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**Original Article**

# Clinical and Laboratory Findings of Visceral Leishmaniasis in Children Hospitalized in Mashhad, Northeastern Iran: A Twenty-Year Retrospective Study

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***Abstract***

***Background:*** Over the last decade, a few cases of visceral leishmaniasis (VL) have been reported in some provinces of northeastern Iran. We aimed to investigate clinical and laboratory findings of VL among children who admitted to the pediatric ward in a referral hospital in Mashhad, northeastern Iran.

***Methods:*** A retrospective study, between 1997 and 2017, was performed on the data sheet registered for children with confirmed VL at the referral Emam Reza Hospital in Mashhad. Hematological and biochemical profiles of the patients were analyzed.

***Results:*** A total of 35 children with VL, confirmed by the presence of amastigotes of *Leishmania* in Giemsa stained smears of the bone marrow, had been recorded through 20 yr. The mean age of patients was 3.7±4 yr. The majority of the patients suffered from hepatosplenomegaly (100%, n=35/35), followed by prolonged fever and pallor (91%, n=32/35), weight loss (85%, n=30/35). The main laboratory findings were anemia (94.1%), leukopenia (52.9%) and thrombocytopenia (70.5%). Almost one-third (37.1%; 13/35) of VL patients inhabited in rural areas of the Bojnoord district as a known VL endemic focus in northeastern Iran.

***Conclusion:*** Our preliminary data showed that the origin of VL is still in some districts other than Mashhad, where VL just will be diagnosed.

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## Introduction

Visceral leishmaniasis (VL) also, named kala-azar is a neglected protozoan disease that transmitted by female *Phlebotomus* as the biological vector. Kala-azar (black fever) is the zoonotic infection in humans in Iran. *Leishmania infantum* is the main etiological agent of VL in Mediterranean regions such as Iran (1,2 ). An estimated 50,000 to 90,000 new cases and about 26,000 to 65,000 deaths occur each year worldwide (2). VL is principally endemic in the Northwestern and Southern areas of Iran (1). In addition, over the last decade, several cases were recorded from northeastern Iran, mainly from Bojnoord as the capital of North Khorasan Province (3). The classic clinical features of VL include fever, anemia, ascites, hepatosplenomegaly, weight loss, jaundice (4). Laboratory findings include anemia, thrombocytopenia, pancytopenia, hypergammaglobulinemia, hypoalbuminemia, neutropenia, hyponatremia and elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (5).

VL should be differentiated from all types of leukemia, lymphoma and myeloproliferative disorders and some infectious diseases such as malaria and typhoid (6). The diagnosis of human VL is frequently defined by aggregating clinical signs with parasitological (such as bone marrow aspiration examination), or serological tests (such as direct agglutination test and or rapid diagnostic tests) (1,7).

Little information is known about originating of VL patients in Mashhad as the capital of Khorasan Razavi, northeastern Iran, thus the main purpose of our study was to investigate retrospectively clinical and laboratory findings of VL in children hospitalized in Mashhad, throughout a twenty-year study.

## Materials and Methods

A retrospective study was performed on hospitalized patients during 20 yr with 35 VL patients consecutively admitted to the Emam Reza Hospital, Mashhad, Khorasan-e-Razavi, Iran. All patients were confirmed by the presence of amastigotes in Giemsa stained smears of bone marrow. Clinical and laboratory parameters of the patients were recorded from a patient's medical records. Gender, age, WBC, RBC, HGB, ESR, Hct, PT, PLT, AST, ALT, Na, K, BUN, PR were evaluated.

This study was approved by the Ethic Committee of Mashhad University of Medical Sciences (IR.MUMS.FM.REC.1396.350). For this type of study, formal consent is not required.

The descriptive data analysis was performed throughout the distribution of frequency, mean and confidence interval of 95% (95% CI).

