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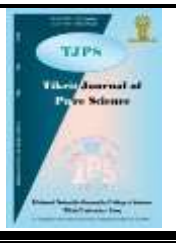

Epidemiological and Biochemical changes of patients infected with *Blastocystis hominis* and Irritable bowel syndrome

Jaber Aowda Kadhim , Hind Salman Jasim

Baquba technical Institute , Middle Technical University , Baquba , Iraq

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Corresponding Author:

Name: Jaber Aowda Kadhim

E-mail: jabarjabar585@gmail.com

Tel:

Abstract

This study was design to examined fecal samples of patients with irritable bowel syndrome (IBS) to detect a relationship between *Blastocystis hominis* infection and IBS. This study was began at January till December 2015 in Kindi teaching hospital / Baghdad .380 patients with IBS and 55 apparently healthy people as control group after making sure of their medical history, clinical and laboratory examinations were done of all subjects which included direct smears examination of stool and culturing of parasite .

Infection of *B. hominis* occurred in males at high rate at October (5.8%) and lowest rate during January (3.7%). Where as in females high rate infection was at August (5.8%) and lowest rate during January (2.1%) *Blastocystis hominis* infection rate in males represent nearly equal in villages and cities (31%,32% respectively) while in females the infection was higher in villages(21%) than in cities (16%).

Incidence of *Blastocystis hominis* infection was the highest (11.0%) at age group 10-15 year. This study also detect that 65.3% of infections were diagnosed by direct smear preparation and 34.7% were diagnosed by culturing.

There was a significant differences of total bilirubin levels in the IBS patient group compared to control group ($p<0.001$).This study represented no significant differences of urea and creatinine levels among patients when compared to control group ($p < 0.05$).

Introduction

Blastocytosis hominis it is common a parasite in the large intestinal of many animals such as (Mammals, birds, Amphibans, Reptiles, Fish, Arthropods, Annelida) and pathogenicity of parasite is still controversial [1]. *B.hominis* considered non-pathogenic protozoan, and can cause intestinal disorders [2,3]. Numerous recent studies strongly suggest that B. hominis is a pathogen [4]. It may associated with diarrhea, dysentery and found in irritatable colon [5] .Role of B. hominis in human disease has been widely debated in the literature during the two last decades, The parasite present in different sizes within the domain (5-30 microns), a refracting light and have oval or spherical shape and has a big vacuole occupy most of the cell while the cytoplasm in therim is compressed in a thin irregular ring contain shiny granules [6] .The most common technique used for diagnosis of this parasite is the permanent stain. The use of xenic cultures in which B. hominis is grown in vitro consider more sensitive in detection [7] .

Many factors affect the intensity and prevalence of intestinal parasitic infection including lack of adequate or proper sanitation facilities, behavioral factors, age, socio-economic factors and geographical factors [8] .

Infection with intestinal parasites has known to cause iron deficiency anemia, growth retardation in children and other physical and mental health problems [9] .

Determination of anthropometric index like body mass index (BMI) to assess under nutrition status and effects of intestinal parasitic infection in the health condition needs research attention [10].

The aim of this study is to detect the prevalence of *B. hominis* in patients and its relation with irritable bowel syndrome.

Objectives

1. Knowledge of statistical prevalence of B. *Hominis* disease among peoples Baghdad in Diyala

governorate, and the percentage according to sex, agee group and region .

2. Know more age gropus and sex, who are suffers from a lack of bilirupin urea and crcatine .

Materials and Methods

The study was carried out in al- Kindi teaching hospital /Baghdad province at the period from . It included 380 patient with IBS disease in kindi Teaching hospital/Baghdad and The outskirts of Baghdad .This study was began January of 2015 to January 2016.

The study population consisted of 380 patients with age ranging from 5 to 60 years , 218 of them were males and 162 were females and 55 apparently healthy people those age and sex matched as a control group. Informational questionnaire for all patients revealed that they coming from different places in Baghdad mainly (Al-Sadder city, Zayonaa ,Palastin street) which classified as urban cities in our study and villages like (Bob- Al-sham and Al-Syradat) which classified as rural places. Endoscopic examination to detect intestinal injury and stool examination were done for all patients.

