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Prevalence and Physiological Effect of Blastocystis Hominis on Lipid Metabolism, Magnesium, and Zinc Levels in Diarrheal Patients

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Abstract

Blastocystis hominis is a microscopic single-celled organism commonly known as a protist or parasite. It was first discovered in the early 20th century by a Scottish scientist named Alexei Pavlovich Alexeieff. Blastocystis hominis is found in the intestines of humans and other animals, where it can colonize the gastrointestinal tract. The objective of this study was to examine the presence of the Blastocystis hominis parasite in the stool samples of patients experiencing diarrhea and to explore its potential physiological effects. The study involved two groups: 1-the patients group, which consisted of 220 samples who suffered from diarrhea, and abdominal pain and 2-the control group, which consisted of 100 samples of healthy individuals. The age range of participants ranged between 4-40 years. The outcome indicated that the vacuolar form was the most common morphological appearance observed in the stool. The result showed non-significant difference was observed (P>0.05) for all ages. Furthermore, a statistically significant difference (P≤0.05) was observed in the infection rate between males (58.00%) and females (42.00%). The physiological examinations were conducted using Spectrophotometer revealed notable findings. There was a significant increase (P≤0.01) in the levels of cholesterol, low-density lipoproteins-cholesterol (LDL), very low-density lipoproteins (VLDL), and triglycerides (TG), in patients with diarrhea infected with B. hominis compared to the control group. Conversely, concentrations of magnesium, zinc, and high-density lipoproteins (HDL) showed a significant decrease (P≤0.01) in the same patient group compared to the control group.

Keywords: Blastocystis hominis, Magnesium, Zinc, Lipid profile.

الخلاصة

المتبرعمة الكيسية البشرية هي كائن مجهري وحيد الخلية يُعرف باسم الاوالي أو الطفيليات. تم اكتشافه لأول مرة في أوائل القرن العشرين من قبل عالم إسكتلندي يدعى Alexei Pavlovich Alexeieff. تم العثور على المتبرعمة الكيسية البشرية في أمعاء البشر والحيوانات الأخرى، حيث يمكنها استعمار الجهاز الهضمي. كان الهدف من هذه الدراسة هو فحص وجود طفيلي Blastocystis hominis في عينات البراز للمرضى الذين يعانون من الإسهال واستكشاف آثاره الفسيولوجية المحتملة. اشتملت الدراسة على مجموعتين: مجموعة المرضى التي تكونت من ٢٢٠ عينة اشتكوا من الإسهال، وآلام البطن، ومجموعة الضبط التي تكونت من ١٠٠ عينة من الأشخاص الاصحاء. كان النطاق العمري للمشاركين ٤-٤٠ سنة. أشارت النتائج إلى أن شكل الفراغ هو المظهر المورفولوجي الأكثر شيوعًا الذي لوحظ في البراز. أظهرت النتائج عدم وجود فروق معنوية (P> 0.05) لجميع الأعمار. علاوة على ذلك، لوحظ وجود فروق ذات دلالة إحصائية (P≤0.05) في معدل الإصابة بين الذكور (٥٨,٠٠٪) والإناث (٤٢,٠٠٪). كشفت الفحوصات الفسيولوجية التي أجريت باستخدام جهاز قياس الطيف الضوئي عن نتائج ملحوظة. كانت هناك زيادة معنوية (P≤0.01) في مستويات الكوليسترول والبروتينات الدهنية منخفضة الكثافة والكوليسترول (LDL) والبروتينات الدهنية منخفضة الكثافة (VLDL) والدهون الثلاثية (TG)، في مرضى الإسهال المصابين بطفيلي B. hominis مقارنة بمجموعة السيطرة. على العكس من ذلك، أظهرت تراكيز المغنيسيوم والزنك والبروتينات الدهنية عالية الكثافة (HDL) انخفاضًا معنويًا (P≤0.01) في نفس مجموعة المرضى مقارنة بمجموعة السيطرة.

INTRODUCTION

Blastocystis parasites are among the most prevalent eukaryotic organisms in human feces worldwide. However, they are more widespread in developing countries (30-100%) compared to developed countries (1.5-15%), which is thought to be due to inadequate personal and environmental hygiene, as well as limited access to safe water supply and insufficient waste removal services [1][2]. Blastocystis exhibits a diverse range of morphological forms throughout its life cycle including vacuolar, cystic, amoeboid, and granular. The vacuolar forms indicates its high degree of polymorphism [3] and its classification within the Stramenopiles group [4].

Blastocystis species demonstrate remarkable genetic diversity with a current total of 26 established subtypes based on the small subunit of the ribosomal RNA gene (SSU rRNA). Each subtype exhibits a distinct distribution pattern and is associated with various host species across the globe [5][6]. Previous studies have indicated that Blastocystis subtypes capable of infecting humans include ST1 to ST10 and ST12. However, it is widely acknowledged that ST1, ST2, ST3, and ST4 are the most identified subtypes in human infections [7-10].

