Seroprevalence of *Leishmania* infection among asymptomatic renal transplant recipients from southern Spain

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Abstract: *Background.* The aim of this article is to assess the seroprevalence of *Leishmania* infection among asymptomatic renal transplant recipients in a population in the south of Spain.
Methods. Serum samples were screened for immunoglobulin-G antibodies against *Leishmania* with an indirect fluorescent antibody test.
Results. Of 625 examined serum samples, 30 (4.8%) samples were positive for *Leishmania* antibodies. Thirteen samples showed titers of 1:80, 15 samples showed titers of 1:160, and 2 samples showed titers of 1:320. None of the patients with positive serology to *Leishmania* showed signs or symptoms compatible with leishmaniasis.
Conclusion. The prevalence of *Leishmania* infection found among asymptomatic renal transplant patients reinforces the need for attention in evaluation of these patients in endemic areas.

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Leishmaniasis refers to a group of vector-borne diseases caused by an opportunistic intracellular protozoan parasite of the genus *Leishmania*, belonging to the family Trypanosomatidae, order Kinetoplastida (1).

The disease is associated with 3 main manifestations: (i) cutaneous leishmaniasis, (ii) mucocutaneous leishmaniasis, and (iii) visceral leishmaniasis (VL) also known as kala-azar (2). VL is considered to be a well-established zoonotic disease in the area of the Mediterranean basin, especially in southwestern Europe, where approximately 700 cases per year appear and the clinical form is frequently associated to immunosuppression (3, 4).

The actual prevalence of leishmaniasis in Spain is not completely known (5); however, in our geographical area of southern Spain, studies published in 1996 of the Alpujarra region (Granada province) and Axarquia region (Malaga province) of rural populations, using the leishmanin skin test (LST) (test cell immunity), reported average positivity rates >40% in both studies

(6, 7). Nationwide, more recent studies in areas close to ours, such as the study performed on blood donors from Ibiza (Balearic Islands, Spain) in 2004, indicated a high rate of asymptomatic infection on the island, and another study in 2008 from Balearic Islands confirms this high prevalence (8, 9).

Apart from these studies, the most studied populations have been human immunodeficiency virus (HIV)-infected individuals and intravenous drug users in our environment (southern Spain) (10–12). Not many publications have reported the known prevalence of *Leishmania* in special groups, such as transplant recipients, oncology patients, or other immunodeficient non-HIV groups, in Spain and the Mediterranean region.

Leishmaniasis is not a common disease among transplant recipients, but with this growing pool of transplant survivors and increasing migration dynamics, the numbers of infected cases among transplant recipients are steadily increasing, especially among renal transplant recipients mainly in southern Europe (13), which is considered to be an additional challenge (14, 15).

Transmission of VL to this specific population occurs through different routes: *Leishmania* transmission by sandflies after transplantation, when the specific group of population is living in an endemic area or makes a short stay in an endemic area represents a route of transmission (16, 17). Also, it may have originated from a donor with an undiagnosed infection, or a recrudescence or latent infection in a previously infected recipient during a period of immunosuppression. Infection can also be acquired through blood transfusion (18).

Aim of study

Considering these findings, the present study was undertaken to assess the seroprevalence of *Leishmania* infection among asymptomatic renal transplant recipients in Granada (Southern Spain) while they are tracked post transplant.

Material and methods

This study was approved by the Ethical Committee of Research of the University Hospital Virgen de las Nieves (Granada, Spain), before initiation of data collection. Serum samples from 625 individual renal transplant patients were collected for 1 year (April 2013–April 2014). Age, gender, cause of the transplant,

survival time after transplantation, and type of immunosuppressive therapy were recorded. Any individuals with fever or infectious disease were excluded from the study. All patients were living in neighboring Granada provinces for at least the last 10 years, for which our hospital is a referral center for renal and liver transplantation.

To investigate *Leishmania* infection, peripheral blood was collected into sterile Vacutainer™ tubes. Samples were centrifuged at 4°C and stored at −80°C until serological tests were performed. All patients had a low level of immunosuppression at the time of blood sampling. Serum samples were screened for leishmaniasis with indirect fluorescent antibody (IFA) test. The IFA test for determination of antibodies against *Leishmania infantum* (*Leishmania* IFI IgG Kit, Vircell, Santa Fe, Granada, Spain) was performed according to the manufacturer's instructions.

When samples were positive with a titer of 1:80, a 2-fold serum dilution was done until samples reached 1:1280 dilution. As shown in the kit instructions, IFA test is considered positive with promastigotes fluorescence, with a cutoff dilution of 1:40 owing to the endemicity of the area of study. This result is considered as indeterminate in this assay (19).

Results

During the study period, 625 patients with a mean time of 10 years from transplantation to the last review were recruited at their transplant consultation. Median age distribution was 49 years (range 11–81 years). According to gender, 225 (36%) were female and 400 (64%) were male. Regarding the distribution by race, the Caucasian race was predominant at 621 (99.4%), within which 17 were of Romani ethnicity, and 4 (0.6%) were black. The etiology of renal failure that caused the transplantation was as follows: glomerular disease 174 (27.8%), unknown 116 (18.6%), cystic disease 98 (15.7%), interstitial disease 84 (13.4%), vascular disease 81 (13%), diabetes 27 (4.3%), and 45 other causes (6.1%).

