Molecular Identification of Cryptosporidiosis in Diabetes and Cancer patients

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KEYWORDS
Immunocompromised, Nested-PCR, Diabetes, Cryptosporidium spp

ABSTRACT
Cryptosporidium spp., a primary intestinal parasite and obligate intracellular parasite, infects a wide range of vertebrates, including human. The current study aimed to determine the infection rate of Cryptosporidium spp. among immunocompromised patients attending the Diabetes and Endocrinology Centre and Oncology Department at Al-Diwaniya Teaching Hospitals in Al-Diwaniya Providence, Iraq. The research period extended from the first of October 2023 until the end of February 2024. Eighty-seven stool samples were obtained from immunocompromised patients of various ages and both genders who attended the hospital. Stool samples were examined using the nested PCR technique. The results showed an overall infection rate of 22.98%. Researchers found that the infection rate was 20.83% among diabetic patients and 25.64% among cancer patients. Male patients had a recorded infection rate of 23.07%, whereas females recorded 22.85%. A high infection rate (60%) was observed among patients under the age of 15 and among those coming from rural areas, reaching 34.88%. Additionally, it was found that 42.42% of the infected individuals had contact with animals.

1. Introduction

Cryptosporidium is an obligate intracellular parasite infecting various vertebrates, including humans, causing cryptosporidiosis, especially in individuals with weakened immune systems, such as AIDS patients (Dabrowska et al., 2023). Its infection remains a major public health concern, mostly in developing nations, due to various aspects that might contribute to the spread of this parasite among humans, particularly among toddlers and immunocompromised patients (Khan et al., 2019).

Several species of parasites are pathogenic to humans, but the most common are C. parvum and C. hominis, which are accountable for 95% of human infections. C. hominis primarily affects humans, while C. parvum has an animal origin and can be transmitted between humans and animals (Ryan et al., 2021). The parasite finishes its life cycle in a single host, reproducing both sexually and asexually in the host's intestines (Tandel et al., 2019).

Cryptosporidiosis is caused by consuming water and food contaminated with oocysts deposited in the feces of infected hosts via the oral-fecal pathway. (Ghazy et al., 2015). The primary site of infection is the small intestine, whether in immunocompetent individuals or immunocompromised ones. Additionally, the parasite can infect the epithelial cells of the respiratory system, causing respiratory cryptosporidiosis (Sponseller et al., 2014; Varughese et al., 2014).

The severity of the clinical symptoms of the disease varies from one person to another with regards to the immune status of the infected person, the impact of the infection in intestinal cells and the intensity of the infection of the parasite infection. According to Kotloff (2017), watery diarrhea is considered a significant hallmark of this disease.

Generally, many acute symptoms appear on the onset of infection. These include intestinal colic as a result of inflammation of intestines and stomach, vomiting, nausea, and fever. Basically, the disease generally runs its course for two weeks, after which the patient spontaneously recovers(Yang et al., 2017). The disease, however, may persist for a long duration and develop into a chronic type characterized by severe life-threatening diarrhea in immunosuppressed patients such as cancer patients. (Caner et al., 2020). Objectives of this study were to Molucular diagnosis of cryptosporidiosis infection in immunosuppressed patients in Al- Diwaniya city , and to investigate of some variables associated with Cryptosporidiosis infection

2. Methodology

Sample Collection

Eighty-seven stool specimens were collected from a period between October 1st, 2023, and late days of February 2024 from immunosuppressed patients attending the Diabetes, Endocrine, and Oncology departments in Al-Diwaniya Teaching Hospital in Al-Diwaniya Governorate. The samples were categorized according to immunological status, gender, age, residency, and contact with animals.

The samples were collected in sterile, dry, and clean plastic containers with tight lids to prevent desiccation and preserve moisture. Each container was labelled with a sample number corresponding to a questionnaire designed specifically for each patient. The samples were stored frozen at -22°C until Nested-PCR analysis was performed.

Molecular Diagnosis

Ribosomal DNA (SS rRNA) was extracted from 87 stool samples depleted of oxygen using the Presto™ Stool DNA Extraction Kit. The extraction procedure was conducted according to the manufacturer's instructions (Geneaid, Taiwan). Subsequently, the extracted DNA was purified, followed by the utilization of the Nested-PCR technique to amplify the small subunit ribosomal DNA (ssrRNA gene).

Primers for the Nested PCR reaction were designed to detect Cryptosporidium spp. in this study according to the small subunit ribosomal DNA (ssrRNA genes) diagnostic gene using NCBI-Genbank (AF442484.1) and designed with a +3 extension. The primers were supplied by ScientificResercher Co Ltd in Iraq, as indicated in Table 1. The Nested PCR reaction products were analyzed using electrophoresis on an agarose gel.

Table 1. Utilized primer sequences

Primer	**Sequence 5'-3'**		**Size**
PCR ssrRNA gene	F	ATTGGAGGGCAAGTCTGGTG	726bp
	R	TCCACCAACTAAGAACGGCC	
Nested PCR ssrRNA gene	F	CGCGGTAATTCCAGCTCCAA	533bp
	R	TCAGCCTTGCGACCATACTC	

Statistical analysis

The SPSS (Statistical Package for the Social Sciences) was employed in the current statistical evaluation of the research. The Chi-square test was employed to compare percentages, considering $P \leq 0.05$ as statistically significant (Nuchjangreed, 2018).

