



RESEARCH

The prevalence of *Cryptosporidium* spp. and other intestinal parasites in elderly patients

Yaşlı hastalarda *Cryptosporidium* spp. ve diğer bağırsak parazitlerinin yaygınlığı

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Abstract

Purpose: Cryptosporidiosis is a self-limiting infection in individuals with immune competence, but it can have serious and life-threatening consequences in the elderly and immune-compromised individuals. In this study, we aimed to investigate the prevalence of *Cryptosporidium* spp. and other intestinal parasites and their relationship with symptoms in elderly patients.

Materials and Methods: The distribution of *Cryptosporidium* spp. and other intestinal parasites was prospectively evaluated in elderly patients admitted to different clinics of the university hospital between September 2018 and September 2019.

The study group included 40 elderly patients (18 females and 22 males; mean age: 73.35 ± 6.55 years) with gastrointestinal complaints and a control group consisting of 44 elderly patients without gastrointestinal complaints. Stool samples were examined using wet mount, and Kinyoun acid fast staining.

Results: The most frequently isolated parasites were amoeba cysts and trophozoites in elderly patients. *Cryptosporidium* spp. cysts were detected in 9 (22.5%) of patients with gastrointestinal symptoms, and in 13 (40.6%) of those with other complaints and in the control group. *Cryptosporidium* spp. cysts were found more common in those with a solid stool consistency than others with a watery stool consistency.

Conclusion: Cryptosporidiosis could be a life-threatening condition in a high-risk population, such as the elderly with co-morbidities, and physicians should have increased awareness.

Keywords: *Cryptosporidium*, elderly, parasite.

Öz

Amaç: Kriptosporidyoz, bağışıklık yetkinliğine sahip bireylerde kendini sınırlayan bir enfeksiyondur, ancak yaşlı ve bağışıklığı baskılanmış kişilerde ciddi ve hayatı tehdit edici sonuçlara yol açabilir. Bu çalışmada yaşlı hastalarda, *Cryptosporidium* spp. ve diğer bağırsak parazitlerinin prevalansını ve semptomlarla ilişkilerini araştırmayı amaçladık.

Gereç ve Yöntem: Eylül 2018 ile Eylül 2019 tarihleri arasında üniversite hastanesinin farklı kliniklerine başvuran yaşlı hastalarda *Cryptosporidium* spp. ve diğer bağırsak parazitlerinin dağılımı prospektif olarak değerlendirildi.

Çalışma grubuna gastrointestinal şikayetleri olan 40 yaşlı hasta (18 kadın ve 22 erkek; ortalama yaş: 73,35 ± 6,55 yıl) ve gastrointestinal şikayeti olmayan 44 yaşlı hastadan oluşan kontrol grubu dahil edildi.

Gaita örnekleri ıslak baki ve modifiye Kinyoun asit hızlı boyama kullanılarak incelendi.

Bulgular: En sık izole edilen parazitler amip kistleri ve trofozoitleri oldu. Gastrointestinal semptomları olan hastaların 9 (% 22.5)' unda ve diğer şikayetleri olanlar ile kontrol grubunun 13 (% 40.6)' ünde *Cryptosporidium* spp. kistleri saptandı. *Cryptosporidium* spp. kistleri katı dışkı kıvamında olanlarda sulu dışkı kıvamına sahip olanlara göre anlamlı derecede daha yaygın bulundu.

Sonuç: Kriptosporidyoz, altta eşlik eden hastalık varlığında yaşlılar gibi yüksek riskli bir popülasyonda hayatı tehdit eden bir durum yaratabilir ve doktorlar bu konuda farkındalığı arttırmalıdır.

Anahtar kelimeler: *Cryptosporidium*, yaşlı, parazit.

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Received: 25.08.2023 Accepted: 12.12.2023

INTRODUCTION

Gastrointestinal infections associated with fluid depletion and poor hospital progress are an important cause of morbidity and mortality in the elderly. This may be due to a weakened immune system, hypochlorhydria, intestinal motility disorders, malnutrition, or underlying chronic diseases¹. Elderly people are also more susceptible to enteric infections^{2,3}. *Cryptosporidium* spp., are enteric zoonotic parasites. However, cryptosporidiosis can spread from person to person, and the most common species in humans are *C. parvum* and *C. hominis*⁴. One study suggested that the elderly have a higher risk of contracting a *Cryptosporidium* infection with a shorter incubation period³.

