE protein profiles analysis.

2. Materials and Methods

2.1. Stool Samples Collection

The samples were included 380 stool samples which divided into 280 samples from patients who severe from digestive complaints, severe abdominal pain and diarrhea, and another 100 samples were collected from apparently healthy individuals who underwent a normal or mandatory health examination (asymptomatic) from Diwanyah Teaching Hospital and private clinics in Diwanyah City from (March to December 2022). The stool samples were placed in clean plastic containers. The patients names, ages, sex and collection dates also recorded. The characterization stool samples is also recorded such as texture, mucus, fatty blood as well as color, for example, yellow, brown, semi-brown and green mucus, and blood that confirms the condition of the disease.

2.2. Stool Examination

All stool samples were examined microscopically by the direct method using low power objective lens (10 X); suspected parasites were examined using the high objective lens (40 X) for identification of B. hominis and for the presence of other intestinal parasites. Two types of direct stool wet smears were done for each specimen at the same time, one with normal saline (0.85 %) and the second with Lugol´s iodine (5%). The detected oocysts of Blastocyst hominis were identified according to their Morphological descriptions [15].

2.3. Culture Method

All condition of culture was worked under sterilization in laminar flow to prepare Jones's medium [16].

2.4. Soluble Antigen Protein Extraction of Blastocyst Hominis

The soluble antigen protein was extracted from Blastocyst hominis parasite culture isolates according [17] and done as following methods:

The Blastocyst hominis culture samples were harvested by centrifuge at 5000rpm for 5 min then discarded the supernatant. Blastocyst hominis cells pellets were wash twice in 1x PBS buffer and then , stored at − 80 °C overnight . then Blastocyst hominis cells pellets thawing at 37 °C in a water bath. 4-ml of PBS (pH: 7.2) was added to the vial contain glass beads, vortex was done for (10min). 3ml lysis buffer (0.5%Nonidet P40, 10Mm Tris-HCL , Aprotinen 0.1 U/ml ,1% Triton X -100)was added and vortex for 2 min. The suspension was taken and sonication for (15min) intervals (30secound) the speed was 20Khz by sonicater apparatuse. After that Centrifugation was done to the suspension by cold centrifuge for(30 min) the speed was (10000 rpm).

The supernatant was taken as source of soluble antigen protein. Bradford protein assay was used to measure the concentration of proteins according to method described by [18] and done as microassay procedure. The antigens were kept at − 20 °C till use.

2.5. SDS PAGE Electrophoresis Technique

SDS-PAGE is technique that used sodium dodecyl sulfate and polyacrylamide gel to separation of protein molecules according to their molecular weight (kDa). The Blastocyst hominis soluble antigen protein was extracted were analysis by SDS-PAGE technique according to method descripted by [19]. And done as following steps:

2.5.1. SDS-PAGE gel Cast Preparation

The SDS-PAGE gel was prepared using (Mini-PROTEAN Tetra Cell Cast. Bio Rad. USA). The SDS-PAGE gel consist from two gel layers as following:

2.5.1.1. Separating Gel

The separating gel was prepared in 10ml sterile tube at 15% due to small molecular weight of Blastocyst hominis protein size is ~100-10 kDa as following table (1):

Table 1. SDS PAGE Separating gel solution.

Separating gel solution	Volume
Purified distal water	1.1ml
30% polyacrylamide	2.5ml
1.5 M Tris pH 8.8	1.25ml
10% SDS	50µl
10% APS	50µl
TEMED	5µl
Total volume	5ml

The Mini-PROTEAN Tetra Cell glass plates, combs, and spacers were washed and cleaned and assembling on Mini-Protean System

Casting Stand then separating gel solution was added 1.1 ml ddH2O at first, then 2.5ml 30% polyacrylamide solution, then 2.5 ml of 1.5 M Tris pH 8.8 and mixed well by vortex, then 50µl SDS and 50µl APS were added and finally 5µl TEMED was added and mixed the mixture well by vortex and let stand for 2 min then pour in glass plates cast into line of Stacking gel and immediately 0.2ml isopropanol was added to prevent air bubbles formation in up line of the separating gel left to 30min to solidified.

2.5.1.2. Stacking Gel

The stacking gel was prepared at 6% as following table (2):

Table 2. SDS PAGE stacking gel solution.

Stacking gel solution	**Volume**
Purified distal water	1.1ml
30% polyacrylamide	0.4ml
0.5 M Tris pH 8.8	0.5ml
Stacking gel solution	**Volume**
10% SDS	20µl
10% APS	20µl
TEMED	2µl
Total volume	~2ml

The stacking gel solution was prepared in 10ml sterile tubes by added 1.1 ml ddH2O at first, then 0.4ml 30% polyacrylamide solution, then 0.5 ml of 0.5 M Tris pH 6.8 and mixed well by vortex, then 20µl SDS and 20µl APS were added and finally 2µl TEMED was added and mixed the mixture well by vortex and immediately poured in glass plates cast into up line of stacking gel and immediately fixed the comb and left to 30min to solidified. The gel cast can be used directly or store for 1 week at 4ºC until used.