IFA testing was performed on 625 serum samples; 30 samples were positive, 13 showed titers of 1:80, 15 showed titers of 1:160, and 2 showed titers of 1:320. Fourteen additional samples showed titers of 1:40, considered as indeterminate. The characteristics of patients with *Leishmania* antibodies in relation to the variable studied are shown in Table 1. The prevalence was 4.8%. The immunosuppressive treatments after transplantation for patients with positive serology included the following regimens: anti-thymocyte globulin (administered only during the first 3 months after

Characteristics of patients (*n* = 30) grouped by antibody titer against *Leishmania infantum*

Antibody titer (*n*)	1:80 (*n* = 13)	1:160 (*n* = 15)	1:320 (*n* = 2)
Age in years (range)	44.15 (29–66)	49.33 (29–67)	45.50 (43–48)
Gender			
Male	6 (46.2)	9 (60)	2 (100)
Female	7 (53.8)	6 (40)	0 (0)
Race			
Mediterranean white	13 (100)	14 (93.3)	2 (100)
Nordic white	–	1 (6.7)	–
Renal failure type			
Unknown	4 (30.8)	3 (20)	1 (50)
Glomerular	5 (38.5)	3 (20)	–
Vascular	2 (15.4)	1 (6.7)	–
Systemic	2 (15.4)	–	–
Interstitial	–	2 (13.3)	1 (50)
Cystic	–	3 (20)	–
Diabetes	–	2 (13.3)	–
Survival time in years (range)	2.64 (1.58–3.72)	2.20 (0.3–3.67)	1.52 (0.44–2.61)

All patients had a low level of immunosuppression.

Table 1

transplantation) + mycophenolate mofetil (MMF) + prednisone in 10 patients (30.3%); tacrolimus + MMF + prednisone in 8 patients (2.6%); prednisone + MMF + basiliximab in 5 patients (16.6%); tacrolimus (once daily) + basiliximab + MMF + prednisone sodium in 4 patients (13.3%); and basiliximab + cyclosporine + MMF + prednisone in 3 patients (10%). All patients had a low level of immunosuppression at the time of blood sampling. No differences existed between the immunosuppressive treatment among patients with negative serology and positive serology. No patient developed leishmaniasis disease during the study period or to date.

Discussion

Although VL is a rare disease among transplant patients, the current situation has been changing, and the number of published cases has increased in recent years (20). Worldwide, >100 VL cases following transplantation have been reported, predominantly described in renal transplantation (15, 19, 20).

The prevalence of asymptomatic infections of *Leishmania* species varied from 0.6% to 71% in endemic areas (21). To detect asymptomatic carriers, direct method (polymerase chain reaction [PCR], culture, microscopic examination) or indirect methods (IFA test, direct agglutination test, enzyme-linked immunosorbent assay [ELISA] with single or crude *Leishmania* antigens, Western blot [WB], or immunochromatographic techniques) can be used, but no gold standard test is available to identify asymptomatic infection with high sensitivity and specificity (21, 22).

The prevalence of *Leishmania* infection in our study is in agreement with previous studies in different geographical areas of our country, Balearic Islands and Castilla León, with results of prevalence of 3.1% and 4.9% in blood donors and in the general population respectively (9, 23).

More recent studies, like the one published in the province of Seville on a small population that included intravenous drug users and non-drug injectors, where the frequency of *Leishmania* seropositivity was high, showed prevalence of infection by *Leishmania* of 24% using PCR-ELISA. This study showed that a remarkable proportion of asymptomatic *Leishmania*-seropositive individuals at risk for parenterally transmited infections carry *Leishmania* kinetoplast DNA (kDNA) in blood (11).

Some studies have been made in southern Spain in asymptomatic HIV population diagnosing by amplifica-

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tion of kDNA from peripheral blood showing a prevalence of 30.8% (28/92) infection by *Leishmania*. No patients showed positive results by other techniques, such as ELISA, WB, or LST. Conversely, patients with a negative PCR result showed prevalence values, respectively, of 3.5%, 2.4%, and 4.3% when tested by ELISA, WB, and LST (12).

In other zones endemic for *L. infantum*, such as Brazil, studies have recently been published that raise interest in the detection of asymptomatic infection in the transplant population living in endemic areas, as well as the need to use more sensitive techniques for screening this population group, such as the research of Clemente et al. (19) in 2014 on asymptomatic *Leishmania* infection among liver donors and recipients. The results of this study show a prevalence of *Leishmania* infection of 1.5% using serological techniques, which increased to 7.5%, 8.9%, and 5.9%, respectively, in blood samples, liver samples, and splenic samples, using molecular methods (19).

The results obtained in our study (4.8%) using a unique serological technique may be underestimated, because the IFA test has less sensitivity than other indirect assays (ELISA) (21). The use of 2 serological methods could slightly increase the proportion of asymptomatic carriers detected (24, 25). On the other hand, antibody detection may not reflect a chronic infection, but more a recent contact with parasite followed by cure; PCR assay would have greater sensitivity (26) than the indirect method, and reflects actual parasitism rather than a previous infection by *Leishmania*. Different studies highlight the added value of using a combination of tests (molecular and serological) to increase the capacity to detect asymptomatic *Leishmania* infections (21, 22).

At present, it is not possible to predict exactly when and who among the asymptomatically infected people will develop VL disease (27). Reasons causing an infection to remain asymptomatic or progress to VL are likely the result of a complex interaction between environment, parasite, and host-related factors (28).

Studies of the risk of progression from infection to disease have yielded contradictory results based on serological status; however, the data revealed strong associations between the magnitude of positive serology and risk of progressing to symptomatic VL (29).

International guidelines for the management of transplant recipients recommend specific serology in donors and recipients from endemic areas, regardless of laboratory test limitations and availability (30).

Given that patients, such as transplant recipients, are susceptible to develop leishmaniasis, and the clinical manifestations in these patients can be serious, it seems advisable to perform a screening of serological status in this population group and a more intense monitoring of patients with positive serology with high titers to prevent possible development of disease.

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