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Original Article

Visceral Leishmaniasis in Ardabil Province, Northwest of Iran: A Retrospective Epidemiological, Clinical and Paraclinical Study (1985-2018)

Soheila Molaei [1,2], Hamed Zandian [3], Akbar Molaie [4], Telma Zahirian [3], Mehdi Mohebali [5,6], Zabih Zareie [5], Babak Nakhostin [2], Dariush Emdadi [2], Behnaz Akhoundi [5,6], Seyyedeh-Negar Modares-Sadrani [2], Hamed Abdollahzadeh [2], Zahra Rakhshidan [2], Ezati Ezati [2], Zahra Heidari [7]

1. *Zoonoses Research Center, Ardabil University of Medical Sciences, Ardabil, Iran*
2. *Deputy of Health, Ardabil University of Medical Sciences, Ardabil, Iran*
3. *Social Determinants of Health Research Center, Ardabil University of Medical Sciences, Ardabil, Iran*
4. *Shahid Madani Hospital, Tabriz University of Medical Sciences, Tabriz, Iran*
5. *Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
6. *Center for Research of Endemic Parasites of Iran, Tehran University of Medical Sciences, Tehran, Iran*
7. *Department of Medical Parasitology and Microbiology, Ardabil University of Medical Sciences, Ardabil, Iran*

***Corresponding Author:** Email: s.molaei@arums.ac.ir

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Abstract

Background: We aimed to investigate the epidemiological, clinical and paraclinical aspects of Visceral Leishmaniasis in Ardabil Province, from 1986 to 2018.

Methods: The disease has been diagnosed by a serological validate test as Direct Agglutination Test, parasitological methods, and clinical manifestations. The data were collected and then analyzed.

Results: Overall, 2824 out of 69007cases (4.1%) had anti-*Leishmania* antibodies at titers≥1:3200. The majority of VL cases belonged to Meshkin-Shahr, and then Germi County. The percentages of both male and female cases were 52.8 % and 46.6%, respectively. Approximately 78.1% of cases were less than 5 yr old. The high incidence rate of the disease was recorded in the years 1987 and 2003. 48.6%, 3.2%, and 1.9% of the patients had low titer (1:3200), high titer (1:102400), and suspicious titer (1:1600), respectively. Out of 1200 bone marrow aspiration, 23% were positive, and 77% were negative. Fever, paleness, and hepatosplenomegaly were the main symptoms in patients. Moreover, abdominal swelling, edema, weight loss, lymphadenopathy and jaundice were some other symptoms in the patients. Among biochemistry factors, the levels of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, triglyceride and cholesterol have been increased. The death rate was estimated to be 2.8%, mainly related to the Ardabil and Germi cases.

Conclusion: Kala-Azar is still one of the most important infectious diseases in Ardabil Province, and is responsible for considerable death rate in infants. More attention should be done to parasite control and early diagnosis.

Keywords: Visceral leishmaniasis; Epidemiological; Clinical; Paraclinical; Iran

Introduction

Leishmaniases are a group of zoonotic infectious diseases caused by the *Leishmania* genus, which is an obligate intracellular parasitic protozoon. Over 20 *Leishmania* species, known to be infective to humans, cause different clinical effects; self-limited cutaneous lesions to most severe systemic visceral form among them, known as Kala-Azar. Overall, 700000 to 1 million new cases and some 26000 to 65000 deaths occur annually due to VL infection. In 2017, 98 countries reported endemic for leishmaniasis; however, over 90% of global VL cases were recorded in seven countries, including: Brazil, Kenya, Somalia, India, Ethiopia, Sudan and South Sudan (1). Iran is one of the endemic countries for zoonotic, and anthroponotic cutaneous leishmaniasis (CL) and zoonotic visceral leishmaniasis (VL). The main reservoir host of VL in Iran is dogs, though cats are considered as the main reservoir in feline VL (2,3). Since the reservoirs are mostly in villages, VL is more common in rural than urban areas (4).

