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ORIGINAL ARTICLE

Alteration of Direct Agglutination Test (DAT) results in Iranian Kala-Azar patients: a case series

Soheila Molaie[1] · Mehdi Mohebali[2] · Mohamad-Reza Abai[3] · Akbar Molaie[4] · Behnaz Akhoundi[2] · Eslam Moradi Asl[5]

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Abstract The early diagnosis of visceral leishmaniasis (VL) using Direct Agglutination Tests (DAT) and its treatment and control are essential actions taken in rural health centers in endemic foci of the infection based on the national protocols set by the Iranian Ministry of Health and Medical Education. Eleven clinically confirmed VL patients with typical VL symptoms and negative results of DAT, admitted to the pediatrics department of Valiasr Hospital in Meshkinshahr underwent parasitological tests. 7 of the total of 11 patients had positive result of bone marrow puncture smears and all of them had negative results of DAT. Thus factors that had led to false negative DAT results were examined. The patients' blood samples were collected in microhematocrit tubes using the finger prick technique, centrifuged and their plasma then separated. The complete medical history of the patients was taken using a questionnaire. The laboratory staff therefore checked the quality of anti-leishmania antigen, materials and equipment used. The patients' medical history showed that they had all been administered corticosteroid medications such as dexamethasone or hydrocortisone prior to visiting the laboratory. The DAT was repeated in these patients 2–3 weeks after their last administration of corticosteroids. The antibody titers were positive this time. A total of 3 of the collected specimens (27.3 %) showed a titer of 1.3200, 5 (46 %) showed a titer of 1.1600 and 3 (27.3 %) a titer of 1.800. Due to the effects of some medications, particularly corticosteroids, on serological tests, the patients' full medical history should be taken prior to performing this test and physicians working at endemic regions of this infection should be notified about these drug interactions.

Keywords Visceral leishmaniasis · Direct Agglutination Test · False negative · Corticosteroid

Introduction

Mediterranean visceral leishmaniasis (VL) or Kala-Azar is a vector-borne parasitic disease with at least six endemic foci in northwestern and southern of Iran (Mohebali 2013). Wild carnivores (such as foxes and jackals) and domestic dogs act as the main sources of the transmission of Leishmania infantum while wild rodents (such as the gray hamster) act as secondary sources of its transmission in Iran (Mohebali et al. 2005, 2004). VL is transmitted by various species of phlebotomine sand flies (Azizi et al. 2008; Oshaghi et al. 2009; Absavaran et al. 2009; Rassi et al. 2012a, b). The laboratory diagnosis of VL is critical due to the high fatality rates of the infection (up to 100 %) if left untreated (Mohebali 2013). Parasitological, serological and molecular methods are used for the diagnosis of human VL (Mohebali et al. 2006). DAT is a serological test available for the diagnosis of VL that is highly specific and

✉ Soheila Molaie
smolaie83@gmail.com

[1] Kala-Azar Laboratory, Health Center, Ardabil University of Medical Scince, P.O. Box 56617-45378, Meshkinshahr, Iran

[2] Department of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[3] Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[4] Shahid Madani Hospital, Tabriz University of Medical Sciences, Tabriz, Iran

[5] Health Care Center of Meshkinshahr, Ardabil University of Medical Sciences, Meshkin-shahr, Iran

sensitive, cost-effective, reliable, safe and adaptable to microtiter plates (Edrissian et al. 1996). Any factor that interferes with the results of DAT can cause negative false results and lead to errors in the diagnosis of the infection. This present study was conducted in Kala-Azar laboratory of Meshkinshahr Health Center due to the of negative DAT results in eleven clinically and parasitology confirmed VL patients. It should be noted that 7 of the total of 11 cases had positive parasitological test results.It may showed low sensitivity of bone marrow puncture smears versus serological tests.

Materials and methods

Study area

The present study was conducted in Meshkinshahr, Ardabil province, northwest of Iran, where VL is endemic. Meshkinshahr is a region in central north Ardabil located at an altitude of 1490 m above sea level, at the longitude of 47.190N and 48.170E and the latitude of 38.570N and 38.130E. A total of 42 % of Meshkinshahr's population resides in urban areas, 58 % lives in rural areas, and a small population has instead adopted a nomadic lifestyle.

Questionnaires were used to record the personal information, clinical data and symptoms of the VL patients admitted to Valiasr Hospital and Kala-Azar laboratory.

Blood sampling

For the blood sampling, the middle finger of the patient was disinfected with 70 % alcohol and then punctured by lancets after the alcohol had dried. The blood samples were then taken to the laboratory in microhematocrit tubes, centrifuged at 3000 rpm for 5–10 min and then had its plasma separated for the DAT.

