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Original Article

Demographic Characteristics besides Clinical and Laboratory Manifestations of Children with Visceral Leishmaniasis in Rasht, Northern Iran

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Abstract

Background: Visceral leishmaniasis (Kala-azar) is an inherently dangerous and progressive disease that can be seen in many parts of the world, including Iran. Therefore, we decided to conduct this study on children with Kala-azar in Rasht-Iran.

Methods: In this descriptive study, the hospital records of all children with Kala-azar disease admitted to 17 Shahrivar Hospital, Rasht- Iran from 2009 to 2021 were reviewed. Required information were collected from patients' records and descriptive statistical analysis was done using SPSS version 24 statistical software.

Results: A total of 22 children with visceral leishmaniasis were admitted to 17 Shahrivar Hospital during the study period. The average age of the children was 2.93 ± 3.32 years. Most of them (59.1%) were boys and lived in the foothills (59.1%). The most common season for children to get the disease was spring (45.5%), and the average duration of the disease until hospitalization was 16.5 ± 13.54 days. In this study, the most common clinical manifestations included fever (90.9%), splenomegaly (77.3%), hepatomegaly (50%), weakness and restlessness (27.3%), and vomiting (18.2%). The most common laboratory findings were anemia (90.9%), leukopenia (59.1%), increased erythrocyte sedimentation rate (ESR) (75%), and increased C-reactive protein (CRP) (75%). 72.7% of the children were treated with liposomal amphotericin and others with glucantime, which were all successful.

Conclusion: The results of our studies were consistent with most studies in Iran and other countries. These findings can help in the diagnosis and management of children with Kala-azar and better control of the disease in the province.

Introduction

Leishmaniasis is one of the world's health problems, especially in tropical and subtropical countries. Visceral leishmaniasis (Kala-azar), or black disease, is a parasitic disease that is caused by different species of protozoa called *Leishmania* (*L. donovani, L. infantum, and rarely, L. tropica*).

In endemic area, Kala-azar usually progresses slowly and is even asymptomatic in many cases. However, malnutrition and immunodeficiency diseases, especially HIV, can lead to rapidly progressive and dangerous disease. In addition, in people who get this disease for the first time and in sporadic cases, Kala-azar is more acute and severe (1, 2).

Clinical manifestations of visceral leishmaniasis are irregular and fluctuating fever (sometimes up to 40 °C) accompanied by chills and restlessness, abdominal pain, anorexia and weight loss, splenomegaly and sometimes hepatomegaly (and as a result, the enlargement of the patient's abdomen), anemia, and decrease in the number of other blood cells (leukopenia, thrombocytopenia), and sometimes darkening of the skin and edema of the face, hands, and feet. This disease is one of the causes of severe splenomegaly (Huge splenomegaly) and is a potentially fatal disease that can be seen in epidemic, endemic, or sporadic forms in all parts of the world.

Leishmania, the causative agent of the disease and its carrier, is different depending on the geographical region (3). Endemic Mediterranean type visceral leishmaniasis occurs in countries around the Mediterranean Sea, Southern Europe, North Africa, the Middle East, the Caucasus and Central Asia, and infects a large number of people, most of whom are children, every year. The Mediterranean type of the disease is also common in Iran. The main cause of Mediterranean Kala-azar is *L. infantum*, although in some cases it is also caused by *L. tropica* (4). *L. infantum* is transmitted by a special species of sand fly of the genus *Phlebotomus*. The reservoir of this parasite, which is common among animals and humans, is wild canids (5).

Kala-azar is known as a common disease in Ardabil province and Fars, especially among nomads (4), but North Khorasan province has been proposed as a more recent focus (6). In general, one out of every 50 cases of new infection due to mosquito bites will lead to Kala-azar, so asymptomatic infection is more common than Kala-azar disease. Some studies have shown that even in areas where canines are reservoirs of Kala-azar, asymptomatic individuals are involved in the chain of transmission. Therefore, in addition to determining the prevalence of the disease, determining the spread of asymptomatic cases also helps to better identify the burden of visceral leishmaniasis and its control methods (7, 8).

It is very important to take measures to reduce the prevalence of leishmaniasis in Iran, and in this regard, knowing the burden of the disease and its clinical manifestations and epidemiological characteristics is one of the most important measures. Therefore, considering the importance of the disease and its potential risks and the lack of studies on it in Guilan Province-Iran, we decided to investigate the clinical manifestations and epidemiological characteristics of visceral leishmaniasis in children with Kala-azar hospitalized from 2009 to 2020 in the 17 Shahrivar Teaching Hospital of Rasht as the only subspecialty referral hospital for children in Guilan province.

