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Failure to Detect *Leishmania* in the Blood of Patients with Old-World Cutaneous Leishmaniasis: Implications for Blood Donation

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Abstract. Cutaneous leishmaniasis (CL) is endemic in Israel, caused mainly by *Leishmania major* (*L. major*) and *L. tropica*. In addition, returning travelers import another leishmanial species such as *L. braziliensis.* Although we are dealing with a skin disease, the blood bank in Israel does not accept blood donations from people infected with CL in cases of multiple lesions due to the possibility of transfusion. Our purpose was to investigate the prevalence of *Leishmania* in the blood of patients with active or previous CL. This pilot study screened patients with active or previous CL for parasites in their blood. All patients were infected in Israel or were returning travelers with leishmaniasis acquired in Latin America. Patients were seen at the Sheba Medical Center. In addition, patients were seen at their homes in *L. tropica* and *L. major* endemic regions in Israel. Blood samples were taken from each patient for culture and polymerase chain reaction (PCR). Altogether 62 blood samples were examined (*L. tropica* = 26, *L. major* = 33, and *L. braziliensis* = 3). Twenty-seven patients had an active disease and 35 were recovered. All blood cultures and PCR were negative for parasites except one blood sample that was PCR positive for *L. braziliensis*. The findings of our study, although a small sample, suggest that people with active or recent CL caused by *L. major* and *L. tropica*, do not harbor parasites in their blood. Thus, their exclusion from blood donation should be revisited. Further studies are needed with larger sample size and highly sensitive tests.

INTRODUCTION

Leishmaniasis is a vector-borne disease caused by several species of these protozoan parasites.[1] Cutaneous leishmaniasis (CL) is the most common form of the disease.

Cutaneous leishmaniasis is endemic to Israel and is increasing in its frequency. Historically, CL in Israel has been attributed almost exclusively to *Leishmania major* (*L. major*).[2] *Leishmania major* exists mainly in the Southern part of Israel (Negev and Arava). However, over the last two decades, CL due to *L. tropica* has been increasingly reported in several other regions of Israel including greater Jerusalem, and northern and eastern Israel. Cutaneous leishmaniasis caused by *L. tropica* heals more slowly and is relatively resistant to treatment in contrast to *L. major*.[3,4]

In addition, returning travelers import another leishmanial species such as *L. braziliensis* mainly from the Amazon region of Bolivia.[5]

Recently, attention has been focused on iatrogenic causes of leishmaniasis by the transfusion-transmitted route.[6,7] In infected human hosts, the overwhelming majority of *Leishmania* organisms reside within reticuloendothelial cells and do not circulate freely in the blood. A concern was raised that maybe at the time of blood collection, organisms that are present inside monocytes of infected donors could eventually emerge as free amastigotes that may transform into promastigotes and are able to survive outside the cell in the stored blood.[8] The evidence of human visceral leishmaniasis (VL) cases of transfusion-transmitted leishmaniasis (TTL) is scarce.[6,7,9–12] Evidence of TTL from patients with CL is almost nonexisting.[13]

The blood bank in Israel does not receive blood donations from people with CL in cases of multiple lesions either active or healed lesions. Our hypothesis was that people with Old-World CL caused by *L. major* and *L. tropica* do not carry the parasites in their blood.

The purpose of the present study was to investigate the prevalence of *Leishmania* in the blood of patients with active or recovered CL caused by *L. major* and *L. tropica* or *L. braziliensis.*

PATIENTS AND METHODS

This pilot study examined patients with an active or a previous history of CL infection in Israel. The patients were seen at the Dermatology Department or Center for Geographic Medicine and Tropical Diseases, Sheba Medical Center, or their residences in three known CL foci (Peduel in the Shomron—endemic for *L. tropica*, and Ze'elim or Revivim in the Negev endemic for *L. major*).

Cutaneous leishmaniasis was diagnosed when cutaneous lesions (ulcers, nodules, or papules) clinically compatible with leishmaniasis were noted, and a polymerase chain reaction (PCR) assay tested positive for *Leishmania*, or a smear or biopsy specimen showed *Leishmania* amastigotes.

All participants signed an informed consent and filled out a demographic questionnaire. Blood samples were taken from each patient for culture and PCR.

Conventional internal transcribed spacer 1 region (ITS1) PCR was used in this study. The ITS1 PCR was previously validated and used in the diagnosis of Leishmania from suspected patients and in several studies.[14,15] For DNA extraction, a high pure PCR template preparation kit (ROCHE, Roche Diagnostics GmbH, Sandhofer Strasse 116 68305

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Mannheim Germany Cat. No. 11 796 828 001) was used. The kit is used to extract DNA from human whole blood material. The primers used in the study according to previously validated published article[14] and they are LITSR: 5'CTGGATCATTTTCCGATG and L5.8S: 5'TGATACCACTT ATCGCACTT.

Leishmania culture was performed by seeding 100 μL of the purified peripheral blood mononuclear cells (PBMCs) into 1 mL of 10% rabbit blood agar semisolid medium containing 200 IU/mL penicillin and 200 μg/mL streptomycin (Teva). The cultures were examined microscopically for parasites at increasing time intervals and were considered negative after 1 month without any growth. The ribosomal internal transcribed spacer region 1 (ITS1) PCR was used for the molecular detection of the parasites.[15]

The study was approved by the institutional review board (protocol approval no. 0482-13).