Cryptosporidiosis is transmitted by the fecal-oral route of infective *Cryptosporidium* spp. oocysts and can cause waterborne outbreaks. Its importance in public health is primarily due to the small size of *Cryptosporidium* oocysts (4–6 µm), which are too small to be retained by sand filters at water treatment plants. They are also highly resistant to disinfectants, including those used to disinfect water, such as chlorine (e.g., 3% hypochlorite, iodine compounds, phenols, glutaraldehyde and quaternary ammonium compounds)⁵. One of the main symptoms of the disease is watery diarrhea. Although *Cryptosporidium*-related disease affects all people, some groups are likely to develop more serious disease. Symptoms can be severe in people with an immune deficiency, such as AIDS patients and the malnourished, and could lead to serious or life-threatening disease. The disease is usually self-limiting in immune-compromised people^{6,7}. It is generally a pathogen not recognized in the elderly and testing for *Cryptosporidium* is not routinely required by clinicians. *Cryptosporidium* tests are considered when treatments for diarrheal disease are unsuccessful⁸.

The elderly population is increasing worldwide. Thus, the importance of studies on older age groups is increasing. *Cryptosporidium* cysts can also be detected in solid stools without diarrhea in high-risk populations such as the elderly with comorbidities. Therefore, immuno-compromised patients with persistent gastrointestinal symptoms should be investigated for suspected parasitosis.

The aim of this study was to investigate the frequency of *Cryptosporidium* spp. and other intestinal parasites among elderly patients according to gastrointestinal symptoms.

MATERIALS AND METHODS

The study protocol complies with the Declaration of Helsinki (1964). This research was approved by Medicine Faculty Ethics Committee (Meeting No: 2018/13, Meeting Date: 25 July 2018, Decision No: 20) at Kahramanmaraş Sütçü İmam University. Additionally, written informed consent was obtained from all participants.

Study design and sample

We conducted a cross-sectional study at Kahramanmaraş Sütçü İmam University in the eastern Mediterranean region of Turkey. Stool samples taken from patients aged 65 and over between September 2018 and September 2019 were examined in the clinical microbiology laboratory.

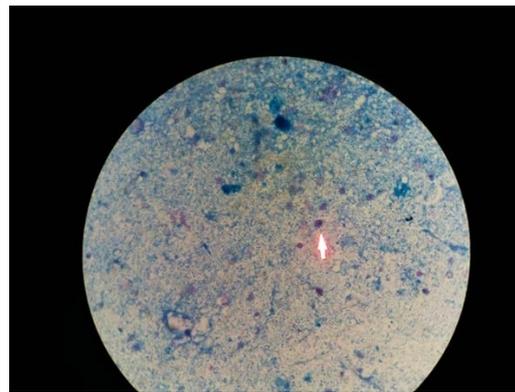


Figure 1. *Cryptosporidium* spp. cysts were detectable using the modified Kinyoun acid fast staining method ($\times 100$).

A total of 84 patients were included in the study. The study group included 40 elderly patients (18 females and 22 males; mean age: 73.35 ± 6.55 years) who were undergoing stool examinations due to gastrointestinal symptoms. The control group included 44 elderly subjects (18 females and 26 males; mean age: 71.59 ± 7.28 years), who were healthy or who applied to the clinic with other complaints. The under 65 years of age and those without gastrointestinal symptoms were excluded from the study.

Parasite examination

Initial parasite examination was evaluated based on stool consistency. Fresh samples were collected and examined the same day by our experienced laboratory staff using wet mounts and modified Kinyoun acid

fast staining (MKAFs) to determine the presence of *Cryptosporidium* spp. and other pathogenic parasites (Figure-1).

Statistical analysis

Power analysis was used to determine the size of the study. Considering the statistical parameters in the reference studies⁹, it was planned to recruit a total of 78 individuals, 39 for each group, with a test power of 0.80, α :0.05 first type error level and β : 0.20 second type error level.

The Shapiro–Wilk test was used to check the normality of the variable distribution. Comparisons of age were made with the independent samples t-test. Frequency distributions between qualitative variables were evaluated by the chi-square or Fisher’s exact test. Chi-square test and Fisher exact test were used to examine the frequency distribution of bacteria between the patient and control groups in the elderly population. Chi-square test and exact test were used to compare the patient and control groups according to their demographic characteristics. Independent samples t test was used to compare the patient and control groups according to their ages. A

p-value ≤ 0.05 was considered significant. IBM SPSS version 22 software (IBM SPSS for Windows version 22, IBM Corp., Armonk, NY, USA) was used to evaluate the data.

