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Examination of *Giardia intestinalis* with Direct Microscopy and Direct Fluorescent Antibody in Patients with Diarrhea

Giardia intestinalis'nin İshalli Hastalarda Mikroskopi ve DFA Yöntemiyle Araştırılması

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ABSTRACT

Objective: In this study, our objective was to compare direct microscopic examination and direct fluorescence antibody (DFA) method for *Giardia* diagnosis in stool samples and to evaluate the possible risk factors related to *Giardia* infections.

Methods: Stool samples of 185 patients with diarrhoea collected between June 2019 and July 2019 in Erzurum Yakutiye Research Hospital were included in the study. Microscopic examination of the samples was performed with native-lugol, and they were subsequently scanned by the indirect fluorescent assay microscope using the DFA method at 100-200X magnification. In addition, all patients filled a questionnaire prepared to determine the possible risk factors related to *Giardia* infection.

Results: The age of the 185 participating patients who belonged to different groups was between 0 and 94 years. *Giardia* spp. cysts were detected in five stool samples (2.7%) using direct microscopic examination. Nine samples (4.9%) were DFA-positive. The incidence of giardiasis was noted to be 7.5% in children, 3.8% in adults, 7.3% in people living in rural areas, 2.9% in people living in urban areas, 10% in people having pets and 4.2% in people who do not have pets.

Conclusion: By taking the DFA method as a reference, the sensitivity and specificity of the microscopic examination were found to be 44.4% and 99.4%, respectively. The *Giardia* positivity rate was higher in children, those living in rural areas, those having pets and those using well water as drinking water.

Keywords: *Giardia* spp., diarrhoea, direct fluorescent antibody, direct microscopy, risk factor

ÖZ

Amaç: Bu çalışmada ishalli dışkı örneklerinde *Giardia*'nın teşhisinde, direkt mikroskobik bakı yöntemi ile direkt flöresan antikor (DFA) yönteminin karşılaştırılması ve ayrıca *Giardia* enfeksiyonları için olası risk faktörlerinin araştırılması amaçlanmıştır.

Yöntemler: Haziran-Temmuz 2019 tarihleri arasında Erzurum Yakutiye Araştırma Hastanesi; farklı kliniklerinden 185 hastanın ishalli dışkıları çalışma materyali olarak kullanıldı. Laboratuvara gelen örneklerde; öncelikle native-lügol ile mikroskobik bakı, sonrasında DFA yöntemi kullanılarak IFA mikroskobunda X100-200 büyütmede tarandı. Ayrıca *Giardia* enfeksiyonunun olası risk faktörleri araştırmak için hastalardan anket formu doldurmaları istendi.

Bulgular: Yaşları 0-94 arasında değişen ve farklı gruplarda yer alan bu hastalara ait 185 fekal örneğin 5'inde (%2,7) direkt mikroskopi ile *Giardia* spp.'ye ait kistler görüldü. DFA yöntemiyle örnekleri 9'unda (%4,9) pozitiflik saptandı. Giardiyozis yaygınlığı çocuklarda %7,5, yetişkinlerde %3,8, kırsal bölge de yaşayanlarda %7,3, şehirde yaşayanlarda %2,9, evcil hayvan sahiplerinde %10 ve evcil hayvanı olmayanlarda %4,2 idi.

Sonuç: DFA yöntemi referans alındığında mikroskobi yönteminin duyarlılığı %44,4, özgüllüğü ise %99,4 olarak hesaplandı. Çocuklar, kırsal bölgede yaşayanlar, evcil hayvan besleyenler ve içme suyu olarak kuyu suyu kullananlarda *Giardia* pozitifliği daha yüksek bulundu.

Anahtar Kelimeler: *Giardia* spp., ishal, direkt flöresan antikor, direkt mikroskopi, risk faktörü

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INTRODUCTION

Giardia intestinalis (known also as *G. lamblia or G. duodenalis*) is one of the ten enteric parasites, which are most common among people worldwide (1). *Giardia*, which was first identified by Antony van Leeuwenhoek in 1681, is a whip-shaped and flagellated parasite and has only trophozoite and cyst forms (2). Contaminated water and food are the main risk factors for *Giardia* infections. Poor living conditions, being a large family, contaminated environment, usage of raw sewage and low socioeconomic class are other related risk factors (3,4). *Giardia* infection may also be transmitted directly from infected pets or wild animals (5). Although *Giardia* infections may be asymptomatic in humans, they may also lead to different clinical courses extending from mild diarrhea to severe malabsorption (6). Conventional microscopic methods are usually used for the diagnosis of *Giardia* in the laboratories. However, the error margin of the examination of only one stool sample is relatively high if the parasite concentration is low, the quality of the microscopic examination is poor, and the parasite is hidden due to the intermittent shedding and bile pigments (7). Three stool samples should be examined to increase sensitivity (8). For the diagnosis of *Giardia*, serological methods such as ELISA and direct fluorescence antibody (DFA), which are based on the detection of the parasite antigens in the stool samples, are also used besides the conventional methods (2,9).

