

## Visceral Leishmaniasis Hospitalization in Ardabil Province, Northwest of Iran

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**Abstract:** In endemic zones like Iran where *L. infantum* causes visceral leishmaniasis the great majority of cases tend to be children and 25-40% of all infections in Iran occur in Ardabil Province. this study was out in order to identify clinical laboratory presentations and demographic characteristics of infected patients. In this descriptive cross-sectional study all children hospitalized with a kala-azar diagnosis from Ardabil province were enrolled into the study in a given period of time. One hundred and twenty patients were enrolled among whom 10 case were excluded because lack of definitive diagnosis criteria. A questionnaire was filled out for each patient to collect data in three main areas as demographic information, clinical presentation and laboratory findings. Data were entered into the computer and analyzed through SPSS 13 statistical software package. Boys constituted 62 cases (56.4%) and the rest were girls. 62.7 % of cases were under two years of age and 39 % were infants. The highest hospitalization rate was recorded in winter. Only in 37.8% of cases, the disease was diagnosed within one month after symptomatic presentation of disease. There was a previous infection history in 8.5% of cases and a familial history of Kala-Azar in 6.3 % of patients. The main reason for a medical referral was fever (72.5%), abdominal swelling (20%) and abdominal pain (7.5%). The minimum and maximum expected rate of anemia among patients was 84-90%. Thrombocytopenia was found in 60.8% and Leukopenia was observed in 24.8 %. It mean ESR was 60.92±36. There was a 5.5 % mortality and 2.7% relapse or treatment failure in this study. The was a delayed diagnosis in many of the cases. No extra ordinary clinical presentation or laboratory findings was found. A distribution pattern change in endemic zones has occurred.

**Key words:** Kala-azar, visceral leishmaniasis, leishmania infantum, Iran

### INTRODUCTION

The annual occurrence of Human Visceral Leishmaniasis (HVL) cases worldwide is 500,000<sup>[1]</sup>. *Leishmania infantum* infections are responsible for Visceral Leishmaniasis (VL) in at least 70 countries. In most endemic areas, it is widely believed that domestic dogs (*Canis familiaris*) are the principal hosts<sup>[2]</sup>.

Leishmaniasis is an important health problem in the Islamic Republic of Iran. Visceral Leishmaniasis (VL), or kala-azar, is seen sporadically all over the Islamic Republic of Iran and is of the Mediterranean type. In endemic zones like Iran where *L. infantum* causes Visceral Leishmaniasis (VL) the great majority of cases tend to be children<sup>[3-7]</sup>

In Iran according to the reports of Iranian Center for Disease Control, the rate of visceral leishmaniasis has ranged from 0.43 per 100000 in 1998-99 to 0.3 per 100000 in 2002-3, and 25-40% of all infections in Iran occur in Ardabil Province<sup>(5)</sup>. Several serologic tests have been used for Kala-azar diagnosis along with clinical presentations. IFA, ELISA, DAT, LATEX

AGGLUTINATION TEST are some of these all of which are tested for kala-azar diagnosis in Adabil province. DAT test is an easy to use one having a high sensitivity and specificity recommended by world health organization<sup>[8]</sup>.

Considering the importance of visceral leishmaniasis, this study was retrospectively carried out in order to identify clinical laboratory presentations and demographic characteristics of infected patients.

### MATERIALS AND METHODS

In this descriptive cross-sectional study all children hospitalized with a kala-azar diagnosis from Ardabil province were enrolled into the study in a given period of time. The names of all children who were treated or died due to this disease from December 1996 to August 2003 were collected from three hospitals of Ardabil province of Iran namely Ali-Asgar, Sabalan and Arta hospitals. In addition, to make the list complete the names of all children diagnosed to have kala-azar and registered at district health centers were added to the list if led to

hospitalization. 128 patients were eventually identified, among which the medical files of 120 cases were available. Among patients with available files, 10 cases lacked kala-azar diagnosis criteria as parasites in bone marrow aspiration or characteristic clinical symptoms (fever, splenomegaly, cytopenia and weight loss) along with positive Direct Agglutination Test (DAT). So, they were removed from the study either.

It should be mentioned that DAT has a high specificity as much as 100% and in symptomatic persons has a 98% sensitivity and 100% positive predictive value. (6). The minimum normal rate of hemoglobin was considered as 10 g dL<sup>-1</sup> in infants and 11 g dL<sup>-1</sup> in other children in our study.

A questionnaire was filled out for each patient to collect data in three main areas as demographic information, clinical presentation and laboratory findings. Data were entered into the computer and analyzed through SPSS 13 statistical software package. This research was approved in committee of ethics in Azad University of Ardabil.

