Investigation of intestinal parasites in dialysis patients

Gulkan Karadag, MD, Gulden S. Tamer, MD, PhD, Erkan Dervisoglu, MD.

ABSTRACT

الأهداف: دراسة الطفيليات المعوية والمحرضة في مرضى الكلى ومقارنة الطرق المستخدمة في التشخيص.

الطريقة: أجريت دراسة عشوائية اشتملت على مرضى الكلى. أجريت هذه الدراسة في قسم الميكرولوجيا، مستشفى الأبحاث، كلية الطب في جامعة قوجا، قوجا، تركيا خلال الفترة من يونيو 2012م ومارس 2013م. تم تشخيص 142 مريض في المراحل الأخيرة للمرض وخضع للغسيل و 150 شخص سليم في هذه الدراسة. كما تمت دراسة اختبارات ثلاثية الألوان، اختبارات ثلاثية الألوان المحدثة، وصامد الحمض، وطريقة كاليسفور واستيدين التثقل في عينات البراز. استخدمت طريقة اليزا لفحص المستضد في البراز وتشخيص مكرويات الأبواغ، وانتان النسج، والمعوية.

النتائج: ظهرت الطفيليات في 62 مريض كلى 43.7% و 19 من مجموعة التحكم 12.7%. كما أن العوامل الطفيلية في مرضى الكلى كانت انتان المتبرعمة الكيسيه 39.9%، المعوية 8.5%، النسج 2.1%، ومكرويات الأبواغ 2.1%. كما أن معدل الإصابة بالطفيليات لاستيدين التثقل كانت أعلى من المحلول الطفيلي $p>0.05$.

خاتمة: أنه من الضروري عمل فحوصات مخبرية للبراز لحماية مرضى الكلى المصابين بالإسهال من الالتهابات الطفيلية وذلك باختبارات ثلاثية الألوان، الملونة المحدثة، وصامد الحمض، وطريقة كاليسفور، وطريقة أليسا التي تكشف المستضد في البراز.

Objectives: To search for the opportunistic and other pathogenic intestinal parasites in dialysis patients, and to compare the methods used for diagnosis.

Methods: This is a randomized study, which recruited participants from the dialysis patients. The study was carried out in the Department of Microbiology, Research Hospital, School of Medicine in Kocaeli University, Kocaeli, Turkey between June 2012 and March 2013. One hundred and forty-two patients were diagnosed with an end-stage renal failure, which underwent dialysis, and 150 healthy volunteers were enrolled in the study. Native-lugol, formol ethyl acetate sedimentation method, trichrome, modified trichrome, acid fast, and Calcofluor staining methods were applied to the stool samples. For the diagnosis of *Cryptosporidium spp., Giardia intestinalis (G. intestinalis)*, and *Entamoeba histolytica (E. histolytica)*, commercially available ELISA kits were used, which detect antigen in the stool.

Results: Parasites were found in 62 of the dialysis patients (43.7%) and 19 of the control group (12.7%). The most encountered parasitic agents in the dialysis patients were *Blastocystis spp.* (23.9%), *G. intestinalis* (8.5%), *E. histolytica* (2.1%), *Microsporidia spp.* (2.1%), and *Cryptosporidium spp.* (2.1%). The parasite detection rate of the formol ethyl acetate sedimentation method was found to be higher than native-lugol ($p<0.05$).

Conclusion: To protect the dialysis patients with diarrhea from parasitic infections, it is important to carry out interval stool examinations with trichrome, modified trichrome, acid fast, and Calcofluor staining methods, and the ELISA method, which detects antigen in the stool.

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From the Departments of Microbiology (Karadag, Tamer), Internal Diseases (Dervisoglu), School of Medicine, Kocaeli University, Kocaeli, Turkey.

