

Investigation of Visceral Leishmaniasis and Coinfection of Intestinal Parasites in HIV-Positive Patients

HIV Pozitif Hastalarda Visseral Leishmaniasis ve Intestinal Parazitlerin Koenfeksiyonunun Araştırılması

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Özet

Amaç: Paraziter enfeksiyonlar, insan immün yetmezlik virüsü (HIV) ile enfekte olgular gibi immünsüpresif olgularda daha yüksek oranlarda görülmektedir. Buna ek olarak, immünkompetan olgularla karşılaştırıldığında immünsüpresif hasta grubunda daha ciddi bir klinik seyre yol açmaktadır. HIV pozitif bireylerde parazitlerin patojenitesi artmakta ve mortalite ile sonuçlanabilen değişen şiddetteki klinik semptomlar görülebilmektedir. Visseral leishmaniasis (VL) vektörle bulaşan bir hastalık ve her iki enfeksiyonun endemik olduğu özellikle Güney Avrupa ve Afrika'da HIV/ Acquired Immune Deficiency Syndrome (AIDS) vakalarında görülen önemli bir fırsatçı enfeksiyondür. Toxoplazmosis, cryptosporidiosis, isosporiasis, cyclosporiasis, amebiasis, giardiasis, plasmodium ve strongyloidiasis olmak üzere çeşitli parazitler bu hasta grupları için önemli risk faktörü oluşturan enfeksiyonlardır.

Gereç ve Yöntemler: Çalışmamızda HIV pozitif olguların serum örneklerinden serolojik olarak visceral leishmaniasis ve gaita örneklerinden konvansiyonel yöntemlerle parazitler enfeksiyonlar araştırıldı.

Bulgular: Çalışmaya dahil edilen 42 olgunun 5'i kadın idi. Bu olguların ortalama yaşı 38.7 (19-66) idi. HIV pozitif olgulardan alınan dışkı örneklerinin 5'inde Blastocystis spp tespit edildi. Serum örnekleri ile çalışılan rK39 dipstick testi sonucunda bir olguda çok zayıf bant elde edilirken, diğer testlerin hiçbiri pozitif bant vermedi.

Sonuç: HIV pozitif olgularda subklinik olarak seyredilebilen bu gizli enfeksiyonların bölgesel prevalansının bilinmesi önemlidir. Ancak literatürde ülkemizdeki durum ile ilgili yeterli veri bulunmamaktadır. Bu çalışmada bölgemizde HIV enfeksiyonu olan kişilerde enterik parazitler enfeksiyon prevalansı düşük bulunmuştur ve VL tespit edilmemiştir.

Anahtar kelimeler: Bağırsak Parazitleri, HIV, Visseral Leishmaniasis

Abstract

Objective: Parasitic infections are more frequently observed in immunosuppressed cases, such as those infected with human immunodeficiency virus (HIV). Furthermore, compared to immunocompetent cases, a more severe clinical course is observed in the immunosuppressed patient group. HIV-positive individuals have increased pathogenicity of parasites and may exhibit a wide range of clinical symptoms of varying severity that may result in mortality. Visceral leishmaniasis (VL) is a vector-borne disease and an important opportunistic infection seen in HIV/Acquired Immune Deficiency Syndrome (AIDS) cases, most notably in Southern Europe and Africa, where both infections are endemic. Several parasites namely toxoplasmosis, cryptosporidiosis, isosporiasis, cyclosporiasis, amebiasis, giardiasis, plasmodium, and strongyloidiasis are infections posing significant risk factors for these patient groups.

Materials and Methods: In this study, visceral leishmaniasis was investigated serologically in serum samples and parasitic infections were analyzed by conventional methods in fecal samples from HIV-positive cases.

Results: 5 of the 42 cases included in the study were female. The mean age of these cases was 38.7 years (19-66). Blastocystis spp was detected in 5 fecal samples obtained from HIV-positive cases. The rK39 dipstick test with serum samples yielded a very weak band in one case, while none of the other tests yielded a positive band.

Conclusion: The regional prevalence of these latent infections, which can be subclinical in HIV-positive patients, is important to know. However, the literature lacks sufficient data on the situation in Turkey. In this study, the prevalence of enteric parasitic infections in people with HIV infection in our region was found to be low and VL was not detected.

