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Visceral leishmaniasis in adults from southern Turkey: evaluation of epidemiologic, clinical, laboratory and treatment outcome characteristics of 26 cases

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PURPOSE / OBJECTIVES

Leishmaniasis is a vector-mediated protozoan infection that is endemic mainly in developing countries. The causative microorganism, the genus *Leishmania*, is a flagellated protozoan with more than 20 species and is transferred to humans and animals during blood sucking by females of arthropod vectors (*Phlebotomus* spp in the Old World and *Lutzomyia* spp in the New World) in the sand fly species. Infection in humans can occur zoonotic (where dogs act as reservoirs) or anthroponotic. Leishmaniasis, as a neglected infection, is an important cause of morbidity and mortality, especially in poor regions of the world. Leishmaniasis occurs in 102 countries around the world; It has been reported that the disease is seen in 2 million people every year, and it causes the death of 20,000-40,000 people every year. The number of people living at risk of leishmaniasis all over the world is around 955 million people (566 million people are at risk of VL, 399 million people are at risk of CL). A significant portion of VL cases reported globally are detected in countries with coastlines to the Mediterranean. WHO determined countries with high disease burden for CL and VL in 2015 and included Turkey among the high-risk countries for CL in the published report. Turkey has a special position for vector-mediated diseases due to its geographical location, and CL and VL are among the diseases that are endemic in Turkey. Although Turkey is not counted among the countries with a high burden of VL disease, this deadly disease poses a risk for those living in Turkey, and the number of VL cases reported from Turkey for 2016 is 37, and the number of cases increased compared to previous years. Cases intensify within the country, especially in the Mediterranean and Aegean regions. VL cases in Turkey are mainly caused by *Leishmania infantum*, but it was reported that *L. donovani* was also found to be the causative agent among VL cases seen in Turkey in 2014.
Most of the VL cases occurring in Turkey, one of the Mediterranean countries, are in the pediatric age group, and reports of adult cases are relatively few. Here, we aimed to examine the epidemiological and clinical features and laboratory findings of 26 adult VL cases who are residents in the Çukurova region, located in the southern part of Turkey.

MATERIAL & METHODS

In this study, 26 adult cases with the diagnosis of VL who were followed up and treated by Çukurova University Faculty of Medicine Infectious Diseases and Clinical Microbiology Department between 2002 and 2019 were retrospectively analyzed. Approval for this study was obtained from the Çukurova University Faculty of Medicine Non-Interventional Practices Ethics Committee.
For the clinical for diagnosis of VL, each of the international disease criteria (38 ° C or higher fever, hepatosplenomegaly, weight loss, anemia, leucopenia, polyclonal hypergammaglobulinemia, lymphadenomegaly in at least two) plus at least one of the following is required: amastigotes seen in the bone marrow (BM) smear or tissue biopsy (bone marrow or lymph node), dipstick test for antibodies against rK39 antigen or PCR positivity for VL, or growth of Leishmania promastigotes in NNN (Novy-MacNeal-Nicole) medium. Demographic information of the cases, epidemiological data such as the city and geography of residence, the dates of application and diagnosis, the time between applying to a health institution and diagnosis after the first symptoms appear, the area of expertise applied for, disease symptoms, physical examination findings, underlying disease and immunosuppression status, laboratory values, the method used to confirm the diagnosis of VL, the drug used for treatment, the treatment scheme and duration, information on improvement in symptoms and signs after treatment (time for fever, liver and spleen size to return to normal, etc.), prognosis, control bone marrow examination, information about the patients' condition during the control visit between three and six months, and relapse assessment was recorded.

RESULTS

The patients comprised 22 males (84.6 %) and 4 females (15,4%) with mean age 40±15 (min17-max70). Of the patients, 80.8% were from Adana, which is a developed and 6th biggest city of Turkey, the rest of the patients were from Mersin (%7.7), Hatay (%7.7), and Osmaniye (%3.8). Most of the patients (88.5%) were residents of rural area. Employment of the patients is as follows: Farmer (23.1%), worker (19.2%), self-employed (15.4%), clerk (7.7%), student (3.8%), and unemployed (26.9%). The distribution of the patients in years is given Figure 1. The number of cases detected monthly in a year period is presented Table 2. Incubation period of the disease were between 4-365 days (mean 91±SD17). According to the medical records, only 19.2% (n=5) of the patients made their first admission to our clinic. Most of the patients (80.8%, n=21) admitted to various outpatient clinics of Internal Medicine. The applied branches in order of frequency are as follows: Internal Diseases Hematology (46.2%), General Internal Diseases Polyclinic (11.5%), Internal Diseases Oncology (7.7%), Internal Diseases Gastroenterology (7.7%), Internal Medicine Rheumatology (7.7%), Family Physician (7.7%). The time from the first symptom to the patient's admission to the health institution ranged from 4-365 days and the mean was 93 (SD±84) days. The mean time from the first symptom to diagnosis was 99 days (SD±87). Fever was the most frequently reported “first symptom” in patient history records and was detected in 72% of patients; this was followed by fatigue (20%), abdominal pain (4%) and cough (4%), respectively. Fever and chills were recorded at any time in the history or physical examination of all patients. The clinical symptoms of the patients are given in Table 1 in order of frequency. The physical examination and radiological examination findings of VL cases are presented in Table 2. On physical examination, body temperature was the lowest 37°C, the highest 40°C, and the mean was 38.8°C (±0.7). Clinical and laboratory findings of our patients were presented in the table. Immunosuppression (steroid use) were detected in 20% of the patients. Treatment (n=25) were constituted by liposomal amphotericin B (LAmB) in 20 patients (80%), by conventional amphotericin B in one patient (4%) and by meglumin antimoniate in four patients (16%). All patients were treated successfully except one who died due to sepsis and pulmonary thrombus on seventh day of the hospitalization. The numbers of the VL cases per year between 2002-2019 are presented in Figure 1. The frequency of the VL cases per month in a year period is shown in Figure 2.