Methods

In this retrospective descriptive study, the hospital records of all children with Kala-azar hospitalized from 2009 to 2020 at the 17

Shahrivar Teaching Hospital in Rasht-Iran were analyzed.

The inclusion criterion was the final diagnosis of kalazar in the patients' records, which was established based on bone marrow aspiration and/or specific serology tests including Enzyme-linked immunosorbent assay (ELISA) test (specifications: *L. infantum* promastigote antigen, Alkaline phosphatase conjugate anti-human IgG made by Sigma-Aldrich Co., P-Nitrophenyl Phosphate or PNP substrate diluted by diethanolamine buffer, the results were read with the help of an ELISA reader at a wavelength of 405 nm); and immunofluorescence assay (IFA) test (specifications: euroimmun Kit made by medizinische Labordiagnostik Co., Reference range or cut-point: >1/160). The exclusion criterion was the incompleteness of the record data.

The required data, including age, sex, duration of hospitalization, duration of illness until hospitalization, place of residence, season, clinical and laboratory manifestations, type and results of treatment, and short-term complications, were entered into the checklist and descriptive statistical analysis was done using SPSS version 24 software. Quantitative variables were described using mean and standard deviation, and qualitative variables were described with number and percentage. The personal information of the patients was considered confidential.

The Ethics Committee approved this study at Guilan University of Medical Sciences (Ethics Code: IR.GUMS.REC.1399.166).

Results

The hospital records of 22 children with Kala-azar admitted to the 17 Shahrivar Teaching Hospital were reviewed. All patients were diagnosed by observing amastigotes of *Leishmania* in Giemsa stained smears of the bone marrow. ELISA test was performed for 6 patients and IFA test was also performed for 5 patients, all of which were positive.

The average age of children was 2.93 ± 3.32 years, with a range of one month to 12 years. The demographic and epidemiological characteristics of the patients are shown in Table 1

Table 1: Demographic and epidemiological characteristics of the patients

Demographic categories		*Number*	*Percent*
Age groups (yr)	<1	9	40.9
	1-5	9	40.9
	>5	4	18.2
Gender	Female	9	40.9
	Male	13	59.1
Place of residence	Urban areas	14	63.6
	Rural areas	8	36.4
Geographical situation of residence	Foothill areas	13	59.1
	Plains areas	9	40.9
Geographical direction of residence	East	6	27.3
	Center	13	59.1
	West	3	13.6
Season	Spring	10	45.5
	Summer	4	18.2
	Autumn	3	13.6

	Winter	5	22.7

The average duration from the onset of the disease until hospitalization of the affected children was 16.5 ± 13.54 days (between 1 and 60 days).

As seen in Fig. 1, the most common clinical manifestations included: fever (20 cases, 90.9%), splenomegaly (17 cases, 77.3%), hepatomegaly (11 cases, 50%), weakness (6 cases, 27.3%), and vomiting (4 cases, 18.2%).

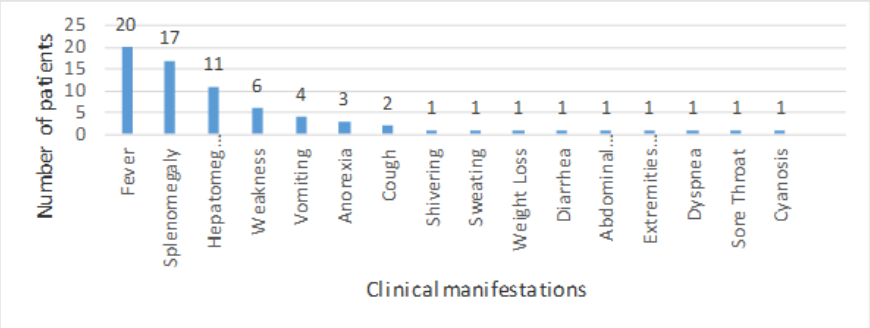

Fig. 1: Frequency of clinical manifestations in the studied children with Kala-azar

Anemia (20 cases, 90.9%), leukopenia (13 cases, 59.1%), increased erythrocyte sedimentation rate or ESR (15 cases, 75%), and increased C-reactive protein or CRP level (15 cases, 75%) were the most common laboratory findings in the patients (Table 2)