In this study, the objective was to compare the direct microscopy using native-lugol and DFA in the diagnosis of *Giardia* and to evaluate the risk factors of giardiasis with the help of a questionnaire filled by the patients.

METHODS

The stool samples of 185 patients with diarrhea, who were referred from different clinics of the Erzurum Yakutiye Research Hospital between June 2019 and July 2019, were included in the study. The microscopic examination of the samples admitted to the laboratory was done with native-lugol and then the remaining stool samples were stored at -20 °C for the examination with DFA. Meriflour *Giardia/Cryptosporidium* (made in the USA) kit was used for DFA. The results obtained using positive and negative controls according to the recommendation of the manufacturer and screened under x100-x200 magnification for each well. The slides showing fluorescence were confirmed under higher magnification. The prepared stool specimens, which contained green-apple colored samples with a size between 8-12 µm and the characteristic cyst morphology, were considered positive for the presence of *Giardia* spp. Necessary approval form for study was taken from patients. In addition, we let the patients fill questionnaires and tried to determine the possible risk factors related to giardiasis.

Statistical Analysis

The statistical analysis was performed to determine the relationship between the different patient groups (grouped for the age, drinking water source, etc) for the examined parameters. The SPSS software package (v.22.0, SPSS Inc.) was used for all analyses. The p-values <0.05 were considered significant according to the results of the Pearson's chi-square test.

RESULTS

Our study involved 185 patients, who were between the ages of 0-94 years and complained of diarrhea. 97 of the patients were male and 88 of them were female. The age of 53 patients was between 0-14 years and the remaining 132 patients were older than 15 years. In our study, we observed cysts belonging to *Giardia* spp. in 5 of the 185 stool samples (2.7%) with direct microscopy. On the other hand, 9 stool samples (4.9%) were DFA positive. Although four of the five samples diagnosed positive with the direct microscopy were also positive on DFA, the remaining one sample was DFA negative. On the other hand, five samples, which were negative on the microscopic examination, were positive on DFA. The sensitivity, specificity, positive predictive value, and negative predictive value of the direct microscopic examination were determined with the reference to the DFA method (Table 1).

The possible risk factors for the *Giardia* spp. prevalence determined with the data obtained through the used questionnaire were listed in Table 2.

One of the samples containing *Giardia* spp. under the IFA microscope (DFA method) was shown in Figure 1.

DISCUSSION

In this study, we determined the prevalence of *Giardia* spp. in individuals, who had applied to our hospital with the complaint of diarrhea and compared the direct microscopic examination with the DFA for *Giardia* diagnosis. In addition, we evaluated the data obtained for the possible risk factors related to giardiasis. In Turkey, studies focused on *Giardia* infections have mostly an epidemiological design. In our study, the prevalence of *Giardia*

Table 1. Comparison of the results of the DFA method and direct microscopic examination, and sensitivity and specificity level of the direct microscopic examination

Tested	DFA (+) n	DFA (-) n	Total
Direct microscopy positive	4	1	5
Direct microscopy negative	5	175	180
Total	9	176	185
The evaluation of the direct microscopy			
Sensitivity	44.4%		
Specificity	99.4%		
Positive predictive value	80.0%		
Negative predictive value	97.0%		

DFA: Direct fluorescence antibody

Table 2. Possible risk factors for the *Giardia intestinalis* infection

Risk factors	n (185)	Positive (%)	p
Gender			
Male	97	6.2%	0.299
Female	88	3.4%	
Age groups			
0-14 years	53	7.5%	0.236
Over 15	132	3.8%	
Living area			
City	103	2.9%	0.150
Rural	82	7.3%	
Pets			
Yes	20	10%	0.252
No	165	4.2%	
Drinking-water supply			
Bottled water	15	0%	0.324
Tap water	138	4.3%	
Well water	32	9.4%	

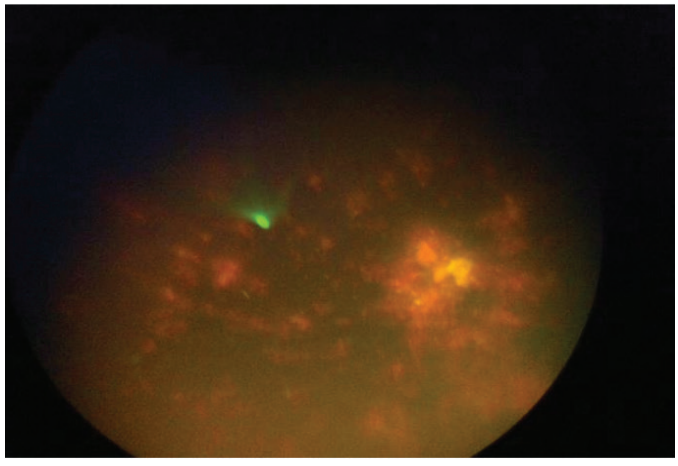

Figure 1. Picture of one of the samples with *Giardia* spp. detected with DFA (x20)

DFA: Direct fluorescence antibody

determined 2.7% with direct microscopy and 4.9% with DFA method. Regarding the studies, which had been conducted with direct microscopy in different regions of our country, the *G. intestinalis* prevalence was 1.2% in the study conducted by Arserim et al. (10) in Izmir, 5.7% in the study conducted by Oncel (11) in Şanlıurfa, 9.4% in the study conducted by Cengiz et al. (12) in Van and 1.45% in the study conducted by Baştemir et al. (13) in Manisa.