RESULTS

The most frequently isolated intestinal parasite among geriatric patients was amoeba cysts. Amoeba cysts were detected in 35% (n = 14) of those with diarrhea, while this rate was 6.8% in patients without symptoms. Amoeba cysts were detected in 35% (n = 14) of those with diarrhea, while this rate was 9.4% (n = 3) in patients without symptoms. Amoeba cysts were not detected in the control group. *Cryptosporidium* spp. was found in 22.5% (n = 9) of patients with diarrhea, in 40.6% (n = 13) of those with other complaints, and in the control group. Stool consistency was 70.3% (n = 26) watery in patients with gastrointestinal symptoms, while stool consistency was 29.7% (n = 11) solid in patients without symptoms (Table 1). No significant difference in gender was observed between the groups.

Table 1. The frequency of parasites examined in an elderly population according to gastrointestinal symptoms and stool consistency

Analysis of Stool Specimen		Patient				p	Control Group				p
		Symptom Yes		Symptom No			Symptom Yes		Symptom No		
		n	%	n	%		n	%	n	%	
Fecal Consistency	Solid	11	29.7	14	45.2	0.189*	0	0.0	10	83.3	-
	Watery	26	70.3	17	54.8		0	0.0	2	16.7	
Amoeba cysts	Positive	14	35.0	3	9.4	0.011**	0	0.0	0	0.0	-
	Negative	26	65.0	29	90.6		0	0.0	12	100.0	
<i>Cryptosporidium</i> spp.	Positive	9	22.5	13	40.6	0.097*	0	0.0	5	41.7	-
	Negative	31	77.5	19	59.4		0	0.0	7	58.3	

* Chi-square (χ^2) test was used for comparisons of categorical data from two independent groups. **Exact test; α : 0.05.

A correlation was found between the presence of parasites and the presence of gastrointestinal symptoms ($p < 0.001$). In patients with gastrointestinal symptoms, distributional differences were observed according to stool consistency and parasite status ($p = 0.019$). *Cryptosporidium* spp. cysts in patients with gastrointestinal symptoms were highly positive in those with a solid stool consistency,

compared to those with a watery stool consistency ($p = 0.022$) (Table 2).

A distributional difference was found between the clinic to which the patient applied and symptom status ($p = 0.001$). Patients with symptoms were mostly often referred to the emergency department or the gastroenterology service.

Table 2. Demographic features of the elderly patients according to symptoms

Parameter		Gastrointestinal Symptoms				Other Clinical Complaints					
		Stool Consistency				Stool Consistency					
		Solid		Watery		Solid		Watery			
		n	%	n	%	n	%	n	%		
Clinic ^a	Emergency	3	27.3	11	42.3	0.170	0	0.0	0	0.0	0.053
	Internal medicine	0	0.0	1	3.8		2	8.3	5	26.3	
	Dermatology	0	0.0	0	0.0		5	20.8	4	21.1	
	Endocrinology and metabolic disease	0	0.0	0	0.0		1	4.2	0	0.0	
	Infectious disease and clinical microbiology	1	9.1	5	19.2		2	8.3	1	5.3	
	Gastroenterology	5	45.5	7	26.9		0	0.0	1	5.3	
	Surgery	2	18.2	0	0.0		0	0.0	0	0.0	
	Nephrology	0	0.0	0	0.0		3	12.5	0	0.0	
	Neurology	0	0.0	0	0.0		0	0.0	1	5.3	
	Hematology-oncology	0	0.0	2	7.7		1	4.2	5	26.3	
Control group	0	0.0	0	0.0	10	41.7	2	10.5			
Amoeba cysts ^a	Positive	1	9.1	13	50.0	0.019*	0	0.0	3	15.8	0.044*
	Negative	10	90.9	13	50.0		24	100.0	16	84.2	
<i>Cryptosporidium</i> spp. ^b	Negative	6	54.5	23	88.5	0.022*	13	54.2	12	63.2	0.553
	Positive	5	45.5	3	11.5		11	45.8	7	36.8	
Gender ^b	Male	5	45.5	17	65.4	0.259	10	41.7	15	78.9	0.014*
	Female	6	54.5	9	34.6		14	58.3	4	21.1	
Age ^c	Mean±SD	72.55±4.55		74.19±7.42		0.500	71.00±6.52		72.47±8.26		0.533

SD: Standard deviation; *Exact test; ^bChi-square (χ^2) test; ^cIndependent samples t test; α : 0.05*

DISCUSSION

Structural and functional changes in most organs, including the gastrointestinal tract (GT), occur with aging¹⁰. The physiological changes of aging cause significant changes that increase infection rates. The most important of these are changes in the immune system¹¹. An increase in parasitic infections has been observed in the elderly in nursing homes due to the long-term co-existence of chronic diseases and their weakened immune systems¹².

