



## INVESTIGATION OF THE FREQUENCY OF *CRYPTOSPORIDIUM SPP.* AND *GIARDIA INTESTINALIS* IN PATIENTS WITH SUPPRESSED IMMUNE SYSTEM

### BAĞIŞIKLIK SİSTEMİ BASKILANMIŞ HASTALARDA *CRYPTOSPORIDIUM SPP.* VE *GIARDIA INTESTINALIS* SIKLIĞININ ARAŞTIRILMASI

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#### Abstract

**Objective:** *Giardia intestinalis* and *Cryptosporidium spp.* are important agents causing diarrhea in developing and developed countries. If untreated, these agents may cause severe life-threatening diarrhea, especially in immunocompromised people and children. This study aimed to investigate the frequency of *Cryptosporidium spp.* and *G. intestinalis* in the stool samples of immunocompromised patients who applied to Harran University Research and Application Hospital.

**Methods:** This study was carried out with a total of 100 patients with immunosuppression and diarrhea who were admitted to our hospital between January 2017 and January 2019. *Cryptosporidium spp.* and *G. intestinalis* antigens were investigated in stool samples of the patients by immunochromatographic (IC) method and oocyst, and cyst-trophozoite were examined with Native-Lugol and Kinyoun acid-fast staining.

**Results:** *G. intestinalis* was detected in eight (8%) patients. Seven of them (14%) were pediatric patients (n=50) and one (2%) was an adult female patient (n=50). *Cryptosporidium spp.* was detected in 3 (3%) patients. Of these, two (4%) were children patients and one (2%) was an adult female patient. Our results also demonstrated that both *Cryptosporidium* and *Giardia* were more found in immunocompromised patients, especially in pediatric ones (4% and 14% respectively).

**Conclusion:** Although the cost of the IC method, which detects antigens in stool samples is high, it is easy to work with, provides early results and it also helps to overcome the difficulties in active diagnostic methods. Despite the limited number of investigated samples, our findings showed that the incidence of infections from these two parasites is higher than in other studies conducted in Turkey or European countries.

**Keywords:** *Giardia intestinalis*, *Cryptosporidium spp.*, chromatographic method, immunosuppressed, diarrhea.

#### Öz

**Amaç:** *Giardia intestinalis* ve *Cryptosporidium spp.* türleri gelişmiş ve gelişmekte olan ülkelerde ishale neden olan bağırsak parazitleridir. Özellikle bağışıklık sistemi baskılanmış hastalarda ve çocuklarda tedavi edilemediğinde ölümcül olabilmektedir. Bu çalışmada Harran Üniversitesi Araştırma ve Uygulama Hastanesine başvuran bağışıklık sistemi baskılanmış hastaların dışkı örneklerinde *Cryptosporidium spp.* ve *G.intestinalis* sıklığının araştırılması amaçlanmıştır.

**Yöntem:** Bu çalışma Ocak 2017 ve Ocak 2019 yılları arasında hastanemize başvuran bağışıklık sistemi baskılanmış ve ishal şikâyeti olan toplam 100 hasta ile gerçekleştirildi. Hastaların dışkı örneklerinde immünokromatografik (İK) metot ile *Cryptosporidium spp.* ve *G. intestinalis* antijenleri arandı ve nativ-lugol ve kinyoun asit fast boyama ile ookist, kist-trofozoit açısından incelendi.

**Bulgular:** Toplam sekiz (%8) hastada *G. intestinalis* saptandı. Bunlardan yedisi (%14) çocuk hasta (n=50) ve biri (%2) yetişkin (n=50) bir kadın hasta olduğu tespit edildi. Hastaların 3 (%3)'ünde ise *Cryptosporidium spp.* görüldü. Bunlardan ikisi (%4) çocuk ve biri (%2) yetişkin kadın hastalar idi. Sonuçlarımız hem *Cryptosporidium* hem de *G. intestinalis*'in bağışıklık sistemi baskılanmış çocuk hastalarda (sırasıyla %4 ve %14) daha fazla saptandığını göstermiştir.

