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Prevalence of Blastocystis hominis in patients with diabetes mellitus in Balad City , Salahaddin Province, Iraq

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Article Informations

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A B S T R A C T

Blastocystis sp. is anaerobic,global, eukaryotic, found in the human gastrointestinal tract. The purpose of the current study is to estimate the prevalence of *B. hominis* infection in people with diabetes mellitus. This study is conducted on a total of three hundred (300) individuals, ranging between 4 and 70 years, included (150) diabetic group and (150) non diabetic group. Samples are taken between November 2022 and May 2023. The practical portion and the collection of samples have been done in laboratories of General Balad Hospital in Balad City; Salahadin; Iraq. The study include the collection of 2 ml of venous blood, which is used to measure the HbA1c ratio with stool sample. This is used to examine the presence or absence of *B.hominis* by microscopic examination after it had been cultured. Modified Jones' Medium has reportedly been used for *Blastocystis sp.* in vitro culture. Only 80 diabetic patients (50 male, 30 female) were infected with *B.hominis* after the laboratory diagnosis, and only 50 people (13 male, 37 female) among the healthy participants have *B.hominis*. This study demonstrate that the DM group had a higher prevalence of this protozoan than the non-DM group. Also, it notice that there is a link between the presence of *Blastocystis* and other participant characteristics (risk factors). Most results in the present study are display a highly significant difference between both genders, residence, source of drinking water and keeping animals ($p<0.05$),but there is no statistically correlation with infection by this parasite among the classes of age and educational level ($p>0.05$) .

Introduction

Blastocystis sp. is anaerobic, global, eukaryotic, found in the human gastrointestinal tract[1]. In both developing and wealthy nations, *Blastocystis* is the parasite that is most frequently isolated from samples of human feces. 3.3% of developed nations experience infection rates, while 53.8% of underdeveloped nations do[2]. A Russian doctor named Fedor Aleksandrovich Lesh discovered *B. hominis* in 1870 [3], and Brumpt picked the Scientific name in 1912 [4]. It comes in a variety of morphological shapes. The morphologies that have been best described thus far are vacuolar, granular, ameboid, and cystic[5]. The cyst, which can range in size from 6 to 40 µm and is the most common variety observed in human feces[6], it is transmitted mostly through fecal-oral methods and is made worse by unsanitary settings[7]. Blastocystis life cycle already has close contact with animals. According to studies, eligible hosts can become infected with Blastocystis by ingesting raw aquatic plants that have been contaminated with cysts or drinking untreated water. Recent research has shown that dirty hands can act as a vehicle for the direct contact transmission of cysts from infected people or from contaminated soil [8]. The cysts only become vegetative forms in the appropriate host after ingestion. In the large intestine, the cyst form is excystrated to release the vacuolar form. Any of the other types can change from the vacuolar forms. The amoeboid, avacuolar, and multi-vacuolar forms of diarrhoea are frequently observed, raising the hypothesis that these forms may contribute to the pathophysiology [9]. According to recent studies ,the parasite is only transmitted in its cyst stage[10]. According to certain research, the most significant factor in the transmission of the parasite is contaminated water [11]. Diarrhoea, stomach discomfort, and vomiting are the most typical symptoms of blastocyst infection. Protease-secreting ameboid forms may be the most deadly, according to certain case reports. These forms are typically observed during a patient examination in stool samples from symptomatic patients. Cysteine proteases induce the production of interleukin-8 by mucosal cells. This process results in dehydration and intestinal irritation in people who are affected. The protease decrease secretory IgA, aiding immune evasion and parasite survival [12]. In general, the prevalence is larger in developing nations (22.1–100%) than in developed nations (0.5-23.1%) [13]. This variation could be attributed to various levels of hygiene standards, animal exposure, ingestion of tainted food or drink, and improper waste disposal [11]. According to reports, a variety of variables, including host immune state, geographic location, age, and dietary habits, may have an impact on the prevalence of Blastocystis sp. infection [11,14]. Intestinal parasite infections in patients with diabetes mellitus can cause more severe complications [15].

