Serological Assessment for *Leishmania donovani* Infection in Blood Donors of Sunsari District, Dharan, Nepal

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Abstract Visceral leishmaniasis (VL) is a major vector-borne disease caused by *Leishmania donovani*, after replication of the parasites in macrophages, mononuclear phagocytic system. VL is endemic in 12 districts of central and eastern Terai lowlands of Nepal bordering North Bihar, India with an estimated 8 million population at risk. In addition, VL endemicity is also extending to new endemic regions like Dharan from its classical rural foci. Hence, we aimed to detect the evidence of *Leishmania donovani* infection in the blood samples received from blood donors of Sunsari district, Dharan, (eastern Nepal), a region endemic for human VL. Sera from 507 asymptomatic blood donors were subjected to serological screening for anti-*Leishmania donovani* antibodies. Direct agglutination test (DAT) was performed on the sera. Out of 507 donors, majority (78.50 %) were male. Among the donors, 472 (93.10 %) belonged to age group 18–45 years where as 35 (6.90 %) to age group >45 years. Circulating anti-*Leishmania* antibodies were detected in 5 (1 %) out of 507 healthy, Human Immunodeficiency Virus types 1 and 2 (HIV 1 and 2), Hepatitis B Surface Antigen (HBsAg), anti-Hepatitis C Virus (anti-HCV)-negative, and Syphilis non-reactive donors. All the seropositive cases were male and

belonged to the age group 18–45 years. The result suggests that there is an immediate need of screening asymptomatic blood donors for *leishmania* seropositivity especially in endemic areas.

Keywords Asymptomatic blood donors · DAT · Dharan · Visceral leishmaniasis

Introduction

Visceral leishmaniasis (VL) also known as Kala-azar (KA) is a major vector borne parasitic disease with fatal outcome if left untreated [1]. In the Indian subcontinent it is caused by *Leishmania donovani*, a member of *Leishmania donovani* complex whose transmission is believed to be only anthroponotic in the region [2]. Sandfly is the vector whereas man is the usual reservoir. Sandfly is infected by sucking blood of patient of leishmaniasis, which then transmits the infection to another person by its bite. In human host, parasites are engulfed by macrophages which are then carried by the blood stream to distant organs like spleen, liver and bone marrow. There they cause marked hyperplasia of reticuloendothelial cells [1].

The first case of VL in Nepal was officially recorded in 1980 [3] and is now endemic in [1] 2 districts of eastern and central Terai lowlands bordering North Bihar, India with an estimated 8 million population at risk. Since 2005, Nepal participates in a regional Kala-azar elimination initiative with a target of reducing the incidence rate below 1:10,000 per year by 2015 [4].

VL remains occult in individuals with normal immune systems and appears to be very common in most of the VL endemic foci [5]. Much attention has been invited on preventable and iatrogenic causes of leishmaniasis that

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include transfusion transmitted forms. The American Association of Blood Banks has recommended ban on blood donations from a selected group (soldiers returning to the United States from VL endemic parts, specially countries of middle East) to prevent transfusion transmitted Leishmaniasis [6]. Hence, this study was conducted to detect the evidence of *L. donovani* infection in the blood samples received from blood donors living in recent peri-urban focus-Dharan, eastern Nepal.

Materials and Methods

The study was conducted in Kala-azar laboratory of department of Microbiology at B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal.

Human Sera

Human serum samples from 507 asymptomatic volunteer blood donors from Sunsari district, Dharan (eastern Nepal), a region endemic for human VL, were analyzed from June to December 2010.

Human blood samples (5 ml each) were collected from the Blood Bank of Nepal Red Cross Society, Dharan, Sunsari branch. Serum was separated from each blood sample and stored at -20°C for DAT analysis at BPKIHS. Informed consents were obtained from all the participants and the ethical committee of the institution approved the study.

Serological Study

The presence of anti-*Leishmania* antibodies in all the serum samples was determined by DAT. DAT was performed as described elsewhere [7] using freeze dried antigen suspension of trypsin-treated and coomassie brilliant blue stained *Leishmania* promastigotes (purchased from ITG-Belgium). The sensitivity and specificity of the test kit were claimed to be 90–100 and 80–95 % respectively [8].