**Sonuç:** Dışkı örneklerinde antijen saptayan İK metodu maliyeti yüksek olmasına rağmen çalışması kolay, erken sonuç vermesi ve etkensel tanı yöntemlerindeki zorluklara yardımcı olduğu görülmektedir. Sınırlı sayıda araştırılmış örnek bulunmasına rağmen bulgularımız bu iki parazitten kaynaklı enfeksiyonların Türkiye'de veya Avrupa ülkelerinde yapılan diğer çalışmalardaki görülme sıklığından daha yüksek olduğunu gösterdi.

**Anahtar kelimeler:** *Giardia intestinalis*, *Cryptosporidium spp.*, kromatografik metot, bağışıklık sistemi baskılanmış, ishal.

**Introduction**

Human intestinal parasites are seen at different rates among various geographical regions in our country. The reasons are climate, and environmental factors, as well as socio-economic level, education and culture level, living habits, inadequacy in wastewater treatment facilities, and unhealthy environmental conditions.<sup>1</sup> It is known that *Giardia intestinalis* is among the most common parasites in our country.<sup>2</sup>

*G. intestinalis* and *Cryptosporidium spp.* are among the important agents responsible for endemic and epidemic diseases caused by contaminated water and food, therefore, detection of carriage in food workers is very important for the protection of public health. Both protozoans are transmitted by the fecal-oral route by consuming contaminated water and food. Infections are transmitted by thick-walled oocyst for *Cryptosporidium spp* and cyst for *G. intestinalis*.<sup>3-5</sup> Both parasites are seen more frequently in immunocompromised patients and children.<sup>6</sup>

*Cryptosporidium* genus protozoa are absolute intracellular parasites common in the world. The disease caused by *Cryptosporidium* is called cryptosporidiosis.<sup>3</sup> *Cryptosporidium* species cause two different types of infections in humans: intestinal and lung infections. The diagnosis of this disease is made by the detection of oocysts in the stool samples of the patients. The clinical picture varies according to the type, age, and immune status of the infected hosts. Sometimes it causes cholera-like diarrhea and can be life-threatening.

It is one of the most common protozoan diseases in immunocompromised patients (especially HIV-positive patients) and people with low socioeconomic status.<sup>6,7</sup> The parasitic infection caused by the *G. intestinalis* parasite is called Giardiasis. *Giardia* infections can be seen in all age groups worldwide, especially in childhood and in patients with a suppressed immune system.<sup>8,9</sup> The diagnosis of giardiasis is made by detecting cysts or trophozoites in stool samples of patients.<sup>10</sup>

In this study, it was aimed to investigate the incidence of *Cryptosporidium spp* and *G. intestinalis* parasites in stool samples of patients with immunocompromised diarrhea by immunochromatographic (IC) method.

**Methods**

**Study Groups**

The study group included immunocompromised oncology patients and intensive care patients with diarrhea who were admitted to Harran University Research and Application Hospital between January 2017 and January 2019. *Cryptosporidium spp* and *G. intestinalis* antigens were searched in the stools of the patients. This study was conducted with a total of 100 patients, 50 pediatric patients, and 50 adult patients. This study was approved by the Harran University Non-Interventional Clinical Research Ethics Committee (Approval number HRU/16/05/19).

**Analysis of Stool Samples**

Antigens of the parasites were searched in stool samples with the *Crypto + Giardia* Combo Card test (CerTest Biotec S.L., Spain). The tests were performed and interpreted according to the manufacturer's instructions. If there is a green line in the C control region of the reaction band and a red line in the T region on the side where *Giardia* is located, it will be evaluated as *Giardia* positive. *Cryptosporidium* will be

considered positive if there is a green line in the C control region of the reaction band and a red line in the T region on the side with *Cryptosporidium*. However, as a result of the formation of green and red lines in all C and T regions, both *Giardia* and *Cryptosporidium* will be evaluated as positive. The green line on both sides only in the C control region indicates that both parasites are negative. Staining preparations were prepared for the samples that were positive in both methods. The parasites were analyzed in terms of trophozoite, cyst, and oocyst by Kinyoun-acid-fast and Native-Lugol methods.