2.5.1.3. Proteins Samples Preparation

The protein samples were thawing on ice and about 15-20µg was placed in 0.2ml tube with added (1:1) 2X Laemmli SDS Protein Loading Buffer that contains (375mM Tris.HCl, 9% SDS, 50% glycerol, 0.03% bromophenol blue) then added 9% ß-mercaptoethanol (9% V/V) as reducing agent . Then the mixture tubes were placed in thermal block at 95ºC for 5 min for denaturation of protein folds and left to cool at RT for 5min . After that, the tubes were centrifuged for 5min.

2.5.1.4. Samples Loading and Electrophoresis

The gel cassette was removed from the casting stand and place it in the electrode assembly with the short plate on the inside and filled with 1x running buffer and the comb was removed carefully. Then 20µl proteins sample were loaded and 5µl prestained Protein Ladder for first well by using long pipette tip.

After that, the electrodes cover was applied on Mini-PROTEAN Tetra Cell electrophoresis apparatus and power supply was running in two running time as following:

- 60 volt for 30min for stacking of protein folds.
- 200 volt for 35min for separating of protein bands.

2.5.1.5. Protein Bands Detection

After finish the electrophoresis running time, the gel cast was removed and the gel removed carefully from glass and washed twice by distal water to removed access SDS running buffer, then gel was transferred into staining buffer solution container and placed in rocker shaker for overnight.

After that, the gel was removed from staining buffer solution washed twice by distal water and transferred into destaining buffer solution container for at less two hours, or until Coomassie Brilliant Blue removed from the

gel and only proteins band appeared. Then the proteins bands were investigated on white light viewer.

3. Results and Discussion

The phenotypic characterization of Blastocystis hominis genotypes ST3, ST1, and ST7 was not identified by investigation under light microscope as showed in figure (1), (2) and (3).

The SDS-PAGE protein profiles analysis results of symptomatic and asymptomatic Blastocystis hominis positive subtypes isolates were showed the presence high variable numbers of both high and low weight (KDa) protein bands in Blastocystis hominis positive ST3, ST7, and ST1 subtypes. The Blastocystis hominis positive subtype ST3 isolates were showed 19 protein bands in symptomatic patients isolates which ranged from (200KDa-17KDa) and 17 protein bands in asymptomatic patients isolates which ranged from (135KDa-17KDa). The Blastocystis hominis positive subtype ST7 isolates were showed 13 protein bands in symptomatic patients isolates which ranged from (80KDa-30KDa) and 8 protein bands in asymptomatic patients isolates which ranged from (80KDa-30KDa). The Blastocystis hominis positive subtype ST1 isolates were showed 16 protein bands in symptomatic patients isolates which ranged from (180KDa-30KDa) and 13 protein bands in asymptomatic patients isolates which ranged from (80KDa-30KDa) as showed in figure (4).

Figure 1. Microscopic image of positive culture stool samples that showed heavy growth of Blastocystis hominis subtype 1 infection at (Magnification field 400X).

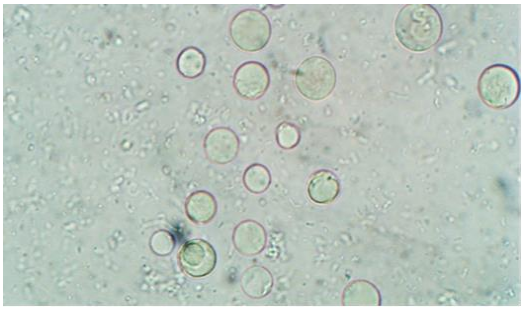


Figure 2. Microscopic image of positive culture stool samples that showed heavy growth of Blastocystis hominis subtype 3 infection at (Magnification field 400X).

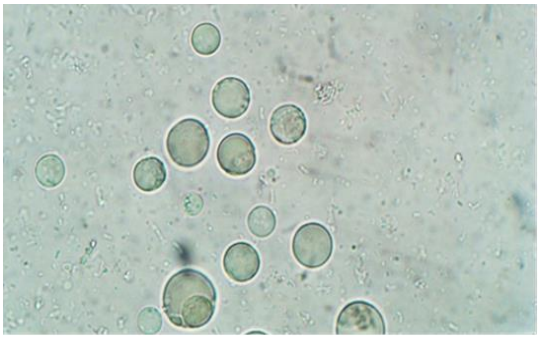


Figure 3. Microscopic image of positive culture stool samples that showed heavy growth of Blastocystis hominis subtype 7 infection at (Magnification field 400X).

