Chinese Journal of Evidence-Based Medicine

MOLECULAR DETECTION AND RELATIONSHIP OF *BLASTOCYSTIS HOMINIS* AND *GIARDIA LAMBLIA* IN DIARRHEAL PATIENTS AT WASIT PROVINCE, IRAQ

.

Prof. Dr. Abdulsada A. Rahi
Suadad B. Khairi [2]
[1,2] Department of Biology, College of Science, Wasit University

Abstract:

Blastocystis hominis and *Giardia lamblia* are a massive, unicellular intestinal protozoan parasite that live all over the world. It is connected to a wide range of clinical diseases and its pathogenicity is unknown.. The objective of this investigation was to ascertain the molecular prevalence of *Giardia lamblia* and *Blastocystis hominis* in a group of patients who exhibited symptoms and those who did not, and to evaluate the correlation of *B. hominis* and *G. lamblia* using the traits of the patient as potential indicators of Blastocystosis and Giardiasis. Human fecal specimens were gathered, and a molecular test was conducted using Nested polymerase chain reactions (PCR) targeting *B. hominis* and *G. lamblia* DNA was carried out to look for intestinal parasites. The prevalence of parasitic illnesses showed promise of Nested-PCR; 85 from 135 (62.9 %), while negative results was 50(37.1%). *Blastocystis hominis* was the prevalent parasite 58 (43.0%), followed by co-infection15(11.1%) and *G. lamblia* 12 (8.8%).

Among the patient variables examined, age indicated a high level of infection with two parasites. *Blastocystis hominis* and co-infection (47.9%) and (12.5%) in age (6-17) years old. Infection with was more common in females than in males where *B. hominis* (44.4%) but in *G. lamblia* infection males was higher than females (9.7 %), even though the numbers for male and female were comparable with co-infection (11.1%). The prevalence rate of infection with *B.hominis* and *G. lamblia* in rural area was higher than urban (50.0%) and (16.7%) respectively .

According to the current study, diarrhea and stomach discomfort were the most prevalent symptoms among all patients with various infection with (46.7%) and (53.6%) respectively . According to education levels of parents patients , positivity was found more frequently in level of don't have fathers education when infection with *B. hominis* (41.9%) and *G. lamblia* (9.8%) . According to a university investigation, the prevalence of *B. hominis, G. lamblia* and co-infections was associated with family size at ($P < 0.001$). The findings indicated that infections are common in families made up of more than 5 person in all parasites (47.7%), (9.9%) and (11.7%) respectively . Both symptomatic and asymptomatic results in patients at Wasit province have shown that *Blastocystis hominis* is still a common intestinal parasite.

Keywords: *Giardia, Blastocystis*, molecular epidemiology, Diarrhea .

Introduction

Human parasitizing protozoa have been identified in more than 15 genera; some are thought to be naturally occurring commensals, while others are linked to intestinal illnesses that manifest as symptoms in the afflicted individuals. Most prevalent and widely distributed protozoans worldwide are *Giardia, Entamoeba, and Blastocystis* (Ministerio, 2015). Typically, the presence of feces in the soil and food, the availability of drinking water, the usage of wastewater, the lack of proper sanitation, and unstable socioeconomic conditions are all associated with these parasite infections. Consequently, In populations when these characteristics are present, maintaining the

Vol. 20, No. 1. (2024)
E ISSN: 1672-2531

prevalence of intestinal parasite infections generates a latent risk of dynamic transmission among its inhabitants (Ortiz *et al*., 2012).

Blastocystis is a genus of intestinal parasitic protists with a single cell that is frequently found in humans and has been identified in a variety of species, including non-human primates (NHPs), birds, rodents, carnivores, and animals classified as mammals Cladopods (Hublin *et al* ., 2021) . The pathogenic potential of *Blastocystis* is still debatable; some research indicates that infection with *Blastocystis* is associated with irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) and cutaneous allergic disorders (Aykur *et al* ., 2022). However, Recent studies on the micro-biome indicate that *Blastocystis* may actually be a helpful commensal (Deng *et al* ., 2022) .