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Address correspondence and reprint request to: Dr. Gülden S. Tamer, Department of Microbiology, School of Medicine, Kocaeli University, Umuttepe Campus, Kocaeli, Turkey. Tel. +90 (262) 3037446. Fax. +90 (262) 3037003. E-mail: guldensonmez@hotmail.com

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Increased life expectancy and elongation of human life have also increased the frequency of diseases such as chronic renal failure (CRF).[1] The fact that patients with CRF have a disorder in their immune system was first noticed by Dammin et al[2] in 1957 by recognizing that the skin homograft in these patients were long-lived. Progressive and irreversible loss of renal functions causes accumulation of urea in the body, where in turn uremia gives rise to changes in natural and gained immunity. The CRF has negative impacts on neutrophil chemotaxis, phagocytosis, and bactericidal actions and T cell function. The problems in maturing of the T lymphocytes increase susceptibility to infections. Results show that CRF may be accepted as a gained immune deficiency.[3] Patients with a suppressed immune system catch parasitic infections more easily. It is known that cellular immunity plays a more important role in defence against parasitic diseases.[4] Suppression or unwell functioning of the immune system causes the increase of the pathogen effects of the parasites, which are especially affected by cellular immunity, and formation of severe clinical pictures. Generally, the average age of patients diagnosed with CRF and end-stage renal failure (ESRF) is high, and they have additional health problems, such as diabetes. When the infection risks and related complications of these patients, who are open to infections, are considered, studies towards preventing infections are very important. In patients with suppressed immunity, research of any factors that cause opportunistic infections, such as *Cryptosporidium*, *Cyclospora*, *Isospora belli*, and *Microsporidia*, and other pathogenic intestinal parasites like *Blastocystis spp*, *Dientamoeba fragilis (D. fragilis)*, *Entamoeba histolytica (E. histolytica)* and *Giardia intestinalis (G. intestinalis)* is important.[5,6] In this study, we searched for the pathogenic intestinal parasites in patients who underwent hemodialysis and peritoneal dialysis treatment by using different diagnostic approach. Healthy volunteers were used as the control group. The study allowed reaching a conclusion that using different methodological approaches in diagnosis of intestinal parasitic agents were crucial and should not be underestimated. We investigated immune suppressed patients caused by dialysis and compared several different methods for detecting parasitic infections. Other studies also investigated the effects of immune suppression on parasitic infections. However, many of these studies were performed with cancer patients, HIV positive patients, and others.[5,7-9] Some other studies also used dialysis patients in their studies to reveal prevalence of intestinal parasites. These studies used a few of the methods available for diagnosis of parasites, which made them likely to miss present infections. The main objective of this study was to reliably determine the prevalence of intestinal parasitic infections. The second objective of this study was to highlight the importance of using combination of different methodological approaches in diagnosis of intestinal parasitic infections to prevent failure of diagnosis.

Methods.

Patients. The study was carried out in a medical school in Kocaeli University between June 2012 and March 2013. One hundred and forty-two patients who underwent dialysis due to the diagnosis of ESRF, were enrolled to the study. One hundred and fifty healthy volunteers were enrolled to the study as the control group. The approval for the study was granted by the University Ethics Committee. Principles of Helsinki Declaration were followed. Of the patients 62 were male and 80 were female. Of the control group, 62 were male and 88 were female. Dialysis patients who did not use parasitic drugs for treatment within a month prior to study were excluded. Dialysis patients who were receiving treatment for more than 5 years were included to the study. The control group was consisted age- and gender- matched healthy individuals. A questionnaire was handed out to participants of the study who signed the consent forms. The questions included the age, gender, education status, the number of people living at home, the place of residence, the house of residence, hand washing habits, the drinking water source, any complaints that might be associated with parasite in the stool, and the period of dialysis. The stool samples were taken to the laboratory within the possible shortest period of time, and were first examined macroscopically. Direct preparations were prepared with native-lugol (NL). The samples were also examined with formol ethyl acetate sedimentation method, and 5 preparations were prepared from each stool sample. The preparations were stained with modified trichrome, modified acid fast (MAF), and trichrome staining techniques, and examined under a ×100 immersion lens. The preparations stained with Calcofluor were examined under a fluorescence microscope at 470 nm wavelengths. A portion from each stool sample was stored at -20°C to be used in ELISA later. The ELISA tests were performed using commercially available kits for *Giardia spp* (*Giardia* 2nd Generation, Diagnostic Automation Inc, CA, USA), *E. histolytica* (WAMPOLE *E. histolytica* II ELISA, TechLab, USA), *Cryptosporidium spp (Cryptosporidium* (fecal), Diagnostic Automation Inc).