Laboratory diagnosis of *B. hominis* disease depends on direct Microscopic examination of parasite on stool sample. Where was expected many different morphologic form [11].

1- Vacuolar form which is very common .
2- Granular form .
3- Amoebic form .
4- Cystic form have 4 nuclei with peripheral cytoplasmic rim.

Direct Lugol's iodine preparation [12] and examined under a low power 10x and high power 40x.

Culture of feces

Using of the formalin- ether and sedimentation technique then culture technique to detect trophozoites in the Jones culture medium that specific for *B.hominis* as following [13]:

1- taking 5mg of stool sample and put them in the dish from Jones medium
2- The use of starch which supports good growth of *B.hominis* .Incubated culture media in incubator at temperature 37c and examined after 96 hours .

Diazo method:

Measurement of total biliruininthe blood serum by Electrical auto - analysis system

Measurment direct bilirubin in serum blood used

Direct bilirubin and total bilirubin generation direct reaction with 3.5-Diclorophenyl diazomiumin isolated acid form red colour of azobilirubin Bilirubin + 3.5 DPD ⟶ azobilirubin.

Measurement of total serum bilirubin, direct bilirubin, Uoric acid creatinine were done by using system Equipment processed by the company (Roche Diagnostics GmbH, Germany).This system include random and continuous access, sample selective analyser ,integration of four measuring principles that were absorbance photometry (used for above tests) , turbidimetry, fluorescence polarimetry, ion-selective electrode potentiometry.

Also in this study found there are significant different in bilirubin and no significant different uoric acid and creatinine.

Statistical analysis:

Results were expressed as the mean ± SD for continuous variables. Uni-variate analysis was performed using the independent sample t-test. The Pearson chi-square test were also used whenever appropriate. Statistical interpretation of data was performed by using the com-puterized software program SPSS version 10.0 (SPSS Inc., Chicago, IL)

Results

Irritable bowel syndrome participant were 218 males and 162 females with age range from 5 -60 years old. Healthy control were 35 males and 20 females that age matched with case group. Clinical symptoms of the IBS patients were reported respectively: abdominal pain (43.2%), diarrhea (25%) and constipation (7.8%).

B. hominis was detected in 57% of males and in 43% of females in IBS group. October reported high number of infection in males (5.8%) and August showed high number of infection in females (5.8%) , while January reported lowest number of infection in both males and females (3.7%,2.1% respectively), (Table1).

Table (1) :Distribution of *Blastocystis hominis* during months of year.

1	Month of Year	Patient number	Male	Prevalence of infection %	Female	Prevalence of infection %
2	January	22	14	3.7	8	2.1
3	February	36	16	4.2	20	5.3
4	March	29	20	5.0	9	2.3
5	April	30	18	4.7	12	3.1
6	May	31	20	5.7	11	4.2
7	June	36	16	4.2	20	4.3
8	July	30	17	4.0	13	3.4
9	August	41	19	5.0	22	5.8
1	September	30	20	5.2	10	2.6
1	October	34	22	5.8	12	3.2
1	November	30	19	5.0	11	2.9
1	December	31	17	4.5	14	3.8
1	Total	380	218	57%	162	43%

Its clarified that *B. hominis* infection rate increased in villages(52%) rather than that in cities (48%). *B. hominis* infection rate in males represent nearly equal in villages and cities (31%,32% respectively) while in females the infection was higher in villages(21%) than in cities (16%), (Table2).

Table (2): Geographical distribution of *Blastocystis hominis* in cities and villages.

Place of living	Number of examined samples	Prevalence of infection %	Infected Males	Prevalence of male infection %	Infected Females	Prevalence of female infection %
villages	199	52	119	31	80	21
Cities	181	48	120	32	61	16
total	380	100	239	63	141	37%

Its cleared from results of table (3) that (10 – 15) years age group was at highest percentage (11.0 %)of infection with *B. hominis* ,while lower percentage occurred at age group range from 40-45(7.4%).