**Statistical Analysis**

SPSS (Statistical Package of the Social Sciences) Demo Ver 22 (SPSS Inc., Chicago, IL, USA) program was used for statistical analysis of the data, and the Pearson chi-square test was used. Analysis results were considered statistically significant in the presence of  $p < 0.05$ .

**Results**

Of the 100 patients included in the study, 60 were female and 40 were male. The ages of the patients were between 1-89 years and the mean age was 38.21. While 50 of the patients (14 male and 36 female) were children aged 1-18 years, the remaining 50 patients were adults over the age of 19 (26 male and 24 female).

Similar clinical symptoms such as nausea vomiting, fever, and abdominal pain have been observed in patients with giardiasis and cryptosporidiosis. Although we examined the stools of patients with enteritis, in some cases more than one clinical symptom such as nausea, vomiting, abdominal pain, cystitis, anemia and fever was simultaneously seen with enteritis. Different clinical manifestations (cystitis, anemia, chest pain) were also observed in patients with giardiasis.

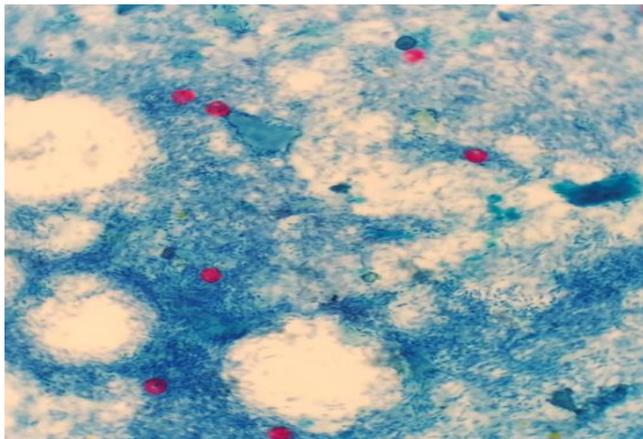
*G. intestinalis* was seen in 8 (8%) of the patients and *Cryptosporidium spp.* was observed in 3 (3%) patients (Table 1). While 7 of these patients were children between the ages of 1-12, one was a 59 years old patient. In addition, *Cryptosporidium spp.* antigen was detected in 3 patients; 2 of these patients were children between the ages of 1-12, and the other was 43 years old female patient (Table 1). No *Giardia/Cryptosporidium* coinfection was found in our study. According to our results, a statistically significant difference was observed at higher rates in children than in adults ( $p < 0.05$ ). In addition, there was no statistically significant difference between intestinal parasitic infections and gender ( $p = 0.228$ ).

**Table 1.** The numbers of *G. intestinalis* and *Cryptosporidium spp* detected according to age groups and gender

Age groups	<i>G. intestinalis</i>		<i>Cryptosporidium spp</i>	
	Male	Female	Male	Female
1-12 years	3	4	1	1
13-18 years	-	-	-	-
19-30 years	-	-	-	-
31-40 years	-	-	-	-
41-50 years	-	-	-	1
51-60 years	-	1	-	-
Above 61 years	-	-	-	-
<b>Total</b>	<b>3</b>	<b>5</b>	<b>1</b>	<b>2</b>