RESULTS

Between the years 2014 and 2016, 62 CL patients (55% [$N = 34$] male and 45% [$N = 28$] female) were recruited to the study. Twenty-six of the patients were infected with *L. tropica*, 33 with *L. major*, and 3 with *L. braziliensis*. Among the patients, 35 (56%) presented with two or more lesions, while the remaining 27 only had one lesion (Table 1). Blood samples were taken from all the patients. Twenty-seven patients had an active disease and 35 were recovered. In the group of Old-World leishmania (*L. major* and *L. tropica*), blood PCR and cultures were all negative. Among the three patients with *L. braziliensis* (all imported from Bolivia), one patient had a positive PCR for *L.* braziliensis (culture was not performed).

DISCUSSION

The prevalence of *Leishmania* in the peripheral blood of CL patients with active or healed lesions caused by *L. major*, *L. tropica,* and *L. braziliensis* was investigated using PCR and parasite culturing. Previous studies demonstrated that asymptomatic people in areas where *Leishmania* is endemic may present transient parasitemia.[16] The PCR has been effectively used to detect parasites in these cases,[14] but the inability to distinguish between dead and live parasites is one limitation of this method. Culture techniques overcome this problem, as only viable amastigotes can result in a positive parasite culture.[17,18] In this study, neither positive PCR nor culture positive were found in peripheral blood of patients with Old-World CL, but there was one blood culture that was positive in a returning traveler with *L. braziliensis*.

Transfusion-transmitted diseases comprise several pathogens that are transmitted by blood transfusions, mainly viruses and parasites, while the transmission of *Leishmania* parasites species through transfusion using blood from healthy nonimmune suppressed people is relatively rare, it is probably underestimated.[19] Transfusion-transmitted leishmaniasis is of special concern in regions where VL caused by either *L. infantum* (*syn. chgasi*) or *L. donovani* is endemic. During the last 70 years, there have been about 10 probable cases only of TTL worldwide.[9,10,20]

TABLE 1
Demographic data of patients with cutaneous leishmaniasis

	L. major	*L. tropica*	*L. braziliensis*
Total cases	33	26	3
Males	16 (48%)	15 (58%)	3 (100%)
Females	17 (52%)	11 (42%)	0 (0%)
Active cases	6 (18%)	18 (69%)	3 (100%)
Single lesion	10 (30%)	14 (54%)	3 (100%)
≥ 2 lesions	23 (70%)	12 (46%)	0 (0%)

In the case of CL, the presence of parasite DNA in patient peripheral blood is still a matter of some controversy. Reports describing the identification of parasite DNA in blood from patients with New World *Leishmania* (*Viannia*) subgenus are mixed. Several researchers have attempted parasite isolation from peripheral blood, but only very few have succeeded. In one study, *Leishmania* (*Viannia*) DNA was detected by PCR in the blood of 2 (3.4%) out of 59 patients whose skin lesions were positive for amastigotes.[14,21] Another study found leishmanial DNA not only in patients presenting with active lesions, but also in blood from healed individuals, as well as asymptomatic skin-test-positive residents of endemic areas. In this study, overall 26.2% of the 225 examined samples were positive for parasite DNA.[22] However, another study in South America failed to confirm the presence of *Leishmania* (*Viannia*) DNA in the blood of 60 CL patients.[23] The hematogenous spread of New World CL might explain the presence of parasites in the patients' blood, as was detected in one patient of our cohort with *L. braziliensis*.

However, the detection of *Leishmania* in peripheral blood of patients with Old-World CL is not well established.[13] One report in 1992 describes two Lebanese patients with chronic CL with positive blood cultures for *Leishmania* (species unknown).[24]

However, these cases were considered very unusual.[21] A major concern regarding TTL arose following Operation Desert Storm in 1991 when several cases of viscerotropic leishmaniasis caused by *L. tropica* were reported in the U.S. military personnel.[8,25] Further concern regarding TTL arose when 22 cases of CL were reported in the U.S. military personnel deployed to the Middle East during 2002–2003, primarily to Afghanistan, Iraq, and Kuwait.[20] Although no cases of TTL were ever described in the United States, the AABB (American Association of Blood Banks) and the U.S. Armed Services Blood Program Office of the Department of Defense (DOD) implemented policies preventing prospective blood donors from donating blood for 12 months after leaving one of these countries.[26] This was taken as a precautionary measure against the possibility that blood-borne amastigotes might be undetected, resulting in TTL. However, no case showed isolation of parasites from peripheral blood. Subsequently, the AABB eliminated the requirement for this delay, and it is not in place in civilian blood centers. However, armed forces blood programs continue to maintain this deferral even though reports of atypical leishmaniasis in returning military personnel have subsided.[26]

Cutaneous leishmaniasis caused by *L. major* and *L. tropica* is highly endemic in Israel,[27] since the year 1993, even though it is highly uncommon, the blood bank in Israel does not accept blood donations from people infected with CL showing multiple lesions.

Surprisingly, an article published in 2011 by Lebanese researchers[13] on a group of 162 biopsy-proven, untreated patients with CL, described a parasite isolated in the blood

and blood components of 50 patients (30.9%). From the 28 isolates that were positive in both skin and blood, 8 isolates were *L. major* and 2 were *L. tropica*. The remaining isolates were *L. infantum*. Isolated parasite species were characterized by isoenzyme electrophoresis.[13] This method is obsolete and is no longer in standard use.

Our study contradicts these unusual findings reported from Lebanon. The findings of our study, although a small sample, confirmed by PCR, suggest that people with active or recent CL caused by *L. major* and *L. tropica*, even those with multiple lesions, do not harbor parasites in their blood. Therefore, the decision to exclude them from donating blood should be revisited. It is a further support due to the lack of reports on TTL transmitted in patients with CL. However, patients with active lesions positive for *L. braziliensis* should be avoided from donating blood during the active phase of the disease.

In any case, the introduction of leukodepletion filters to the blood bank, and using it at the time of collection should avoid any risk of TTL, even in endemic areas for VL where *L. infantum* is present.

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