3. Results and discussion

Cryptosporidium spp. Infection According to Immunological Status

In this study, 87 stool samples from diabetic and cancer patients were detected using Nested PCR technique. The results revealed an overall infection rate of 22.98%. Furthermore, the current study showed an infection rate among diabetic and cancer patients of 20.83% and 25.64%, respectively.

The statistical analysis outcomes indicated no significant difference between the immunological statuses examined in the study regarding the infection with Cryptosporidium spp. at a significance level of $P \leq 0.05$, as shown in Table (2).

Table (**2**): Cryptosporidium parasite infection according to immune status

Characteristics		**Examined No.**	**Positive No.**	**%**	**X^2**	**P value**
Immune status	Diabetes	48	10	20.83	0.281	0.869(NS)
	Cancer	39	10	25.64		
	Total	87	20	22.98		

NS: No significant difference.

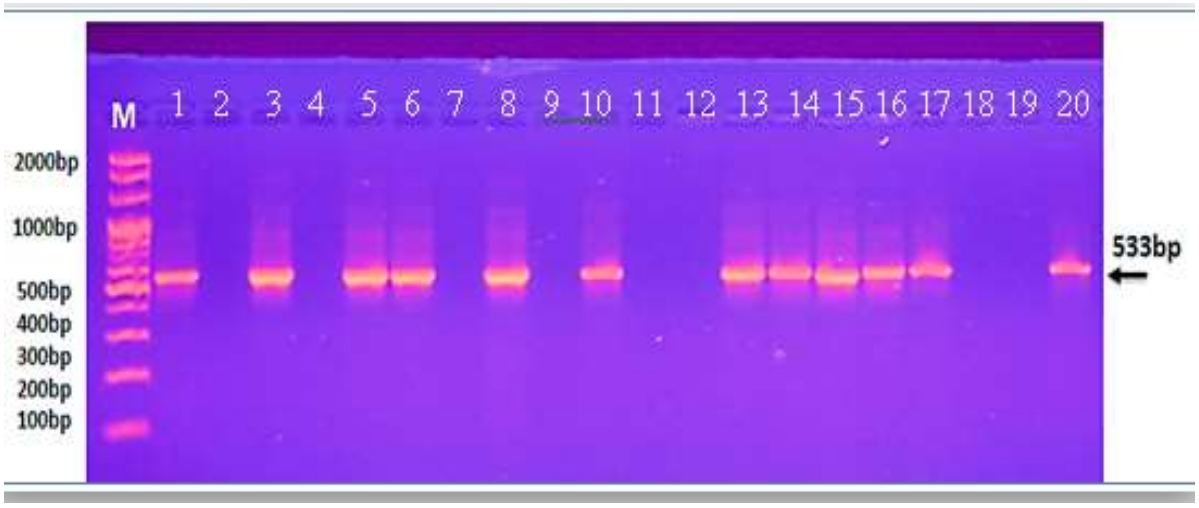

Figure 1: Electrophoresis of nested PCR product on an agarose gel for the small subunit gene of ribosomal RNA (ssrRNA) for Cryptosporidium parasite species in human stool samples. Path M (100 - 2000 base pairs) represents the leader marker, and paths (1 - 20) represents some positive samples at (533 base pairs)

Cryptosporidium spp. Infection According to Gender

The current results showed similar infection rates of Cryptosporidium spp. among males (23.07%) and females (22.85%), with statistical analysis indicating no significant differences in infection between genders at a significance level of P≤0.05, as listed in Table (3).

Table (**3**): Cryptosporidium parasite infection according to sex

characteristics		**examined** No.	**Positive** No.	%	X^2	P value
sex	Male	52	12	23.07	0.001	0.981(NS)
	Female	35	8	22.85		
	Total	87	20	22.98		

NS: No significant difference.

Cryptosporidium Spp. Infection According to Age Groups

This study showed that infection rates were high among patients aged 1–15 years (60%), followed by the age group (60–46 years) with an infection rate of 25%. The age group (75–61) years recorded 21.87%, followed by the age group (45–31) years with a rate of 14.28%. The age group (16–30) years did not record any infections. Statistical analysis results indicated no significant differences in infection rates among age groups at a significance level of P≤0.05, as listed in Table (4).

Table (**4**): The relationship between Cryptosporidium infection and the age of patients

Age group	**examined No.**	**Positive No.**	**%**
1 – 15	5	3	60
16 – 30	4	0	0
31 – 45	14	2	14.28
46 – 60	32	8	25
61 – 75	32	7	21.87

Total	87	20	22.98
X^2	5.75		
P value	0.218(NS)		

NS: No significant difference.