Giardia lamblia is an intestinal parasite that is common throughout the world and affects a wide variety of vertebrates. Clinical symptoms of infections, such as oily diarrhea, abdominal cramps, nausea, and wasting, can affect both humans and animals. These symptoms pose a substantial risk to human health and cause large financial losses for farms (Hatam *et al* ., 2023). according to genetic sequences of *Giardia* at small subunit rRNA (*SSU* rRNA), eight assemblages (A–H) have been identified, and it was shown that assemblages A and B have zoonotic potential. *Giardia* Multi-locus genotyping (MLG) is increasingly being used to genetically characterize isolates from humans and animals (Ryan *et al* ., 2021). The most reliable method for giardiasis diagnosis in clinical settings is microscopy; nevertheless, PCR-based methods are becoming increasingly used (Showgy and Staffan , 2024) .

Materials and Methods

Stool specimens collection

A total of 135 stool samples from patients with diarrhea at Al-Karamah and Al-Zahra Teaching Hospital were collected at random at Wasit province from October 2022 to October 2023 . Prior to the examination of the fecal samples, a unique questionnaire was created to record all patient data pertinent to the several epidemiological variables such as the patient's name, age, gender, location, type of abdominal pain, and father's educational attainment. Stool samples were taken from patients in sterile universal screw-cap bottles and stored in freeze (- 20 C) until used in PCR method.

Nested-PCR for detection of *B. hominis and G. lamblia*

Frozen stool samples was carried out by PCR thermocycler and GoTaq®Green Master Mix kit (Promega, U.S.A) according to manufacturer's instructions in final volume 25 µl reactions. The eluted DNA was stored at 20℃ for PCR assays. Extracted DNA for *B. hominis* was amplified using the primers:

Reverse primer TCCACCAACTAAGAACGGCC and
Forward primer AGAGTGTTCAAAGCAGGCGT.
While extracted DNA for *G. lamblia* was amplified using
Reverse primer ATTGACAGAGGCGGTCTTGG and
Forward primer CAAGGACGAAGCCATGCATG.

The amplified PCR products were separated on a 1.5% (w/v) agarose gel (Promega), stained with ethidium bromide (Promega), and seen under ultraviolet light after the PCR reaction and reaction conditions were completed. For each PCR reaction, there were positive and negative controls.

Statistical analysis

Vol. 20, No. 1. (2024)
E ISSN: 1672-2531

The statistical program for social sciences (SPSS) version 2 and Microsoft Office Excel 2010 were utilized in the collection, compilation, analysis, and presentation of the data. To look at mean differences between more than two groups, a one-way ANOVA test was used, provided the variable was normally distributed. The chi-square test can be used to examine associations between any two categorical variables. The level of significance was established using a P-value of less than 0.05, and a 0.01 or less level indicated high significance (Daniel, 2018) .

Results

The molecular approach was utilized in the current investigation of nested PCR to discover the DNA of *Intestinal Parasites* in stool samples. The positive result of PCR was 85 from 135 (62.9%), while negative results was 50(37.1%), as shown in figure (1).