Table 2: Laboratory changes in the studied children with Kala-azar

Laboratory Parameter Status		Number	Percent
The number of red blood cells/mm^3 (according to age)	Normal	2	9.1
	Decreased (Anemia)	20	90.9
	Total	22	100
The number of white blood cells/mm^3 (according to age)	Normal	9	40.9
	Decreased (Leukopenia)	13	59.1
	Increased (Leukocytosis)	0	0
	Total	22	100
The number of blood platelets/mm^3	Normal	9	40.9
	Decreased (Thrombocytopenia)	13	59.1
	Increased (Thrombocytosis)	0	0
	Total	22	100
CRP (mg/dl)	Normal	5	25
	Increased	15	75
	Total	20	100
ESR (mm/h)	Normal	5	25
	Twenty To Fifty	11	55
	Above Fifty	4	20
	Total	20	100

The majority of children were treated with liposomal amphotericin (16 cases, 72.7%), and the rest were treated with Meglumine antimoniate (Glucantime). In all cases, fever stopped within a week or less, and the average time for fever to return to normal was 2.55 ± 1.19 (between 1 and 6) days. The number of leukocytes returned to normal in 12.53 ± 10.54 (between 3 and 47) days. One of the patients had an underlying disease (Hemophagocytic lymphohistiocytosis -HLH). No medication complications or deaths occurred during hospitalization in the patients.

Discussion

Indeed, it is very important to take measures to reduce the prevalence of leishmaniasis, and in this context, knowing the burden of the disease, its clinical manifestations, and its epidemiological aspects is one of the most important measures. In this study, the hospital records of 22 children with Kala-azar admitted to the 17 Shahrivar Teaching Hospital were reviewed. The majority of affected children were under the age of 5 years. The results of most similar studies in Iran also show the high prevalence of this disease in children under 5 years of age. In a study in Ardabil-Iran, 38 patients (95%) were under 6 years of age, of which 15 (37.5%) were under 1 year of age (9). In the study in Shiraz-Iran, the affected children were in the age group of 3.5 months to 7 years (10). The mean age of patients with kala-azar hospitalized in Emam Reza Hospital in Mashhad, who were reviewed by Shamsian et al., was 3.7±4 years (11). Nourian et al. reported that only 7.9% of hospitalized patients with visceral leishmaniasis in Mofid Hospital in Tehran were over 10 years old (12). In addition, studies from other countries showed similar results (1). For example, in a study conducted in the south of France in people with leishmaniasis infection, the rates of infection in children under 5 years of age and under one year of age were 84% and 17%, respectively (13). In another study conducted in Turkey between 1981 and 2001, the average age of patients with Kala-azar was 3 years, and in another study in southern Greece between 1986 and 1998, patients with visceral leishmaniasis had an average age of 2.5 years (14, 15).

In addition, according to the results obtained from the present study, the number of affected boys was higher (59.1%), which was consistent with the results obtained in Choobineh et al.'s study in Tehran (16). This is probably because boys are more in contact with polluted sources and use open and less clothing than girls are. In addition, in the study of Rafeey et al. in Ardabil, 60% of the patients were male. The authors of the article considered the cultural tendency of families to follow-up treatment and hospitalization of male children as the reason for the higher prevalence of the disease in boys (9). The higher number of male patients has not been shown in all studies (12).

In our study, the majority of affected children (59.1%) lived in the foothills and were from the center of Guilan province (The cities of Rasht, Rostamabad, Rudbar, and Manjil). Kala-azar disease is more common in nomads because they have the two factors of contact with carnivores as a reservoir and contact with sandfly as a vector of the disease at the same time. For this reason, the main foci of visceral leishmaniasis in Iran include the Zagros and Sablan ranges (Meshgin shahr County and Mughan Desert), and the disease mainly occurs in villagers and nomads living in those areas (4), but North Khorasan province as a more recent focus has been proposed, too (6, 11).

In the current study, the average duration of the onset of the disease until hospitalization was 16.5 days. This interval in other studies was longer than in our study. In the Altaf's study in Pakistan, the average time from the onset of the disease to diagnosis was 45 days, with the explanation that the average age of

the children in this study was 17 months (17). On the other hand, in Singh's study in Nepal, 35% of children aged 2 to 14 years had a delay of more than 6 months until diagnosis (18). The two factors of the lack of opportunity for patients to visit a doctor during working seasons, and the lack of anorexia may be the causes of delays in diagnosis or patient referral. In addition, in younger children, who made up the majority of our patients, the disease occurred more acutely and progresses faster, which could lead to earlier referral of patients (1).