In the Mediterranean Basin, especially in Iran, *L. infantum* is the agent of VL, which can lead to the infants' infection and consequence fatality in case untreated. *L.donovani* is the agent of anthroponotic VL, not reported in Iran, although reported in vectors (5). VL has been recorded sporadically across the country; however, East Azerbaijan and Ardabil in the northwest, Fars in the south, Lorestan in the west, Khuzestan in the southwest, and North Khorasan in the northeast are the main infected regions (4, 5-7). The clinical symptoms of VL are different in each patient, which in turn differ from asymptomatic to restricted infection and lethal forms (8,9).

The control of the disease is based on epidemiological figures in endemic cities, identified by parasite strain, clinical form, and environmental parasite distribution (8, 10).

The main purpose of this study was to clarify the epidemiological condition, clinical and paraclinical features of VL in Ardabil as one of the main infected regions in Iran during 1985-2018.

Materials and Methods

The study area, data accessibility and analysis

Ardabil Province is located in a prairie with the same name, between Talesh and Sabalan Mountains in the northwest of Iran. The center of the province is Ardabil City, and according to the latest administrative divisions, it includes 10 cities (Ardabil, Bilasavar, Parsabad, Aslandoz, Khalkhal, Sareyn, Givi, Nir, Namin and Meshkin-Shahr) (Fig.1).

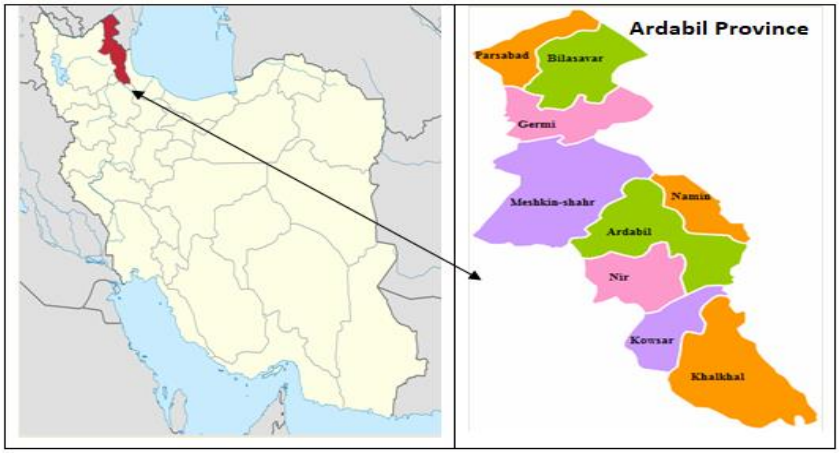

Fig. 1: The Geographical location of Ardabil Province in Iran map and its cities

In the present retrospective study, the data over three decades, from 1985 to 2018, were collected from the Disease Management Center of Ardabil University of Medical Sciences, Ministry of Health Communicable Disease Unit, Leishmania laboratories, and Hospital records. In the present research, the following factors as laboratory (serological and parasitological), demographic (age and gender), geographical (place of residence and season) and sampling (active or passive) were considered. The hematological and biochemical parameters, including White Blood Cell (WBC), Hematocrit (HCT), Hemoglobin (HB), Platelet (PLT), Reticulocyte count (RETIC%), Prothrombin time (PT), Partial Thromboplastin time (PTT), Erythrocyte Sedimentation Rate (ESR), C Reactive protein (CRP), Blood sugar (BS), Blood urea nitrogen (BUN), Creatinine (Cr), Triglyceride (TG), Cholesterol (CHOL), High-density lipoprotein (HDL), Low- density lipoprotein (LDL), Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Bilirubin total (BILI T), Bilirubin direct (BILI D), Albumin (Alb), Albumin/Globulin (Alb/Glb), Sodium (Na), Potassium (K), Calcium (Ca), and Phosphor (P) were surveyed.

