

DETECTION OF *Blastocystis hominis* AMONG PATIENTS ATTENDING HOSPITALS OF DUHOK CITY – KURDISTAN REGION – IRAQ

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ABSTRACT

Blastocystis hominis is a common intestinal parasite causing diarrhea in human and animals in developing countries. The present cross-sectional study was carried out during the period from November 2017 to August 2018 in Duhok city - Kurdistan region of Iraq. A total 579 stool samples from both gender and different ages (one year->60 years) suffering from primary gastrointestinal disorders who attended Azadi Teaching, Golan, and Heevi Teaching Pediatric Hospitals in Duhok City – Kurdistan Region.

The stool samples were divided into two portions, the first portion was cultured in Ringer's solution containing 10% horse serum and 0.05% asparagine and incubated at 37 °C for 3–4 days, then a specimen from the culture was screened under the light microscope (40X) for the detection of *Blastocystis*. While the second sample was mixed with physiological saline (0.85%) and Lugol's iodine, and then examined under the light microscope with objective lens (40X) for the detection of other intestinal protozoa. Identification of parasites was done according to morphological features.

In this study 16.93% (98 of 579) of the enrolled patients were positive for the cysts of *B. hominis* in their stools. The age group > 60 years showed the highest rate of infection (21.28%), while the lowest rate was 13.4% in the age group from 31-40 year, with non-significant difference ($P>0.05$) between them. The rate was higher (20.79%) in the children group versus adults (16.11%) with also non-significant difference ($P>0.05$) between both groups.

Furthermore, males showed higher rate (62.25%) of infection versus females (37.75%), but this rate was statistically non-significant ($P>0.05$). Regarding other recorded intestinal protozoa, *Entamoeba histolytica* was recorded at a rate of 11.23 % of the positive cases (98 patients) with *B. hominis*. Statistical analysis did not show any significant difference ($P >0.05$) with this association. *Giardia lamblia* was reported in 8.16% of positive cases with *B. hominis*, this association was statistically significant ($P <0.05$).

KEYWORDS

INTRODUCTION

Blastocystis hominis is a common obligate anaerobic intestinal parasite, and it has widespread geographical distribution (Yoshikawa *et al.*, 2007). It is usually isolated from human and many other animals including pigs, cattle, poultry, dogs, cats, and insects (Abe *et al.*, 2002; Tan, 2008; Alfellani *et al.*, 2013). The first description of *B. hominis* was in 1912 by Brumpt, but the name was later changed to *Blastocystis* species due to an indistinguishable difference embedded in the isolates obtained

from humans from those found in animals (Silberman, *et al.*, 1996).

A true pathogenic state of *Blastocystis* species still has been debated (Sadaf *et al.*, 2013). Although many research have given credit to *Blastocystis* species as pathogens which can be caused many gastrointestinal symptoms such as abdominal pain, diarrhea, constipation, nausea, flatulence, vomiting, and fatigue (Andiran *et al.*, 2006). Furthermore, other reports noted that *Blastocystis* species may play an important role in another gastrointestinal illness such as irritable bowel syndrome (Hussein *et al.*, 2008).

Blastocystis species has various morphological forms which can all be found in the stool including, vacuolar, a vacuolar, multivacuolar, granular, ameboid trophozoite stage (Mac Pherson and Mac Queen, 1994; Stenzel and Boreham, 1996; Zhang *et al.*, 2007). The vacuolated type is considered a dominant form (Yoshikawa *et al.*, 2004). Previous studies verified water resistant thin-walled cysts responsible for autoinfection which present in feces, in contaminated water and food (Basak *et al.*, 2014).

Diagnosis of *B. hominis* routinely by direct microscopy is a critical task due to the size of the cysts which measures 3 to 10 μm (Tan, 2004). Moreover, the polymorphic nature of the organism in wet mounts can lead to confusion with yeast, or fat globules (Stenzel *et al.*, 1994). Therefore, the use of trichrome-stain, Giemsa stain, and iron hematoxylin is recommended for stool examination for the identification of *B. hominis*. In vitro cultivation was useful to confirm the presence of *B. hominis* because of its specificity and sensitivity (Stenzel *et al.*, 1997).