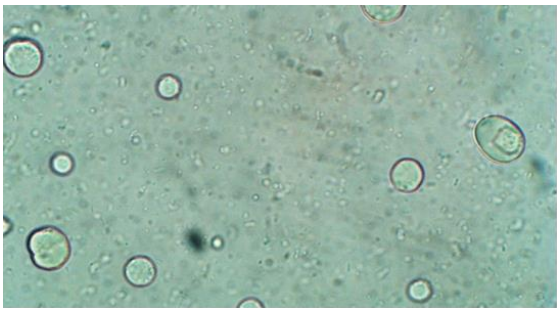
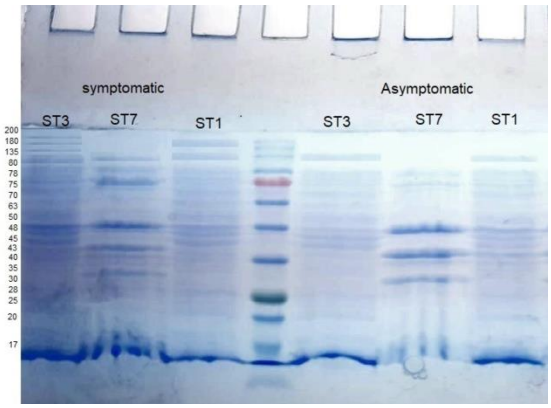


Figure 4. The SDS-PAGE electrophoresis image that showed protein profiles analysis in symptomatic and asymptomatic Blastocystis hominis positive subtypes ST3, and ST7, and ST1 isolates and showed the presence (16) both high and low weight (KDa) protein bands ranged from (80KDa-80KDa).



Statistical analysis of protein profiles revealed significant differences in symptomatic and asymptomatic Blastocystis hominis positive subtypes isolates. In Blastocystis hominis subtype 3 was showed high significant differences at (P value 0.0034) and found (76.42%) in symptomatic isolates and (45.37%) in asymptomatic Blastocystis hominis subtype 3 isolates as showed in table (3).

The Blastocystis hominis subtype 7 was showed non-significant differences at (P value 0.1092) and found (61.54%) in symptomatic isolates and (38.46%) in asymptomatic Blastocystis hominis subtype 7 isolates as showed in table (4).

The Blastocystis hominis subtype 1 was showed significant differences at (P value 0.0193) and found (66.12%) in symptomatic isolates and (39.06%)in asymptomatic Blastocystis hominis subtype 1 isolates as showed in table (5).

Table 3. The SDS PAGE protein profiles analysis in symptomatic and asymptomatic Blastocystis hominis positive subtype 3 isolates.

Protein band No.	MW of bands (kDa)	Symptomatic group (146)	Asymptomatic group (29)
1	200 kDa	60 (41.10%)	0(0.0%)
2	180 kDa	94 (64.38%)	0(0.0%)
3	135 kDa	120 (82.19%)	4(13.79%)
4	80kDa	136 (93.15%)	26 (89.66%)
5	78 kDa	120 (82.19%)	10 (34.48%)
6	75 kDa	140 (95.89%)	24 (82.76%)
7	70 kDa	133 (91.10%)	26 (89.66%)
8	63 kDa	110 (75.34%)	6 (20.69%)
9	50 kDa	100 (68.49%)	3 (10.34%)
10	48 kDa	146 (100%)	29 (100%)
11	45 kDa	146 (100%)	29 (100%)
12	43 kDa	146 (100%)	29 (100%)
13	40 kDa	86 (58.90%)	8 (27.59%)
14	38 kDa	90 (61.64%)	6 (20.69%)
15	35 kDa	123 (84.25%)	2 (6.90%)
16	30 kDa	120 (82.19%)	5 (17.24%)
17	25 kDa	140 (95.89%)	20 (68.97%)
18	20 kDa	50 (34.25%)	15 (51.72%)
19	17 kDa	60 (41.10%)	8 (27.59%)
Total	19	76.42%	45.37%
T-test			
P value	0.0034	** HS.	

NS: not significant at P > 0.05; S: significant at P ⩽ 0.05; HS: highly significant at P ⩽ 0.01.

Table 4. The SDS PAGE protein profiles analysis in symptomatic and asymptomatic Blastocystis hominis positive subtype 7 isolates.

