Original Article

Prevalence of *Blastocystis hominis* in Patients with Type II Diabetes Mellitus in Kirkuk City, Iraq

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Abstract

Background: *Blastocystis hominis* is a prevalent human and animal bowel protozoan parasite, which is the most widespread eukaryotic organism in the human intestines. Intestinal parasite usually infects immunocompromised individuals; one of these is type II diabetic mellitus (TIIDM). **Aim:** The aim of the study was to assess the rate of infection with *B. hominis* in patients suffering from TIIDM. **Materials and Methods:** The current study is conducted on 50 participants (20 males and 30 females) (27 diabetic and 23 nondiabetic [control]), aged 25–75 years, diagnosed by a consultant physician, according to the laboratory diagnosis hemoglobin A1c (HbA1c), from November 2021 to March 2022. Two milliliters of venous blood was collected for the evaluation of the ratio of HbA1c with a sample of stool for the diagnosis of the presence or absence of *B. hominis.* **Results:** About the age, the highest age group in the TIIDM was 61–75 years, whereas in the control, the highest age group was 46–60 years. About education, 17 samples were from noneducated patients in the TIIDM group and 13 samples in the control group. About residence, 16 were rural residences in the TIIDM group and 23 urban samples in the control group. About autoimmune, 14 samples of diabetic patients infected with another autoimmune disease and 20 samples were not infected in the control group. About *B. hominis*, all diabetic patients were infected with *B. hominis*, but in the control group, just two persons have *B. hominis* infection. **Conclusions:** *B. hominis* was more common in the patients with diabetics than the controls. To better understand the relationship between possible risk factors and *Blastocystis* infection, the sample size must be increased, and the survey area must be expanded.

Keywords: *Blastocystis hominis*, HbA1c, type II diabetes mellitus

Introduction

The infection of intestinal parasites has a very wide distribution, and the propagation is high in the developing countries. Simultaneously, these countries have increasingly higher levels of public health problems related to noncommunicable diseases, such as diabetes mellitus (DM). *Blastocystis spp.* is an enteric protozoan found in both humans and animals[1] and is a common anaerobic unicellular protozoan of the human intestine worldwide. In humans, the prevalence of *Blastocystosis* was higher in the developing countries (53.8%) than in the developed ones (3.3%). It was considered as a commensal organism in fecal samples.[2] It has a variety of morphological forms. The most well-known forms are vacuolar, granular, ameboid, and cystic.[3] The cyst is the most common form found in human stools, and its size ranges from 6 to 40 μm,[4] and the transmission occurs mainly through fecal–oral route, which is amplified in poor sanitary conditions. The most significant issue in the parasite's spread is contaminated water.[5] The cyst was proposed as the infectious phase in a life cycle for *Blastocystis* spp. When cysts are consumed, the parasite undergoes excystation in the large bowel and evolves into vacuolar forms. These vacuolar aspects divide into ameboid or granular shapes via binary fission. Then, before cyst defecation in the feces, encystation may happen while crossing the colon.[6] Extraintestinal abnormalities related to *Blastocystis* infection, such as cutaneous lesions, include pain, bloating, and bowel problems.[1]