The data analysis was performed using Microsoft Excel 2013 and SPSS software (ver. 26.0, Chicago, IL, USA). *P*-value of <0.05 was considered as significant for the evaluation in descriptive statistics and Chi-square tests.

Results

Epidemiological findings of the VL cases

Over the years between 1985 and 2018, 69007 samples were screened for Kala-Azar in Ardabil Province, Iran. Overall, 2824 samples were positive and confirmed by serological or parasitological tests. The highest number of samples and cases were recorded in Meshkin-Shahr, Germi, and then Ardabil with seroprevalence of 65%, 31% and 3.5%, respectively. During 2014-2018, 13 further cases (0.46%) were reported in Parsabad County. The average seroprevalence of the disease was calculated to be 4.1% over 1985-2018. The highest seroprevalence of VL was observed in the years of 1990 and 1991, while the lowest was recorded in 2013 (Table 1). Approximately 85% of cases were from rural areas. The highest reports in urban regions were related to Ardabil City (38%). The percent of active and passive samples were 60.4% and 39.6%, respectively. The highest rate of active sampling was from Moghan and Meshkin-Shahr. No active sampling was reported in Ardabil City so far, and all of the cases were related to the hospitalized patients (Table 2).

Out of 2824 VL cases, 1628 (58%) were male, and 1196 (42%) were female. In Meshkin-Shahr and Germi, most cases were males; however, in Parsabad, all cases were females except one case. Regarding to the age aspect, 26.14% of the patients were under 1-year-old, while 51.99% were 1- 5 yr old. The monthly distribution of the disease showed that the most distribution records were in February, then in Apr and Oct. However, the lowest incidence of VL was recorded in Jun and July. Since the population census of the considered areas was not recorded exactly in the years during 1985-2000, and those data were not reliable, the incidence of the disease was calculated after 2000. The annual average incidence of VL was 0.63 per 10,000. As shown in Fig. 2, the number of VL cases increased during 1985-1998; however, it declined except in 2002. The peak incidence of VL was observed in 2004 with 2.41 cases per 10,000.

Table 1: The distribution of approved VL cases in Ardabil Province during 1985-2018