In brief, the samples were diluted in physiological saline (0.9 % NaCl) containing 0.78 % β -mercaptoethanol. Two-fold dilution series of the sera were made in a V-shaped microtitre plate with the necessary positive and negative controls on each, starting at a dilution of 1:100 and going up to a maximum serum dilution of 1:102,400. Fifty μl DAT antigen (concentration of 5×10^7 parasites per ml) was added to each well containing 50 μl diluted serum and the results were read after 18 h of incubation. The test was read visually against a white background and the end-point titre was taken as the last well where agglutination was seen. A sample was considered positive if it had a titre

$\geq 1:1600$ (threshold chosen as *Leishmania* infection marker in the Kalanet project at BPKIHS, Dharan, Nepal).

Results

Out of 507 volunteer blood donors, majority (78.50 %) were male. Among the donors, 472 (93.10 %) belonged to age group 18–45 years where as 35 (6.90 %) to age group >45 years. The median age of the donors was found to be 27 years (Range 22–32 years).

Circulating anti-*Leishmania* antibodies were detected in 5 (1 %) out of 507 healthy, hepatitis B virus surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV), human immunodeficiency virus types 1 and 2 (HIV 1 and 2) negative, and syphilis non-reactive donors. All the positive cases were males. Among the positive sera, 2 were with antibody titre 1:3,200, other 2 with antibody titre 1:6,400 and a single sample represented serum antibody titre of being more than 1:6,400 against VL.

In addition, 2 of the sera were found to be with antibody titre 1:200, other 4 with antibody titre 1:400, and 1 with antibody titre 1:800, while rest of the sera showed no signs of agglutination (Table 1).

Furthermore, none, among the positive subjects, reported a history of suffering or having a relative suffering from VL and to have a family history of VL.

Moreover, with the constraints of time, money, and other germane resources follow-up could not be done but all positive subjects were advised to undergo parasitological diagnosis for the confirmation of VL and to initiate appropriate treatment.

Discussion

This study describes the results of a survey of asymptomatic *L. donovani* human infection in Sunsari district, Dharan of eastern Nepal, which is considered an area of endemicity, characterized by a low incidence of officially reported VL cases. According to our study, 5 (1 %) out of 507 healthy human blood donors were found to be positive for anti-*Leishmania* antibody by DAT.

Asymptomatic *Leishmania* infections are described in various foci over the world, and the ratio of asymptomatic to clinical cases has varied from 1:2.4 to 18:1; with a ratio of 8.9:1 in India and Nepal [9]. And hence such large numbers of latent leishmania infections raise the questions on whether there is any clinical relevance of asymptomatic infection for an individual, and whether they play any role in disease transmission. If asymptotically infected persons are infectious for sandflies, theoretically they could contribute to an epidemic [10]. In addition, Hasker et al.

Table 1 Age-wise, sex-wise distribution of the participants and result of serology of VL along with Ab titres among the positives

	Number (N = 507)	Percentage (%)
<i>Age-wise distribution of the participants</i>		
18–45	472	93.10
>45	35	6.90
<i>Sex-wise distribution of the participants</i>		
Male	398	78.50
Female	109	21.50
<i>Serology of Visceral Leishmaniasis</i>		
Antibody titre <1,600	502	99.0
Antibody titre ≥ 1,600	5	1.00
<i>Antibody titres among the positives</i>		
No agglutination	495	97.62
=1:200	2	0.40
=1:400	4	0.78
=1:800	1	0.20
=1:3200	2	0.40
=1:6400	2	0.40
>1:6400	1	0.20

2014 found out a strongly increased risk of progressing to disease among DAT and/or rK39 seropositives with high titers [11].

