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Prevalence and Clinical Features of *Blastocystis hominis* Infection among Patients in Sebha, Libya

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انتشار عدوى المُتَبَرْعِمَةُ الكيسِيَّةُ البَشَرِيَّة وصفاتها السريرية عند المرضى في سبها (ليبيا)

محمد عبد السلام الفلاني، عبد الحفيظ خان، رقية محمد الجازوى، مبروك خليفة زايد ومحمود على الفرجانى

الملخص: الهدف: تحديد انتشار المُتَبَرْعِمَةُ الكيسِيَّةُ البَشَرِيَّة ومعرفة الاختلاف الموسمي فيها. وكذلك تقييم الحالة السريرية والعلاجية للمرضى الليبيين المصابين بها. **الطريقة:** أخذنا عينات من براز 3645 لتقصي حالة المُتَبَرْعِمَةُ الكيسِيَّةُ البَشَرِيَّة باستعمال محلول ملحي نظامي ومحلول اليود. تم وصف الحالة السريرية ل 108 مريضا مصابا بالمُتَبَرْعِمَةُ الكيسِيَّةُ البَشَرِيَّة فقط. عولج خمسين مريضا ممكن كانت لديهم أعراضا بعقار الميترونيدازول (1500 ملجم) لمدة سبعة أيام. وبعدها أعيد فحص البراز مرة أخرى. **النتائج:** وجدت المُتَبَرْعِمَةُ الكيسِيَّةُ البَشَرِيَّة في 969 (26.58%) من مجموع عينات البراز البالغة 3645. كانت العدوى أكثر حدوثا في الصيف منه في الشتاء في السنوات الثلاث بفارق إحصائي معتد (P=0.05). في دراسة مسبقة ل 108 مريضا. كانت الأعراض الأكثر شيوعا مع الفحص الموجب للبراز هي الإسهال (84.94%). ألم البطن (66.66%). الغازات (17.20%) والتقيؤ (16.12%). كان عدد خلايا المُتَبَرْعِمَةُ الكيسِيَّةُ البَشَرِيَّة أكثر عند المرضى الذين يظهرون أعراضا عن غيرهم (9.20 خلية في مجال التكبير 40 مرة مقابل 4.06 للآخرين). وبفرق إحصائي معتد(P<0.001) . شفي المرضى المصابون بالمُتَبَرْعِمَةُ الكيسِيَّةُ البَشَرِيَّة بعقار الميترونيدازول خلال فترة اسبوع. **الخلاصة :** ربما تكون العدوى بالمُتَبَرْعِمَةُ الكيسِيَّةُ البَشَرِيَّة عند مراجعي العيادة الخارجية لها علاقة بالمناخ. حيث أن مناخ سبها الحار والجاف قد يساعد على تكاثر وانتقال المُتَبَرْعِمَةُ الكيسِيَّةُ البَشَرِيَّة. والتي ربما تلعب العدوى بها دورا في بعض الحالات المرضية التي تسبب أعراضا هضمية.

مفتاح الكلمات: المُتَبَرْعِمَةُ الكيسِيَّة. التغيرات الفصلية. الإسهال. الزرع.

ABSTRACT ***Objective:*** To determine the prevalence and seasonal variation, and to assess the clinical manifestations and treatment of blastocystosis in Libyan patients. ***Methods:*** Three thousand six hundred and forty five stool samples were screened for Blastocystis hominis using normal saline and iodine solution preparations. The clinical features of 108 patients were described, in whom *B. hominis* was the only parasite isolated. Fifty symptomatic patients were treated with 1500 mg metronidazole daily for 7 days and their stools were re-investigated for *B. hominis*. ***Results:*** *B. hominis* was found in 969 (26.58 %) of 3645 stool specimens examined. The infection of *B. hominis* was significantly more ($p < 0.05$) in summer than in winter over a three year period. In a prospective study of 108 patients, the most common symptoms with stools positive only for *B. hominis* were diarrhoea (84.94 %), abdominal pain (66.66 %), flatulence (17.20 %) and vomiting (16.12 %). High concentration of *B. hominis* cells were found more in symptomatic patients than asymptomatic ones (9.20 cells per 40 X field versus 4.06 respectively) with statistically significant differences ($p < 0.001$). Patients with *B. hominis* responded to metronidazole and were fully cured after 7 days. ***Conclusion:*** The occurrence of *B. hominis* infections in outpatients are probably related to weather conditions, with the suggestion that the hot, dry weather of the Sebha region favors the development and transmission of this organism. *B. hominis* infections might have a role in some pathological conditions, resulting in gastrointestinal symptoms.