## Results

Bone marrow aspiration revealed the presence of *Leishmania* amastigotes in all patients. The mean age of patients was 3.7±4. Twenty-four (68%) of the patients were male and others 11 (32%) were female. The male to female ratio was 2.2 and there was no statistically significant difference in gender. All patients had hepatosplenomegaly with one fatal outcome in our patients. The fever did not exist in all patients. The clinical manifestation of all patients in this study was summarized in Table 1. We do not have any information about the duration of the disease before VL diagnosis in our patients.

**Table 1:** Clinical manifestation of pediatric visceral leishmaniasis in Mashhad, northeastern Iran, during 1997-2017

| *Clinical manifestations* | *NO. of patients* | *Percentage (%)* |
|---|---|---|
| Hepatosplenomegaly | 35 | 100 |
| Prolonged fever | 32 | 91 |
| Pallor | 32 | 91 |
| Weight loss | 30 | 85 |
| Sweat | 26 | 74 |
| Jaundice | 21 | 60 |
| Anorexia | 17 | 48 |

Almost one–third (37.1%; 13/35) of VL patients lived in rural and or nomadic areas of Bojnord district as a known VL endemic focus in northeastern Iran.

Confidence interval obtained for HGB: (95% CI, 1.78 to 3.38), Hct: (95% CI, 2.89 to 10.41), ESR: (95% CI, 63.31 to 16.73), AST: (95% CI, 104.58 to 10.933), Na: (95% CI, 3.59 to 8.632), BUN: (95% CI, 4.79 to -15.74). Laboratory findings of VL patients were summarized in Tables 2 and 3.

**Table 2:** Biochemical parameters of pediatric visceral leishmaniasis in Mashhad, northeastern Iran, during 1997- 2017

| *Parameters* | *Patients (N=35)* |
|---|---|
| BUN (mg/dl) | 7.87±2.74 |
| AST(IU/L) | 40.5(23-117) |
| ALT(IU/L) | 21.25±13.31 |
| Na(mEq/L) | 135.82±3.12 |
| K(mEq/L) | 4.40±0.69 |

**Table 3:** Hematological parameters of pediatric visceral leishmaniasis in Mashhad, northeastern Iran, during 1997- 2017

| *Parameters* | *Patients (N=35)* |
|---|---|
| HGB (g/dl) | 8.56±1.60 |
| Hct(g/dL) | 26.25±6.27 |
| ESR (mm/hr) | 52(18.5-80.50) |
| RBC ($10^6$cells/µL) | 3.58±0.72 |
| WBC ($10^3$cells/µL) | 3643.60±2575.39 |
| PLT ($10^3$cells/µL) | 131040±102826 |
| PT(Sec) | 13.20(12.93-17.50) |

HGB hemoglobin, Hct hematocrit, ESR erythrocyte sedimentation rate, RBC red blood cell, WBC white blood cell, PLT platelet, PT prothrombin time. The numerical values are presented as means± SD.

## Discussion

In this hospitalized based study, clinical, hematological and biochemical features of 35 VL patients were investigated for twenty years in Mashhad, northeastern Iran. It is noteworthy that Imam Reza Hospital is an only VL referral hospital in Khorasan Province where all cases of VL are admitted here.

During the last 20 yr, the incidence rate of hospitalized VL patients was decreased in comparison with the last decade in Northeastern Iran. One hundred four VL patients have been reported (92% were less than ten yr old) in Khorasan Province between 1982- and 1996 (3). It seems that the municipal program to control the stray dog population has been influenced on reducing the number of affected subjects.

In Mashhad, a study showed that 1.04% (2/192) of stray dogs were infected by *L. infantum* (8). In addition, the seroprevalence of VL among dogs and humans was reported 7.9%, 2.4% respectively in this area (1). In another study in northeastern Iran, the seroprevalence of VL was recorded 31/3798 (0.8%) by direct agglutination test (DAT) (1). The incidence rate of human infection in northwestern Iran is 2.8% as the highest rate record (9). *Ear-*

*ly and accurate diagnosis of VL generally increases the chances for successful treatment, better care and helps avoid misdiagnosis, which may lead to death* (10).