City / *Year*	*Total No cases/No DAT (+)/Percent (%)*	*Pars Abad No cases/No DAT (+)/Percent (%)*	*Moghan District (Bila savar, Germi) No cases/No DAT (+)/Percent (%)*	*Meshkin- Shahr No cases/No DAT (+)/Percent (%)*	*Ardabil No cases/No DAT (+)/Percent (%)*
1985-86	2855/109/(3.81)	0/0/(17)	1300/10/(9.2)	1555/99/(90.8)	0/0/(17)
1987	3120/183/(5.86)	0/0/(17)	1520/24/(13.1)	1600/159/(86.9)	0/0/(17)
1988	2872/218/(7.59)	0/0/(17)	958/27/(12.4)	1259/190/(87.1)	655/1/(0.5)
1989	3227/199/(6.14)	0/0/(17)	1120/52/(26.3)	1440/145/(72.8)	667/(2/1)
1990	3925/372/(9.47)	0/0/(17)	1455/166/(44.6)	1785/198/(53.2)	685/8/(2.2)
1991	2910/410/(14)	0/0/(17)	1150/168/(41)	1240/230/(56.3)	520/11/(2.7)
1992	3300/227/(6.87)	0/0/(17)	1600/105/(46.2)	1200/115/(50.7)	500/7/(3.1)
1993	2253/128/(5.68)	0/0/(17)	955/56/(43.7)	920/70/(54.7)	378/2/(1.6)
1994	2069/88/(4.25)	0/0/(17)	820/22/(25)	960/65/(73.9)	289/1/(1.1)
1995	2166/72/(3.32)	0/0/(17)	987/25/(34.7)	969/45/(62.5)	210/2/(2.8)
1996	1944/66/(3.39)	0/0/(17)	910/29/(43.9)	845/35/(53.1)	189/2/(3)
1997	1984/59/(2.97)	0/0/(17)	977/9/(15.3)	829/47/(79.7)	178/3/(5)
1998	1979/111/(5.32)	0/0/(17)	820/29/(26.1)	990/78/(70.3)	169/4/(3.6)
1999	2048/111/(5.41)	0/0/(17)	950/47/(42.3)	920/61/(55)	178/3/(2.7)
2000	2164/45/(2.13)	0/0/(17)	955/4/(8.9)	1020/40/(88.9)	189/1/(2.2)
2001	2047/81/(3.95)	0/0/(17)	966/37/(45.6)	889/42/(51.9)	192/2/(2.5)
2002	2069/77/(3.72)	0/0/(17)	929/12/(15.6)	950/51/(66.2)	190/14/(18.2)
2003	1961/32/(1.63)	0/0/(17)	901/1/(3.1)	880/28/(87.5)	180/3/(9.4)
2004	2164/50/(2.31)	0/0/(17)	972/17/(34)	970/22/(44)	222/11/(22)
2005	1966/20/(1.02)	0/0/(17)	897/3/(15)	810/17/(85)	259/0/(17)
2006	1897/14/(0.74)	0/0/(17)	898/2/(14.3)	820/11/(78.6)	179/1/(7.1)
2007	1666/16/(0.96)	0/0/(17)	610/3/(18.8)	900/11/(68.7)	156/2/(12.5)
2008	1731/25/(1.44)	0/0/(17)	622/13/(52)	930/10/(40)	179/2/(8)
2009	1811/25/(1.38)	0/0/(17)	672/4/(16)	855/20/(80)	284/1/(4)
2010	1623/14/(0.86)	0/0/(17)	466/2/(14.3)	902/10/(71.4)	255/2/(14.3)
2011	1598/12/(0.75)	0/0/(17)	519/2/(16.65)	837/8/(66.7)	242/2/(16.65)
2012	1761/9/(0.51)	0/0/(17)	629/1/(11.1	867/7/(77.8)	265/1/(11.1)
2013	1742/3/(0.17)	0/0/(17)	759/1/(33.3)	735/2/(66.7)	248/0/(17)
2014	1513/9/(0.56)	12/2/(22.2)	684/2/(22.2)	655/4/(44.5)	162/1/(11.1)
2015	1206/9/(0.75)	15/3/(33.3)	457/0/(17)	559/4/(44.5)	175/2/(22.2)
2016	1313/14/(1.06)	10/4/(28.6)	498/0/(17)	618/4/(28.6)	187/6/(42.8)
2017	1051/8/(0.75)	5/2/(25)	355/0/(17)	539/3/(37.5)	152/3/(37.5)
2018	1072/8/(0.78)	12/2/(25)	458/2/(25)	436/4/(50)	166/0/(17)
Total	69007/2824/(4.1)	54/13/(0.46)	28769/876(31)	31684/1835/(65)	8500/100/(3.53)

Table 2: The frequency of VL according to DAT titer, gender, sampling type, and location distribution in Ardabil Province during 1985-2018

City Year	Total No cases/No DAT (+)/Percent (%)	Pars Abad No cases/No DAT (+)/Percent (%)	Moghan District (Bila savar, Germi) No cases/No DAT (+)/Percent (%)	Meshkin- Shahr No cases/No DAT (+)/Percent (%)	Ardabil No cases/No DAT (+)/Percent (%)
1985-86	2855/109/(3.81)	0/0/(17)	1300/10/(9.2)	1555/99/(90.8)	0/0/(17)
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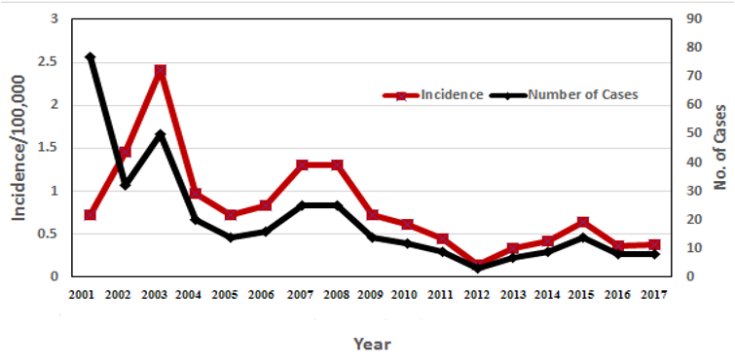