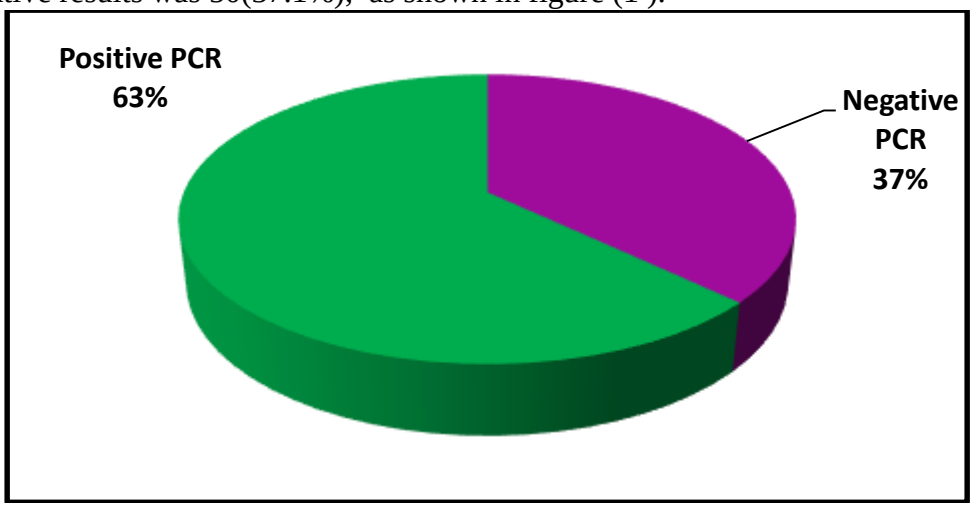

Figure (1): Frequency distribution of patients according to results of PCR results.

The present result showed the *18S ribosomal RNA* gene for detection *B. hominis* were reported in 58 (43.0%) of patients and the *18S ribosomal RNA* gene for detection *G. lamblia* showed in 12 (8.8%). Furthermore, mixed infection was observed 15 (11.1%) of patients have *G. lamblia and B. hominis* mixed infections as show in table (1) .

Table (1): The results of Nested-PCR for the detection of 18S rRNA of intestinal parasite

Total number	***B. hominis***		***G. lamblia***		**Mixed**		***P***
	n	***%***	***n***	***%***	***n***	***%***	
Nested PCR results							
135	58	43.0	12	8.8	15	11.1	0.001 ¥ S

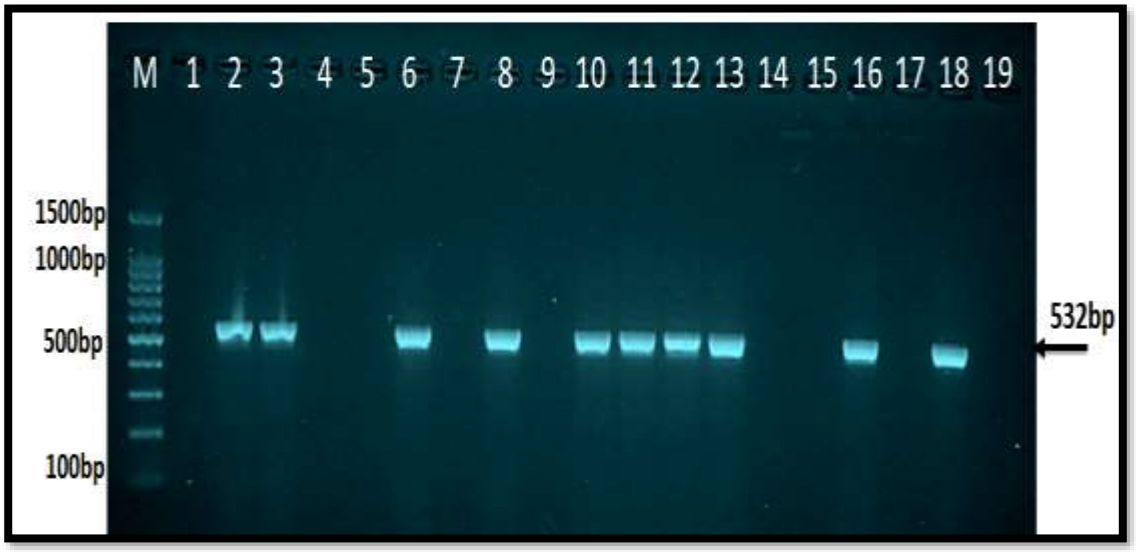

Figure (2): *Blastocystis hominis* was found in stool samples from diarrhea patients using Nested-PCR product analysis of the small subunit ribosomal RNA gene, as demonstrated by the agarose gel electrophoresis image. M (Marker ladder 100-1500 bp). Lane (1-19) showed some positive *B. hominis* samples at 532 bp PCR product size.