DAT test

V-shaped microtiter plates were used to perform the DAT. The Leishmania infantum antigens for this study were prepared in the Parasitology Laboratory, School of Public Health, Tehran University of Medical Science (Edrissian et al. 1996). The principal phases of the procedure for preparing the DAT antigen were mass production of promastigotes of leishmania infantum (Iranian strain) in RPMI 1640 medium, supplemented with 10 % fetal bovine serum, trypsinization of the parasites, staining with Coomassie Brilliant blue and fixing with 2 % formaldehyde(Mohebali et al. 2005; el Harith et al. 1989; Harith et al.1986).

At first, serum dilutions were made from 1.800 and 1.3200 titers. Samples with a 1.800 titer were diluted further for an end-point dilution of 1.102400. The negative and positive controls were tested in each plate. The results were read after 12–18 h incubation in a wet room at room temperature. The titer was defined as the highest dilution at which agglutination was still visible in comparison with positive and negative controls. Compact blue dots were scored as negative and large diffuse blue mats as positive (Harith et al. 1986).

Results

In the present study, of the total of 11 confirmed VL cases, 8 (72.7 %) were male and 3 were female (27.3 %). Four (36.4 %) of the patients were under the age of 2, 5 were aged 2–5 (45.5 %) and 2 were aged over 5 (18.1 %). Patients admitted to the laboratory had shown general flulike symptoms, such as anorexia, malaise, fever and diarrhea, as well as typical VL symptoms, such as fever, anemia and splenomegaly. Seven (64 %) of the patients were hospitalized and 4 (36 %) were referred to the laboratory. The patients' clinical manifestations had created the expectation of finding positive DAT results; however, all the samples showed negative reactions. The Pediatricians at Valiasr Hospital of Meshkinshahr insisted on their diagnosis of visceral leishmaniasis. In seven patients, the bone marrow aspiration confirmed the diagnosis. After the occurrence of false negative DAT, the quality of all the laboratory material, equipment, buffers, diluents and antigens was controlled and the DAT was repeated, showing a negative reaction.Then investigate history of patients. All the patients had been administered corticosteroid medications such as dexamethasone or hydrocortisone prior to admission to hospital or laboratory. The third DAT was performed 2–3 weeks after the patients' last administration of corticosteroids. Surprisingly, the test showed positive reactions this time. The titers ranged from 1.800 to 1.6400, with 3 cases (27 %) having a titer of 1.800, 5 cases (46 %) a titer of 1.1600 and 3 cases (27 %) a titer of 1.6400 (Table 1).

Discussion

DAT has been used extensively over the last decade for seroepidemiological studies of VL (Mohebali et al. 2005). Features such as high specificity, sensitivity, simplicity, safety, cost-effectiveness, adaptability to microtiter plates and field applicability have led to the extensive application of DAT in VL endemic regions of Iran (Mohebali 2013; Dehkordi et al. 2011). Researchers have examined the

Table 1 Distribution of the VL patients by gender, age group and titer for anti-Leishmania infantum antibodies 2–3 weeks after the last injection of corticosteroids at Valiasr Hospital of Meshkinshahr, Iran

Characteristic	No.	%	Total (%)
Gender			
Male	8	72.7	11/100
Female	3	27.3	
Age group (in year)			
2	4	36.4	11/100
2–5	5	45.5	
≥6	2	18.1	
Titers of antibody			
1:800	3	27	11/100
1:1600	5	46	
1:640	3	27	

factors affecting the sensitivity and specificity of DAT, including technical factors and the quality of materials, equipment, buffers and antigens used (Akhoundi et al. 2012; Mohebali et al. 2011). Researchers have also examined other patient-related factors, including the stage of the disease such as acute, subacute or chronic phases (Harith et al. 1986). All the patients admitted to Kala-Azar Laboratory had presented typical symptoms of VL, but their DAT had shown an unexpected negative resultss. The accuracy of the DAT performed was verified through the control of the anti-Leishmania antigens and the laboratory materials and equipments. The patients' medical history was carefully taken from their parents. The patients had been administered corticosteroids drugs such as dexamethasone and hydrocortisone prior to admission to Kala-Azar laboratory by other physicians to help relieve symptoms such as malaise, fever, rash and allergy. Corticosteroid medications were assumed to have affected the body's immune system, suppressed the B lymphocytes and inhibited the production of antibodies, thereby leading to the DAT showing false negative reactions.

Both cellular and humoral immunity interfere with immunity to Leishmania; however, cellular immunity is more crucial to immunization while humoral immunity is crucial to the diagnosis of sera. The number of leishmaniasis cases associated with immunosuppressants has been increasing over the past 20 years. HIV immunosuppressive agents, immunosuppressive treatments, organ transplantation and neoplastic diseases increase the risk for the Leishmania-infected to develop visceral diseases (Pittalis et al. 2006). Circulating B lymphocytes are reduced with the administration of glucocorticoids, especially through changing the Ab production patterns; low doses of glucocorticoids, however, appear to have little, if any, effect on the production of antigen stimulated antibodies (Tuon et al. 2007).