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DM is a metabolic disorder that is characterized by hyperglycemia caused by deficiencies in insulin secretion, action, or both. Diabetes chronic hyperglycemia is linked to long-term harm, dysfunction, and failure of several organs, particularly the eyes, kidneys, nervous system, and circulatory system.[7] DM is a mute killer; many sufferers become sensible they have DM only when they faces difficulty. Interference for preventing DM and controlling difficulties is poor, causing insufficient sensibility among the public. The administration of diabetes is based on many factors such as diet, drug therapy, physical exercise, and other lifestyle changes. There are two types of diabetes: type I DM is also known as insulin-dependent diabetes, when the pancreas is not able to make insulin, whereas type II diabetes mellitus (TIIDM) occurs when pancreas does not make enough insulin.[8] Diabetes is likely one of the oldest illnesses. It was first mentioned in an Egyptian article approximately 3000 years ago.[9] TIIDM has two categories, diabetes with fatness and without fatness. TIIDM patients suffering from fatness usually develop resistance to endogenous insulin because of the conversion in the receptors of the cell, and this is related to the distribution of abdominal fat. In TIIDM patients without fatness, some insulin resistance occurred at the postreceptor levels added to inadequacy insulin production.[10] Many herbs and plants play an important role in improving glycemic and lipid profiles, as well as oxidative stress markers in DM. One of these is chamomile, which is high in phenolic compounds and terpenoids and appears to be an effective treatment for chronic conditions such as diabetes.[11] Chamomile plays an important role in decreasing the rate of infection in diabetic patients with intestinal parasites because of its role against the toxicity of these parasites.[12]

Bowel parasites and diabetes have been studied extensively, owing primarily to the possibility of systemic infection in diabetes, which allows for the development of more severe parasitic diseases.[13] In people with diabetes, both innate immune response deformities and adaptive immune response dysfunction (including T cells) are thought to be responsible for immunological weakness against pathogens.[1] Many things may play an important role in decreasing the intensity of chronic disease (DM). As an example, prevalence studies have linked dietary intake of phenolic-rich foods to a decreased prevalence of the emergence of several chronic conditions; phenolic compounds have an important role against intestinal parasite; and polyphenols are known for their antioxidant activities.[14] A study associated between *Blastocystis* and DM found that the most predominant intestinal parasite detected among diabetics was *Blastocystis hominis* (29%).[15]

The aim of this study was to assess the rate of *B. hominis* infection in patients suffering from TIIDM.

Materials and Methods

Study population

The current study is conducted on 50 Iraqi participants (20 males and 30 females) (27 with TIIDM and 23 nondiabetic [control]), aged 25–75 years, and diagnosis by a consultant physician, according to the clinical signs and laboratory diagnosis hemoglobin A1c (HbA1c), during the period between November 2021 and March 2022. The practical part was conducted at the Kirkuk city in laboratories of Azadi Teaching Hospital. Data collected from patients include name, age, gender, education, residence, and other autoimmune.

Stool samples

The samples were collected from patients complained of gastrointestinal disorders, and the symptoms covered diarrhea, abdominal pain, cramp nausea, loss of weight, and sometimes vomiting. Immediately after receiving the watery and loose stool specimen, physiological saline solution and buffered methylene blue were employed to obtain wet-stained slide preparations.[16] Formalin (10%) was added to the remaining portions of these stool specimens and to the soft stool specimens. The formalin-added specimens were further handled in the Parasitology Department, Faculty of Veterinary Medicine. They were subjected to the modified Ritchie concentration method to have wet slide preparations stained with Lugol's iodine solution. The stained preparations were closely examined under a binocular light microscope looking for the protozoa *B. hominis*.[17]

Blood collection

The blood samples were collected by vein puncture with plastic disposable syringes, 2 mL of blood from each patient. This blood was put in an ethlylene diamine tetraacetic acid tube for the detection of the ratio of HbA1c.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 42446 on 9/12/2021 to get this approval.

Results

A total of 27 TIIDM samples were taken (nine samples from males and 18 from females), whereas 23 samples were taken from healthy participants (controls) (11 samples from males and 12 from females). About the age, the highest age group in TIIDM was 61–75 years,

Table 1: Relationship between HbA1c and gender

Risk factor	Diabetic patients		Nondiabetic patients	
	Frequency	Mean	Frequency	Mean
Gender				
Male	9	10.28	11	4.454[b]
Female	18	10.99	12	3.750[b]
P value = 0.0002				
HbA1c	27	9.83 ± 0.75	23	6.84 ± 1.06
P value = 0.0001				