Key words: Blastocystis, Seasonal variation, Culture, Diarrhoea.

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Infection by *Blastocystis hominis* occurs all over the world, but is commonly found in developing countries.[1,2] Practicing physicians and gastroenterologists usually have low awareness of the association of *B. hominis* with human disease. These infections are overlooked in clinical laboratories. The Centre for Disease Control and Prevention also considered it to be a pathogenic protozoan.[3] *B. hominis* is now increasingly recognized as a possible cause of gastrointestinal disorders.[4-7] A subgroup of *B. hominis* could possibly be pathogenic in some patients.[8] The specific pathogenic potential of *B. hominis* has not been defined.[9-12]

The results of a recent study in Sebha, Libya, revealed that *B. hominis* was the most frequent isolate in all stool specimens submitted for analysis,[13] but the clinical significance of *B. hominis* infection in the Libyan population has not been documented so far.

In the present study, the aim was to investigate the prevalence of *B. hominis* among patients attending the Central Laboratory in Sebha, Libya and to describe the clinical presentations of patients parasitized only with *B. hominis* infection in Libyan communities.

METHODS

This prospective study was performed in Sebha city, Fezzan Province, South-Western Libya. This region is characterized by a hot and dry climate. It is an arid and desert area with a population of over one hundred thirty thousand. Most people work in agriculture.

A total of 3,645 stool samples were collected (from the beginning of January to the end of December 2005) from outpatients attending the Central Laboratory in Sebha, Libya, for routine examination of intestinal parasites. Soon after the collection of stool specimens, two faecal smears were prepared (normal saline and Lugol's iodine) from each sample. These preparations were examined under both a low power (10 X) and a high power (40 X) of microscope to detect possible *B. hominis.*

The seasonal prevalence of *B. hominis* was recorded among 8,089 patients, who attended the Central Laboratory in Sebha, over a 3 year period from January 2003 to December 2005.

A prospective study on 108 patients parasitized exclusively with *B. hominis* was performed. Clinical information on patients with stools which tested positive for *B. hominis* was obtained by sending a survey questionnaire to practicing physicians at the Al-Jadeed clinic in Sebha, Libya. The numbers of *B. hominis* cells in faecal materials were counted with a 40 X field microscope.

Ninety three patients harboured only *B. hominis* and presented with gastrointestinal symptoms (diarrhoea with or without abdominal pain and flatulence or vomiting) were treated for seven days with metronidazole 500 mg three times per day for 7 days. Only fifty patients returned back for a follow up examination. Stool samples from these patients were re-examined for *B. hominis* after completion of the therapy.

The positive rate of *B.hominis* was expressed as a percentage (number, sex, seasonal variation, diarrhoea etc) and statistical analysis was carried out using the independent sample *t*-test. *P*-values of < 0.05 and 0.001 were considered statistically significant.

RESULTS

The prevalence of *B. hominis* was found to be 26.58 % among outpatients at the Central Laboratory in Sebha. Of the 3,645 patients examined, 1,925 (52.81 %) were males and 1,720 (47.18 %) were females. A total of 558 (28.98 %) males and 411 (23.89 %) females were harbouring *B. hominis*. The highest positivity (10.28 %) rate was found in the 21 to 40 age group.

The seasonal variation of *B. hominis* among patients attending the Central Laboratory in Sebha is presented in Table 1. The prevalence of *B. hominis* was found to be significantly more ($p<0.05$) in summer

Table 1 : Seasonal variation of** B. hominis **among patients attending the Central Laboratory in Sebha

Year	Summer season*		Winter season**		Total investigated	
	No.	No. Positive	No.	No. Positive	No.	No. Positive***
2003	2572	458 (17.80)	1855	279 (15.04)	4427	737 (16.64)
2004	2264	497 (21.95)	1398	267 (19.09)	3662	764 (20.86)
2005	2336	640 (27.39)	1309	329 (25.13)	3645	969 (26.58)

Figures in parentheses indicate percentages.
*** April to October.**
**** November to March.**
***** $p < 0.05$ versus comparison with summer and winter season.**

Table 2: *Clinical Manifestations of 93 Symptomatic Patients*

Clinical symptoms	No. of cases
Diarrhoea	79 (84.94)
Cramping abdominal pain	62 (66.66)
Flatulence	16 (17.20)
Vomiting / Nausea	15 (16.12)

Parasitized exclusively by *B. hominis*.
Figures in parentheses indicate percentages.

than in winter.