Further studies on the subject are recommended to confirm the findings of the present study. Physicians working in endemic regions must be familiar with leishmaniasis and their diagnostic tests and should also avoid the administration of medications such as glucocorticoids prior to performing serological tests.

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References

Absavaran A, Rassi Y, Parvizi P, Oshaghi MA, Abai MR, Rafizadeh S, Mohebali M, Zarea Z, Javadian E (2009) Identification of sand flies of the subgenus Larroussius based on molecular and morphological characters in north western Iran. Iran J Arthropod Borne Dis 3(2):22–35

Akhoundi B, Mohebali M, Edrissian GH, Eslami MB, Keshavarz H, Malekafzali H, Rokni MB (2012) Preparation and evaluation of a glycerol-preserved direct agglutination antigen for long-term preservation: a comparative study of the detection of anti-Leishmania infantum antibodies in human and dog. Asian Pac J Trop Med 5(2):117–120

Azizi K, Rassi Y, Javadian E, Motazedian MH, Asgari Q, Yaghoobi-Ershadi MR (2008) First detection of Leishmania infantum in Phlebotomus (Larroussiuss) major Diptera:Psychodidae) fromIran. J Med Entomol 45:726–731

Dehkordi AS, Rassi Y, Oshaghi M, Abai M, Rafizadeh S, Yaghoobi-Ershadi M, Mohebali M, Zarei Z, Mohtarami F, Jafarzadeh B, Ranjbarkhah A, Javadian E (2011) Molecular detection of Leishmania infantum in naturally infected Phlebotomus perfiliewi transcaucasicus in Bilesavar district, Northwestern Iran. Iran J Arthropod Borne Dis 5(1):20–27

Edrissian GH, Hajjaran H, Mohebali M, Soleimanzadeh G, Bokaei S (1996) Application and evaluation of direct agglutination test in sero diagnosis of visceral leishmaniasis in man and canine reservoirs in Iran. Iran J Med Sci 21:119–124

el Harith A, Slappendel RJ, Reiter I, van Knapen F, de Korte P, Huigen E, Kolk AH (1989) Application of a direct agglutination test for detection of specific anti-Leishmania antibodies in the canine reservoir. J Clin Microbiol 27(10):2252–2257

Harith AE, Kolk AHJ, Kager PA (1986) A simple and economical direct agglutination test for sero-diagnosis and sero-epidemiological studies of visceral leishmaniasis. Trans R Soc Trop Med Hyg 80(4):583–587

Mohebali M (2013) Visceral leishmaniasis in Iran: review of the epidemiological and clinical features. Iran J Parasitol 8(3):348–358

Mohebali M, Javadian E, Yagobi-ershadi Akhavan AA, Hajjaran H, Abai MR (2004) Characterization of Leishmania infection in rodents from endemic areas of the Islamic Republic of Iran. East Meditterr Health J 19:591–599

Mohebali M, Hajjaran H, Hamzavi Y, Mobedi I (2005) Epidemiological aspects of canine visceral leishmaniosis in the Islamic Republic of Iran. Vet Parasitol 129(3–4):243–251

Mohebali M, Edrissian GH, Nadim A, Hajjaran H, Akhoundi B, Hooshmand B, Zarei Z, Arshi S, Mirsamadi N, Manouchehri Naeini K, Mamishi S, Sanati AA, Moshfe AA, Charehdar S, Fakhar M (2006) Application of direct agglutination test (DAT)

for the diagnosis and sero-epidemiological studies of visceral leishmaniasis in Iran. Iran J Parasitol 1(1):15–25

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(2):67–74

Oshaghi MA, Ravasan NM, Javadian E, Mohebali M, Hajjaran H, Zare Z, Mohtarami F, Rassi Y (2009) Vector incrimination of sand flies in the most important visceral leishmaniasis focus in Iran. Am J Trop Med Hyg 81(4):572–577

Pittalis S, Nicastri E, Spinazzola F, Ghirga P, De Marco M, Paglia MG, Narciso P (2006) Leishmania infantum leishmaniasis in corticosteroid-treated patients. BMC Infect Dis 6:177–181

Rassi Y, Abai MR, Oshaghi MA, Javadian E, Sanei A, Rafidzadeh S, Arzamani K (2012a) First detection of Leishmania infantum in Phlebotomus kandelakii using molecular methods in north-eastern Islamic Republic of Iran. East Mediterr Health J 18:387–392

Rassi Y, Sanei Dehkordi A, Oshaghi MA, Abai MR, Mohtarami F, Enayati A, Zarei Z, Javadian E (2012b) First report on natural infection of the Phlebotomus tobbi by Leishmania infantum in northwestern Iran. Exp Parasitol 131:344–349

Tuon FF, Sabbaga Amato V, Floeter-Winter LM, de Andrade Zampieri R, Amato Neto V, Siqueira Franca FO, Shikanai-Yasuda MA (2007) Cutaneous leishmaniasis reactivation 2 years after treatment caused by systemic corticosteroids—first report. Int J Dermatol 46(5):628–630