The prevalence of *B. hominis* is higher in developing countries (30 -50%) than in developed countries (1.5 – 10%), the reason might be due to poor hygiene, close contact to animals and consumption of contaminated food and water (Ustun and Turgay, 2006). Infection with *Blastocystis* can be linked to host factors such as age, gender, and level of education as well as exposure factors such as hygiene, the source of water supply, and contact with animals (Duda *et al.*, 1998; Suresh *et al.*, 2005). Furthermore, higher risks of infection and high prevalence have been identified in food and animal handlers, providing conclusive evidence on its zoonotic potential (Yoshikawa *et al.*, 2009; Parkar *et al.*, 2010). In this study, the clinical significance and prevalence of *B. hominis* were investigated to determine the significance of the disease in Duhok province.

MATERIALS AND METHOD

The present cross-sectional study was carried out during the period from November 2017 to

June 2018 in Duhok city- Kurdistan region of Iraq. Stool samples were collected from 579 Patients of both gender and different ages (1 year to > 60years) suffering from primary gastrointestinal disorders such as abdominal pain, epigastric pain and diarrhea who attended Azadi Teaching, Golan, and Heevi Pediatric Teaching Hospitals. Each enrolled patient was provided with a questionnaire form describing the patients' socioeconomic, residency, and any previous diseases. The patients were divided into seven age groups. Furthermore, these groups were divided into two major groups according to their ages, the children group (1-13 years) and the adult group (More than 14years). Stool samples were transferred to the parasitology laboratory of General Azadi Teaching Hospital where each sample was divided into two portions, the first portion was cultured in Ringer's solution containing 10% horse serum and 0.05% asparagine and incubated at 37 °C for 3 – 4 days (Dogruman *et al.* 2010), from this culture wet mounts were prepared for microscopic examination using 40 X objective lens for the detection of *Blastocystis*. The second part of the specimen was mixed with physiological saline (0.85%) and Lugol's iodine, and then examined under the light microscope with objective lens (40 X) for detection of other intestinal protozoa. Identification of parasites was done according to morphological features (Stenzel and Boreham, 1996)

All data were analyzed using statistical package, SPSS (IBM Corporation, New York, NY, USA) Version 24.0.

RESULTS

The results of this study revealed that 16.93% of the patients were positive for *B. hominis* in their stools. It was also shown that the age was not a determining factor that can profoundly contribute to the infection process because non-significant difference ($P > 0.05$) was found between the age groups as far as infection rate was concerned (Table. 1).

Table (1): Infection with *B. hominis* according to age

Age Groups/Years	Patients No. Examined	Positive		Negative	
		No.	%	No.	%
1-10	92	18	19.57	74	80.43
11-20	102	18	17.65	84	82.35
21-30	93	15	16.13	78	83.87
31-40	97	13	13.40	84	86.60
41-50	83	12	14.46	71	85.54
51-60	65	12	18.46	53	81.54
> 60	47	10	21.28	37	78.72
Total	579	98	16.93	481	83.07

Non-significance differences $P > 0.05$.

Regarding to the results of the two main groups, the rate of the infection in the children group was higher (20.79%) as compared to adult

group (16.11%), but statistically the difference between both groups was non-significant ($P > 0.05$) as indicated in table 2.

Table (2): Infection with *B. hominis* according to Adults and Children

Group	Patients No. Examined	Positive		Negative	
		No	%	No	%
Adults	478	77	16.11	401	83.89
Children	101	21	20.79	80	79.21
Total	579	98	16.93	481	83.07

Non-Significance differences $P > 0.05$.

Moreover, it was found that males represented a percentage of 62.25 % of all positive cases while the females represent 37.75

%, but statistically this difference was non-significant ($P > 0.05$) between both genders (Table 3).