Approximately 99% of VL cases in Iran occur under 12 yr old with a mean age of 4 yr (1). In this study, the minimum and maximum age group range was four months and 20 yr, respectively, with 92% of cases under six yr.

Most studies, similar to our study, showing that males are at higher risk for VL as compared to females with a ratio of about 2:1 (1,11). It seems that more outdoor activities and sex-associated hormone in males to be linked with this phenomenon (12). The mortality rate of VL in Iran was reported from 2.8% to 5.3% (1) while, in our study was one out of 35 cases (4%).

Any liver infection, specific or non-specific can elevate the level of ALT and AST. However, ALT and AST were evaluated in most VL patients in the world because amastigotes invade the liver and cause hepatocyte damage. It seems that AST is the more reliable item for confirmation of VL and it is elevated in more cases in comparison with ALT (5, 13). In this study, ALT was not given as a clue reliable laboratory test in the diagnosis of VL. It proposes to measure the serum albumin factor evaluation instead of ALT measurement. Because of hypoalbuminemia was announced as the forecasters of death in VL patients with an odds ratio of 6.4 (11).

Decreasing Na serum level has been described as the reliable diagnostic laboratory item in VL patients in comparison with K, Ca, Cl and Mg electrolytes (13). Hyponatremia is presented as one of the mortality risk factors in human VL (14). Hyponatremia in 94.6% and hypokalemia in 26% of VL patients were reported. In this study, Na level decreased in all patients and, it is strong significantly associated with VL infection. Hyponatremia in all fifty-five of VL patients was seen significantly compared to twenty normal individuals (15). Although biochemical tests showed normal Na serum level in 2.5 yr- old boy who infected by *L. infantum* (2). K serum level measurement is not as an authentic laboratory test in the diagnosis of VL in agreement with our results (13).

Pancytopenia was introduced as an important laboratory finding in VL patients (16). None of the patients was immunocompromised in our study, and interestingly RBC, WBC, and Platelet counts were decreased significantly compared with the healthy group. Thrombocytopenia was reported as predictors of death in VL patients (17). Thrombocytopenia and anemia, both in 80.4% and leucopenia in 43.1% were recorded in Italian patients infected with *L. infantum* (18). We found a significantly higher level of erythrocyte sedimentation rate (ESR) among patients probably because of releasing of acute phase reactants (5).

Evidence-based studies confirmed VL in Iran frequently occurs in nomadic populations (19). Moreover, some shreds of evidence showed that the majority of patients infected to VL in nomadic areas of Bojnoord, North Khorasan Province, where VL is endemic (3, 20).

Some limitations of our study are as follows, all patients were confirmed microscopically using bone marrow aspiration, but unfortunately, there are not available bone marrow abnormality patterns. Moreover, another limitation is a small sample size and lack of registered comprehensive data on the patient's sheet.

## Conclusion

Originating of VL is still in some districts other than Mashhad. As a whole, clinicians should be aware of reliable and available laboratory tests such as microscopic examination of bone marrow smears in VL patients particularly in a none-endemic area.

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## Conflict interest

Non-declared.

## Abbreviation

VL= Visceral leishmaniasis, ESR= Erythrocyte sedimentation rate, ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, WBC= White blood cells, RBC= Red blood cells, HGB= Hemoglobin, Hct= Hematocrit, PT= Prothrombin time, PLT= Platelet, Na= Sodium, K=Potassium, BUN= Blood urea nitrogen, RR= Respiratory rate.