Diabetes mellitus (DM) is a category of metabolic illnesses where a person has excessive blood sugar levels for one of two reasons: either the body does not create enough insulin or the cells do not react to the insulin that is produced[16]. The characteristic Symptoms of polyuria, polydipsia, and polyphagia are produced by this elevated blood sugar level[17]. Diabetes is believed to be a long-lasting, chronic issue that gradually increases blood pressure, malfunctioning, and dysfunction in numerous organs; as a result, it increases a person's susceptibility to a variety of illnesses, particularly infections [18]. Immuno suppresse patients with persistent diarrhoea frequently had *C. parvum, B. hominis,* and *I. belli* [19]. These opportunistic parasites, however, are uncommon in immunocompetent patients [20]. *Blastocystis hominis* parasite is revealed in many cases with uncontrolled Diabetes. There have been reports of immunosuppression in DM patients[21].

118 people with diabetes (patient group) and 118 people without diabetes (control group) were studied by Mohtashamipour et al. to determine the frequency of intestinal parasites. He claimed that *B.hominis*, a type of intestinal parasite, may pose a risk for developing DM in patients[22]. *Blastocystis hominis* was the most common intestinal parasite found in diabetics (29%) according to research linking this parasite to the disease[23]. The four drugs metronidazole, tinidazole, albendazole, and nitazoxanide are the most frequently used to treat intestinal parasites., however resistance to these medications has increased recently [24]. The need for safer and more effective alternative therapies is driven by the risky side effects and potential development of drug resistance to the conventional medication, metronidazole (MTZ); therefore, recent study have examined the effectiveness of several plant and herbal extracts against this parasite [25]. Researcher have underlined the necessity for accurate Blastocystis testing to diagnose patients and to separate treatments that completely eradicate the organism from those that just temporarily relieve symptoms. Researcher has underlined the necessity for accurate Blastocystis testing to diagnose patients and to separate treatments that completely eradicate the organism from those that temporarily relieve symptoms [26]. The purpose of the current study is to estimate the prevalence of *B. hominis* infection in people with diabetes mellitus

Materials and methods:

Study population:-

The current study was conducted at The General Balad Hospital in Salahaddin Province, Iraq, on 300 Iraqi patients (4–70 years old), 150 patients with Diabetes Mellitus , and 150 individuals who appeared to be in good health (control group) during the period from November 2022 to May 2023. Each patient included in this trial had blood and stool samples taken.

Collection of samples

A-Collection of blood samples :-

A plastic disposable syringes were used to pierce veins in order to collect 2 ml of blood. This blood was put to an EDTA tube to measure the HbA1c ratio by using Afias instrument.

B-Collection of stool samples

In clean, dry, wide-mouth plastic containers with tight lids, fresh feces samples weighing about 2 g each were collected. The patient's name, serial number, and the date of collection were printed on labels that were attached to each container. The detection of *Blastocystis sp*. involved wet mounts made of one drop of saline or iodine with stool sample (50 mg) was examined microscopically under 400x and 1000x objective lenses. The detection was also involved in vitro cultivation of the stool on modified Jones' medium[27]. 5 ml of Jones' medium was added to sterile tubes containing each feces sample, and the tubes were incubated at 37 °C for two to three days [28]. The culture was examined 24, 48, 72, and 96 hours after the first batch. With the use of a sterile Pasteur pipette, a single drop was taken from the tube and examined, first under (10x) and then (40x) magnification. After 96 hours, the culture was ruled negative and rejected due to the lack of *Blastocystis* sp. 1 ml of the positive culture sample was transferred to new media using a sterile Pasteur pipette while maintaining complete sterility (inside the UVR-Laminar flow hood) for 48–72 hours [29].

Statistical Analysis:

Data were gathered, calculated, tabulated, and statistically analysed by using a statistical computer program SPSS .Also chi square test was used to find significant differences at the level of probability 0.05.