The pathogenicity of the parasites that belong to the genus Blastocystis is still debatable because these parasites have been reported in both asymptomatic and symptomatic individuals [2][11][12]. However, the lack of symptoms in subjects carrying these parasites does not mean that these parasites are not pathogenic[13][14]. Because some subtypes (STs) of Blastocystis sp. are not pathogenic such as ST2, while ST1 is pathogenic to humans [15][16]. Moreover, numerous epidemiological studies have documented a significantly elevated prevalence of Blastocystis among individuals diagnosed with irritable bowel syndrome (IBS) [17]. The presence of the parasite is frequently associated with a range of symptoms including nausea, urticaria, fever, diarrhea, vomiting, anorexia, cramps, flatulence, discomfort, and abdominal pain. The illness can manifest as either an acute or chronic condition, with symptoms persisting over an extended period, sometimes lasting for several years [18][19].

MATERIALS AND METHODS

Study was conducted between November 2022 and March 2023 at Al-Kadhimiya Teaching Hospital and Al-Shaheed Mohammed Baqir Al-Hakeem Hospital in Baghdad, Iraq. The study involved two groups: the patient group, which consisted of 220 samples from patients who complained of diarrhea and abdominal pain, and the control group, which consisted of 100 samples. Samples were collected from individuals of varying age groups (4-40 years) encompassing both sexes. Stool examinations were performed using microscopy, and laboratory examinations were conducted to assess physical characteristics such as consistency, presence of mucus, and color.

Microscopic Examination of Stool Samples

The stool samples collected were subjected to direct microscopy using double wet preparations of 9% NaCl and 2-5% Lugol's iodine.

Blood Samples and Biochemical Tests

For the blood samples, gel-containing tubes were used to facilitate clotting. After allowing the samples to clot for 20 minutes at room temperature, they were centrifuged at 3000 rpm for 5 minutes to separate the serum. The obtained serum from each patient and control group was then transferred to three Eppendorf tubes using a pipette and stored in a refrigerator at -20°C for subsequent analysis. The analysis included the measurement of serum levels of magnesium and zinc, as well as the lipid profile comprising total cholesterol, triglycerides, HDL, LDL, and VLDL by using spectrophotometer with 500 nm wavelength.

Statistical Analysis

The statistical software SAS (2018) was utilized to analyze the impact of different groups (patients and control) on the study parameters. T-tests were employed to compare means and determine statistical significance. Additionally, the chi-square test was employed to compare percentages with probabilities of 0.05 and 0.01.

RESULTS AND DISCUSSION

The current study involved the collection of 220 stool samples from individuals presenting with gastrointestinal symptoms including acute diarrhea and abdominal pain. Microscopic examination, utilizing Lugal's Iodine Stain and direct wet smears, were performed on all samples. Among the examined cases, 100 were found to be positive for

Blastocystis hominis based on microscopic examination, while 120 cases were negative results. The microscopic examination involved observing the samples under a microscope at both low power (x40) and high power (x100). The observed forms of Blastocystis hominis exhibited a characteristic vacuolar morphology, characterized by a central body or vacuole surrounded by a thin cytoplasmic rim containing up to six nuclei. This study categorized participants into four age groups. The first age group (4-10 years) had 16 (16%) individuals in the control group and 19 (19%) individuals in the patient group. The second age group (11-20 years) consisted of 23 (23%) individuals in the control group and 27 (27%) individuals in the patient group. The third age group (21-30 years) included 34 (34%) individuals in the control group and 32 (32%) individuals in the patient group. The fourth age group (31-40 years) comprised 27 (27%) individuals in the control group and 22 (22%) individuals in the patient group. The P-value for all age categories was more significant than 0.05. The mean±SE (mg/dL) of the control group was (19.53±2.06), while the mean ± SE (mg/dL) of the patient group was (17.02±1.37), with a *P-value* greater than 0.05 as shown in Table 1.

Table 1. Distribution of sample study according to age in control and patients' groups.

Variables	Control without Blastocystis / No (%)	Patients infected with Blastocystis / No (%)	P value
Average age (years ± SE)	19.53±2.06	17.02±1.37	0.302 NS
4–10 yrs.	16 (16.00%)	19 (19.00%)	0.612 NS
11–20 yrs.	23 (23.00%)	27 (27.00%)	0.571 NS
21–30 yrs.	34 (34.00%)	32 (32.00%)	0.805 NS
31–40 yrs.	27 (27.00%)	22 (22.00%)	0.475 NS
Total	100	100	

*NS: Non-Significant.