Figure (3): Agarose gel electrophoresis experiment that demonstrated the use of Nested-PCR product analysis of the 18S rRNA gene to identify *G.lamblia* in stool samples from diarrhea patients. M (Marker ladder 100-1500 bp). Lane (1-22) showed some positive *G.lamblia* samples at 586 bp PCR product size.

Age group was one of the primary determinants of the observed variations in percentages of *G.lamblia* , *B. hominis* and co-infections. The highest frequency of *B. hominis* and there were co-infection infections found in the age category of 6–17 years (47.9%, 12.5%) compared to other age

groups while in *Giardia* the highest rate in age <6 (18.3%) . Gender differences were seen in infection rates, with women reporting a higher infection incidence with *B. hominis* while men recorded a higher infection rate with *G.lamblia*, but in co-infection the rate was similar in both sexes.

Giardia lamblia and *B. hominis* infections were more prevalent in rural (50.0 %, 16.7%) than urban , While in co-infection there was no infection. Abdominal discomfort was the most prevalent clinical symptom linked to diarrhea (46.7%) , (10.5%) (13.3%) in *B. hominis* , *G.lamblia* and co-infection respectively. According to education levels of parents patients, *B. hominis* and co-infection. Positive traits were observed more commonly in those without fathers education levels. (45.2% and 11.9%) but in *G.lamblia* found in level have fathers education (9.8%) .

According to a university investigation, the prevalence of *G. lamblia* and co-infections was associated with family size at ($P < 0.001$). The findings indicate that there is a significant rate of parasite infection in families with more than five members. The findings from the multivariate regression analysis as shown in (Table 2).

Table 2 Association of different variables with positive cases of *B.hominis*, *G.lamblia* and co-infection by PCR

Variables	Examined number	*B.hominis*	*G.lamblia*	Co-infection	*P*
Age groups in years					
< 6	16	7(43.8%)	3(18.8%)	1(6.3%)	0.078
6-17	48	23(47.9%)	3(6.3%)	6(12.5%)	0.001
≥18	71	28(39.4%)	6(8.5%)	8(11.3%)	0.001
Gender					
Male	72	30(41.7%)	7(9.7%)	8(11.1%)	0.001
Female	63	28(44.4%)	5(7.9%)	7(11.1%)	0.001
Residency					
Urban	129	55(42.6%)	11(8.5%)	15(5.6%)	0.001
Rural	6	3(50.0%)	1(16.7%)	0	0.317
Type of stool samples					
Diarrhea	28	15(53.6%)	5(15.2%)	4(14.3%)	0.001
Watery	54	25(46.3%)	6(11.1%)	5(9.3%)	0.001
Mucoid	20	7(35.0%)	3(15.0%)	0	0.206
Normal	33	11(33.3%)	1(3.6%)	3(9.9%)	0.065
Abdominal pain					
Yes	105	49(46.7%)	11(10.5%)	14(3.3%)	0.001
No	30	9(30.0%)	1(3.3%)	1(3.3%)	0.001
Father educations					
Yes	93	39(41.9%)	9(9.8%)	10(10.8%)	0.001
No	42	19(45.2%)	3(7.1%)	5(11.9%)	0.001
Family size					
<5	24	5(20.8%)	1(4.2%)	2(8.3%)	0.179
≥5	111	53(47.7%)	11(9.9%)	13(11.7%)	0.001

Discussion

The recent study indicated that across all age groups, the highest rate of *B. hominis* infection with significant in (P < 0.002) , this result was agreed with Darwish *et al*., (2021) in Syria and Gabr *et al* ., (2018) also found the spread of *B. hominis* higher in Egypt across various age groups. While this result disagreed with a number of studies ; (Abu sheishaa *et al* ., 2022) in Egypt , (Wakid *et al* ., 2022) in Saudi Arabia reported no infection in < 20 years and > 60 years . The numerous reservoirs of this parasite, which raise the risk of infection in humans, are the source of its widespread proliferation (Yunus *et al* ., 2015) .