Fig. 2: The incidence of VL cases per 10 000 humans, 1985-2018, Ardabil Province

Serological and Clinical findings of the VL cases

In the present study, DAT was positive with a lowest titer (titer of 1:3200), highest titer (titer of 1:102400) and suspicious titer (1:1600) in 48.6%, 3.2% and 1.9% of the cases respectively. These cases became positive after 2-3 wk with a titer of 1:3200. Out of 1200 cases with an aspiration of bone marrow, 277 cases (23%) were positive, and 77% were negative (Table 2).

The clinical symptoms, such as fever (92.3%), paleness (90%), hepatosplenomegaly (78.9%), anemia (67%), abdominal swelling (59%) and edema (46%) were the most observed signs in the studied patients. The splenomegaly and splenomegaly were individually seen in 47% and 17% of the patients, respectively (Fig. 3). The statistical analysis revealed that there is a positive relationship between DAT titer and clinical signs (P=0.004).

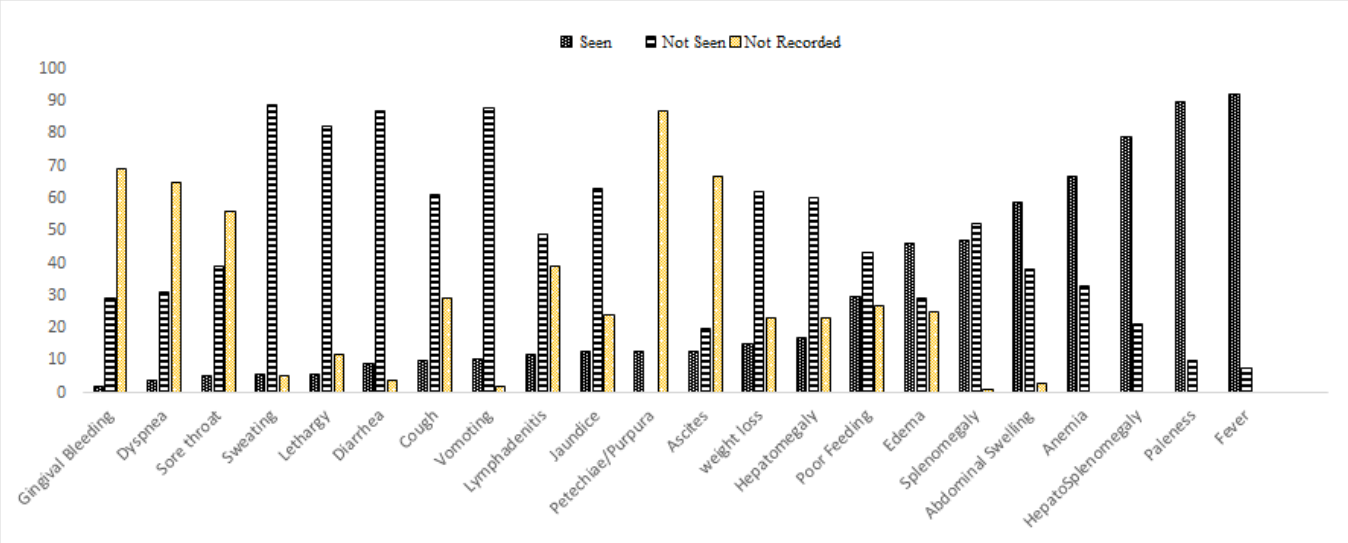

Fig. 3: The clinical signs (%) of VL patients in Ardabil Province during 1985-2018

Hematological and biochemical findings of the VL patients

In the present study, hematological parameters including WBC, HCT, HB, PLT, RETIC%, PT, PTT and ESR were collected. CRP as an essential inflammation factor was considered in this section of the study.