Table (3) : *B. hominis* infection at different age groups.

Age grouping year	Number of examined samples	Prevalence of infection %
5-10	33	8.7
10-15	42	11.0
15-20	41	10.8
20-25	34	9.0
25-30	31	8.2
30-35	35	9.3
35-40	30	7.9
40-45	28	7.4
45-50	40	10.6
50-55	29	7.7
55-60	36	9.4
Total	380	100%

Its showed from table (4) that 280 out of 380 patients were have positive endoscopic cases. 183(65.3%) of that 280 positive cases were positive for *B. hominis* detected by direct stool examination and 97 (34.7%)of them detected by culturing.

Table (4) : Prevalance of infection in both methods of diagnosis of *Blastocystis hominis* (direct smear and culturing) in different months of year.

	Month of Year	Patients	Endoscopic examination		Lab examination			
			Positive cases		Direct smear	%	Technical culture	%
1	January	22	14	5%	10	3.5	4	1.4
2	February	36	30	10.7%	20	7.1	10	3.5
3	March	29	20	7.1%	10	3.5	10	3.5
4	April	30	22	8%	12	4.2	10	3.5
5	May	31	23	8.21%	17	6.0	5	1.7
6	June	36	29	10.3%	20	7.1	9	3.2
7	July	30	22	8%	16	5.7	8	2.8
8	August	41	31	11%	18	6.4	13	4.6
9	September	30	19	6%	15	5.3	4	1.4
10	October	34	25	9%	15	5.3	10	3.5
11	November	30	20	7.1%	16	5.7	4	1.4
12	December	31	25	8.2%	15	5.3	10	3.5
13		380	280	100%	183	65.3	97	34.7

Biochemical tests that done for both IBS patients and control group *B. hominis* detected under microscope (x40) which was unicellular, it was 5-30 microns in diameter, with the usual range being 8-10 microns and was usually spherical. *B. hominis* cyst-like forms had two to four nuclei located in the rim of the cytoplasm.[14]

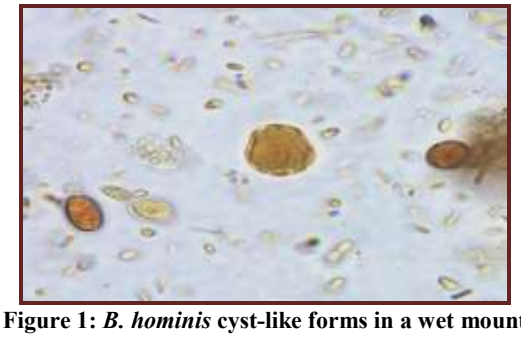

Figure 1: *B. hominis* cyst-like forms in a wet mount treated with lugols iodine(x40)

Discussion

B.hominis is one of intestinal protozoan that found in human with prevalence between 30-50% in the developing countries.[15].

The current study showed there were no significant relation between *Blastocytosis hominis* infection and months of the year (Table 1). Microscopic, colonscopy examination showed that the peak of infection recorded in Autumn season mainly in August (41 patients) then it began to decline in January in males and Females. These results were in agreement with previous study in in 48 states United States and district of columbia [16]. The reason of that variation in infection rate within months of year that activation of virulent parasite during these months may be due to environment condition .

In this study, *B.hominis* infection rate appeared higher in villages (52%) than what found in cities (48%) .This results were accordant with other study in the Irbed region of northern Jordan [17] and that may due to inadequate sanitation standards .

Its cleared from table (3) that percentage of *B. hominis* infection was high at age group (10 – 15) years which resemble the result of study at Sao Paulo State in Brazil [18] and that because malnutrition which lead to disturbance in immunity at that period of life.

There are several methods commonly used to detect human infection with B. hominis. All are based on the demonstration of parasite in fecal samples [19].