Regarding of *G. lamblia* the findings were similar to (Al- Difaie , 2016) who discovered that the highest rate of age-related to 2-10 years (20%) in Iraq, However, the proportion was less than (Kasaei *et al* ., 2018) that he recorded high rate (55. 6%) in age 1-7 years in Iran. Gender-specific variations in infection prevalence rates are evident, and this result was identical to (Magda and Donia, 2022 ; Magda and Ghaidaa , 2022) at Wasit province. However, the current outcome disagreed with (Bassam *et al* ., 2023) in Wasit province found the higher infection rate in males (4.7%) than females(3.1%) . The incidence of B. *homonis and G. lamblia* infections in rural areas was greater than in urban areas., with significant differences at (P< 0.001) and these result agreed with (Magda and Donia , 2022) in Wasit province . Also in the same province (Magda and Ghaidaa , 2022) discovered that the infection rate was higher in rural than in urban areas (5.45%) and (1.81%) but it disagreed with (Firas, 2023) in Diwanyiah, Iraq .

Diarrhea and stomach pain were the most prevalent symptoms among all patients this results were in accordance with that reported in Dohuk province (Al Saeed *et al*., 2013) and Hammood *et al*. (2016) in Kirkuk-Iraq. Emerging infections such as intestinal protozoa are thought to play a significant role in diarrheal illness epidemics in developing countries where deteriorating water quality is an ongoing issue (Sarkari *et al* ., 2016). Our research revealed a strong correlation between the fathers' educational attainment and the prevalence of parasites. These results agreed with (Mohammad *et al* ., 2017) in Malaysia and study in China (Deng *et al* ., 2020) reported the same results in level of don't have fathers education than level have fathers education. The results of present study were revealed a relationship between family size and the occurrence of co-infections , *B. hominis and G.lamblia* at (P < 0.001) . The findings indicated a high rate of infection in a family with more than five members across all parasite species. This result was identical to (Mohammad *et al* ., 2017) in Malaysia but not compatible with (Correa *et al* ., 2020) in Brazil.

Conclusion:

Blastocystis hominis is seen as a contributing factor to human illness in individuals who experience recurrent symptoms. Molecular methods are required to identify the path and source of infection because of the high danger of zoonotic transmission.

References

Abu sheishaa ,G. A. , Haitham, K. A., Khairy, A. M. and Nasr, A. (2022). *Blastocystis* Species Prevalence and Associated Patient Characteristics as Predictors Among a Cohort of Symptomatic and Asymptomatic Egyptians . Al-Azhar International Medical Journal . 4 (2023) 112-118 .

Al Saeed, A. T., Ismaeel, Q. I. and Ahmed, G. F.(2013). "*Blastocystis hominis* infections among patients in Duhok City / Kurdistan Region – Iraq". The quality management journal, 9 (16), 161 (2013).

Aykur, M., Camyar, A., Turk, B.G., Sin ,A.Z. and Dagci ,H. (2022) Evaluation of association with subtypes and alleles of *Blastocystis* with chronic spontaneous urticaria. Acta Trop.231:106455.

Bassam ,A. A. , Baraa , A. H. and Taqi ,M. J.(2023) . Direct smear detection of parasitic infection cause diarrhea in patients of Wasit Province, Iraq. J. An. For Res. 6(1): 3249-5.

Corrêa, C.R., Ana, P. O., Érica, B. D. and Semíramis, G.(2022) . Genetic analysis of Giardia duodenalis isolates from children of low-income families living in an economically successful region in Southeastern Brazil .
Rev Inst Med Trop São Paulo. 62:e20 .

Daniel , W.W.(2018) . Biostatistics : A foundations for Analaysis in the Health Sciences , John Wiley and Sons New Yourk .

Darwish , B., Ghalia , A., and Samar ,A.(2021) . Detection of *Blastocystis* in stool isolatesusing different diagnostic methods .J. Research Square .P: 1-12.

