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ORIGINAL ARTICLE

Seroprevalence of *Leishmania* infection among the healthy blood donors in kala-azar endemic areas of Iran

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Abstract Visceral leishmaniasis (VL) is a serious health problem in Iran. The disease is mainly transmitted by sand fly bites, but its transmission through transfusion in endemic areas may also occur. The current study aimed to determine the prevalence of *Leishmania* infection among blood donors in VL-endemic areas in south of Iran. A total of 2003 healthy blood donors from blood service centers in five VL-endemic districts in Fars province, southern Iran, were enrolled in the study. The blood samples were assessed for antibodies against *Leishmania infantum* by direct agglutination test (DAT). Seropositive subjects were tested for the presence of *L. infantum* DNA in their buffy coat by the molecular method. Socio-demographic features of the subjects were also documented during sample collecting. The mean age of participants was 36.3 (SD = 10.7 years). Male constituted 94.7 % of the subjects while only 5.3 % of donors were female. Twenty-eight blood donors (1.4 %) were positive for *Leishmania* infection by DAT. Only one of these seropositive donors was positive for *Leishmania* infection by polymerase chain reaction. A significant correlation was found between age, the place of residence and seropositivity to *Leishmania* ($P < 0.05$). Findings of this study revealed that the prevalence of *Leishmania* infection among blood donors in transfusion centers in the VL-endemic areas in Iran is relatively high. These asymptomatic blood donors may constitute a risk of transmitting of VL to susceptible recipients.

Keywords *Leishmania* · Blood donors · Prevalence · Iran

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Introduction

Visceral leishmaniasis (VL) is a protozoan disease caused by *Leishmania donovani* complex including *Leishmania infantum* in Middle East and Mediterranean area and *L. donovani* in the Indian subcontinent and Eastern Africa (Desjeux 2004).

Cutaneous and VL are present in most of (14 of the 22) countries of the Eastern Mediterranean Region of the World Health Organization (Postigo 2010). Iran is an endemic country for both cutaneous and VL (Motazedian et al. 2002; Davami et al. 2010; Mohebali et al. 2011). VL is a major health problem in northwest and southern of the country. Dogs are the main reservoirs of the diseases, although feline leishmaniasis has also been reported from the region and cats may also be involved in the epidemiology of the disease (Hatam et al. 2010; Sarkari et al. 2009). The main foci of the disease in southern Iran are Kazeroun, Nourabad, Firouzabad and Darab districts in Fars province (Fakhar et al. 2008; Sarkari et al. 2010, 2012; Mohebali et al. 2011). Based on a hospital records, 260 cases of VL have been recorded during 2001–2009 from these regions (Sarkari et al. 2012).

Transmission of *Leishmania* through transfusion has been reported from different VL-endemic areas of the world (Cohen et al. 1991; Mauny et al. 1993; Cummins et al. 1995; Singh et al.1996). Many of frequent recipients of transfusion are patients undergoing treatment for malignancy, recipients of transplants, infants, and patients on steroid. These populations are at risk of acquiring VL through transfusion due to their immunosuppression status (Cardo 2006).

Blood donors in VL-endemic areas are healthy individual who may have *Leishmania* infection without having an apparent sign or symptoms. These asymptomatic infected subjects may transmit leishmaniasis to susceptible recipients through transfusion (Cardo 2006).

Studies carried out on healthy blood donors in VL-endemic areas have revealed different rate of *Leishmania* infection in these asymptomatic donors. In Southern France, *L. infantum* has been reported in 76 of 565 asymptomatic blood donors and cultures of blood have been positive in 9 donors (Fichoux et al. 1999). Rate of *Leishmania* infection in asymptomatic blood donors in an endemic area of Spain was reported to be 2.4 % by ELISA and 7.6 % by Western blot (Riera et al. 2004). Another study in Spain by Riera et al. reported a seropositivity rate of 3.1 % for *Leishmania* infection in blood donor (Riera et al. 2008). A low prevalence (0.3 %) of *L. donovani* among blood donors has been reported in a VL-endemic area in Bangladesh (Huda et al. 2013).