As shown in Fig.4, the results revealed abnormal levels of HCT, HB and WBC in 59% of the patients (53% with decreased and 6% with increased). Thrombocytopenia was observed in 70% of the patients, while thrombocytosis was reported in 5% of them. Furthermore, 52% and 60% of the considered cases showed abnormal PT and PTT results, respectively. In peripheral blood smear of the patients, lymphocytosis, neutropenia, anisocytosis, poikilocytosis, polychromasia, and tear-drop cells were also recorded (Data not shown).

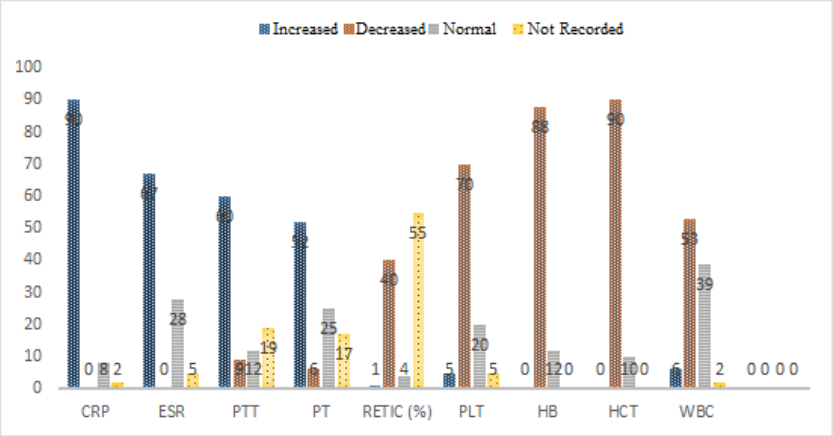

Fig. 4: Hematological features (%) of VL patients in Ardabil Province during 1985-2018.
WBC: White Blood Count, HCT: Hematocrit, HB: Hemoglobin, PLT: Platelet, RETIC (%): Retic Count, PT: Prothrombin time, PTT: Partial thromboplastin time, ESR: erythrocyte sedimentation rate, CRP: C Reactive Protein

The biochemical analysis showed that the levels of liver enzymes, AST and ALT, were abnormal in 79% and 45% of the cases, respectively. The level of LDH was abnormal in 47% of the VL-infected cases (Fig.5).

The highest death rate (~3%) was recorded in Ardabil and Germi Cities (Data not shown).

There was statistical association between clinical symptoms like splenomegaly (P=0.003), hepatomegaly (P=0.004), hematological parameters like WBC (P=0.001), PT (P=0.001), PLT (P=0.001), biochemical parameters like LDH (P=0.001) and Bilirubin (P=0.003) with titer of DAT.

