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 hematoxylins 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 > 60 years) 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 14 years). 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

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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients No. Examined</th>
<th>Positive No.</th>
<th>Positive %</th>
<th>Negative No.</th>
<th>Negative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>478</td>
<td>77</td>
<td>16.11</td>
<td>401</td>
<td>83.89</td>
</tr>
<tr>
<td>Children</td>
<td>101</td>
<td>21</td>
<td>20.79</td>
<td>80</td>
<td>79.21</td>
</tr>
<tr>
<td>Total</td>
<td>579</td>
<td>98</td>
<td>16.93</td>
<td>481</td>
<td>83.07</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Gender</th>
<th>Patients No. Examined</th>
<th>Positive No.</th>
<th>Positive %</th>
<th>Negative No.</th>
<th>Negative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>344</td>
<td>61</td>
<td>62.25</td>
<td>283</td>
<td>58.84</td>
</tr>
<tr>
<td>Female</td>
<td>235</td>
<td>37</td>
<td>37.75</td>
<td>198</td>
<td>41.16</td>
</tr>
<tr>
<td>Total</td>
<td>579</td>
<td>98</td>
<td>100.0</td>
<td>481</td>
<td>100.0</td>
</tr>
</tbody>
</table>

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).
**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 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 (Boorom 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

| **Table (4): Mixed infection of *B. hominis* and *E. histolytica*** |
|---------------------------------|-----------------|-----------------|-------|
| **Entamoeba histolytica**       | Positive No. & % | Negative No. & % | Total |
| Blastocystis hominis            |                 |                 |       |
| 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*** |
|---------------------------------|-----------------|-----------------|-------|
| **Giardia lamblia**             | Positive No. & % | Negative No. & % | Total |
| Blastocystis hominis            |                 |                 |       |
| 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.
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.

**REFERENCES**


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الخلاصة

من الأوالي الطفيلية المعوية الشائعة السببية للإسهال في الإنسان، Blastocestis hominis يعتبر الاجريت الدراسة الحالية في الفترة من تشرين الثاني 2017 وليالي اب 2018 في مدينة دهوك في أقليم كوردستان العراق. تضمنت الدراسة جمع وفحص 579 عينة غنائي من كلا الجنسين وبأعمار مختلفة تراوحت بين ستة 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 إلى جزئين، الجزء الأول مستخدماً لمستخدماً باستخدام تقنية الزرع، حيث تم زراعته في وسط مكون من محلول زنك يحتوي على 10% مصل خيل المربط بالحرارة، بالإضافة إلى 0.05% من الأسبراجين، وتم الحضن في درجة حرارة 37°C لمدة 3-4 أيام، ومن ثم تم التشخيص باستخدام المجهر الضوئي بقوة 40X للتحري عن وجود الطفيلي. أما الجزء الثاني من العينة فقد تم التحري عن وجود الطفيلي فيه تبعاً للصفات المظهرية للطفيلي باستخدام المحلول الفضلي (85%) وكذلك باستخدام صبحة الوكال ايوهين ومن ثم الفحص المجهر بقوة 40X.

تظاهرت نتائج الفحص ووجود اصابات بلغ نسبتها 16.93% وقد ظهرت اعلى نسبة للإصابة ضمن الفئة العمرية الأكثر من ستين سنة و كانت بنسبة 21.28%. في حين ان أقل نسبة للإصابة كانت ضمن الفئة العمرية بين 31-40 سنة مع عدم وجود أي فروقات معنوية في نسب الإصابة بين الفئات العمرية المختلفة عند نسبة استحالي (0.5). P>0.05. جدير بالذكر، انه بالرغم من وجود فرق ملحوظ في نسب الإصابة بين فئة البالغين (16.11%) وفي الاطفال (20.79%)، الا انه لم تسجل فروقات معنوية بينهما عند نسبة الاستحالي (0.5). P>0.05. أما بالنسبة لحالات الإصابة المشتركة للطفيلي مع طفيليات معوية أخرى، فقد ظهرت Entamoeba سالبة (11.23 %) حالة اصابة مشتركة بين الطفيلي و B. hominis والبالغة 98 حالة مع عدم وجود فروقات معنوية للإصابة بين الطفيلي، في حين حالات الإصابة المشتركة بين B. hominis و Giardia lamblia وقد ظهرت (8.16%) حالة اصابة مشتركة مع وجود فرق معنوي بين الطفيلي عند الاستحالي (P<0.05).