Results

Stool samples from 150 diabetic patient (who had glycated haemoglobin (HbA1c) level > 6.5 %) , and 150 non-diabetic patients served as the control group (who had HbA1c level < 6.5 %) . Both were examined microscopically for the presence of *Blastocystis .hominis* parasite . The microscopic examination either for the direct iodine smear from the stool, or direct wet mount from in vitro cultured media for the stool samples . The cultivation was done on Jones' medium, which is the preferred medium for this parasite's growth[27,28]. The number of diabetes patient feces samples that tested positive for *B.hominis* was just 80. In addition, there detected parasites in just 50 samples from non-diabetics. The vacuolar form was the most often seen form in our study , followed by the granular form see Figure-1.

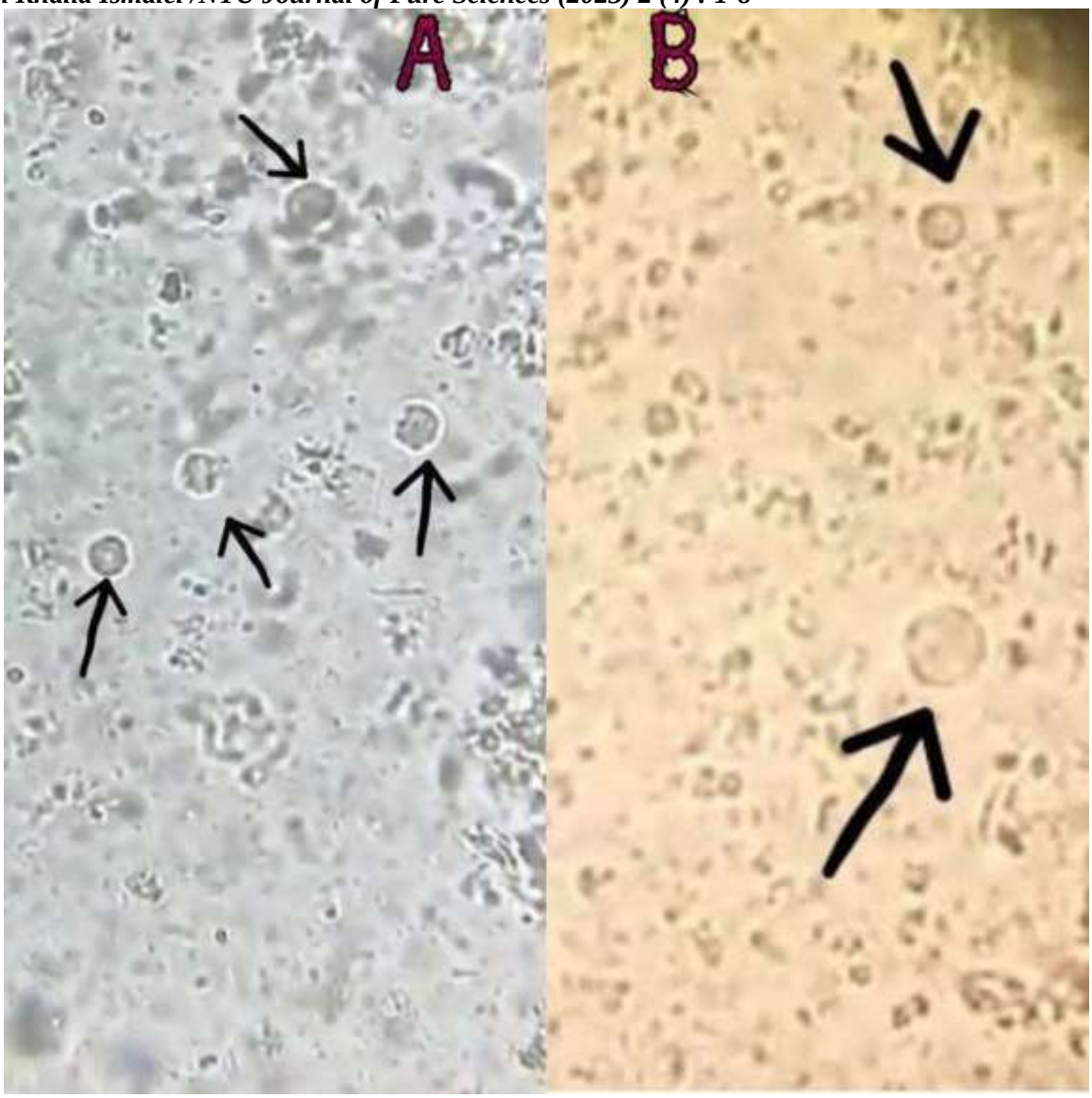

Figure 1: (A) numerous vacuolar and granular forms in the direct smear from the culture (Black arrow) (40x). (B) vacuolar forms in the direct iodine smear (Black arrow) (40x).

Table 1: Prevalence of Blastocystis hominis in patients stool samples with diabetes mellitus in Balad City , Salahaddin Province, Iraq.

No.	Risk factor	No. of Diabetic patients positive with parasite	%	P- Value	No. of Non diabetic patients positive with parasite	%	P- Value
1	**Gender**						
	Male	50	62.5	0.01*	13	26.0	0.01*
	Female	30	37.5		37	74.0	
2	**Age**						
	4-14	10	12.5		5	10.0	
	15-24	8	10.0		9	18.0	
	25-34	9	11.3	0.548	6	12.0	0.01*
	35-44	8	10.0		12	24.0	
	45-54	16	20.0		10	20.0	
	55-70	29	36.2		8	16.0	
3	**Residence**						
	Urban	14	17.5	0.01*	7	14.0	0.01*

	Rural	66	82.5		43	86.0	
4	**Level of Education**						
	Non-educated	46	57.5	0.446	31	62.0	0.01*
	Primary	16	20.0		11	22.0	
	High	18	22.5		8	16.0	
5	**Source of drinking water**						
	Treated	28	35.0	0.01*	26	52.0	0.01*
	Untreated	52	65.0		24	48.0	
6	**Keeping Animals**						
	Yes	58	72.5	0.01*	42	84.0	0.01*
	No	22	27.5		8	16.0	