Keywords: HIV, Intestinal parasites, Visceral Leishmaniasis

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INTRODUCTION

Throughout the world, an estimated 36.9 million people worldwide are infected with human immunodeficiency virus (HIV), 75% of whom are aware of their HIV status and only 21.7 million are receiving treatment. Every year, approximately 1.8 million new infected cases and 940 thousand deaths are observed in the world (1). Turkey is among the countries with low prevalence of HIV infection, but the number of new cases reported each year is growing. Opportunistic parasitic infections in persons with suppressed cellular immunity, such as HIV infection, differ from the usual infections in ordinary hosts (2). Cryptosporidiosis can lead to diarrhea in both immunosuppressed and healthy hosts. In healthy people, it leads to diarrhea with profuse watery diarrhea, abdominal pain, nausea and vomiting, which disappears by itself in 5-6 days. In immunosuppressed patients, it leads to more severe infections with a longer duration of symptoms (2,3). *Isospora Belli* also leads to an infection similar to cryptosporidiosis. Symptoms other than the intestinal tract may also occur. Mediastinal lymph nodes, mesenteric and periaortic lymph nodes may be affected in patients with Acquired Immune Deficiency Syndrome (AIDS) (2). Microsporidiosis, the infection in humans, was identified with the AIDS epidemic. This generates a broad spectrum of infections in the immunosuppressed host. The infection can range from asymptomatic to fatal. This is especially more common in patients with cellular immunodeficiency (2). *Entamoeba histolytica* has high prevalence rates in men who have sex with men and in AIDS cases. It may cause acute or chronic diarrhea (4). *Giardia lamblia*, is a parasitic infection that may lead to acute or chronic diarrhea. It may cause malabsorption and chronic diarrhea. In untreated cases, excretion with feces can take a very long time. It is also a common factor in immunosuppressed patients, especially in AIDS patients, and in case of long-term steroid use (3). Strongyloidiasis is an intestinal nematode causing infection in an immunosuppressed host. In these cases, chronic or subclinical infection may continue for months and may be life-threatening (5).

Visceral leishmaniasis (VL) is a vector-borne, opportunistic protozoal infection characterized by targeting the reticuloendothelial system with an increased rate in HIV-infected cases. HIV/VL coinfection is prevalent in areas where both diseases are endemic, especially in Southern Europe and Africa (6-8). VL infection observed in HIV-infected cases may present with fever, pancytopenia associated with splenomegaly, generalized lymphadenopathy, and burnout syndrome similar to non-HIV-infected cases (9). Most individuals have an asymptomatic infection in areas where visceral leishmaniasis is endemic, and the presence of HIV coinfection increases the risk of developing active VL.

Further, atypical, severe clinical course and higher rates of non-response to VL treatment (independent of the treatment regimen used) have been reported in HIV co-infected cases, and high relapse rates and mortality have been especially witnessed in cases not receiving anti retroviral treatment (ART). The incidence of VL has decreased significantly with the use of ART treatment in HIV-infected patients (10-12). The rapid spread of HIV infection around the world, on the other hand, is accelerating the incidence of severe VL in non-endemic regions and changing the epidemiology of the disease. This not only complicates the eradication of VL, but also may result in delays in diagnosis and treatment, especially in regions where the disease is not endemic, and may result in increased morbidity and mortality. For this reason, regional prevalence of this latent infection in HIV-positive cases must be known. In the literature, despite the fact that there are many studies reported from different countries on this subject, there is insufficient data in our country. This study aimed to investigate parasitic infections in people living with HIV followed up in our clinic.

MATERIALS AND METHODS

Workgroup

The study included fecal and serum samples from a total of 42 cases admitted to the Infectious Diseases Department of Hatay Mustafa Kemal University, Faculty of Medicine. Samples were sent to the Research Laboratory of the Department of Parasitology.

Microscope examination and staining methods

The fecal samples were firstly evaluated macroscopically for color, consistency, presence of blood and mucus, and presence of helminth adults and segments. Following the macroscopic examination, the samples were examined microscopically using the nativ-lugol method. In the diagnosis of organisms difficult to be identified by direct fresh examination, Trichrome staining method, which is one of the permanent staining methods, was used. At the same time, the Kinyoun Acid Fast Staining Method, frequently used in the diagnosis of intestinal coccidian parasites (*Cryptosporidium*, *Cyclospora*, and *Cystoisospora*), which are of great importance in immunocompromised cases, was utilized. Modified Formol Ethyl acetate precipitation method was also used.

Serological testing

Blood samples were centrifuged and serum was separated. All serum samples were tested with the commercially prepared rK39 dipstick test (InBios International, USA) and analyzed for *Leishmania* positivity. The tests were conducted in accordance with the protocol included in the tests.