The current study aimed to evaluate the prevalence of *Leishmania* infection among the healthy blood donors in a VL-endemic area in Iran.

Materials and methods

Study population and sample collecting

Blood samples were taken from 2003 healthy blood donors from blood service centers in five VL-endemic districts in Fars province. The centers were in Kazeroun, Jahrom, Darab, Firouzabad and Nourabad districts. Socio-demographic features of the subjects were also documented during sample collecting. Ethical approval of the study was given by the ethics committee of Shiraz University of Medical Sciences.

Serological test

Sera were obtained from the fresh whole blood of the blood donors. Moreover, buffy coat was obtained from each blood sample for subsequent DNA extraction. Sera and buffy coats were kept at −20 °C until use. Sera samples were tested for anti-*Leishmania* antibodies by direct agglutination test (DAT). The DAT antigen was prepared in our serology laboratory in the department of parasitology and Mycology at Shiraz University of Medical Sciences. Mass cultivated *L. infantum* (Lon-49, Iranian strain) was used for preparation of DAT antigen. DAT was performed as previously described (Sarkari et al. 2010). Briefly, samples were diluted in serum diluent (physiological saline) containing 0.78 % β-mercaptoethanol and 0.2 % gelatin. Two-fold dilution series of the sera were made in a V-shaped microtitre plate, starting at a dilution of 1:50 and going up to a serum dilution of 1:6,400. DAT antigen (50 µl) was added to each well containing 50 µl of diluted serum. After 2 min of gentle shaking on a flat surface, the plate was covered with a lid and checked after overnight incubation at room temperature. An antibody titer of 1:800 and above was considered positive.

DNA extraction and PCR

DNA was extracted from the buffy coat of the samples. DNA of each sample was extracted using proteinase K and lysis buffer followed by phenol/chloroform/isoamyl extraction. Absolute ethanol was used to precipitate the DNA. Precipitated DNA was re-suspended in 100 µl of double distilled water and stored at 4 °C until use. Polymerase chain reaction (PCR) was performed as described by Rahbarian et al. (2009). The primers which were used are: LeishF (5′-CAA CAC GCG GCC TCC TCT CT-3′) AND LeishR (5′-AAA AGG TTG TCN GGG 3′). The thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles including denaturation at 94 °C for 35 s, annealing at 60 °C for 35 s, extension at 72 °C for 45 s, and final extension at 72 °C for 5 min. PCR products were separated by electrophoresis in 1.5 % agarose gel stained with ethidium bromide.

Analysis of data

The findings were analyzed by SPSS software (version 17), with a P value <0.05 considered as statistically significant. Chi squared and Fisher exact tests were used to compare the prevalence values relative to the characteristics of the donors.

Results

The study population included 2003 blood donors, consisting of 1896 (94.7 %) male and 107 (5.3 %) female, with age ranging from 17 to 66 years. The mean age of participants was 36.3 (±10.7) years. Most of the subjects were aged 25–35 years. Participants were blood donors from five blood transfusion centers in five different districts (all known as VL-endemic areas) in Fars province. From the total of 2003 blood donors which were recruited, 28

blood donors (1.4 %) were seropositive for *Leishmania* infection by DAT. Out of 28 positive samples, only one of the donors was positive for *Leishmania* infection by PCR. A significant correlation was found between the age of participants and seropositivity to *Leishmania* ($P < 0.05$).

Considering the residence of the blood donors, Firouzabad had the highest rate of infection (3 %), followed by Nourabad (1.8 %), Kazeroun (1.7 %), and Jahrom (0.5 %) districts. The differences in *Leishmania* seropositivity and residence of the participants were statistically significant ($P < 0.05$). The highest rate of infection (1.9 %) was found in the age group of 46–55 years while the lowest seropositivity was seen in the age lower than 25 years. The differences between age group and *Leishmania* infection were statistically significant ($P < 0.05$).

While most of the donors (71.9 %) were given blood on a regular basis, or had the history of blood donation, 28.1 % of them had no experience of blood donation before. No correlation was found between *Leishmania* seropositivity and ABO blood group ($P > 0.05$). Moreover, no association was found between *Leishmania* seropositivity and the occupations of participants ($P > 0.05$). Table 1 shows the socio-demographic characteristics of blood donors and relative seropositivity to *Leishmania* in this study.