The same letters mean there are no differences between them under the level of significantly 0.05. The different letters mean there is a difference between them under the level of significantly 0.05

HbA1c = hemoglobin A1c

Table 2: Relationship between HbA1c and age

Risk factor	Diabetic patients		Nondiabetic patients	
	Frequency	Mean	Frequency	Mean
Age				
G1 (30–45 years)	2	9.68[b]	7	3.880[c]
G2 (46–60 years)	12	10.009[ab]	11	4.093[c]
G3 (61–75 years)	13	11.603[a]	5	4.362[c]
P value = 0.0008				
HbA1c	27	9.83 ± 0.75	23	6.84 ± 1.06
P value = 0.0001				

The same letters mean there are no differences between them under the level of significantly 0.05. The different letters mean there is a difference between them under the level of significantly 0.05

HbA1c = hemoglobin A1c

Table 3: Relationship between HbA1c and education

Risk factor	Diabetic patients		Nondiabetic patients	
	Frequency	Mean	Frequency	Mean
Education				
Educated	10	10.975[a]	13	4.160[b]
Noneducated	17	10.621[a]	10	3.991[b]
P value = 0.0009				
HbA1c	27	9.83 ± 0.75	23	6.84 ± 1.06
P value = 0.0001				

The same letters mean there are no differences between them under the level of significantly 0.05. The different letters mean there is a difference between them under the level of significantly 0.05

HbA1c = hemoglobin A1c

Table 4: Relationship between HbA1c and residence

Risk factor	Diabetic patients		Nondiabetic patients	
	Frequency	Mean	Frequency	Mean
Residence				
Urban	11	11.237[a]	23	4.087[b]
Rural	16	10.418[a]	–	–
P value = 0.00003				
HbA1c	27	9.83 ± 0.75	23	6.84 ± 1.06
P value = 0.0001				

The same letters mean there are no differences between them under the level of significantly 0.05. The different letters mean there is a difference between them under the level of significantly 0.05

HbA1c = hemoglobin A1c

Table 5: Relationship between HbA1c and patients with autoimmune disease

Risk factor	Diabetic patients		Nondiabetic patients	
	Frequency	Mean	Frequency	Mean
Patients with autoimmune				
Has autoimmune	14	10.186[a]	3	4.467[b]
Has no autoimmune	13	11.361[a]	20	4.029[b]
P value = 0.0009				
HbA1c	27	9.83 ± 0.75	23	6.84 ± 1.06
P value = 0.0001				

The same letters mean there are no differences between them under the level of significantly 0.05. The different letters mean there is a difference between them under the level of significantly 0.05

HbA1c = hemoglobin A1c

whereas in the control samples, the highest age group was 46–60 years. About education, 17 samples were without education in the TIIDM group and 13 samples were with education in the control group. About residence, 16 were rural residences in the TIIDM group and 23 urban samples in the control group. About HbA1c, the TIIDM group showed a significant ($P < 0.05$) increase of HbA1C levels (9.83 ± 0.75) compared with the group without TIIDM (controls) (6.84 ± 1.06) as shown in Tables 1–5.

About *B. hominis*, all the diabetic patients (27) were infected with *B. hominis*, but in the control group, just two persons have *B. hominis*; this indicates that this may due to generalized immunity weakness and gut microbiota alterations.