Demographic data and laboratory

Demographic data such as age, gender were recorded in our study. Some other parameters (such as HIV RNA, CD4 cell count, hemoglobin, platelets, AST, ALT, LDH, total bilirubin, and protein) which are analyzed by routine standard laboratory techniques in HIV cases were also analyzed in our study.

Statistical analysis

Statistical analysis were performed using the SPSS software, version 23.0 for Mac (SPSS Inc, Chicago IL, USA). Categorical variables were compared using Chi-square or Fisher's exact test. Non-normally distributed continuous variables were compared using Mann—Whitney U test between treatment groups. Wilcoxon signed rank test was used for the comparison of non-normally distributed variables at different time points in each treatment group. A p value of <0.05 was considered statistically significant.

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the Hatay Mustafa Kemal University Medical Faculty Clinical Research Ethics Committee with the resolution of the research protocol numbered 2019/17. In addition, a consent form was obtained from all subjects in this study. This study was funded by Hatay Mustafa Kemal University Scientific Research Projects Coordination Unit (Project no: 19.M.014).

RESULTS

Fecal and blood samples were collected from 42 cases admitted to the Infectious Diseases Outpatient Clinic of Hatay Mustafa Kemal University Faculty of Medicine and included in the study. The mean age of the cases was 38.7 (19-66) years and 88.1% (n = 37) were male. **Table 1** shows the demographic characteristics and laboratory findings of the cases.

The rK39 dipstick test with serum samples yielded a very weak band in one case, while none of the other tests yielded a positive band (**Figure 1**).

It was found that the case with a weak positive band was a 39-year-old male, CD4 (30%) was 448 cells and HIV RNA was 12390. In addition, the physical examination of the case was normal. No clinical condition related to VL was detected in the follow-up of the case. Blastocystis spp was detected in 5 fecal samples obtained from HIV-positive cases. No intestinal parasites were found in the samples of other cases (**Figure 2**).

Table 1. Demographic and laboratory characteristics of the cases

| | (n=42) |
|--------------|--------------------|
| Age, years | 38.7 (19-66) |
| Gender, male | 28 (82.3) |
| Protein g/dL | 7 (5-9) |
| CD4 hücre/μL | 360 (3-932) |
| HIV-RNA | 202338(329-186200) |
| WBC, μL | 6950 (3890-10860) |
| PLT, μL | 234 (15-460) |
| AST, U/L | 23 (12-44) |
| ALT, U/L | 23(11-42) |
| LDH, U/L | 189 (110-331) |
| Bil, mg/dL | 0.6 (0.2-1.2) |

HIV RNA: human immunodeficiency virus ribonucleic acid, WBC: white blood cell, PLT: platelet, AST: aspartate aminotransferase, ALT: alanine aminotransferase LDH: lactic dehydrogenase, Bil: bilirubin, n: number of patients



Figure 1a. rK39 dipstick test results of some cases



Figure 1b. rK39 dipstick test kit for the patient with a very weak band

DISCUSSION

To date, VL-HIV coinfection has been reported in at least 33 countries. 25% to 70% of these reported cases are adult cases (13,14). In our region, leishmaniasis has been reported as sporadic in pediatric cases (15). In our country, there is very limited data on the detection of VL in HIV-infected cases. In a study conducted by Özkan et al (16), serologically positive in one of 79 cases. It was determined that the positive case was a 49-year-old male living in the Mediterranean region. The researchers considered that the possible route of transmission could be by midge bite or blood transfusion (16). In a meta-analysis of adult visceral leishmaniasis cases conducted in our country, the positivity rate of rK39 rapid antigen test was found to be 86.6% (13-15). In 88.7% of the cases, the diagnosis was made with the appearance of amastigotes in bone marrow aspiration and/or biopsy (17). In our study, a weak band was observed with the rK39 rapid antigen test in only one case. However, no biopsy was performed because of clinical and laboratory incompatibility and also because the patient did not consent to bone marrow biopsy. The case continues anti-retroviral therapy (ART) treatment and no VL-related clinical findings were detected during follow-up.