Table (3): Infection with *B. hominis* according to Gender

Gender	Patients No. Examined	Positive		Negative	
		No	%	No	%
Male	344	61	62.25	283	58.84
Female	235	37	37.75	198	41.16
Total	579	98	100.0	481	100.0

Non-significance differences $P > 0.05$.

Co-infection may occur in many cases of parasitic infections with variable frequencies, as shown in table (4) in 11.23% of the cases co-infection with *Entamoeba histolytica* was observed which was statistically non-significant

($P > 0.05$). On the other hands, in 8.16% of the cases co-infection with *Giardia lamblia* was recorded, despite to their lower frequency, the difference between them was statistically significant ($P < 0.05$) as indicated in table (5).

Table (4): Mixed infection of *B. hominis* and *E. histolytica*

	<i>Entamoeba histolytica</i>			
		Positive No. & %	Negative No. & %	Total
<i>Blastocystis hominis</i>	Positive No. & %	11(11.23)	87 (88.77)	98
	Negative No. & %	51 (82.25)	430 (74.26)	481
	Total	62	517	579

Non-significance differences $P > 0.05$.

Table (5): Mixed infection of *B. hominis* and *Giardia lamblia*

	<i>Giardia lamblia</i>			
		Positive No. & %	Negative No. & %	Total
<i>Blastocystis hominis</i>	Positive No. & %	8 (8.16)	90(91.83)	98
	Negative No. & %	10 (55.55)	471(81.34)	481
	Total	18	561	579

* Significance differences $P < 0.05$.

DISCUSSION

The results of the present study showed that the rate of blastocystosis among residents of Duhok city within different ages was 16.93%, and this rate of infection is about 3 folds of the previously recorded rate in the same city by Al-Saeed *et al.* (2013), which was 5.08%. Despite to the development in the hygienic education and the drinking of bottled water, the rate of the infection is increased, and this can be attributed to several reasons, such as the diagnostic techniques used in this study, since many studies indicated that the *Blastocystis* infections are difficult to be detected microscopically in stool specimens by direct method because of their numerous morphological forms (Boorum *et al.*, 2008), the high variation in the size and the similarity of the parasite to a fat cell (Stensvold *et al.*, 2009); therefore the culture is the most efficient method for the identification of *Blastocystis* species (Dogruman *et al.*, 2010). Noteworthy mentioning, this high variation in the rate of infection can be attributed to the different identification techniques (Dogruman *et al.*, 2010; Padukone *et al.*, 2018).

In the current study, the age was not considered as a significant factor for the variation in the rate of infections, even though, the highest rate of infection was recorded in ages above >60 years. This may be due to the presence of other diseases among these ages such as irritable bowel syndrome (Wawrzyniak *et al.*, 2013) in addition to the diminished

immunity that accompanies advanced ages (Rodriguez *et al.*, 2013). Similarly, Suresh and Smith (2004) reported high rates among these ages. Moreover, the age group 1-10 years was only second to the elderly group in term of infection rate which may be explained by unawareness to water and food hygiene. The lowest infection rate was detected in the age group 31-40 years.

The higher rate of infection among the children group may be attributed to the use of public water supply for drinking and outdoor eating especially in the school and neglecting personal hygiene. These results are consistent with studies of Raof and Abdul-Rahman (2011) in Baghdad, who reported the highest rate of infection within the age group 5-10 years. Also, Mohammed and Ali (2015) in Sulaimaniya showed that infection was prevalent in children of both genders aged 10-12 years. Furthermore, Mahmood and Khudher (2016) stated that the age group between 6-10 years is the most susceptible for infection.

Despite of the variation in the infection rate between males (62.25%) and females (37.75%), but this difference was statistically non-significant ($P > 0.05$) between both gender, consequently the gender is not a potential determining factor for the infection with *B. hominis*. This is consistent with the results of Mohammed and Ali (2015) in Sulaimani city, Mahmood and Khudher (2016) in Samarra city, Noor *et al.* (2007) and Mohammad *et al.* (2017) in Malaysia, and Leelayoova *et al.* (2008) in

Thailand, all of them stated that there were non-significant differences between both genders as far as infection rate was concerned.