The DFA method used in the *Giardia* diagnosis has a very high sensitivity. Garcia and Shimizu (9) reported a 100% sensitivity level for DFA. In Turkey, there are only a limited number of studies focused on the comparison of the direct microscopic examination and DFA in *Giardia*'s diagnosis. Regarding this limited literature; Kuştimur et al. (14) reported that DFA was useful to determine the protozoa and could be helpful in the routine laboratory practice. Bayramoğlu et al. (15) used DFA in their study for *G. intestinalis* diagnosis with food employees and reported 54.1% sensitivity and 100% specificity for the direct microscopy. In the USA, Alles et al. (16) conducted a study, in which DFA was used as a reference, and they reported the sensitivity and specificity rates of the direct microscopy as 66.4% and 100% respectively. In Egypt, El-Nahas et al. (17) used DIF (Direct Immunofluorescence Assay) as a reference and reported 76.9% and 100% for the sensitivity and specificity of the direct microscopy respectively. In our study, we found that the sensitivity and specificity of the direct microscopy were 44.4% and 99.4% respectively for the detection of the *Giardia* spp. based on DFA as a reference.

It has been reported that Diarrheal disease is the leading cause of death and illness for children under five years of age in developing countries (18). There are several studies focused on the risk groups for giardiasis. Several factors such as gender, childhood-adulthood, living in the rural and urban areas, source of drinking water, and the presence of pets and education level of parents were evaluated in these studies. Julio et al. (19) showed in Portugal that the positivity rate for *G. intestinalis* was 6.8% in children aged 0-15. Sagabiel et al. (20) from Germany showed that the positivity rate was 1.5% among children aged 0-6 years and Kramar et al. (21) from Russia showed that the same rate was 31.9% among children aged 0-5 years. In our study, the *Giardia* prevalence was 8.2% among children aged 0-14 years. The same rate was 3.3% among adults. The prevalence of *Giardia* is usually higher in children compared to adults. This may be explained by the relatively poor personal hygiene in childhood.

The rate of *Giardia* is higher in people living in rural areas compared to urban areas. Julio et al. (19) found a *Giardia* positivity of 5.3% and 7.4% in people living in urban and rural areas respectively. Naz et al. (4) showed that the *Giardia* positivity was 7.3% and 12.3% in people living in the urban and rural areas respectively. In our study, the positivity rate was 2.9% and 7.3% in people living in the urban and rural areas respectively. Thus, our results were consistent with the results of other studies and in all studies, the positivity rate was higher in people living in rural areas. The close relationship between nature and house environment in rural areas and consequently higher contact with the *Giardia* species may explain this finding.

Giardia species may cause infections in animals in close contact with humans. In Pakistan, Naz et al. (4) found that the *Giardia* prevalence was 13.8% in people with a pet, while the same rate was 9% in people not having a pet. In Malesia, Choy et al. (22) reported a *Giardia* prevalence of 12.4% and 9.6% for people with and without a pet respectively. In our study, the same rates were 10% and 4.2% respectively. These findings showed that giardiasis is considerably higher in people living with pets.

CONCLUSION

The direct microscopy in the diagnosis of *Giardia* is a valuable method because of its rapid and easy implementation and the possibility of the detection of other parasites besides *Giardia*. However, low specificity is the limitation of this method. On the other hand, the high sensitivity of DFA is considered the positive aspect of this method. However, the high cost and requirement of a highly-equipped laboratory environment are the disadvantages of DFA. We conclude that the development of rapid and cost effective immunological diagnostic tests, which may detect simultaneously several parasites, will be very useful. We

considered children, people in rural areas, people having pets, and people using well water are groups more under risk of giardiasis.

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* Ethics

Ethics Committee Approval: Our study was approved by the Ethics Committee for Clinical and Laboratory Research in the Medical Faculty of Atatürk University (approval date: 30.05.2019, no: 55).

Informed Consent: Patient consent form and questionnaire were prepared.

Peer-review: Internally peer-reviewed.

* Authorship Contributions

Concept: A.Y., H.U., Design: A.Y., Data Collection or Processing: A.Y., H.U., Analysis or Interpretation: A.Y., H.U., Literature Search: A.Y., H.U., Writing: A.Y., H.U.

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