HIV/Leishmania co-infection is reported with increasing frequency, especially in Southern Europe. Since 1998, 1911 new cases have been reported in Spain, France, Italy and Portugal (18). More than 85% of co-infected cases are young adult men aged 20-40 years, although Mediterranean-type VL occurs mostly in children (19,20). In a study conducted by Köse et

al. it was determined that 25% of leishmaniasis cases reported in adult cases in Turkey were immunocompromised, but none of them were coinfecting with HIV (17). In our study, no HIV co-infected case was detected. In the light of these results, we consider that the use of serologic tests as a confirmatory test in cases living with HIV without a compatible clinical picture and a history of exposure suggestive of visceral leishmaniasis is not a correct approach.

Serum collected from 49 HIV/AIDS cases in VL endemic areas of Iran was screened using a direct agglutination test (DAT) and nine cases (18.4%) were serologically positive and all seropositive cases showed clinical signs and symptoms. In this study conducted in north-eastern Iran, it has been demonstrated that this is an opportunistic disease that may occur in HIV-positive patients (21). This study is the first research conducted in our region. In our study, 42 cases were screened with the rK39 dipstick test and a very weak band was obtained in one case. No VL clinical signs and symptoms were detected in this case. An increase in the incidence of VL-HIV co-infection was observed in the Mediterranean basin in the early 1990s, with cases peaking in 1997. With the use of antiretroviral therapy the prevalence of VL-HIV co-infection has decreased significantly in the Mediterranean basin (22).

Akgül et al (23) screened 90 HIV/AIDS cases and found intestinal parasites in 18 (20%) cases by microscopic methods. Intestinal parasites were detected in 33 (36.7%) cases using molecular methods (23). In our study, intestinal parasites were detected in 5 (12%) cases

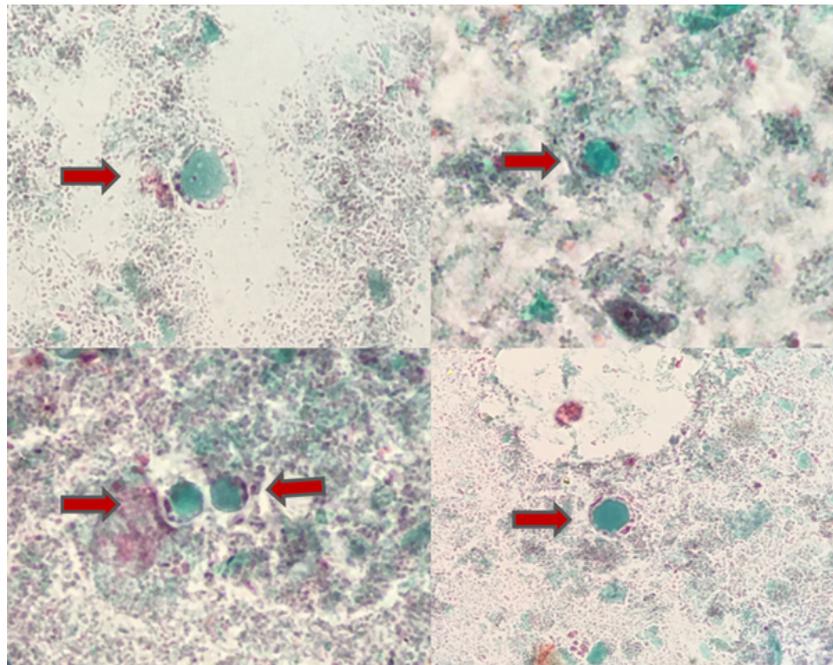


Figure 2. Blastocystis spp (Trichrome stain, 100x)

using conventional methods. Uysal et al²⁴. screened 115 cases for *C. cayetanensis* and *Cryptosporidium* using both conventional and molecular methods and found parasites in three cases using conventional methods and six cases using molecular methods (24). The results of this study showed that the PCR method was faster and more sensitive than microscopic methods. We believe that it may be appropriate to use molecular methods in screening for intestinal parasites in people living with HIV in developing countries such as our country.

The limitations of this study are that it was a regional study and the number of cases was small.

As a result, the regional prevalence of these latent infections, which can be subclinical in HIV-positive patients, is important to know. However, the literature lacks sufficient data on the situation in Turkey. We also think that the use of serologic tests to confirm HIV/VL coinfection is not the right approach in our region. There is a need for more multicentered studies with more cases on this subject.

Ethical Approval: The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the Hatay Mustafa Kemal University, Medical Faculty Clinical Research Ethics Committee with the resolution of the research protocol numbered 2019/17. In addition, consent forms were obtained from all patients included in the study. This study was supported by Hatay Mustafa Kemal University Scientific Research Projects Coordination Unit (Project number: 19.M.014).

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