The coexistent of *E. histolytica* and *G. lamblia* with *B. hominis* in the present study at the rates of 11.23% and 8.16%, respectively, is in accordance to previous studies performed in various Iraqi cities regardless to the infection rates, since variable rates were recorded in these studies. In Duhok City the co-infection of *G. lamblia* with *B. hominis* was 39.4% (Al-Saeed and Issa 2006), while in Baghdad Nayef *et al.* (2011) observed mixed infection of *E. histolytica* and *B. hominis* in 71% and *G. lamblia* with *B. hominis* in 29% of the tested samples. While, Mahmood and Khudher (2016) reported a lower rate in Samarra city, which were 3.78% coexistent of *B. hominis* with *E. histolytica* and 2.27% with *G. lamblia*. Furthermore, in several other studies also variable coexistent rates of *B. hominis* with *E. histolytica* and *G. lamblia* were reported, as that carried out by Diarthini *et al.* (2018) in Dukuh village, Karangasem regency-Bali- Indonesia, they observed that 33.3% of total samples have co-infection with both *Blastocystis* spp. and *Giardia lamblia*, while ; Forsell *et al.* (2016) in Zanzibar, Tanzania mentioned that two parasitic infection (*Blastocystis* spp. and *Giardia lamblia*) were identified in 56 % of the candidates patients. On the other hand, Duda, *et al.* (2015) in Szczecin, Poland reported only single infection with *B. hominis* without co-existence of any other parasite in their samples.

In conclusion: Age and genders were not determining factors for infection with this parasite, furthermore, the GIT environment can be suitable for the infection of a great number of parasites concomitantly.

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پوخته

Blastocystis hominis دهیته هژمارتن ئیک ژ هه بوئین سه ره تایی بین مشه خور بین بهر به لاف ئه وین دبنه ئه گهری چیبونت زک چوونئ ل ناف مروقان. ئه ف خاندنه هاتیه ئه نجامدان د ماویئ دنافهرا چریا دووی سالا 2017 تا ته باخا سالا 2018 ل باژیری دهوکی ل هه ریما کوردستانا عیراقی. دقئ خاندنی دا 579 نمونه بیین ده ستناقا گران هاتنه کومکرن ژ هه ردوو ره گهزا وش ژیی ئیک سالی تا پتر ژ شیست سالیئ ژ نه خوشین گازنده ژ شه پرزه بونا هناقا وزک چوونئ دکهن ل نه خوشخانا نازادی یا فیرکرنئ ونه خوشخانا هیقی یا زاروکا و نه خوشخانا گولان ل باژیری دهوکی.

نمونه هاتنه دابهشکرن بوو دوو به شا، به شئ ئیکئ هاته جاندن دناف تیکه لی بیک دهیت ژ شله یی رینگه ر دگه ل 10% ژ سیره می خوینا هه سپا یی هیوا برکری ب گهرمی، دگه ل 0.05% ئه سپه راجین و دهیته گرکه تن د پلا گهرماتیئ C37 بو ماوی 3 تا 4 روژا و پاشی دهیته تاقیکرن ب مایکروسکوبی ب وردبنا ب هیزا X40، و به شئ دووی دهیته تیکه ل کرن دگه ل شله یی فزیولوجی 85% و رهنگ کرن ب لوگال ایودین پاشی دهیته تاقیکرن ب مایکروسکوبی ب وردبنا ب هیزا X40 بو ده ستنیشانکرنا هه بوونا مشه خوری.