Discussion

B. hominis is a prevalent human and animal intestinal protozoa parasite.[5] This parasite is thought to be the most widespread eukaryotic organism in the human intestines.[18] Some developing countries have reported prevalence rates of up to 60%.[5] Intestinal parasite infects immunocompromised individuals; one of these is TIIDM.[19] The most important factors that promote TIIDM infection with intestinal parasites are generalized immunity weakness, a reduction in T-cell response caused by humoral immunity diseases, innate immunity deregulation, metabolic derangement of various organs, gut microbiota changes, a lack of health education, and poverty; inadequate sanitation[20,21] is considered the most important reason for diabetic patients to get infected with *B. hominis*, which appeared in the result of this study. In Table 1, regarding the gender, the mean of infection with *B. hominis* in the male with diabetics is 10.28, which is higher than the ratio of infection in the healthy male, which is 4.454; also in female with diabetics, the ratio of infection with *B. hominis* is 10.99, which is higher than 3.750 in the healthy female. This is nearly in agreement with a previous study,[22] which showed the most common infection was *B. hominis* (14 cases), which is similar to a study conducted in two nearby cities in Tehran (Karaj and Savojbolagh), Iran, in which patients with DM had a higher rate of bowel parasitic infections than the control subjects (5.6 vs. 10.0%).[23] The results were consistent with Ali *et al*.[24] who reported that the common intestinal parasites recorded in the current study, in which intestinal protozoan parasites the highest rate of protozoan infection being for *B. hominis*, and there is no significant difference between the male and female diabetic participants at the level (*P* 0.05), and no difference between healthy people (control) (male and female) at the level of *P* 0.05. Also no difference between healthy people (control) (male and female) under the level 0.05. In Table 2, regarding the age factor also, there was a difference between all three groups (G1, G2, G3) between patients with diabetic and healthy people (male and female), in which the highest ratio was in group G3 (61–75 years), which may be due to the fact that the elderly people do not concern with their food or water, which results in the transmission of many types of parasite; among them was *B. hominis*, which is in disagreement with the study by Duda *et al.*,[25] which indicates no relation between age and infection. Between three groups of diabetics (G1, G2, and G3), we noticed that there is a significant difference between these groups under the level 0.05, but in groups of healthy people, there was no significant difference between them under the same level. In Table 3, in our study, educated people with diabetic were more susceptible to infection with *B. hominis* with a high difference than healthy people with the ratio of 10.975:4.160, respectively, which is primarily because diabetes can cause immune dysfunction, resulting in the emergence of the more severe intestinal parasites.[13] Also diabetic people with poor educational background have a greater ratio for infection with *B. hominis* than the noneducated healthy people with the ratio of 10.621:3.991, which is nearly in agreement with the study by Ambachew *et al.*[26]; the result showed no significant difference between educated and noneducated in the diabetic patients group under the level 0.05; also no significant difference between the educated and noneducated in the healthy people group (control) under the same level, and this referred to that there is no relationship between education and infection with *B. hominis*. In Table 4, regarding the residence, the

result indicates that the ratio of diabetic patients live in the urban area infected with *B. hominis* is higher than those of healthy people; also between the same group (diabetic patients), the ratio of diabetic patients lives in the urban area was higher than those live in the rural, which is in disagreement with this study by Waly *et al.*,[15] which showed significantly more parasitic infection in diabetic patients inhabiting in rural areas (77.3%) than in urban ones (22.7%), and there is no relationship between residence and infection with *B. hominis*. In Table 5, regarding the autoimmune disease, patients who have another autoimmune disease (cancer, Rheumatism, thyroid gland disease, etc.) as well as diabetic are more susceptible to be infected with *B. hominis* than healthy people because of the same reasons mentioned above, which is in agreement with another study done in Egypt, which reported that the patients with DM have a high risk of parasitic infection,[23] and there was no significant difference between patients who have autoimmune and patients who have not of the same group (diabetic patients) under the level 0.05, and for the healthy people it is the same under the level 0.05.

Conclusions

This is the first report on the occurrence of *B. hominis* in diabetic patients in Kirkuk city, Iraq. This protozoan was more common in diabetics than in nondiabetics (control). Despite the results indicate no link between *Blastocystis* infection and potential risk factors, potential risk factors for *Blastocystis* infection should not be ruled out. To better understand the relationship between possible risk factors and *Blastocystis* inflammation, the sample size must be increased, populations examined, and the survey area expanded.

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Conflicts of interest

There are no conflicts of interest.

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