Statistical analysis. Analysis was carried out in a computer environment using the Statistical Package for

Social Sciences version 20 (SPSS Inc, IBM, Chicago, IL, USA). During the analyses, chi-square test was used in dependent groups, and $p<0.05$ values were accepted as significant.

Results. The average age of patients who underwent dialysis treatment was 52.2 (±12) for males and 52.2 (±13) for females. The average age was 47.7 (±11.9) for males and 48.3 (±10) for females in the control group. The study group and the control group were similar in gender distribution. The rate of parasite infection in dialysis patients was 43.7%. This ratio was higher in patients who underwent dialysis than the control group ($p=0.000$). The parasite rates determined in the study and the control groups are shown in **Table 1**. When the answers given to the questionnaire were reviewed, no meaningful relationship was found between the frequency of parasites and age ($p=0.22$), gender ($p=0.512$), number of occupants in the house ($p=0.445$), place (village/city) ($p=0.086$), and the house (detached/block of apartments) of residence ($p=0.535$), drinking water ($p=0.205$), animal feeding habits ($p=0.411$), and educational status ($p=0.854$). Although there is no meaningful relationship between the dialysis period and frequency of parasites, the frequency of parasites was higher in patients in the study group. Parasites were determined in 16 of the 21 dialysis patients who answered the questions related to hand washing habits, and 14 patients stated that "they sometimes wash their hands," and 18 patients stated that "they sometimes use soap during hand washing". A meaningful relationship was found between the habit of hand washing, regular soap use, and frequency of parasites ($p=0.000$). Positivity of *G. intestinalis* and *Blastocystis spp.* was found significantly higher in dialysis patients ($p=0.012$) compared to the control group ($p=0.002$). Although the number of positive cases was less for *Cryptosporidium spp, E. histolytica, Microsporidium spp,* and *D. fragilis* the fact that they were determined only in dialysis patients was considered to be meaningful. The types of parasites detected in the study, and the corresponding percentages were shown in **Table 2**. When the cases in the study group infected with multiple parasites were examined, infections of *Blastocystis spp.* plus *Escherichia coli (E. coli)* and *Blastocystis spp plus G. intestinalis* (4 patients) and *Blastocystis spp plus E. coli* (2 patients)

Table 1 - Frequency of parasites in patients who underwent dialysis and the control group.

Groups	Positive	Negative	Total	*P*-value
Study	62 (43.7)	80 (56.3)	142 (100)	0.000
Control	19 (12.6)	131 (87.4)	150 (100)	
Total	**81 (27.7)**	**211 (72.3)**	**292 (100)**	

Table 2 - Types of parasites detected and its corresponding percentages .

Parasite	Study group	Control group	Total
	n (%)		
Blastocystis spp	34 (23.9)	16 (10.6)	50 (34.5)
Giardia intestinalis	12 (8.5)	3 (2.0)	15 (10.5)
Entamoeba histolytica	3 (2.1)	- -	15 (2.1)
Microsporidium	3 (2.1)	- -	3 (2.1)
Cryptosporidium	3 (2.1)	- -	3 (2.1)
Dientamoeba fragilis	2 (1.4)	1 (0.7)	3 (2.1)
Escherichia coli	4 (2.8)	3 (2.0)	7 (4.8)
*Other	4 (2.8)	1 (0.7)	5 (3.5)
Total	**65 (45.7)**	**24**	

*Other: *Endolimax nana, Entamoeba hartmanni, Iodamoeba butschlii*

Table 3 - Parasites determined with the Native-lugol and formol ethyl acetate sedimentation methods.