Not:*P-value <0.05 considered statistically significant difference .

Discussion

Blastocystis sp. is an intestinal protozoan and most commonly reported in human in children and adults in developing country, has a world-wide distribution with prevalence of 30% to 60 % in developing country and 1,5% to 20% in developed country [31,32]. The number of people dying from parasitic and infectious diseases is significant and steadily rising [33].Diabetic patients should have regular parasite infections examined because there is a strong link between diabetes and gastroenteritis brought on by intestinal parasites [34]. Due to a weak ened immune system, diabetic patients are more likely to contract an intestinal parasite [35,36]. It's also possible that this is because some of the study participants were not fully aware of how serious their conditions were, so they neglected to eat a healthy diet and take their prescribed medications on time to boost their immunity and reduce their vulnerability to parasite infection.

Based on the data in Table-1, the findings of this investigation, which concur with those of a study conducted earlier in 2023[37] and the findings from these studies [38-42], showed that parasite infection in diabetic patients is higher than in the control group (non-diabetic group). Contrarily, to a researcher [43] discovered that people with diabetes mellitus (T1DM + T2DM) have a lower chance of developing intestinal parasitosis .In the current study, it was evident that the prevalence rate of *B.hominis* infection in the diabetic group was 62.5% (50/80) for males and 37.5% (30/80) for females, respectively. This ratio is different from those in Kirkuk/Iraq [44] that found the percentage of infected females with parasite was higher than percentage of males . The rate of prevalence in the current study is consistent with a study that was conducted in 2020 that confides the ratio of infected males was higher than infected females[45].