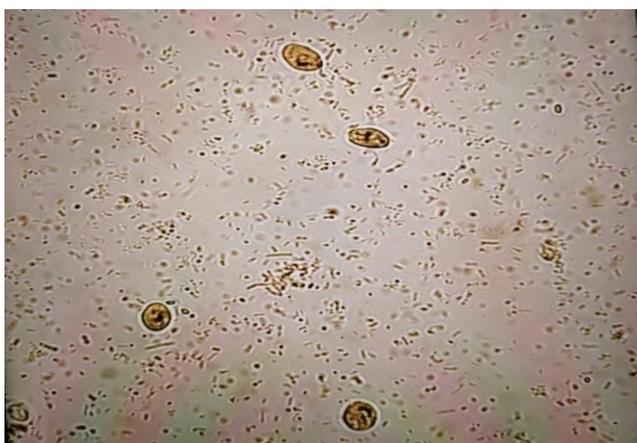
*G. intestinalis* was seen in 4 (8%) and *Cryptosporidium spp.* in one (2%) of 50 oncology patients. *G. intestinalis* was seen in 4 (8%) and *Cryptosporidium spp.* in 2 (4%) of 50 intensive care patients. No statistical difference was found in the analysis for a possible relationship between *G. intestinalis* and *Cryptosporidium spp.* intensive care and oncology patients ( $p>0.05$ ).

Stool samples of positive patients were examined for cyst-oocyst with different staining methods (Native-Lugol, Kinyoun acid-fast). In Kinyoun acid-fast staining, oocysts of *Cryptosporidium spp.* were seen in 3 (3%) patients (Figure 1). While 2 (4%) of the patients were children between the ages of 1-18, one (2%) was a female patient in 43 years old age. In the preparations examined in Lugol, oocysts could not be detected because they seemed too small and resembled yeast cells.



**Figure 1.** *Cryptosporidium* oocysts (red) with Kinyoun acid-fast staining method ( $\times 100$ )

*G. intestinalis* cysts were seen in 8 (8%) patients with the Native-Lugol method and it was detected in different densities in each patient (Figure 2). Cysts were seen in 3 (3%) 1-2 year old patients, 4 (4%) in 3-11 years old patients, and one (1%) in 51-60 years old patient. Trophozoites were also detected in some patients with cysts. In our study, it was observed that the rate in immunosuppressed children was much higher than in immunosuppressed adult patients.



**Figure 2.** The cyst form of *G. intestinalis* using the Native-Lugol method ( $\times 40$ )

## Discussion

The prevalence of *Cryptosporidium* in humans varies between 0.4-46.6% in studies conducted in different parts of the World. The rates of *G. intestinalis* in humans in recent

studies are between 0.8-54.8% in Turkey. Thus, the importance of cryptosporidiosis and giardiasis is increased.<sup>11-13</sup> Infections caused by *Cryptosporidium spp.* and *G. intestinalis* are thought to constitute two of the first three pathogens among the most common protozoan infections that cause diarrhea in humans in the world, especially in developing countries.<sup>14,15</sup>

In study conducted at the Child Protection Agency in Şanlıurfa, the examination of stool samples of 46 healthy children revealed that the detection rate of *G. intestinalis* was 19.6%. *Cryptosporidium spp.* was not found at all.<sup>16</sup> In our study, stool samples were taken from 50 children, and *Cryptosporidium spp.* was found in 4% and *G. intestinalis* was found in 14%. The high incidence of *Cryptosporidium spp.* has shown that the infection risk of is higher in immunocompromised children. Although their study group consisted of only immunocompromised patients, the presence of *G. intestinalis* antigens in both studies indicates that healthy individuals are also at risk.

In a study conducted in Egypt, the most common parasite in immunocompromised patients was *G. lamblia* with a prevalence of 10%, and *C. parvum* at 7%.<sup>17</sup> In our study, only 8% *G. intestinalis* and 3% *Cryptosporidium spp.* were found in immunocompromised patients. Although they investigated only one species of *Giardia* and *Cryptosporidium spp.* in their studies it was seen that our prevalence was lower.