Discussion

The current study is the first report of *Leishmania* infection among healthy blood donors in south of Iran which aimed to assess the rate of *Leishmania* infection in blood donors and also to evaluate the presence of *Leishmania* in buffy coat of seropositive subjects. Samples have been taken from healthy volunteer blood donating people living in VL-endemic areas of Fars province, south of Iran.

Findings of this study revealed that more than one percent of healthy blood donors in VL-endemic areas of Iran are infected with *Leishmania*. So, the blood recipients, especially those with immunosuppressed status and infants, in VL-endemic areas of Iran might be at risk of acquiring VL through transfusion.

The ability of an organism to survive in the stored blood contributes to its transmission through transfusion. Recent studies carried out on blood donors from different VL-endemic areas of the world have revealed a relatively high rate of *Leishmania* infection in healthy blood donors (Luz et al. 1997; Fichoux et al. 1999; Riera et al. 2004; Colomba et al. 2005; Cardo 2006; Riera et al. 2008; Scarlata et al. 2008).

More than 90 percent of *L. infantum* and also *L. donovani* infection do not progress to clinically apparent diseases, in spite of the persistence of visceral infection (Engwerda et al. 2004). As a result, in any given VL-endemic area, there are a vast majority of infected people; among them are blood donors, which would be remained undetected.

It has been shown that *Leishmania* can circulate in asymptomatic blood donors more than 1 year after exposure (Fichoux et al. 1999; Cardo 2006). Therefore there is a risk of transmission of *Leishmania* to blood recipients due to its subclinical persistence in infected individuals.

Cases of transfusion transmitted leishmaniasis have been reported from different VL-endemic areas of the world including France, Belgium, Brazil and India (Cohen et al. 1991; Mauny et al. 1993; Cummins et al. 1995; Singh et al.1996). The majority of these infected recipients were patients who received whole blood from donors from the area where VL is endemic.

Support for the view of transmission of *Leishmania* through transfusion also comes from the study which has confirmed the transmission of *Leishmania* through blood transfusion in experimentally infected dogs (de Freitas et al. 2006).

Transmission of *Leishmania* through transfusion requires parasites to be present in the blood, either within infected white blood cells, or as free amastigotes released from phagocytes (Cardo 2006). Furthermore, the parasite should survive during processing and storage of blood. Disruption of monocytes during leukodepletion, or blood fractionation may result in release of amastigotes and could represent a potential transmission risk.

To avoid transfusional transmission of leishmaniasis, deferral of donation for as long as 12 months after returning from VL-endemic and permanent deferral of donation of people with apparent skin infection is taking place in the United States (Cardo 2006).

In our study, presence of antibodies against *Leishmania* was used for determining the prevalence rate of *Leishmania* infection in healthy blood donors. Nevertheless, antibody to *Leishmania* might not be detectable in all of asymptomatic subjects; therefore it may underestimate the prevalence of the *Leishmania* infection in healthy blood donors. In view of that, the real prevalence of *Leishmania* infection in blood donors might be higher than what we are reporting. In keeping with that, a study in Spain evaluated the blood donors for *Leishmania* infection by different methods and demonstrated the infection in 16/656 by ELISA, 50/565 by western blotting and 27/122 by PCR (Riera et al. 2004).

Previous studies demonstrated that *Leishmania* DNA may be present in the blood of healthy people without having any detectable humoral immune responses. In Reiera et al. study, only 2 of 18 PCR-positive donors were seropositive for VL. The lack of humoral immune response in asymptomatic individuals has been reported in Fakhar et al. (2008) study in Iran as well, where the individuals with positive PCR for *Leishmania* were seronegative by serological assays.

Considering the results of the aforementioned studies, some of the seronegative individuals in our study may have *Leishmania* infection.