The laboratory diagnosis of *Cryptosporidium* is usually made referring to acid fast staining or a fluorescent antibody method using a microscopic examination of feces¹³. The polymerase chain reaction (PCR) method is the gold standard for detecting *Cryptosporidium*¹⁴. This study was conducted by using the MKAFs method. MKAFs is a preferred method that can be used when enzyme-linked immune-sorbent assay (ELISA) and molecular tests cannot be performed. Some studies have compared methods to identify the parasite. One study detected *Cryptosporidium* cysts by comparing modified acid fast staining, ELISA and the Cassette Kit. The ELISA was the reference

method and modified acid-fast staining had 50% sensitivity and 100% specificity¹⁵. Gawad et al.¹⁶ detected *Cryptosporidium* using asit fast staining, ELISA and nested PCR. As a result, the staining method had 100% specificity and 45.2% sensitivity. In 89 children with leukemia/lymphoma and diarrhea, cryptosporidiosis was detected as 12.35% by ELISA and 7.86% by MKAFs¹⁷. No cryptosporidiosis was detected in the control group. We detected 27 (32.1%) positive samples out of 84; of these 9 (22.5%) had symptoms, 13 (40.6%) had no symptoms, and 5 (41%7) from the control group had solid fecal consistency. Tamomh et al.¹⁷ detected *Cryptosporidium* using modified Ziehl Neelsen (mZN) in 27.1% of children with diarrhea. Muhammad et al.¹⁸ detected *Cryptosporidium* in 29.7% of children with cancer by using mZN and 25.5% and nested PCR. Ramadan et al.¹⁹ identified *Cryptosporidium* in 15.7% of 83 children with hematological and solid malignancies by using ELISA. We detected *Cryptosporidium* cysts in 3 (3.5%) patients with neoplasm from the oncology-hematology clinic. Shad et al.²⁰ reported that 29% of 124 renal transplant recipients were positive for *Cryptosporidium*, while Arserim et al.¹ found one or more parasites in stool

samples from a nursing home. *Cryptosporidium* spp. (2.4%) as well as *Blastocystis* spp. (13.4%), and *Dientamoeba fragilis* (2.4%) were also detected. Infection with at least one other enteric pathogen has been reported in another study. *Clostridium difficile* and *Giardia lamblia* were reported as other identified enteric pathogens in 42% and 15% of cases, respectively²¹. In the current study, amoeba cysts were detected mainly in symptomatic elderly patients. *Cryptosporidium* spp. causes chronic diarrhea in patients with immune deficiency and is generally not severe in people with normal immunity¹⁶. Symptoms of immune-compromised patients, are mainly nausea, vomiting, abdominal cramps, fever, and watery diarrhea^{17,9}. In this study, 70.3% ($n = 26$) of patients who presented to the clinic had diarrhea. While the amoeba cysts were detected in patients with diarrhea, there was a discrepancy between the symptoms and the presence of *Cryptosporidium* spp. cysts. The reason for the appearance of *Cryptosporidium* cysts in asymptomatic patients may be age-related immune weakness or a concomitant co-morbidity.

Cryptosporidiosis in the elderly can accompany an intestinal obstruction or a non-infectious disease of the digestive system, such as diverticulitis or enteritis/colitis⁹. The reason we detected *Cryptosporidium* cysts in stool samples with a solid consistency may be due to gastrointestinal motility disorders, such as constipation. GT motility in this age group is frequently unregulated due to concomitant neurological, endocrinological, and other diseases. Some drugs used by the elderly can affect motility of the GT, such as anticholinergics, antidepressants, opioid analgesics, and calcium antagonists²².

The study was conducted by adherence to the local laboratory conditions, and the lack of confirmatory tests such as ELISA or PCR tests can be considered as the limitations of the study.

In conclusion; this study revealed the clinical significance and patient symptoms of cryptosporidiosis. Cases were under-diagnosed and others went unreported. Therefore, identifying reservoirs of infection and transmission routes is critical to preventing infection and controlling disease.

Author Contributions: Concept/Design : FO; Data acquisition: HG, BP, AD; Data analysis and interpretation: AD, SI; Drafting manuscript: FO; Critical revision of manuscript: MA; Final approval and accountability: FO, AD, MA, HG, SI, BP; Technical or material support: MA; Supervision: MA, SI; Securing funding (if available): n/a.

Ethical Approval: The study protocol complies with the Declaration of Helsinki (1964). This research was approved by Medicine Faculty Ethics Committee (Meeting No: 2018/13, Meeting Date: 25 July 2018, Decision No: 20) at Kahramanmaraş Sütçü İmam University. Also, written informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support

Acknowledgment: The authors would like to thank Enago (www.enago.com) for the English language review.

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