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Screening of immunocompromised patients at risk of strongyloidiasis in western Turkey using ELISA and real-time PCR

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Background/aim: *Strongyloides stercoralis* causes life-threatening hyperinfection or disseminated strongyloidiasis in immunocompromised patients such as HIV-positive, organ transplantation, and cancer patients. This study investigated the presence of strongyloidiasis in immunocompromised patients for the first time in Turkey.

Materials and methods: Serum and stool samples were collected from 108 patients (25.9% of them were chronic renal failure and 74.1% were renal transplantation patients) who were admitted to Ege University Medical School in İzmir, located in western Turkey. Serum samples were analyzed by ELISA (DRG, Germany) and the presence of 18S rRNA gene of *S. stercoralis* was detected in stool samples by real-time PCR.

Results: The analysis of serum samples showed that only one patient was anti-*S. stercoralis* IgG antibody and real-time PCR positive (0.92%). The patient was treated twice with albendazole (400 mg/day for 3 days) at 2-week intervals. Follow up real-time PCR was negative and the patient became seronegative 6 months after the initial diagnosis.

Conclusion: This screening showed that the prevalence of strongyloidiasis in this small group of patients who were at risk of strongyloidiasis was 0.92%. Overall, the results showed that more systematic studies are required in Turkey to show the prevalence of strongyloidiasis.

Key words: *Strongyloides stercoralis*, ELISA, real-time PCR, diagnosis, Turkey

1. Introduction

Strongyloidiasis is a parasitic disease caused by *Strongyloides stercoralis* and *Strongyloides fuelleborni*. Strongyloidiasis is transmitted through contact with contaminated soil and is endemic in tropical and subtropical regions (1). According to the World Health Organization (WHO), 30–100 million people are infected by *Strongyloides* spp. Moreover, it is stated that most of these cases are associated with immunosuppressive diseases, organ transplants, and hematological malignancies (2).

In immunocompromised patients, *S. stercoralis* leads to severe strongyloidiasis, causing life-threatening infection by dissemination and hyperinfection. *S. fuelleborni*, another species of the same genus, occurs sporadically and has globally minor importance because of geographical restriction (3,4).

Generally, strongyloidiasis is characterized by chronic infection and can be asymptomatic for decades in healthy individuals (2). However, severe strongyloidiasis may also develop when the immune system of asymptomatic individuals is debilitated. Therefore, before initiating immune suppressive treatment, screening for strongyloidiasis can be performed in these patients.

The routine diagnosis of strongyloidiasis depends mainly on stool culture and microscopic, serologic, and molecular assays but none of these assays are regarded as the gold standard (2). In addition, it is known that a combination of diagnostic procedures improves sensitivity and specificity (5,6).

Prevalence rates of strongyloidiasis in Africa, in some regions of South and Central America, in Southeast Asia, and in some regions of Europe have been reported as 0.1%–91.8%, 1.0%–83.3%, 6.6%–29.8%, and 1.4%–28%, respectively (2–7).

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In Turkey, the prevalence of strongyloidiasis varies between 0% and 4.4% based on routine stool examinations. Depending on these limited data, strongyloidiasis commonly occurs in the Black Sea, Marmara, Central Anatolia, and East Anatolia regions and regions that have a temperate climate in Turkey such as the Mediterranean region (8–16). In addition, some reports state that strongyloidiasis in Turkey has been reported in a patient with celiac disease, a patient with Behçet disease, and patients taking corticosteroids (17–20). However, a comprehensive study has not been conducted in immunocompromised patients to determine the risk of hyperinfection or disseminated strongyloidiasis.

Therefore, we aimed to investigate the presence of strongyloidiasis in immunocompromised patients for the first time in Turkey. During the study, serum and stool samples were collected from 108 immunocompromised patients who were admitted to Ege University Medical School in İzmir, located in western Turkey. Serum samples were analyzed by ELISA and the presence of the 18S rRNA gene of *S. stercoralis* was detected in stool samples by real-time PCR.

2. Materials and methods

2.1. Ethics statement and patient samples

The study was approved by the Research Ethics Committee of Ege University Medical School (Approval number: 13-2/15). The written informed consent form was obtained from all study participants or their legal guardians. No participants were under legal age. In addition, demographic, clinical, and epidemiological data were collected.

A total of 108 stool and serum samples were collected between April 2013 and December 2013 from patients with chronic renal failure (n: 28) or renal transplantation patients (n: 80) admitted to Ege University Medical School.

2.2. Microscopy

All stool samples collected from 108 immunocompromised patients were initially examined by phase contrast microscopy (Nikon, Japan). Thereafter, DNA extraction was performed on the samples.