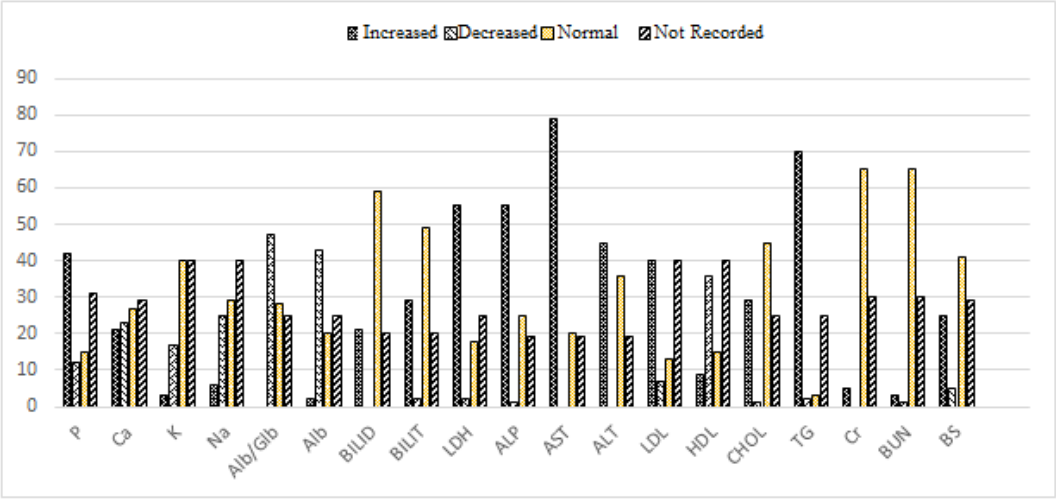

Fig. 5: Biochemical features (%) of VL patients in Ardabil Province during 1985-2018.
BS: Blood Sugar, BUN: Blood Urea Nitrogen, Cr: Creatinine, TG: Triglyceride, CHOL: Cholesterol, HDL: High-density lipoprotein, LDL: Low- density lipoprotein, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase, BILI T: Bilirubin total, BILI D: Bilirubin direct, Alb: Albumin, Alb/Glb: Albumin/Globulin, Na: Sodium, K: Potassium, Ca: Calcium, P: Phosphor

Discussion

During 1985-2018, 2824 cases of VL were recorded in Ardabil Province. According to the previous studies (6, 7, 8), the disease in Ardabil Province was mainly spread in Meshkin-Shahr and Germi. In the present study, Ardabil was found as the main infected City after Meshkin-Shahr from 2009 to 2018. Parsabad is estimated to be another focus of VL infection in the future. It is the border of Armenia and Azerbaijan, which are the main focus for humans, canine VL and vectors (6, 11). Furthermore, the increased travel to Azerbaijan and Armenia countries was an important factor in the distribution of this disease in the studied areas.

According to the current study, two incidence courses were observed: From 1985 to 2012, Meshkin-Shahr had the highest incidence rate; however, from 2013 to 2018, Ardabil had the highest incidence. Additionally, the new foci were observed (12). In this study, four positive cases were reported in Namin, a city located in the west of Ardabil (Data not shown). These patients were hospitalized in Ardabil, and medical history represented that they commute to the endemic areas such as Meshkin-Shahr. However, the recent situational survey of VL showed that Namin has been considered as a new endemic area (12). Seroprevalence of VL varies up to 1%-25% in different areas of Iran (13, 14). The seroprevalence of VL was reported to be 5.1% in 2012 in Ardabil Province; however, the present study confirmed that this figure decreased by 1% (6). Other retrospective studies also indicated that VL has been recorded in other regions of Iran, including Shiraz, Bushehr, and East Azerbaijan (4, 6, 8, 15). During 1986-2009, the rate of disease was more in males than females; however, this rate was equal in both genders in the years between 2010 and 2018. The possible reasons for this issue are 1) the increased population of females in the studied areas and 2) the raised parental awareness and sensitivity about the female gender. In Iran and some other countries, the sex ratio of VL was reported 2:1 (M: F)(9,16).

Analysis of the data showed that approximately 80% of VL cases were 1 to 5-year-old. This is probably related to 1) the great effect of disease agent (*L.infantum*) on infants, 2) the immunity condition of infants and their different immune responses, and iii) the easy accessibility of their body to sandfly bites. It is valuable to consider that *L. major* or *L. tropica* has been recently recorded in the south and central regions of Iran (5).