Ten of our patients (45.1%) were admitted in the spring season. In a study in Iran between 1988 and 2004, most of the patients with Kala-azar were hospitalized in spring (16). In the study of Tamook et al. in 2016 in Ardabil, patients visited the doctor more in winter and spring, and only 37.8% of patients were diagnosed within one month from the onset of disease (19). The reason for the seasonal difference in the prevalence of the disease is probably related to the period of sandfly activity in the region, as well as the climate conditions of the region (16). It should also be considered that in the summer and early autumn seasons, the villagers are busy planting or harvesting, and therefore they may not be able to refer to health care on time.

In the current study, 90.9% of the children experienced fever, 27.3% experienced weakness and lethargy, 18.2% experienced vomiting, and 13.6% experienced loss of appetite. In addition, Body temperature returned to normal in 40% of patients within two days. In the study of Rafeey et al., fever was observed in 100% of patients in Ardabil (9). In other studies, fever was observed in 91% of patients in Mashhad, Iran, in 76.3% of patient in Tehran- Iran, in 100% of patients in Turkey, in 95% of cases in southern Greece, and in 90% of patients in southern France. (11, 12, 14, 15, 20).

Fifty percent of our children had hepatosplenomegaly, and 27.3% of children had splenomegaly alone, which was in line with the results of other previous studies. In general, the results of this study and previous studies showed that in patients with Kala-azar, an enlarged spleen with or without an enlarged liver was a common finding. Rafiei et al. showed that splenomegaly was observed in 100% of patients and hepatomegaly in 77.5% (9). In the study of Shamsian et al. in Mashhad, hepatosplenomegaly was observed in 100% of patients, and in Nourian's study in Tehran, hepatomegaly and splenomegaly was observed in 39.5% and 42.1% of patients, respectively (11, 12). In addition, in the survey conducted in Turkey, splenomegaly was present in 100% of cases, in southern Greece in 95% of cases, and in southern France, splenomegaly was present in 100% of cases (14, 15, 20). It seems that the younger the patient is, the more likely liver and spleen involvement is, and even symptoms such as portal hypertension occur earlier (1, 9). In addition, according to the results of the present study, there were no enlarged lymph nodes in any of the patients, which is in line with the results of most previous studies, so that in the study of Rafiei et al. in Ardabil City, enlarged lymph nodes were observed in none of the patients (9). In a study conducted in Malta, lymphadenopathy was reported in only 4% of cases (21).

Our study showed that 90.9% of the children had anemia, 59.1% had leukopenia, 59.1% thrombocytopenia, 75% increased CRP, and 75% increased ESR, and the patients' white blood cells returned to normal in an average of 12.5 days. In the study of Rafiei et al. in Ardabil, anemia was also found to be common (90%) (9). Other studies have found similar results (11, 15, 20, 21).

In our study, 72.7% of children were treated with liposomal amphotericin. Currently, resistance to classical treatment with pentavalent antimoniate is increasing. Although the rate of resistance in many parts of the world is less than 10%, in some regions, such as India, initial resistance and relapse are reported in 25–50% of cases, which is the reason for the use of alternative drugs such as amphotericin B and its new lipid compounds,

aminosidine and interferon. However, due to the risk of nephrotoxicity caused by amphotericin B, liposomal amphotericin in which deoxycholate is replaced with other lipids is used nowadays. Liposomal amphotericin is well absorbed by the reticuloendothelial system, which is the place of replacement for the organism, but its excretion by the kidney is very small. Liposomal amphotericin B is not only effective in the treatment of visceral leishmaniasis in the Mediterranean area, where infection with *L. infantum* is common, but also has a good effect on other species of *Leishmania* in other geographical areas such as India, where *L. donovani* is usually the cause of the disease. The tolerance of the drug is very good and there are no significant side effects (22, 23).

Conclusion

The average age of Kala-azar in children hospitalized in our hospital was about 3.3 years, and it was more common in boys and foothills residents. The most common clinical manifestations were fever and hepatosplenomegaly, and the most common laboratory finding in children with Kala-azar was anemia. The response to both the main medications of the disease including liposomal amphotericin and glucantime has been good. Considering that our hospital is a referral hospital for children in Guilan Province-Iran, these findings can help in the diagnosis and treatment of children in the province and better control of the disease. It is also suggested that by conducting systematic review studies in the country, the results of studies in different cities of Iran will be evaluated more precisely.

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Conflict of interest

The authors declare that there is no conflict of interest.

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