ئه نجاما خسته رو کو ریژا هه ودانی 16.93% و به رزترین ریژه ئاشکرا بو دناف گروبی ژیی وان پتر ژ شیست سالیئ دا و کیتمترین ریژه دناف گروبی ژیی وان نافهرا 31-40 سالیئ دا، دگه ل نه بوونا هیج جیاوازیه کا واته به خش دناف بهرا ریژا دا ل ده می $P > 0.05$

سه باره ت هه بوونا هه ودانی هه قبه ش یا مشخوری *B. hominis* دگه ل مشه خورین دب یین هناقا، ئه نجاما ئاشکرا کر ب هه بوونا 11 (11.23%) ژ هه ودانی هه قبه ش دنافهرا *B. hominis* و مشه خوری *Entamoeba histolytica* ژ کوی گشتی یین توشبو یین *B. hominis* کو دبنه 98 که س، دگه ل نه بوونا هیج جیاوازیه کا واته به خش دنافهرا ریژا توشبو یی ب هه ردوو مشه خورا دا، لئ هه ودانی هه قبه ش دنافهرا *B. hominis* دگه ل *Giardia lamblia* ئاشکرا بو ل ده ف 8 (8.16%) و دگه ل هه بوونا جیاوازیه کا واته به خش دنافهرا هه ردوو مشه خورا دا ل ده می ($P < 0.05$).

الخلاصة

يعتبر ال *Blastocystis hominis* من الاوالي الطفيلية المعوية الشائعة المسببة للإسهال في الانسان. اجريت الدراسة الحالية في الفترة من تشرين الثاني 2017 ولغاية اب 2018 في مدينة دهوك في اقليم كوردستان العراق. تضمنت الدراسة جمع وفحص 579 عينة غائط من كلا الجنسين وبأعمار مختلفة تراوحت بين سنة وأكثر من ستين سنة لمرضى يعانون من اضطرابات معوية وحالات اسهال من مستشفى ازادي التعليمي ومستشفى هيفي للأطفال ومستشفى كولان في مدينة دهوك. جمعت العينات.

قسمت العينات الى جزئين، الجزء الاول تم تشخيصه باستخدام تقنية الزرع، حيث تمت زراعته في وسط مكون من محلول رنكر يحتوي على 10% مصل خيل المبطل بالحرارة، بالإضافة الى 0.05% من الاسبراجين، وتم الحضان في درجة حرارة 37°C ولمدة 3-4 ايام، ومن ثم تم التشخيص باستخدام المجهر الضوئي بقوة 40X للتحري عن وجود الطفيلي. اما الجزء الثاني من العينة فقد تم التحري عن وجود الطفيلي فيه تبعا للصفات المظهرية للطفيلي باستخدام المحلول الفسلجي (85%) وكذلك باستخدام صبغة الوكال ايويدين ومن ثم الفحص المجهرى بقوة 40X.

اظهرت نتائج الفحص وجود اصابات بلغت نسبتها 16.93% و قد ظهرت اعلى نسبة للإصابة ضمن الفئة العمرية الاكثر من ستين سنة و كانت بنسبة 21.28%، في حين ان اقل نسبة للإصابة كانت ضمن الفئة العمرية بين 31-40 سنة مع عدم وجود اي فروقات معنوية في نسب الإصابة بين الفئات العمرية المختلفة عند نسبة احتمالية $P > 0.05$. جدير بالذكر، انه بالرغم من وجود فرق ملحوظ في نسب الإصابة بين فئة البالغين (16.11%) وفئة الاطفال (20.79%)، الا انه لم تسجل فروقات معنوية بينهما عند نسبة احتمالية $P > 0.05$. اما بالنسبة لحالات الإصابة المشتركة للطفيلي مع طفيليات معوية اخرى، فقد اظهرت النتائج 11 (11.23 %) حالة إصابة مشتركة بين الطفيلي *B. hominis* و الطفيلي *Entamoeba histolytica* وذلك من المجموع الكلي للحالات التي اظهرت ايجابية لتشخيص *B. hominis* و البالغة 98 حالة مع عدم وجود فروقات معنوية للإصابة بين الطفيليين، في حين حالات الإصابة المشتركة بين *B. hominis* و *Giardia lamblia* فقد ظهرت 8 (8.16%) حالة إصابة مشتركة مع وجود فرق معنوي بين الطفيليين عند الاحتمالية ($P < 0.05$).