## References


1. Mohebali M. Visceral leishmaniasis in Iran: Review of the Epidemiological and Clinical Features. Iran J Parasitol. 2013; 8(3): 348-58.
2. Leishmaniasis. Fact sheet [Internet]. Geneva: World Health Organization; 2019 (https://www.who.int/news-room/fact-sheets/detail/leishmaniasis, accessed 14 March 2019).
3. Alavi S, Fata A, Modarresi A. Retrospective study of visceral leishmaniosis during 14 years in Mashhad University of Medical Sciences. Univ Med Sci J. 2002; 45(75): 41-51.
4. Sah SP, Rijal S, Bhadani PP, et al. Visceral leishmaniasis in two cases of leukemia. Southeast Asian J Trop Med Public Health. 2002; 33(1): 25-7.
5. Varma N, Naseem S. Hematologic changes in visceral leishmaniasis/kala azar. Indian J Hematol Blood Transfus. 2010; 26(3):78-82.
6. Fakhar M, Asgari Q, Motazedian MH, et al. Mediterranean visceral leishmaniasis associated with acute lymphoblastic leukemia (ALL). Parasitol Res.2008 ;103(2):473-5.
7. Daneshbod Y, Dehghani SJ, Daneshbod K. Bone marrow aspiration findings in kala-azar. Acta Cytol. 2010; 54(1): 12-24.
8. Moghaddas E, Fata A, Zarean M, et al. Investigation of visceral leishmamiasis among 192 dog carcasses killed by road accidents in Khorasan- e- Razavi during 2014- 2016. Iran J Public Health. 2018; 47:1742-1748.
9. Davies C, Gavgani AM. Age, acquired immunity and the risk of visceral leishmaniasis: a prospective study in Iran. Parasitology. 1999; 119(Pt 3): 247-257.
10. Asgari G, Fakhar M, Motazedian M, et al. Viseral leishmaniasis, an alarming rate of misdiagnosis (letter to editor). Iran Red Crescent Med J. 2007; 9(1): 45-47.
11. Gani ZH, Hassan MK, Jassim A-MH. Outcome of Hospitalized Children with Visceral Leishmaniasis in Basrah, Southern Iraq. MJBU. 2008; 26(2): 121-127.
12. Snider H, Lezama-Davila C, Alexander J, et al. Sex hormones and modulation of immunity against leishmaniasis. Neuroimmunomodulation. 2009;16(2):106-13.
13. Sarkari B, Naraki T, Ghatee MA, et al. Visceral leishmaniasis in southwestern Iran: A retrospective clinico-hematological analysis of 380 consecutive hospitalized cases (1999–2014). PloS One. 2016; 11(3): e0150406.
14. Daher EDF, de Sousa Soares D, Parente Filho SLA, et al. Hyponatremia and risk factors for death in human visceral leishmaniasis: new insights from a cross-sectional study in Brazil. BMC Infect Dis. 2017; 17(1):168.
15. Verde FAL, Verde FAAL, Neto AS, et al. Hormonal disturbances in visceral leishmaniasis (kala-azar). Am J Trop Med Hyg. 2011; 84(5):668-673.
16. Asfaram S, Pagheh A, Fakhar M, et al. Case Series of Visceral Leishmaniasis (kala-azar) in Mazandaran and Golestan Provinces, North of Iran. J Mazandaran Univ Med Sci. 2016; 26(144):373-81.
17. de Queiroz Sampaio MJA, Cavalcanti NV, Alves JGB, et al. Risk factors for death in children with visceral leishmaniasis. PLoS Negl Trop Dis. 2010; 4(11): e877.
18. Cascio A, Colomba C, Antinori S, et al. Pediatric visceral leishmaniasis in Western Sicily, Italy: a retrospective analysis of 111 cases. Eur J Clin Microbiol Infect Dis. 2002; 21(4): 277-282.
19. Asgari Q, Fakhar M, Motazedian H. Nomadic kala-azar in South of Iran. Iran J Public Health. 2006;35(3):85-6.
20. Torabi V, Mohebali M, Edrissian G, et al. Seroepidemiological survey of visceral leishmaniasis by direct agglutination test in Bojnoord district, north Khorasan province in 2007. Iran J Epidemiol. 2009 ;4(3):43-50.