**Findings:** Boys constituted 62 cases (56.4%) and the rest were girls (43.6%). In this study 62.7 % of cases were under two years of age and 39 % were infants. The age histogram of patients is given in (Fig.1).

The highest hospitalization rate was recorded in winter, during which 43 cases (39%) were hospitalized. Spring, fall and summer were in the next ranks. The hospitalization frequency in different months is given in (Fig. 2).

DAT was carried out on 97 cases and the results of 91 cases were positive. Positive results were found in 26 out of 42 cases having a bone marrow aspiration and biopsy.

Up to 60 % of patients lived in Germe and Bilesavar, while 15.9 and 15 % of patients lived in Meshgin-shahr and Ardabil districts respectively. Others lived in Parsabad and Khalkhal districts. The age of subjects ranged from 4 months to 14 years with a mean two years of age.

The time period between starting disease symptoms and diagnosis varied from 2 to 969 days. In fact, only in 37.8% of cases, the disease was diagnosed within one month after symptomatic presentation of disease. There was a sepsis record in 8.5% of cases and a familial history of Kala-Azar in 6.3 % of patients. The main reason for a medical referral was fever (72.5%), abdominal swelling (20%) and abdominal pain (7.5%). Relative frequency of different signs and symptoms of disease is given in (Table 1).

Hemoglobin mean was about 8 g dL<sup>-1</sup> ±1.9 (Mean±SD). The minimum and maximum expected rate of

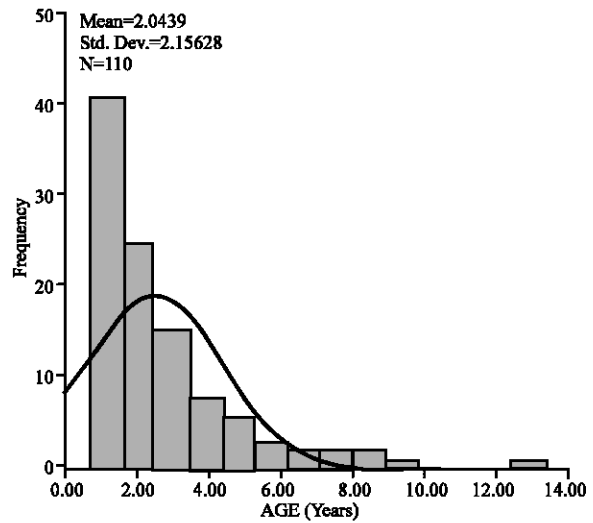


Fig. 1: Age distribution histogram of patients with Kala-Azar

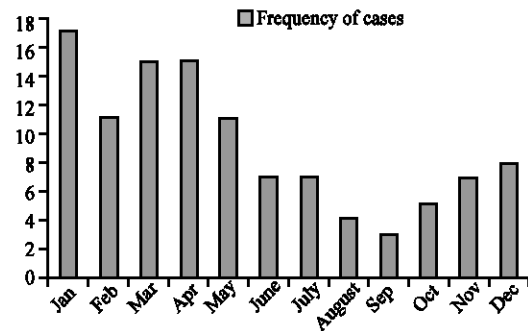


Fig. 2: Monthly distribution of kala-azar hospitalizations

Table1: Relative frequency of different signs and symptoms of Kala-Azar patients

Symptom	Patients with reliable records	Percentage of patients with clinical presentation	Maximum and minimum expected rate
Fever	110	97.3	97.3
Anorexia	70	97.1	61-98
Splenomegaly	110	92.7	92.7
Weight loss	63	93.7	53-96
Clinical anemia	94	85.5	73-88
Heptamegaly	94	57.4	49-63
cough	68	57.4	35-73
Diarrhea	74	35.1	23-56
Bleeding	93	11.8	10-25
Lymphadenopathy	46	8.7	3-82
Edema	93	8.6	7-22
Icteric	94	8.5	7-21
Ascites	94	7.4	6-20

anemia among patients was 84-90%. Thrombocytopenia was found in 60.8% (platelet count below 150,000) with minimum and maximum expected rate of 42-70%.

Leukopenia (white blood cell count below 4000) was observed in 24.8%. The mean ESR was  $60.92 \pm 36$  (Mean  $\pm$  SD). Considering ESR rate over 10 as an abnormal rate for children older than one year and ESR rate over 2 as abnormal for infants, 97.87% of all cases had high ESR rate.

There was a 5.5 % mortality and 2.7% relapse or treatment failure in this study.