The clinical presentations of 93 patients parasitized exclusively with *B. hominis* are shown in Table 2. The most frequent manifestation was diarrhoea (84.94%). Sixty-four (68.81%) patients had two or more gastrointestinal complaints. Twenty-nine patients (31.18 %) had only one clinical feature (25 had diarrhoea and 4 had cramping abdominal pain). Forty-nine patients (52.68 %) had two gastrointestinal complaints (33 had diarrhoea with cramping abdominal pain, 6 had diarrhoea with vomiting, 2 had vomiting with cramping abdominal pain and 8 had cramping abdominal pain with flatulence). Fifteen patients (16.12 %) had three clinical symptoms of blastocystosis (7 had diarrhoea with cramping abdominal pain and vomiting, 8 had diarrhoea with cramping abdominal pain and flatulence).

The intensity of *B. hominis* among symptomatic and asymptomatic individuals is presented in Table 3. The mean number of *B. hominis* was significantly higher ($p<0.001$) in symptomatic patients than in asymptomatic individuals (9.20 ± 2.41 organisms per 40 X field, versus 4.06 ± 1.86 respectively). Two infected persons, in whom *B. hominis* cells were found 10 and 12 per 40 X field, were entirely asymptomatic.

B. hominis were not found in stools from patients upon the completion of the 7 day therapeutic course of metronidazole. All fifty patients showed clinical improvement and were fully cured using 1500 mg of metronidazole daily for 7 days.

DISCUSSION

Physicians usually have low awareness that *B. hominis* is a cause of human disease. The number of infections appears to be high in most populations, however, the frequency is grossly underestimated. Asymptomatic shedding of *B. hominis* provides an appropriate environment for its transmission to other subjects. Recently *B. hominis* has been considered as potential pathogen. [5-7]

Infections of *B. hominis* may be an important pubic health problem in Libyan communities. [30-32] So far, only one study has been carried out on the prevalence of *B. hominis* among patients attending the Central Laboratory in Sebha, Libya.[13] This study was carried out to investigate the prevalence, seasonal variation and the association between the presence of *B.hominis* and gastrointestinal symptoms among patients.

The results of this study show that *B. hominis* was detected in 26.58 % of stool specimens examined. Very similar prevalence have been described in other parts of the world: 25% in Jordan,[3] 32% in Pakistan,[6] 18% in Bethesda,[14] 31% in Egypt,[20] 25.78% in Venezuela,[24] 26.5% in Brazil[29] and 22.9% in Argentina[33] [Table 4]. However, higher prevalence of *B. hominis* has been reported in other countries of the world: 36% in Tanzania,[23] 40.7% in Philippines,[25] 36.9% in Thailand,[26] and 46.9% in Venezuela.[34] In the present study, the prevalence of *B. hominis* was higher than previously reported among the same population. This may be due to improvement in detection of *B. hominis* infection in clinical laboratories, but infections with this organism may have also increased in the population. In the Libyan Arab Jamahiriya, the rapid socio-economic development, agriculture practices and the enormous increase in the number of foreign workers from neighbouring countries may have lead to a substantial increase of intestinal parasites in the country. Prior research has shown that immigrants and refugees from

Table 3: ***Intensity of B. hominis Infection among Blastocystosis Patients.***

Category	No. of cases and (%)	Intensity of *B. hominis*	
No. of organisms	Mean of No. ± SD	No. of organisms	Mean of No. ± SD
Symptoms	93 (86.11)	Positive for *B. hominis* with gastrointestinal symptoms More than 7	9.20±2.41** (8 to 30)*
Symptoms	15 (13.88)	Positive for *B. hominis* without trointestinalsymptoms Less than 5	4.06±1.86 (2 to 12)*

*** Range of No. of *B. hominis* cells per 40 X field.**
**** $p < 0.001$ versus comparison with number of *B. hominis* cells among asymptomatic individuals.**

Table 4: Prevalence of B. hominis in various countries/states of the world.