Deng, L., Wojciech, L., Png ,C.W., Koh, E.Y., Aung ,T.T and Kioh, D.Y.Q.(2022) . Experimental colonization with *Blastocystis* ST4 is associated with protective immune responses and modulation of gut microbiome in a DSS-induced colitis mouse model. Cell Mol Life Sci. 79:245.

Firas , H. N. , Ali, B. M . A. (2023) . The Prevalence Study of *Blastocystis hominis* Isolated from Irritable Bowel Syndrome (IBS) in Diwanyiah City of Iraq . Journal of Survey in Fisheries Sciences . 10(3S) 4008-4014 .

Gabr , N.S., Manal, Z.M., Rabie ,M.M. ,Usama , S.B., Manar , M.S. and Wael , M.A.(2018) . Blastocystis SPP. Infection among IBS patients : various diagnosis methods and epidemiological study . Journal of Egyptian Society of Parasitology , Vol .48, No.1 .P: 119-128.

Hatam , N. K., Carmena, D., Rezaeian, M., Mirjalali, H., Rahimi, H.M., Badri, M., Vafae, Eslahi ,A., Shahrivar, F.F., Rodrigues, Oliveira, S., Pereira, M.L., and Ahmadpour, E.(2023). Gastrointestinal parasites of domestic mammalian hosts in Southeastern Iran. Vet. Sciences, 10(4):261.

Hammood, A. M., Ahmed, B. A., & Salman, Y. J. (2016). *Blastocystis hominis* detection among gastrointestinal disorders' patients in Kirkuk Province using three different laboratory methods. International Journal of Current Microbiology and Applied Sciences, 5: 883-901.

Hublin, J. S.Y., Maloney, J.G. and Santin ,M. (2021) . *Blastocystis* in domesticated and wild mammals and birds. Res Vet Sci. 135:260–82.

Kasei, R., Carmena ,D., Jelowdar, A.and Beiromvard ,M.(2018) . Molecular genotyping OF Giardia duodenalis in children from Behbahan, southwestern Iran. Parasitol Res. 2018;117:1425-31.

Magda A. A. and Ghaidaa M. N., (2022) . Prevalence of intestinal parasitic infection in patients

at Wasit Province, Iraq. International Journal of Health Sciences, 6(S6), 10644–10649.

Magda, A. A. and Donia, F. A. (2022). Detection of *Blastocystis hominis* among Gastrointestinal Disorders Patients in Wasit Province Using Different Laboratory Methods. J. Teikyo medical . Volume 45, Issue 01.P: 5103-5109.

Ministerio, D.(2015). Biblioteca Digital/ RIDE/ VS/PP/ Ecuesta-nacional-de parasitismo-2012-2014.

Mohammad, A.N. , Hesham M. A., Norhayati ,M. and Tengku , S.A. (2017). Prevalence and risk factors of *Blastocystis* infection among underprivileged communities in rural Malaysia. Asian Pacific Journal of Tropical Medicine;10.1016.

Ortiz, C., Lopez, M. C., and Rivas, F. A. (2012). Helminth prevalence in a waste-water plant at El Rosal, Cundinamarca. Rev. Salud Publica (Bogota). 14, 296–304. doi: 10.1590/S0124..

Ryan ,U.M., Feng ,Y., Fayer, R. and Xiao, L. (2021). Taxonomy and molecular epidemiology of *Cryptosporidium* and *Giardia* – a 50 year perspective (1971–2021). Int. J. for Parasitology, 51(13–14), 1099–1119.

Showgy, M.A. and Staffan, S. (2024) . *G iardia* and giardiasis . Molecular Medical Microbiology . Pages 3107-3119 .

Wakid , M.H. Waad ,T.A., Muslim , N.A. and Asmaa, M.E. (2022) . Identification and genetic characterization of *Blastocysits* species in patients from Makkah , Saudi Arabia .J. Infection and drug Resistance . 15.P: 491-501.

Yunus, E. B., Hasan , Y. , Zeynep ,T. C. , and Abdurrahman , E. (2015) . Clinical significance and prevalence of *Blastocystis hominis* in Van, Turkey . J. national center for biotechnology information . 36(9):1118-21 .