Moreover, our seroprevalence data is consistent with recent surveys on healthy subjects in other regions of high endemicity such as Southern Italy (0.75 % [12]), Iran (1.62 % [13]) by indirect fluorescent antibody test (IFAT) and DAT respectively. Taken together, these data prompt a strong suspicion that significant local transmission of *L. donovani* to humans has been ongoing for years in the areas surveyed, suggesting the importance of long-lasting, uninterrupted contact with both the reservoir and the vector. Contrast to our finding, high seroprevalence of *Leishmania* infection were observed in blood donors in various places [14–17]. In this study only men are found to be infected and similar result has been observed by Bern et al. 2005 [18]. Women in these communities wear long dresses which could prove to be protective to some degree from sandfly biting.

Furthermore, a *Leishmania* infected subject may have circulating organisms even after clinical recovery and incubation periods of up to 30 years have been reported for occult *Leishmania* [19], suggesting that transmissibility is long-lasting. In addition to circulating within monocytes, *L. donovani* was reported to circulate extracellularly not only in the blood of patients with symptomatic VL but surprisingly also in the blood of persons with asymptomatic infection [20]. This existence of cryptic *Leishmania* infections, associated with an asymptomatic subclinical period, has been concluded as the main cause of the transmission by blood [17]. In contrast, blood donors are healthy and if infected are asymptomatic, producing the

ideal situation for transmission. Greater than 95 % of *L. donovani* and *L. infantum* do not progress to clinically apparent visceral disease, in spite of the persistence of visceral infection, demonstrating how likely it would be for *Leishmania* to be undetected in an infected donor [4].

Transmission of *Leishmania* by transfusion is most likely to occur in an endemic area because that is where infected donors most often reside. *Leishmania* does produce clinical disease in infants and the immunocompromised as demonstrated in documented cases of transmission by transfusion by different authors [21–23]. Asymptomatic infection is at least 20 times more likely in healthy donors and is a danger to recipients of blood products, because *Leishmania* has been shown to circulate in asymptomatic blood donors more than 1 year after exposure [15], and leishmaniasis can become symptomatic many years after occult infection has been contracted [24].

Similarly, VL transmission in transplant recipients has already been documented with 80 cases so far [25]. In fact, only 12 post-transfusion VL cases have been reported, to our knowledge, since 194 [8, 26] 11 of the above occurred in infants or children. On the other hand, it has been demonstrated that the preparation of blood products using leukoreduction by filtration minimizes the potential risk of transmission of several pathogens (e.g. *Leishmania* and cytomegalovirus) [27].

Finally, assuming a real transmission of *L. donovani* by transfusion, it does not necessarily result in the transmission of the disease. Nevertheless, we propose to extend our study in other endemic areas of our region using more sensitive techniques to achieve a more complete knowledge of our epidemiologic situation about the existence of

asymptomatic *L. donovani* carriers. As the need for control of blood products to prevent transmission of kala-azar is already recognized in the United States [6], our findings emphasize the urgent need for routine preventive blood control for leishmanial infections in areas where human visceral leishmaniasis is endemic. Our findings undoubtedly raise clinical issues concerning the possible screening of both blood/transplant patients and blood/organ/bone marrow donors for asymptomatic leishmaniasis and on the other hand, the need of longitudinal follow-up studies on asymptomatic VL in blood donors in endemic areas and transfused immunosuppressed and pediatric patients.

Limitations of this study rely on our data which were obtained from highly selected groups (blood donors, where male subjects are dominant) rather than randomized data from the general population, so probably are less representative of the true local epidemiological situation. In addition, a positive result in immunological techniques does not necessarily represent an active infection and may only indicate previous exposure or contact with *Leishmania* that is particularly likely in an endemic area. Also, a negative result does not discard infection, mainly in cryptic forms.

Conclusion

Since pathogens are constantly emerging in our blood supply, their spread can be controlled through early response by blood banking community. Thus, this study recommends a complete donor testing to protect recipients of possible transfusion transmitted infections. And in addition, the DAT could be very useful in the detection of subclinical infection and thus the identification of those at risk of VL and of foci of transmission. Data collected using the DAT could therefore facilitate the control and treatment of VL in endemic areas.

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