The study found no significant correlation between age and Blastocystis hominis infection, consistent with previous research conducted by Merza [20] in Duhok City, Bugis [21] in Saudi Arabia, and El Safadi [22] in Lebanon. However, some studies such as Dagci [23], have reported a higher frequency of Blastocystis infection among the 20-29 age group in symptomatic patients. This was also observed in the current study where the infection was most common in the 21-30 age group.

This study observed that among patients with diarrhea and *Blastocystis hominis* infection, males had a higher proportion (58%) compared to females (42%). The *P-values* for gender distribution were 0.0499 for females and 0.033 for males, indicating a statistically significant difference in gender distribution between the healthy population and patients with Blastocystis hominis infection, as shown in Table 2.

Table 2. The distribution of sample study according to Gender in control and patients' groups.

Variables	Control without Blastocystis/No (%)	Patients infected with Blastocystis/ No (%)	P-value
Female	62 (62.00%)	42 (42.00%)	0.0499 *
Male	38 (38.00%)	58 (58.00%)	0.0330 *

* ($P \leq 0.05$)

These findings align with previous studies conducted by Sylla [24], Bugis [21], and Khalili [25], which also reported a higher prevalence of Blastocystis hominis infection in males than females. Specifically, Sylla [24] found a rate of 53.5% for males and 46.5% for females. Bugis [21] reported a rate of 51.6% for males and 48.4% for females and Khalili, [25] found a rate of 61% for males and 39% for females. Overall, comparing the gender distribution in Blastocystis hominis infection across studies emphasizes the significance of considering regional and demographic factors.

Biochemical Parameters

The study's Table (3) indicates highly significant differences ($P \leq 0.01$) in lipid levels between patients infected with the Blastocystis group and healthy control subjects. The patient group exhibited significantly higher levels of cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) while experiencing a significant decrease in high-density lipoprotein (HDL) compared to the healthy control group. These findings align with previous research demonstrating similar alterations in serum lipid levels among individuals with Blastocystis hominis infection.

The observed elevation in serum lipid levels may be attributed to the capacity of Blastocystis hominis to synthesize and secrete lipids. Previous laboratory investigations have demonstrated that axenic strains of Blastocystis hominis exhibit lipid droplets in the culture medium comprising a variety of phospholipids and neutral lipids resembling those found in animal cells. These findings indicate the parasite's ability to generate these lipids [26,27]. Additionally, another study reported increased serum cholesterol and VLDL levels alongside decreased serum HDL levels in patients with Blastocystis hominis infection [28].

Table 3. Comparison between patients and control groups in Lipid profile.

Group	Mean ± SE (mg/dL)				
	Cholesterol (TC)	Triglyceride (TG)	HDL	LDL	VLDL
Patients (100)	201.30± 1.10	258.97± 2.36	23.65± 0.44	125.86± 1.19	51.80± 0.47
Control (100)	159.22± 3.46	157.58± 3.73	33.66± 1.29	94.05± 3.72	31.52± 0.75
T-test	5.757 **	8.457 **	2.185 **	10.691 **	6.189 **
P-value	0.0001	0.0001	0.0001	0.0003	0.0001

** (P≤0.01).

Zinc and magnesium parameters

In this study, the serum levels of magnesium and zinc were measured in patients infected with Blastocystis and compared to a control group. The results revealed a significant decrease in magnesium (0.91±0.03 mg/dL vs. 1.29±0.03 mg/dL) and zinc (9.51±0.10 mg/dL vs. 10.43±0.27 mg/dL) levels among the patient group compared to the healthy controls ($P \leq 0.01$), as shown in Table (4).

These findings align with a previous study by[29], which demonstrated a decline in magnesium and zinc levels in Blastocystis-infected individuals compared to healthy individuals. The decrease in magnesium levels may be attributed to disruptions in enzyme systems such as alkaline phosphatase or ATPase. The observed decrease in zinc levels could be attributed to increased parasite consumption during its growth and zinc deficiency related to impaired immune function and chronic inflammation. Intestinal epithelial damage caused by parasites can lead to malabsorption and deficiencies in essential minerals like magnesium and zinc. These parasites utilize the host's nutrients for their energy needs [30,31]. Insufficient absorption, reduced dietary intake, or overutilization of zinc stores can contribute to zinc deficiency [32].

Table 4. Comparison of serum levels of Zn and Mg between patients and control groups.

Group	Mean ± SE (mg/dL)	
	Zn	Mg
Patients	9.51±0.10	0.91±0.03
Control	10.43±0.27	1.29±0.03
T-test	0.477 **	0.0887 **
P-value	0.0032	0.0075

** (P≤0.01).

CONCLUSIONS

These results emphasize several key aspects, including the predominance of the vacuolar form of Blastocystis hominis, the variation in infection rates between genders, and the influence of Blastocystis hominis infection on lipid metabolism as well as magnesium and zinc levels in patients presenting with diarrhea.

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