## DISCUSSION

Boys in our study constituted 56.4% of Kala-Azar cases. Previous studies have shown that although the disease is more prevalent among boys but girls have higher rates of positive DAT test. That is to say sub clinical forms of visceral leishmaniasis is commoner among girls<sup>[9-11]</sup>. Sharma in his study in India showed that there is no statistically significant sex difference before puberty and after puberty the disease is more common among boys. They attributed their findings to the protective role of female sexual hormones. We think they should have also considered their small sample size and maybe the main cause has been low statistical power of study<sup>[12]</sup>.

In our study 62.7 % of cases were under two years of age and 39 % were infants. Soleimanzadeh a decade prior to our study in meshkinshahr found 17% of patients to be infants. Hasheminasab in his study in Fars province which is the second main endemic area of Kala-Azar in Iran found one third of his patients to be under three years of age. In a study in Malta between 1980 and 1998, 81 diseased children were recorded, whose mean age was 34 months, which is consistent with the results of this study<sup>[13]</sup>. In a study in Turkey, Tanir detected 19 children in a three-year period whose mean age was 36 months and in the study of Cascio in Sicily from 1980 to 2000 on 111 infants which were sequentially hospitalized, the mean age of subjects was 1.7 years<sup>[14]</sup>. No doubt seroepidemiologic studies show a higher mean of age. As in Arshi's study only 2% of cases were seropositive.

In our study, the youngest subject was four months of age. Seemingly, long incubation period, the transfer of acquired immunity from mother to infant in endemic regions and covering the neonate which decreases the chance of the contact between neonate and sand fly contributed to the scarcity of this disease among infants younger than 5 months old.

In this study only in 37.8% of cases, the disease was diagnosed within one month after symptomatic presentation of disease. In a study in Pakistan it was shown that the the mean time between clinical presentation and diagnosis was 45 days<sup>[15]</sup>. On the other hand, in a study Nepal, Singh indicated that the diagnosis delay was over 6 months in 35% of cases<sup>[16]</sup>. This figure

was 42.7 days ( $\pm$  45 days) in the study of Marcia in Brazil<sup>[17]</sup>. Kala-Azar patients may have a big appetite despite weight loss and excessive attenuation, which leads to delay in medical consultation. Also in our study the delay in diagnosis may be associated with the late visit by the physician, because peasants are busy cultivating or harvesting during summer and fall. This assumption strengthens regarding the point that the hospitalization rate was highest in winter and spring. besides, the rate of hospitalization declined from April to June in spring. in a study on 800 patients in Meshkin-shahr, Soleimanzadeh revealed that the highest rate of diagnosis was in January, February, March and April<sup>[18]</sup>.

Kala-Azar is mostly observed among nomads because they are in close contact both with carnivorous as the source and sand fly as the carrier. Therefore, the center of this disease in Iran is Zagros and Sabalan mountains (Meshgin-shahr and Moghan). In this study, over 50% of all patients came from Bilesavar and Germe in Moghan Region which shows a change in disease distribution pattern compared to previous studies which Meshkinshahr had several times more cases than Germe<sup>[6]</sup>. In addition, our findings show that there are new centers of the disease around Ardabil (15% of all patients came from Ardabil). In a study carried out in Somarin of Ardabil, Arshi *et al.* presented a new endemic focus of kala-azar in Iran<sup>[9]</sup>.

From the viewpoint of clinical symptoms, our findings were in line with previous studies and are not discussed more. Secondary infection of skin, respiratory system and middle ear is prevalent among patients with kala-azar. Patients may die due to pneumonia, sepsis, tuberculosis, diarrhea, measles and even anemia and bleeding<sup>[19]</sup>. In this study, diarrhea and cough were reported in 35.1 and 57.4% of cases, respectively. In a study by Marica, infection was totally observed in 10.9% of cases and the most prevalent types were pneumonia, otitis and sepsis which were seen in 66, 18.4 and 8.2% of cases, respectively<sup>[17]</sup>.

In the prospective study of Lita on 50 children in Albania, 26% of cases had secondary infection<sup>[20]</sup>. Mortality rate in our study was 5.5% which is consistent with that of Haidar's study being 5%. In some studies such as Marica the mortality rate was reported as 10.2%<sup>[17]</sup>. It was 2% in the study of Lita which were due to excessive bleeding. The relapse rate was 2.7% in this study. It was 11% in the study of Grech<sup>[13]</sup>.

## CONCLUSION

The was a delayed diagnosis in many of the cases. No extra ordinary clinical presentation or laboratory findings was found. A distribution pattern change in endemic zones has occurred.