The dominant clinical signs of VL in this study were nocturnal fever, hepatosplenomegaly, paleness, and weakness. Moreover, anemia, leukopenia with lymphocytosis, leukocytosis, and thrombocytopenia were also observed in some cases. Most VL patients had anisocytosis, poikilocytosis, polychromasia, and teardrop cells in their peripheral blood smears. The studies in the northwest of Iran also reported some of these clinical signs and hematological disorders (17,18). Anemia and consequently splenomegaly is the result of red blood cell disruption in an enlarged spleen, hyper splenic, hemolysis, and immune complex formation. The study on iron kinetic, lifetime of red blood cells, and survival evaluation prove that hemolysis is the main cause of anemia in VL (19). The reticuloendothelial systems, especially spleen, are reservoirs for the infected cells in VL, therefore, splenomegaly, and hepatomegaly in VL patients are the result of large masses of parasite residence in splenocyte and hepatocytes (20). Moreover, similar results have been shown in VL patients in other foci of Iran and other countries such as Italy and Brazil (21). Similar to other studies (22, 23), this study showed an increase in ESR, probably associated with the release of acute-phase reactants.

The increased levels of liver enzymes were observed in our study. This increase is the result of granulomatous features in hepatocytes and liver dysfunction, directly related to protozoa itself or indirectly to the effect of the immune response of the parasites. In the present work, the cholesterol level was decreased in many VL cases. Cholesterol plays an important role in cell membrane

raft formation, which is essential for Leishmania parasites entry. The decreased levels of albumin, globulin and total protein serum in VL patients are associated with a high parasite burden (22).

In this study, 98.1% of the patients had positive DAT, and 42.5% of them underwent bone-marrow aspiration, of which 23% had amastigote forms of the parasite in their smears. Many studies have also demonstrated that DAT has high sensitivity and specificity for VL detection compared to the BM aspiration (6, 8, 10, 18). In a study conducted on hospitalized VL patients, 58.3% of the cases had positive BM smears (22).

In a study among 146 children suspected to have VL in Iraq, 91.1% were positive by DAT and 84.9% were proved by the examination of BM aspirate. The children less than 2 yr of age were mainly affected (74.3%). In addition, the highest frequency of the disease (18.8%) was recorded in July (23). During 2004-2008, annual cases of VL in Iraq were reported to be 1,711, while 3,481 and 34,918 of VL cases were confirmed in Brazil and India, respectively (24). Another study reported the epidemiological status of six geographic endemic regions of Brazil, East Africa, Nepal and India in the years between 1997 and 2009. The ratios of male/female were calculated to be 1.9%, 1.6%, 1.4%, and 1.4% for Brazil, Sudan, Nepal and India, respectively. The percentage of VL patients who were under 5 and 15 yr old was estimated to be 47.6% and 59.2% in Brazil, 30.0% and 69.2% in Sudan, 6.4% and 33.5% in Nepal, and 10.2% and 48.5% in India (25). In Brazil, during 2001-2014, 47,859 VL was registered. The majority of identified VL cases (70%) in Brazil was related to the urban regions and their ages were under 4 yr old; however, the incidence of VL in adults (more than 40 yr) increased (26).

The annual cases of VL in Ethiopia were 3700–7400 caused by *L. donovani* (27). Similar to the present study, fever, hepatosplenomegaly and anemia are the dominant symptoms of VL infection. In addition, leukopenia, and bone marrow suppression were found in some patients (27).

In this study, the mortality rate was near 3% over the studied period. Jaundice, bacterial infections, dyspnea and cardiologic complications have been reported the cause of death. The age of fewer than 2 yr, anemia, hemoglobin <6 g/dL, splenomegaly, and secondary infection were the main reasons for mortality (28).

Economic status, environmental changes such as deforestation, the building of dams, and irrigation schemes are the main factors in the incidence and prevalence of VL disease. Moreover, asymptomatic and symptomatic reservoirs (29), as well as the frequency of sand flies (30) are important factors in the sustainability of VL infection in the region.

Conclusion

The VL incidence rate has been decreased and controlled over the recent years in Ardabil Province because of conducting control strategies. However, the appearance of new cases from new foci necessitates special strategies and control methods to be implemented in this region. Moreover, new and extensive epidemiological studies on humans, reservoirs, vectors and ecology must be performed to provide more details about the condition for plans.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication, etc.) have been completely observed by the authors. Ethical approval ID: IR. ARUMS.REC. 1398.388).

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Conflict of interest

The authors declare that there is no conflict of interest.

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