Cryptosporidium Spp. Infection Based on Residence

The current study recorded an infection rate among patients coming from rural areas (34.88%), which is higher than the rate observed among patients coming from urban areas, where the rate was 11.36%. The statistical analysis results, with a significance level of P≤0.01, indicated a highly significant difference in infection in favor of patients coming from rural areas, as illustrated in Table (5).

Table (**5**): The relationship between Cryptosporidium infection and residence

Characteristics		**Examined No.**	**Positive No.**	**%**	**X2**	**P-value**
Residence	Urban	**44**	**5**	**11.36**	6.79	0.009(HS)
	Rural	**43**	**15**	**34.88**		
	Total	87	20	22.98		

HS: Highly significant difference.

Cryptosporidium Spp. Infection according to Animal Contact

The findings demonstrated that, with percentages of 42.42% and 11.11%, respectively, patients who had contact with animals had a greater infection incidence than patients who did not. Statistical analysis revealed significantly higher differences in favor of patients with animal contact, as shown in Table (6).

Table (**6**): The relationship between Cryptosporidium infection and contact with the animal

Characteristics		**Examined No.**	**Positive No.**	**%**	**X2**	**P value**
Animal contact	Yes	33	14	42.42	11.34	0.001(HS)
	NO	54	6	11.11		
	Total	87	20	22.98		

HS: Highly significant difference.

Discussion

Infection with Cryptosporidium spp. parasites poses a threat to public health and is a principal reason for diarrhea, potentially endangering the lives of immunocompromised patients (Zhang et al., 2020). Interest in this infection has grown with the increasing number of immunocompromised patients, particularly following the spread of HIV, the virus that causes AIDS (Checkley et al., 2015). Additionally, advancements in organ transplantation procedures, such as liver, heart, lung, and kidney transplants, have led to the emergence of other immunosuppressive conditions, including cancers, autoimmune diseases, and the use of immunosuppressive medications, among others (Ferreira & Borges, 2002).

In this study, the Nested PCR technique was utilized to detect the presence of egg cysts in fecal samples, revealing an infection rate among immunocompromised patients who visited the Diabetes and Endocrine Clinic and the Oncology Department at Al-Diwaniya Teaching Hospital in Al-Diwaniya Governorate (22.98%). This finding is consistent with a previous result in Al-Diwaniya (Mohammed et al., 2016), which recorded 24%,, but it does not align with the 52% reported by Rahi and Khlaif

(2021) in Wasit Governorate, Iraq. Overall, numerous global studies and reports have shown significant variation in the rates of cryptosporidiosis infection (7.5-76.6%) (Alali et al., 2021).

The Cryptosporidium oocysts rate of infection among males was (23.07%), slightly higher than the rate recorded among females (22.85%). In line with these results, Saulawa et al. (2022) reported in Nigeria, 8.7% for males compared to 7.9% for females, using the Nested PCR technique. On the different side, the present findings are not agreed with Alkhanaq and Al-Hadidi (2022), where they recorded 41.2% for females and 38.7% for males. The difference in the infection rate between males and females can be a result of some reasons that vary from contact with other environmental factors that aid in the transmission of parasite egg cysts and exposure to contamination due to dietary habits and consumption of uncovered food from public places and street vendor and interaction with the farm animals. (Rahi & Khlaif, 2021)

Findings suggest that the parasite affects every age group in differing proportions, of which the rate of infection was maximal (60%) in the age group 1–15 years. The arguments that have been fronted to be the reasons for the high infection rates among the children are poor personal hygiene, failure to wash hands before eating, low health awareness of disease risks, playing in soil, and low immune status (Mogalli et al., 2020; Samie et al., 2006; Yu et al., 2004). In Iraq, similar studies were found by Abbas and Al-Shabani (2021) in Diwaniya, Khlaif (2021) in Wasit, and Wahid et al. (2022).

Statistically significant differences were observed in infection rates with Cryptosporidium spp. according to the area of residence, where the highest rate of infection was recorded in patients from rural areas with a rate of 34.88% compared to 11.36% in urban areas. This result agrees with those conducted by Naguib et al. in Egypt during 2018, where the rate was 1.5% in rural areas and 1.2% in urban areas. Actually, it was established that in rural areas, it was at a very high rate of 13.7%, in contrast to that of urban areas, which was 2.3%, similar to the findings by Mohaghegh et al. in Iran. The contributing factors to the high infection rates in rural areas include a lack of access to clean drinking water, poor sanitation, poor health, and poor economic conditions. This is in addition to the poor health and economic conditions, as evidenced in Paredes et al. (2023), and drinking or swimming in contaminated parasite oocysts in rivers and lakes, as noted by Adeyemo (2020).

In the present study, the relationship was established between infection and contact with animals, where a high infection rate was noted amongst the cases in contact with animals, as high as 42.42%, and for non-contact cases, it was low with 11.11%. The results from Qasem et al. (2022) in Yemen were similar, and the infection rate was high among the contacts (73.81%). A study in Iran explained that the high rate of infection among the calves was transferred to the cattle breeders and the families; hence, the rate of infection increased. The close association among humans and cattle was a cause of the zoonotic disease in the study area. (Izadi et al., 2014).

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