Parasite	Native-lugol	Formol ethyl acetate sedimentation	*P*-value
	n (%)		
Blastocystis hominis	38 (66.7)	50 (68.5)	0.000
Giardia intestinalis	7 (12.2)	10 (13.7)	
Entamoeba (E.) histolytica/E. dispar	1 (1.8)	2 (2.7)	
Escherichia coli	6 (10.5)	7 (9.6)	
*Other	5 (8.8)	4 (5.5)	
Total	**57 (100)**	**73 (100)**	

*Other: *Endolimax nana, Entamoeba hartmanni, Iodamoeba butschlii*

Table 4 - The diagnostic methods and its corresponding results for the parasites.

Parasites	Methods					
	NL	Formol ethyl acetate sedimentation	Trichrome	Modified Trichrome	MAF	ELISA
Blastocystis spp	38	50	50	0	0	-
Giardia intestinalis	7	10	10	0	0	15
Cryptosporidium spp	0	0	0	0	2	3
Entamoeba histolytica spp	1	2	3	0	0	3
Microsporidium spp	0	0	0	3	0	-
Dientamoeba fragilis	0	0	3	0	0	-
Total	46	62	66	3	2	21

NL -Native-lugol, MAF - modified acid fast, ELISA - enzyme-linked immunosorbent assay

were detected. Also, *Blastocystis spp* and *G. intestinalis* was detected in one patient in the control group. The parasites detected using the NL and formol ethyl acetate sedimentation methods are given in **Table 3**. In this study, the sensitivity of NL was lower than the sensitivity of the formol ethyl acetate sedimentation method. For the diagnosis of *E. histolytica*, the sensitivity of NL was lower than the sensitivity of formol ethyl acetate sedimentation and trichrome staining method and ELISA. The entire *D. fragilis* trophozoites seen in this study were determined with the trichrome staining method. For the diagnosis of the *Cryptosporidium spp*, ELISA and MAF methods were used. The sensitivity of the MAF method compared to ELISA was determined as 66.7% and specificity as 100%. For the diagnosis of *Microsporidium spp,* modified trichrome and Calcofluor staining methods were used. Three *Microsporidium spp.* seen in dialysis patients were found positive with both methods. The methods used for the diagnosis of parasites were given in **Table 4**.

Discussion. The prevalence of *Blastocystis ssp.* is higher in the developing countries (30-50%) than the developed countries (1.5-10%). *Blastocystis ssp.* is a parasite that is frequently isolated from stool samples of symptomatic and asymptomatic cases, using variable methods. The most frequently used method for diagnosis is light microscopy examination. However, it is stated that the examination of the preparation prepared with trichrome staining method is very valuable.[7,8] Kulik et al[1] studied stool samples of 86 dialysis patients and 146 healthy volunteers with the formol ethyl acetate sedimentation method and Kinyoun staining. They determined *Blastocystis spp* in 18, *Endolimax nana* in 14, and *Cryptosporidium spp.* in 4 dialysis patients. They found frequency of parasites high in the group of dialysis patients. In terms of *Blastocystis* and *Cryptosporidium spp,* they suggested that the patients should be examined for the presence of *Blastocystis* and *Cryptosporidium spp* in routine controls.

Botero et al[9] studied 110 stool samples in patients with suppressed immunity using NL, concentration, culture and specific stains, and found *E. histolytical/E. Dispar* in 11, *G. intestinalis* in 8, *Cryptosporidium spp.* in 4, and *Microsporidia spp.* in 2. While the giardiasis cases are generally observed as asymptomatic, chronic infections occur in people with suppressed immunity.[10] Ulçay et al[11] determined intestinal protozoans that might be a factor of gastroenteritis using NL, trichrome, MAF, ELISA, DFA, and PCR. They determined that protozoans like *G. intestinalis, C. parvum, B. hominis*, and *E. histolytica* might be responsible for extended diarrhea in immune-deficient patients. They concluded that if no factor were determined in the stool with the NL method for the diagnosis, it would be appropriate to use DFA or MAF for the diagnosis of *Cryptosporidium spp.* and ELISA or trichrome staining methods for the diagnosis of *E. histolytica*.