Reference	Country / Locality	% of B. hominis
Wang et a[l2]	Community population in China	3.7
Nimri[3]	Preschool children in Jordan	25.0
Yakoob et a[l6]	Outpatients in Pakistan	32.0
Al-Fellani et al[13]	Outpatients in Libya	18.5
Zierdt[14]	Community population in Bethesda	18.0
Garcia et al.[15]	Outpatients in Los Angeles	12.0
Babock et al.[16]	Population in Nepal	10.0
Markell and Udkow[17]	Outpatients in San Francisco	12.0
Sheehan et al.[18]	Outpatients in New York	11.0
Kain et al.[19]	Outpatients Vancouver	13.0
El Masry et al.[20]	Outpatients in Egypt	31.0
Doyle et al.[21]	Outpatients in Canada	3.2
Martin-Sanchez et al.[22]	Primary school children in Spain	19.4
Gomez Morales et al.[23]	Outpatients in Tanzania	36.0
Requena et al.[24]	Food handlers in Venezuela	25.8
Eleonor et al.[25]	Children in Philippines	40.7
Leelayoova et al.[26]	Army personal in Thailand	36.9
Suresh and Smith[27]	Outpatients in United Kingdom	3.9
Khan and Khalife[28]	Food handlers in Saudi Arabia	8.5
Nascimento and Moitinho[29]	Community population in Brazil	26.5
Present study	Outpatients in Libya	26.58

developing countries have a higher incidence of *B. hominis*.

In the present study, males were more infected (28.98%) with *B. hominis* than females (23.89%). The difference was significant *(p*<0.05). Several studies have reported significantly higher prevalence in male than female patients.[2, 3, 12]

The parasitological examination of faecal samples revealed that the incidence of *B. hominis* is widespread throughout the year with a particular peak in the summer season. The high incidence of *B. hominis* in the Sebha region may be due to the dry climatic conditions that favour the survival and transmission of this organism in the population throughout the year. This is in contrary to other parts of the world, where infections of *B. hominis* are commonest during the premonsoonal months, spring and winter seasons.[16, 20, 27]

The most prominent gastrointestinal symptoms in 93 patients parasitized exclusively by *B. hominis* were diarrhoea (84.94%), cramping abdominal pain (66.66%) and nonspecific gastrointestinal symptoms such as flatulence (17.20%) and nausea or vomiting (16.12%). These symptoms were similar to the one reported previously in patients infected with *B. hominis*. [2-8, 20]

In the present study, the number of *B. hominis* was significantly higher *(p* < 0.001) in the stools of symptomatic patients than in asymptomatic individuals (more than seven cells with mean number 9.20 ± 2.41 versus less than five with mean number 4.06 ± 1.86).

Several studies have reported detecting more than five cells of *B. hominis* per 40 X field in stool samples from symptomatic patients.[3, 7, 20, 35] In addition five or more *B. hominis* cells per oil immersion field (100 X) have been detected in symptomatic patients.[22,36-38] The mean numbers of *B. hominis* cells in the stools of symptomatic patients were significantly higher than asymptomatic patients.[5, 7]

Several studies reported severe symptoms in patients with high numbers of *B. hominis,*[9, 20] however, this correlation was not found in others.[21, 22, 40]

In the present study, among asymptomatic subjects (except two cases) the number of *B. hominis* cells found was less than five per 40 X field. Similarly less

than five organisms (fewer than three) per 40 X microscopic fields among stool samples of apparently healthy subjects have been reported.[3, 24] Large numbers of *B. hominis* were also observed in faeces of some asymptomatic individuals.[12, 22, 41]

In this prospective study, the physicians of Health Centre Al-Jadeed in Sebha successfully treated fifty patients infected with *B. hominis* with metronidazole 500 mg three times per day for 7 days. In all these patients, no other parasites were demonstrated in the faecal specimens. All patients responded to the therapy and symptoms disappeared after 7 to 10 days. Moreover, *B. hominis* was not detected in the stools of patients after 7 days.

This suggests that the gastrointestinal symptoms in all these patients (positive only for *B. hominis*) are probably due to *B. hominis* infection. Metronidazole has been suggested as the first line chemotherapeutic agent for treatment of *B. hominis* infection.[42-44] However, some authors reported that metronidazole did not eradicate the *B. hominis* completely and was effective only in some individuals.[6, 45, 46]

In conclusion, we report that *B.hominis* is a common intestinal protozoa infection in Sebha and with higher prevelance in warm and hot seasons of the year. In patients with blastocystosis, gastrointestinal symptoms are more likely to be associated with intensity of *B. hominis*. Further research to evaluate the pathogenic potential of this organism is needed.

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