In a study conducted in İzmir, *Cryptosporidium spp.*, *Giardia spp.*, and *Blastocystis spp.* were detected commonly in diarrhoeic and immunodeficient patients.<sup>18</sup> In our study, *G. intestinalis* and *Cryptosporidium spp.* antigens were investigated in patients with suppressed immune system and diarrhea, both parasites were found. It has been determined that *G. intestinalis* was more common in previous studies and in our study in Şanlıurfa.

A study conducted in Poland<sup>13</sup> was carried out to determine the prevalence of intestinal micro-pathogens such as *Cryptosporidium*, *Giardia*, *Blastocystis* and *Microsporidia* in hospitalized patients with different immunocompromised immunological conditions. They stated that the patients were sensitive to infections caused by the protozoa in question and their main clinical picture was diarrhea. They stated that protozoan infections are rare in immunocompromised patients. They reported that the overall prevalence of micropathogens among the participants was three times higher in adults (12.5%) than in children (2.3%). Accordingly, while adult patients were mostly *Blastocystis* and *Microsporidia* positive, they stated that they were infected with *Cryptosporidium* species more frequently in children. In our study, *G. intestinalis* was found in 8% of the immunocompromised patients and 14% of them were pediatric patients ( $n=50$ ), while *Cryptosporidium spp.* was found in 3% of them, and again 4% of them were pediatric patients ( $n=50$ ). It was observed that higher rates were observed in pediatric patients compared to adults ( $p<0.05$ ). In addition, no statistically significant correlation was found between the positivity of *Cryptosporidium spp.* and *G. intestinalis* and the gender of the patients ( $p=0.228$ ). In many studies, it has been reported that intestinal parasites are seen at close rates between male and female genders, and no statistically significant difference was found. Considering the 100 immunodeficiency patient group, the differences between the patients were statistically significant ( $p<0.05$ ).<sup>19</sup> In our patients, the main clinical picture was diarrhea, and different clinical symptoms were also detected. In general, it has been observed that *G. intestinalis* is a more common

protozoan parasite (8%) than *Cryptosporidium spp.* (3%), and most of examined subjects are pediatric patients. In a study conducted in Tehran, Iran, the prevalence of intestinal parasite infections was investigated in patients with cancer, organ transplantation, and primary immunodeficiency. In that study, *B. hominis* (16.2%) was the most common parasite followed by *G. lamblia* (12.5%) and *Cryptosporidium parvum* (1.2%) in patients with chronic diarrhea.<sup>20</sup> It was thought that the prevalence of *Cryptosporidium* was lowest since its only one species was investigated.

In a study conducted in 2016 in some developed countries in Europe (Germany, Netherlands, England), the prevalence of *Cryptosporidium* in diarrhea patients admitted to hospitals was investigated. In that study, detection rates of *Cryptosporidium spp.* were determined. They were 1.29% (n=1084) in Germany, 1.93% (n=314) in the Netherlands, and 8.25% (n=4128) in the UK.<sup>21</sup> The higher rate of *Cryptosporidium spp.* found in our study might be due to the inclusion of immunosuppressed patients with diarrhea.

Our results show the incidence only in patients with suppressed immune systems and diarrhea in Harran University Research and Application Hospital. The prevalence of such parasites in other hospitalized patients and the general population in Şanlıurfa region is still unknown.

### Conclusion

It was observed that the incidence of both parasites was higher than that obtained in many studies. The detection rates were 4% and 14% for *Cryptosporidium spp.* and *G. intestinalis*, respectively, especially in the pediatric patients. These results showed that the incidence of both parasites in immunocompromised children was higher than in adult patients. This might indicate that besides suppressed immune system, probably the the lack of hygiene standards in pediatric patients may be an adversely influencing factor.

### Conflict of Interest

None declared.

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### Author Contributions

MB: Conception and design; MC: Data collection; MB, MC, AÖ: Analysis and interpretation of data; MC, AÖ: Literature search; AÖ: Statistical analysis; MB, MC, AÖ: Writing the manuscript; MB, AÖ: Critical revision of the manuscript

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