There is mounting evidence that shows the *Cryptosporidium* infection could occur more frequently in individuals with a deficient immune system. *Cryptosporidium* was found in three-fourths of HIV positive patients with chronic diarrhea.[12,13] Türkçapar et al[14] studied the frequency of *Cryptosporidium* in 74 dialysis patients and 50 healthy volunteers using the MAF method. They determined *Cryptosporidium* oocysts in 15 dialysis patients (20.27%) and both *Cryptosporidium* and *Giardia* cysts in one. Seyrafian et al[15] looked for the frequency of *Cryptosporidium* in hemodialysis patients by using the MAF method. They compared the results obtained from 104 hemodialysis patients with 2 control groups (91 dialysis patient's relatives and 140 healthy volunteers), and detected *Cryptosporidium spp* oocysts in 11% of dialysis patients. This result is higher in comparison to both control groups, but no notable difference was detected between the control groups. Since hemodialysis patients are candidates of organ transplant, the authors stated that it was important to prevent the *Cryptosporidium* infection.

In this study, the frequency of *Cryptosporidium* in dialysis patients was 2.1%, which was determined by using the ELISA and the MAF methods. While there was no *Cryptosporidium spp* in the control group, *Cryptosporidium spp.* was found in 2 patients with diarrhea and in one patient without diarrhea who underwent dialysis. The *E. histolytica* is a protozoan that gains importance because it causes serious clinical pictures in people with suppressed immunity. Ferreira-filho et al[16] studied *E. histolytica/E. dispar* infection on 110 chronic hemodialysis patients. They determined *E. histolytica/E. dispar* in 9 patients (8.2%), *Giardia* in one patient, *Strongyloides stercoralis* in 2 patients, *E. nana* in 6 patients, and *E. coli* in 11 patients. We determined *E. histolytica* in 3 dialysis patients in our study. Microsporidium types are generally a serious factor of diarrhea in patients with suppressed immune system, they may also cause an infection in patients with a regular immune system. Karaman et al[17] reported the frequency of *Microsporidium* in 320 patients diagnosed with cancer using the NL, sedimentation, modified trichrome, and Calcofluor staining methods. They employed 320 people, who were not diagnosed with cancer, as the control group. In the study, the incidence rate of *Microsporidium* was 10.9% in the patients group

and 5.6% in the control group. The authors concluded that intestinal parasites and *Microsporidium* could cause significant disturbance in cancer patients, and it also could have a negative effect in the treatment process.

In this study, modified trichrome and Calcofluor staining methods were applied on all samples. *Microsporidium spp* were found in 3 dialysis patients with diarrhea with both methods, and no *Microsporidium spp* were found in the control group. It was concluded that the possibility of finding *Microsporidium* in dialysis patients with diarrhea should not be ignored.

The prevalence of *D. fragilis,* which is a pathogenic intestinal protozoan, varies between 0.4-17%. The *D. fragilis* is found more than Giardia in patients with diarrhea.[18] The permanent stains necessary for the diagnosis are not used routinely by all laboratories. Munasinghe et al[19] examined the stool of 750 patients with and without diarrhea for the presence of *D. fragilis*, and determined that it occurred more than the *G. Lamblia* infection. In this study, the most common found factor was *Blastocystis spp.* again with 92 cases. We also determined *D. fragilis* in 2 patients with or without diarrhea, and in one person with diarrhea in the control group using the trichrome method.

In conclusion, examination of stool samples of dialysis patients in terms of intestinal parasites at certain intervals with the formol ethyl acetate sedimentation method, trichrome, modified trichrome, acid fast, and Calcofluor staining methods was found to be important in early diagnosis and treatment. This study clearly demonstrated that combinations of methods are needed to provide highly reliable and sensitive diagnosis for intestinal parasites. However, such combinations would be costly for routine work. Therefore, we believe that a single method that can detect parasites with high reliability and sensitivity is still in demand.

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