2.3. *Strongyloides* IgG-ELISA

IgG antibodies against *S. stercoralis* were investigated in the serum of 108 patients using a *Strongyloides* IgG-ELISA kit (DRG, Germany) according to the manufacturer's protocol. Briefly, 100 µL of each serum at dilution of 1/64 was added to microtiter plates coated with *S. stercoralis* antigen, incubated for 10 min at room temperature, and washed three times. The positive and negative control serum of the kit was applied directly without dilution. Then all wells were probed by conjugate provided with the kit, incubated 5 min at room temperature, and washed three times. Bound antibodies were visualized after the

substrate provided with the kit was added to each well and incubated 5 min at room temperature. Finally, the reaction was stopped by adding 100 µL of 0.5 M H₂SO₄ stop solution and the results were evaluated in a microtiter plate reader (Bio-Tek EL x 808, USA) at 450 nm. The test results were interpreted according to the manufacturer's instructions and a serum sample was considered positive if the OD value was >0.200.

2.4. DNA isolation and real-time PCR

DNA was isolated from stools by QIAamp DNA stool mini kit (Qiagen, USA) according to the manufacturer's protocol. During DNA isolation, 200 mg stools were used and samples were eluted with 200 µL of elution buffer.

Real-time PCR targeting the 18S rRNA gene of *S. stercoralis* (GenBank: AF279916) was performed as described previously (21). Briefly, the primers used for amplification of the 101 bp 18S rRNA gene fragment were 5'-GAATTCCAAGTAAACGTAAGTCATTAGC-3' (28 nt, forward primer) and 5'-TGCCTCTGGATATTGCTCAGTTC-3' (23 nt, reverse primer). The hydrolysis probe was FAM-5'-ACACACCGGCCGTCGCTGC-3'-BHQ (25 nt). Each 20 µL of PCR mix included 4 µL of patient DNA template or controls, 1X Taqman Hydrolysis Probe mix (Roche), 0.06 µM each primer, probe 0.1 µM, 2.5 µg of BSA (2 mg/mL). PCR amplification reactions were performed using the following calculated control protocol: 10 min preincubation step at 95 °C, followed by 45 cycles of 10 s at 95 °C, 40 s at 60 °C, and 1 min at 72 °C.

S. stercoralis 18S rRNA gene fragment, which has 101 bp size, was isolated by the above primers and amplification reaction and inserted into pCR 2.1-TOPO plasmid (Invitrogen, USA) according to the manufacturer's protocol. This resulting plasmid containing the 101 bp target gene was sequenced and used as positive control plasmid. Distilled water was used as negative control.

3. Results

Among the 108 patients, 80 of them had undergone renal transplantation and were under immune suppression and the remaining 28 patients had chronic renal failure and receiving hemodialysis. Out of the 28 chronic renal failure patients, 4 of them had taken immunosuppressive treatment. The median age of patients was 44.5 ± 14.3 years (range: 18–77 years) with most being male (n = 63; 58.33%); there were 45 females (41.66%).

Out of the 108 patients, 61 had some accompanying disease in addition to chronic renal failure or renal transplantation. While 20 had type II diabetes mellitus (DM), 38 had hypertension and hyperlipidemia, and three had coronary heart disease.

During sample collection, some clinic symptoms were observed in 96 patients: 34 with high fever, 30 with

diarrhea, 20 with cough, and 12 with sputum problem. In addition, leukocytosis was detected in 18 patients, while leucopenia and eosinophilia were present in five and 10 patients, respectively. The risk factors for acquiring strongyloidiasis such as walking barefoot on soil was present in all patients (100%), farming (40.7%), and travelling to temperate regions (21.3%).

Stool samples belonging to the 108 patients were examined by direct microscopy and none of them contained the parasite, egg, or larvae of *S. stercoralis*. Strongyloidiasis was detected in only one patient by real-time PCR and the patient was also seropositive by ELISA. The patient was born in central Anatolia and had been living in İzmir for 30 years. The patient had type II DM and was being followed up for tuberculosis arthritis. Renal transplantation of this patient was performed in 2001. In addition, the patient had a history of walking barefoot on soil but no clinical symptoms. After diagnosis, the patient was treated twice with albendazole (400 mg/day for 3 days) at 2-week intervals. Follow up real-time PCR and direct microscopy of the stool sample were negative. The patient became seronegative 6 months after initial diagnosis. Overall, the prevalence of strongyloidiasis in this small group of patients who were at risk of strongyloidiasis was 0.92%.

4. Discussion

Infections depending on intestinal parasites are still an important public health problem in developing as well as developed countries. Turkey is a developing country where it presents an appropriate position for parasitic diseases because of its geographic and socioeconomic conditions and climate.

S. stercoralis is one of the most important intestinal parasites because it can cause life-threatening infection in immunocompromised individuals. Chronic infection depending on *S. stercoralis* is commonly asymptomatic and eosinophilia sometimes occurs. However, autoinfection in particularly immunocompromised individuals can cause dissemination or hyperinfection, which have high mortality rates (4,22). Thus, screening for strongyloidiasis in this group of patients is necessary before starting immune suppressing treatment.