Our study showed that 183 out of 280 stool specimens were positive for *Blastocytosis hominis* . Its observed that 183(65.3%) of that 280 positive cases were positive for *B. hominis* detected by direct stool examination and 97 (34.7%)of them detected by culturing.That results incompatible with the results of study that done in Philippines which showed that culturing method detect high number of positive *B. Hominis*[20]. This finding may due to unsuitable condition for culturing , antibiotic interaction, or low number of organism in fecal sample.

In this study, Blastocytosis patients showed significant differences ($p < 0.001$) of total and direct bilirubin levels compared to control group thus may due to the damage of the liver, which leads to the inability of the liver to excrete bilirubin, and the result of obstruction of the bile ducts or as a result of breakage of red blood cells, leading to an increase in bilirubin in the blood .Increased levels of bilirubin in the blood have been shown in many parasitic diseases, as well as Blastocytosis disease and risk factors such as arterial parasites, diabetes, metabolic syndrome and obesity. High serum bilirubin levels may occur because of many factors including smoking, sex, fasting, medicines, race, age. [21,22].

This study represented no significant differences of urea and creatinine levels among patients when compared to control group ($p > 0.05$). This finding is similar to the results from previous study [23] that showed normal levels because intestinal parasitic infections do not usually present with disturbance in renal function.

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دراسة المتغيرات الوبائية والكيموحيوية للمرضى المصابين بطفيلي *Blastocystis hominis* ومتلازمة القولون المتهيج

جابر عودة كاظم ، هند سلمان جاسم

معهد تقني بعقوبة ، الجامعة التقنية الوسطى ، بعقوبة ، العراق

الملخص

صممت هذه الدراسة لفحص عينات البراز للمرضى المصابين بمتلازمة القولون المتهيج للكشف عن العلاقة بين الاصابة بطفيلي ***Blastocystis hominis*** ومتلازمة القولون المتهيج. بدأت الدراسة في شهر كانون الثاني ولغاية شهر كانون الأول من عام 2015 حيث جمعت العينات من مستشفى الكندي التعليمي / بغداد. شملت الدراسة (380) مريض مصاب بمتلازمة القولون المتهيج و(55)شخص كمجموعة سيطرة. بعد التأكد من السجل الطبي والفحوصات السريرية والمختبرية لجميع عينة البحث حيث تم اجراء فحص المسحة المباشرة لعينة البراز بالأضافة الى استنبات الطفيلي على الاوساط الزرعية. اظهرت الدراسة شدة الاصابة بطفيلي **(B.hominis)** كانت باعلى معدل في شهر تشرين الاول (5.8%) في الذكورواقل اصابة في شهركانون الثاني حيث بلغت (3.7%)، أما بالنسبة الى الاناث فكانت اعلى اصابة في شهر آب حيث بلغت (5.8%) واقل معدل في شهر كانون الثاني (2.1%) .

بينت الدراسة نتائج متساوية بالنسبة للتوزيع الجغرافي للاصابة بالطفيلي لدى الذكور بين المناطق الريفية والمدينة حيث كانت النسبة (31%، 32% على التوالي) أما بالنسبة للاناث كانت النسبة الاصابة الاعلى في المناطق الريفية (21%) منه في المدينة (16%) .

اتضح من خلال الدراسة ان نسبة انتشار الطفيلي كان عاليا (**11%**) في الفئات العمرية التي تتراوح بين (**10-15** سنة). في هذه الدراسة تم تشخيص (65.3%) من الاصابات عن طريق فحص المسحة المباشرة للبراز و(34.7%) تم تشخيصها بواسطة الأستنبات على الوسط الزرعي.

كما وبينت الدراسة وجود اختلاف معنوي($P < 0.001$) في مستويات البيليروبين الكلي في مجموعة الاشخاص المصابين بمتلازمة القولون المتهيج بالمقارنة مع مجموعة السيطرة. كما واظهرت الدراسة عدم وجود فرق معنوي في مستويات كل من اليوريا والكرياتنين في المرضى مقارنة مع مجموعة السيطرة ($P>0.05$).