Table 1 Socio-demographic characteristics of blood donors and relative seropositivity to *Leishmania* in Fars province, southern Iran

Characteristics	Frequency (no.)	Percent (%)	Positive for anti-*Leishmania* antibodies		*P* value
			No.	%	
Gender					
Male	1,896	94.7	27	1.4	>0.05
Female	107	5.3	1	0.9	
Age group					
≤25	49	2.9	0	0	<0.05
26–35	632	31.6	7	1.1	
36–45	561	28	8	1.4	
46–55	463	23.1	9	1.9	
56 Through higher	272	13.6	4	1.5	
Residence					
Kazeroun	403	20.1	7	1.7	<0.05
Jahrom	395	19.7	2	0.5	
Darab	402	20.1	0	0.0	
Firouzabad	405	20.2	12	3	
Nourabad	398	19.9	7	1.8	
Educational level					
Uneducated	51	2.6	1	2	>0.05
Primary and secondary level	363	18.2	4	1.1	
Post-secondary level	442	22.2	8	1.8	
High school diploma	685	34.3	9	1.4	
University level	480	24.1	6	1.2	
Occupation					
Employee	548	27.4	8	1.5	>0.05
Business	979	48.9	12	1.2	
Housewives	100	5	1	1	
Student	158	7.9	1	0.6	
Laborer	88	4.4	3	3.4	
Farmer and stockbreeder	94	4.7	1	2.9	
Unemployed	35	1.7	13	17.8	
Blood group					
A	549	27.5	8	1.5	>0.05
B	489	24.5	7	1.4	
AB	131	6.6	2	1.5	
O	823	41.3	11	1.3	

In this study, the proportion of seropositive individuals increased with the increasing of the age of the subjects. This might be contributed to a boosting effect in asymptomatic infected subjects upon repeated exposure. Similar observation has been reported in Hasker et al. (2013) study.

Taken together, findings of this study revealed that healthy blood donors in VL-endemic areas of Iran can be infected with *Leishmania*. Therefore, the possibility of transfusion transmitted leishmaniasis in VL- endemic areas of Iran cannot be ruled out. Further studies are needed to find out the real risk of transfusion transmitted leishmaniasis in VL-endemic areas in Iran.

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References

Cardo LJ (2006) *Leishmania*: risk to the blood supply. Transfusion 46:1641–1645

Cohen C, Corazza F, De Mol P, Brasseur D (1991) Leishmaniasis acquired in Belgium. Lancet 338:128

Colomba C, Saporito L, Polara VF, Barone T, Corrao A, Titone L (2005) Serological screening for *Leishmania infantum* in

asymptomatic blood donors living in an endemic area (Sicily, Italy). Transfus Apher Sci 33:311–314

Cummins D, Amin S, Halil O, Chiodini PL, Hewitt PE, Radley-Smith R (1995) Visceral leishmaniasis after cardiac surgery. Arch Dis Child 72:235–236

Davami MH, Motazedian MH, Sarkari B (2010) The changing profile of cutaneous leishmaniasis in a focus of the disease in Jahrom district, southern Iran. Ann Trop Med Parasitol 104:377–382

de Freitas E, Melo MN, da Costa-Val AP, Michalick MS (2006) Transmission of *Leishmania infantum* via blood transfusion in dogs: potential for infection and importance of clinical factors. Vet Parasitol 137:159–167

Desjeux P (2004) Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 27:305–318

Engwerda CR, Ato M, Kaye PM (2004) Macrophages, pathology and parasite persistence in experimental visceral leishmaniasis. Trends Parasitol 20:524–530

Fakhar M, Motazedian MH, Hatam GR, Asgari Q, Kalantari M, Mohebali M (2008) Asymptomatic human carriers of *Leishmania infantum*: possible reservoirs for Mediterranean visceral leishmaniasis in southern Iran. Ann Trop Med Parasitol 102:577–583

Hasker E, Kansal S, Malaviya P, Gidwani K, Picado A, Singh RP, Chourasia A, Singh AK, Shankar R, Menten J, Wilson ME, Boelaert M, Sundar S (2013) Latent infection with *Leishmania donovani* in highly endemic villages in Bihar, India. PLoS Negl Trop Dis 7:e2053

Hatam GR, Adnani SJ, Asgari Q, Fallah E, Motazedian MH, Sadjjadi SM, Sarkari B (2010) First report of natural infection in cats with *Leishmania infantum* in Iran. Vector Borne Zoonotic Dis 10:313–316