According to the table-1, diabetic patients between the ages of 55 and 70 had the highest rates of *B.hominis* infection which recorded 36.2%,compared to patients aged between the ages of 45 and 54 which recorded 20.0% . The other age groups showed different rates, but it were quite low. This might be caused by the weak ened immune system that occurs with ageing in addition to the presence of diabetes mellitus. The findings were consistent with prior research that indicated *Blastocystis sp*. infections were more prevalent in older age groups, with a mean age of 57 years [46]. Additionally, it disagree with research findings that conducted in 2020 [45] that showed *Blastocystis* infection was higher for the age group > 65 years. However, according to other studies, *Blastocystis sp.* infections are frequent in people under the age of 15 [47, 48]. Because the quantity and kind of patients who participated in each study varied, it may be possible to explain the difference in the results regarding age.

In the current study, rural residents of DM patients had a higher prevalence of parasitic infections (82.5%). Frequently coming into contact with animals and soil, poor hygiene, having a big family, and using contaminated water sources are some causes of this [49]. Individuals who live largely in rural areas of low-income nations frequently harbour several parasite illnesses[50]. Additionally, the present study findings (76.9% vs. 23.1%) were in line with [42], who found that patients in rural areas had a greater rate of Blastocystis infection than those in urban areas. According to [51] ,urban regions saw a higher rate of *Blastocystis sp*. detection than rural areas . Also according to study that was conducted in kirkuk/Iraq [44], *Blastocystis sp.* was more common in urban dwellers and this was disagree with present study results.

As observed in the table-1 , results show that occurrence of *B.hominis* infections with a high percentage represents (57.5%) in non-educated diabetic patients. This was agree with previous investigation which indicated that the rate of infection with parasite was higher in non-educated patient than educated ones due to low hygienic awareness, also the impairment of immune system in diabetic patient giving rise to development of more severe parasitic infections[45,52]. In contrast to this finding ,the presence of intestinal parasites in

diabetes patients was not influenced by their education level, according to a study conducted in Iran [22]. With agreement to our conclusion, a study conducted in 2020 that found poor educational background people with diabetic have greater ratio for infection with *B.hominis* than non-educated healthy People[53].

In undeveloped and emerging countries, poor hygiene and contaminated water and food sources are the main causes of IPs infections [54]. Infections with *Blastocystis sp.* may be brought on by contaminated drinking water. Cysts *of Blastocystis sp.* have been demonstrated to be capable of surviving in water that contains typical levels of chlorine [55]. According to the data in table-1 , 65.0% of diabetes patients drank untreated tap water. This findings concur with those of [56], who came to the conclusion that Blastocystis is common among children in rural areas and that the major determinants of the infection were the sources of drinking water. On the other hand, a researcher reported that the source of water was not substantially related to intestinal parasite infections[22]. *Blastocystis sp*. infections are more likely following contact with animals [45,57]. The high incidence of *Blastocystis sp.* in animal handlers may be a sign of zoonotic transmission [58,59,60]. According to the results of the current study, there was positivity towards keeping animals and having contact with them among each group (diabetic and non-diabetic).The results of diabetic patients had a higher percentage (72.5%) of *B. homoins* parasite infection. This was in line with [22] finding that people who maintained animals at home had a higher infection rate (53.8%).Most results in the present study were display a highly significant difference between both genders, residence, source of drinking water and keeping animals ($p<0.05$), but there is no statistically correlation with infection in this parasite among the classes of age and educational level ($p>0.05$) .

Conclusion

This report is first regarding the prevalence of B. hominis in patients with DM in salahaddin;Iraq. This parasite was more prevalent in the DM than in the non-DM group. Also noticed that there was a link between the presence of Blastocystis and other participant characteristics (risk factors), such as place of residence, water source and keeping animals. So the potential risk factors for Blastocystis infection, should not be excluded. To better understand the association between potential risk factors and Blastocystis infection, it will be necessary to increase the sample size, examine a wide variety of populations, and expand the survey area.

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