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### REFERENCES

1. World Health Organization (WHO), (2000). Leishmaniasis and Leishmania/ HIV coinfection, WHO/CDC/CSR/ISR, pp: 1-2.
2. Gavani S., H. Mohite, G.H. Edrissian, M. Mohebali, and. Davies cr., 2002. Domestic dog ownership in Iran is a risk factor for human infection with leishmania infantum. *Am. J. Trop. Med. Hyg.*, 67: 511-515.
3. Bettini, S., M. maroli and I. Gradoni, 1981. Leishmaniasis in Tuscany (Italy) : IV. An analysis of all recorded human cases. *Transactions of the Royal Society of Trop. Med. Hyg.*, 75: 338-344.
4. Navin, T.R., M. sierra, R. custodio, F. steuerer, C.H. porter and T.K. Reubush, 1985. Epidemiological study of visceral leishmaniasis in Honduras, 1975-1983. *Am. J. Trop. Med. and Hyg.*, 34: 1069-1075.
5. Harith, A. E., A.H.J. kolk, P.A. kager, J. leeuwenburg, F.J. Faber, R. muigai, S. kiugu and J.J. laarman, 1986. A simple and economical direct agglutination test for serodiagnosis and sero-epidemiological studies of visceral leishmaniasis. *Transactions of the Royal Society of Trop. Med. Hyg.*, 80: 593-587.
6. Edrissian, G.H., 1996. Visceral leishmaniasis in Iran and the role of serological tests in diagnosis and epidemiological studies. In *Parasitology for the Twenty First Century* (Ed. Ali Ozcel, M. And Ziyal Alkass, M.); CAB Intl., Wallingford, pp: 63-78.
7. Edrissian, G.H., A. Nadim, A. Alborzi, S. Ardehali, 1998. Visceral Leishmaniasis; The Iranian Experience. *Arch Im Med.*, 1: 22-26.
8. Khorshidian, S.H.H. Ajjaran, M.T. Sarkissian and GH. Edrissian, 1994. Evaluation of ELISA, using intact promastigotes as antigen, For the diagnosis of visceral leishmaniasis. *Iranian J. Med. Sci.*, pp: 19.
9. Arshi, S., M. Mohebali, B. Akhoundi, H. Sadeghi Bazargani, V. Sepehran, Z. Zarei, S. Hajikhani, S.H. Sezavar, 2002. Identification of a new endemic focus of Kala-azar and seroepidemiological study of visceral leishmania Infection in Ardabil province. *J. School of Public Health and Institute of Public Health Res.*, 2: 9-18.
10. Edrissian, G.H., 1990. Kala-azar in Iran. *Med J Islamic Rep Iran.* 4: 235-238.
11. Cambroner Galache, M., 1983 Kala-Azar in childhood. *Anales Espanoles de Pediatria.* 18: 28-32.
12. Sharma, M.C., A.K. Gupta, R. Saran and S.P. Sinha, 1990. The effect of age and sex on incidence of kala-azar. *J. Communicable Dis.*, 22: 277-8.
13. Grech V., J. Mizzi, M. Mangion and C. Vella., 2000. Visceral leishmaniasis in Malta--an 18 year paediatric, population based study. *Arch. Dis. Child. May.* 82: 3 81-5.
14. Cascio, A., C.S. Colomba, M. Antinori, D. Orobello, Paterson and L. Titone., 2002. Pediatric Visceral Leishmaniasis in Western Sicily, Italy: A Retrospective Analysis of 111 Cases. *European J. Clini. Microbiol. Infec. Dis.*, 21: 277-282.
15. Altaf, C., P. Ahmed, T. Ashraf, M. Anwa, and I. Ahmed., 2005. Clinicopathological features of childhood visceral leishmaniasis in Azad Jammu & Kashmir Pakistan. *J. Ayub. Med. Coll. Abbottabad.* 17: 48-50.
16. Singh, K., R. Singh, S.C. Parija, M.M. Faridi and N. Bhatta., 1999. Clinical and laboratory study of kala-azar in children in Nepal. *Trop Pediatr.* Apr. 45: 95-7.
17. Márcia, J.A., G.B. Queiroz, João Alves, B. Jailson, and Correia, 2004. Visceral leishmaniasis: Clinical and Epidemiological features of children in an endemic area. *Jornal de Pediatria*, 80, N 2, pp: 141-146.
18. Soleimanzadeh, G., G.H., A.M. Edrissian, Movahhed-Danesh and A. Nadim., 1993. Epidemiological aspects of kala-azar in Meshkin-Shahr, Iran: human infection. *Bull World Health Organ.* 71: 759-62.
19. Andrade, T.M., E. M. Carvalho and H. Rocha., 1990. Bacterial infections in patients with visceral leishmaniasis. *J. Infect. Dis.*, 162: 1354-1359.
20. Lita, G., F.G. Davachi, H. Sulcebe, Bregu and M. Basha., 2002. Pediatric visceral leishmaniasis in Albania. *Int. J. Infect. Dis.*, 6: 66-68.