Huda MM, Rudra S, Ghosh D, Bhaskar KR, Chowdhury R, Dash AP, Bhattacharya SK, Haque R, Mondal D (2013) Low prevalence of *Leishmania donovani* infection among the blood donors in kala-azar endemic areas of Bangladesh. BMC Infect Dis 13:62

le Fichoux Y, Quaranta JF, Aufeuvre JP, Lelievre A, Marty P, Suffia I, Rousseau D, Kubar J (1999) Occurrence of *Leishmania infantum* parasitemia in asymptomatic blood donors living in an area of endemicity in southern France. J Clin Microbiol 37:1953–1957

Luz KG, da Silva VO, Gomes EM, Machado FC, Araujo MA, Fonseca HE, Freire TC, d'Almeida JB, Palatnik M, Palatnik-de Sousa CB (1997) Prevalence of anti-*Leishmania donovani* antibody among Brazilian blood donors and multiply transfused hemodialysis patients. Am J Trop Med Hyg 57:168–171

Mauny I, Blanchot I, Degeilh B, Dabadie A, Guiguen C, Roussey M (1993) Visceral leishmaniasis in an infant in Brittany: discussion on the modes of transmission out endemic zones. Pediatrie 48:237–239

Mohebali M, Edrissian GH, Shirzadi MR, Akhoundi B, Hajjaran H, Zarei Z, Molaei S, Sharifi I, Mamishi S, Mahmoudvand H, Torabi V, Moshfe A, Malmasi A, Motazedian MH, Fakhar M (2011) An observational study on the current distribution of visceral leishmaniasis in different geographical zones of Iran and implication to health policy. Travel Med Infect Dis 9:67–74

Motazedian H, Noamanpoor B, Ardehali S (2002) Characterization of *Leishmania parasites* isolated from provinces of the Islamic Republic of Iran. East Mediterr Health J 8:338–344

Postigo JA (2010) Leishmaniasis in the World Health Organization Eastern Mediterranean Region. Int J Antimicrob Agents 36(1):S62–S65

Rahbarian N, Mesgarian A, Mahmoudi Rad M, Hajaran H, Shahbazi F, Mesgarian Z, Taghipour N (2009) Identification of *Leishmania* species isolated from human cutaneous leishmaniasis using PCR method. J Res Health Sci 9:48–51

Riera C, Fisa R, Udina M, Gallego M, Portus M (2004) Detection of *Leishmania infantum* cryptic infection in asymptomatic blood donors living in an endemic area (Eivissa, Balearic Islands, Spain) by different diagnostic methods. Trans R Soc Trop Med Hyg 98:102–110

Riera C, Fisa R, Lopez-Chejade P, Serra T, Girona E, Jimenez M, Muncunill J, Sedeno M, Mascaro M, Udina M, Gallego M, Carrio J, Forteza A, Portus M (2008) Asymptomatic infection by *Leishmania infantum* in blood donors from the Balearic Islands (Spain). Transfusion 48:1383–1389

Sarkari B, Hatam GR, Adnani SJ, Asgari Q (2009) Seroprevalence of feline leishmaniasis in areas of Iran where *Leishmania infantum* is endemic. Ann Trop Med Parasitol 103:275–277

Sarkari B, Pedram N, Mohebali M, Moshfe AA, Zargar MA, Akhoundi B, Shirzadi MR (2010) Seroepidemiological study of visceral leishmaniasis in Booyerahmad district, south-west Islamic Republic of Iran. East Mediterr Health J 16:1133–1136

Sarkari B, Hatam G, Ghatee M (2012) Epidemiological features of visceral leishmaniasis in Fars province, southern Iran. Iran J Public Health 41:94–99

Scarlata F, Vitale F, Saporito L, Reale S, Vecchi VL, Giordano S, Infurnari L, Occhipinti F, Titone L (2008) Asymptomatic *Leishmania infantum/chagasi* infection in blood donors of western Sicily. Trans R Soc Trop Med Hyg 102:394–396

Singh S, Chaudhry VP, Wali JP (1996) Transfusion-transmitted kala-azar in India. Transfusion 36:848–849