Protein band No.	MW of bands (kDa)	Symptomatic group (12)	Asymptomatic group (2)
1	80kDa	11(91.67 %)	1(50%)
2	78 kDa	11(91.67 %)	1(50%)
3	75 kDa	4(33.33%)	1(50%)
4	70 kDa	8(66.67%)	0(0.0%)
5	63 kDa	1(8.33%)	0(0.0%)
6	50 kDa	2(16.67%)	0(0.0%)
7	48 kDa	12(100%)	2(100%)
8	45 kDa	12(100%)	2(100%)
9	43 kDa	4(33.33%)	1(50%)
10	40 kDa	9(75%)	0(0%)
11	38 kDa	3(25%)	1(50%)
12	35 kDa	7(58.33%)	0(0%)
13	30 kDa	12(100%)	1(50%)
Total	13	61.54%	38.46%
T-test			
P value	0.1092	NS	

Table 5. The SDS PAGE protein profiles analysis in symptomatic and asymptomatic Blastocystis hominis positive subtype 1 isolates.

Protein band No.	MW of bands (kDa)	Symptomatic group (19)	Asymptomatic group (4)
1	180 kDa	4(33.33%)	0(0.0%)
2	140 kDa	3(15.79%)	0(0.0%)
3	135 kDa	13(68.42%)	0(0.0%)
4	80kDa	17(89.47%)	2(50%)
5	78 kDa	16(84.21%)	1(25%)
6	75 kDa	14(73.68%)	2(50%)
7	70 kDa	15(78.95%)	1(25%)
8	63 kDa	18(94.74%)	1(25%)
9	50 kDa	16(84.21%)	1(25%)
10	48 kDa	19(100%)	3(75%)
11	45 kDa	19(100%)	4(100%)
12	43 kDa	19(100%)	3(75%)
13	40 kDa	15(78.95%)	2(50%)
14	38 kDa	5(26.32%)	3(75%)
15	35 kDa	6(31.58%)	2(50%)
16	30 kDa	2(10.53%)	2(50%)
Total	16	66.12%	39.06%
T-test			
P value	0.0193	* S	

The study of phenotypic characteristics of Blastocystis hominis based protein profiles by SDS PAGE analysis was used to discriminate between pathogenic potential symptomatic and asymptomatic Blastocystis hominis subtypes isolates. Due to the parasite's presence in both symptomatic and asymptomatic individuals, Blastocystis sp. has been the subject of intense controversy in the literature over the past two decades [20]. According to a number of reports, Blastocystis sp. may be linked to some intestinal diseases like irritable bowel syndrome (IBS) [21].

The phenotypic variations between pathogenic and non-pathogenic Blastocystis hominis can be attributed to the phenomena of intra-subtype variation in pathogenicity. The predominant amoeboid forms [22] , the secretion of proteases and other hydrolytic enzymes [22-23], the protein profiles [24-25], the growth kinetics [26], and the surface ultrastructure are the phenotypic differences of

the pathogenic Blastocystis hominis that have been studied the most [27].

The SDS-PAGE protein profiles analysis results of symptomatic and asymptomatic Blastocystis hominis positive subtypes isolates were showed the presence high variable numbers of both high and low weight (KDa) protein bands in Blastocystis hominis positive subtypes. The present results was agreement with study by [28] who explained the degree of heterogeneity of the Blastocystis parasite and its correlation with clinical conditions of infected patients by using SDS-PAGE protein profiles analysis. The present study was showed that protein profiles analysis revealed significant differences in symptomatic and asymptomatic Blastocystis hominis positive subtypes isolates. The most phenotypic diversity was showed in Blastocystis hominis subtype 3 with high significant differences at (P value 0.0034) and found (76.42%) in symptomatic isolates and (45.37%) in asymptomatic Blastocystis hominis subtype 3 isolates. The Blastocystis hominis subtype 7 was showed non-significant differences at (P value 0.1092) and found (61.54%) in symptomatic isolates and (38.46%) in asymptomatic Blastocystis hominis subtype 7 isolates. The Blastocystis hominis subtype 1 was showed significant differences at (P value 0.0193) and found (66.12%) in symptomatic isolates and (39.06%)in asymptomatic Blastocystis hominis subtype 1 isolates. There are no previous studies concerned with protein profiles analysis in Blastocystis hominis subtypes. The Blastocystis hominis Subtype 3 is the predominant Subtype found in most human epidemiological studies [29- 30] . One study suggested that 32 kDa proteases of ST3 could be virulence factors responsible for protein degradation [23] while another study suggested that the 29 kDa Blastocystis antigen could be used as a marker for pathogenicity and differentiate between symptomatic and asymptomatic infections [24].In conclusion, this work is demonstrating the presence of a significant difference in protein profiles between symptomatic and asymptomatic Blastocystis hominis isolates and The pathogenic potential was related especially into asymptomatic Blastocystis hominis subtype 3 isolates

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