In Turkey, a few studies have been conducted to detect the frequency of strongyloidiasis in humans and it is reported that frequency range varies between 0% and 4.4% (8). In a study conducted in Adana, 0.27% of 731 hospitalized patients were diagnosed positive for strongyloidiasis using fecal examination methods (9). In addition, positivity rates of strongyloidiasis in patients presenting to a parasitology laboratory for stool routine examination were 0.94%, 0.22%, 0%, 4.4%, 0.04%, 0.006%, 0.08%, 0.4%, and 0% in Hatay, Adana, İçel, Samsun, Ankara,

Bursa, Van, Eskişehir, and Diyarbakır, respectively (10–16). In addition, some common properties of these studies are as follows: patients at risk for strongyloidiasis are not selected, only microscopic stool examination is used for diagnosis, and finally all positive results detected in these studies were detected during stool routine examinations.

In the present study, we aimed to study the prevalence of strongyloidiasis in immunocompromised patients who also have risk factors such as walking barefoot on soil, farming, and travelling to temperate regions. To investigate strongyloidiasis, ELISA and real-time PCR were performed on serum and stool samples of all patients. Real-time PCR was used to investigate the presence of the 18S rRNA gene of *S. stercoralis* instead of microscopy because PCR methods show excellent sensitivity and specificity compared to microscopic methods (21,23). In addition, ELISA, detecting anti-*S. stercoralis* antibodies in serum samples of patients, was used in combination with real-time PCR to support and increase the positivity.

As a result, the 18S rRNA gene of *S. stercoralis* and anti-*S. stercoralis* antibodies were detected only in one patient's samples (0.92%). This patient had type II DM and was being followed up for tuberculosis arthritis. Renal transplantation of this patient was performed in 2001 during treatment with everolimus (2.5 mg/day), mycophenolate (1080 mg/day) and prednisolone (16 mg/day) for immunosuppression. As the patient was diagnosed with strongyloidiasis, albendazole treatment was initiated. During the follow up of the patient, PCR and direct microscopy of stool sample became negative and anti-*S. stercoralis* IgG antibodies was not detected 6 months after the initial diagnosis. The seronegativity after 6 months may be because of the treatment of active infection and reduction in the total antibody amount due to the continuous immunosuppression of the patient. Another reason for the seronegativity may be the false negative result obtained during the analyses of the follow-up serum sample or a false positive result in the initial analyses of the serum sample as detected by commercial ELISA kit, which can be regarded as a limitation of the study.

In Brazil, *S. stercoralis* was investigated in stool and serum samples of patients with type II DM (n: 78) and controls without type II DM (n: 42). *S. stercoralis* was detected in the stools of 3 patients with type II DM but not detected in the control group. Moreover, anti-*S. stercoralis* antibody was detected in 18 (23%) of 78 patients with type II DM and 3 (7.1%) of 42 control patients without type II DM, which was statistically significant ($P < 0.05$). Interestingly, the *S. stercoralis* positivity in patients with $HbA1c \leq 7$ was 14% and with $HbA1c > 7$ was 9%. Altogether, this study indicated that there was a possible association between positive *S. stercoralis* serology and

diabetes (24). Similarly, our *S. stercoralis* positive patient's HbA1c was 6.2, which supports the finding reported by Mendonça et al. (24).

Molecular methods offer higher sensitivity and specificity for the detection of parasitic infections in stool samples (21,23). In a previous study, the sensitivity and specificity of a real-time PCR method detecting the 18S rRNA gene of *S. stercoralis* were determined in comparison with agar plate culture, formalin ethyl acetate, and Harada Mori microscopy techniques. Analysis of 231 stool samples from patients at risk of strongyloidiasis showed that the sensitivity and specificity of real-time PCR were 93.8% and 86.5%, respectively (25). In another similar study, the 18S rRNA gene of *S. stercoralis* was investigated by real-time and conventional PCR and compared with other parasitological methods such as the Lutz method, Rugai method, and agar-plate culture. Both molecular methods (real-time and conventional PCR) on 100 stool samples were more sensitive and specific compared to other methods. The sensitivity and specificity of real-time PCR were 90% and 85.7%, respectively (23). As a result, molecular methods having high sensitivity and specificity

can be a more effective tool to diagnose strongyloidiasis. In our study, the real-time PCR method detecting the 18S rRNA gene of *S. stercoralis* successively detected strongyloidiasis in one seropositive patient's stool sample.

The detection of a strongyloidiasis case during the screening of a group of immunocompromised patients enabled us to implement timely treatment of the patient. The accumulated epidemiological data and the result of this study show that strongyloidiasis does not seem to be a major health problem in Turkey. Detecting one strongyloidiasis positive patient among 108 patients is the limitation of this study. More systematic studies covering larger patient groups from the whole of Turkey, especially the regions with higher incidence of strongyloidiasis, may be performed to overcome this limitation. In addition, our results support the use of molecular techniques for the diagnosis of strongyloidiasis.

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