RESULTS

Table 1. Clinical and laboratory characteristics of the VL patients.

Characteristics	Case No	%
Symptoms		
Fever	25 (n=25)	100
Chills	25 (n=25)	100
Sweating	24 (n=25)	96.2
Abdominal pain	8 (n=25)	32
Fed up early	5 (n=25)	20
Cough	12 (n=25)	48
Weight loss	21 (n=25)	84
Loss of appetite	19 (n=25)	76
Weakness	21 (n=25)	84
Nausea-vomiting	9 (n=25)	36
Epistaxis	2 (n=25)	8
Dysuria	4 (n=25)	16
Findings on Physical Examination and Radiological Investigation		
Splenomegaly	26 (n=26)	100
Hepatomegaly	23 (n=26)	88.5
Hepatosplenomegaly	22 (n=26)	84.6
Lymphadenomegaly	9 (n=26)	34.6
Ascid	4 (n=26)	15.4
Pallor	18 (n=25)	72
Petechia-purpura	2 (n=25)	7.7
Laboratory Findings		
Anemia	25 (n=25)	100
Leukopenia	25 (n=25)	100
Thrombocytopenia	24 (n=25)	96
Pancytopenia	24 (n=25)	96
Reversed Albumin/ Globulin Ratio	20 (n=23)	87
High C-Reactive Protein	24 (n=25)	96
High Erythrocyte Sedimentation Rate	20 (n=24	83.3

Table 2. Diagnostic methods and laboratory values of the VL cases.

Diagnostic Method	Case No	%
Detection of amastigotes on tissue (Bone marrow/Liver)	20 (n=26)	76.9
rK-39 Dipstick test	19 (n=20)	95
Polymerase Chain Reaction	7 (n=8)	87.5
Leishmania growth on NNN media	0 (n=16)	0

Laboratory Results (n=25)	Mean	SD
Hematological		
White blood cells	2016.8	785.5
Hemoglobin	8.6	1.6
Hematocrit	25.8	4.6
Platelets	85	39
Biochemical		
C-Reactive Protein	94.0	64.2
High Erythrocyte Sedimentation Rate	49	28
ALT	62	54
AST	97	68
LDH	714	743
Creatinine	0.8	0.2
Albumin	24.7	5.7
Total protein	72.7	12.8
Total bilirubin	1.21	1.06
Direct bilirubin	0.53	0.63
Ferritin (n=18)	7337	14869

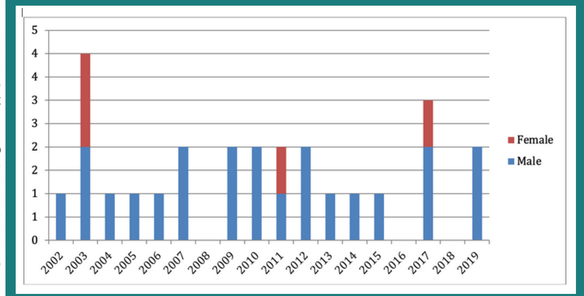


Figure 1. Numbers of the VL cases diagnosed per year between 2002-2019.

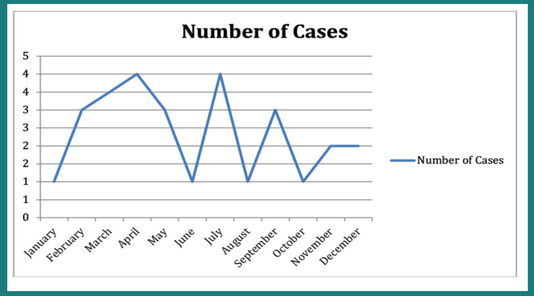


Figure 2. Frequency of the VL cases per month in a year period

DISCUSSION

VL is one of the parasitic diseases that cause the most deaths in the world with its devastating effects especially in poor societies. Leishmaniasis has often been a neglected disease by the authorities and 350 million people around the world live at risk for VL. In geographic regions where VL is relatively rare, the diagnosis of VL is usually delayed or even cannot be established. In recent years, when global travel has intensified, it is worrisome that autochthonous VL cases have been detected in countries that have not been reported before, due to the vector movements caused by global warming and climate change, as well as VL cases developing after travel. Although Turkey is not among the countries at risk for VL, it can be predicted that in addition to the cases reported every year, there may be cases of VL that are overlooked or diagnosed in our country. Turkey has a location where human and animal movements in the reservoir are intense, due to its geographical location that connects three continents, and its neighborhood with Syria, which has been under war conditions for years. For this reason, follow-up and reporting of VL cases are important in terms of public health strategies.
Most of the 26 patients examined in this study were male. There are other studies reporting that VL cases are less common in women. Most of the patients live in rural areas and almost all of them have low incomes. The cases of VL presented here also support that VL is a disease associated with the poor population. The long time elapsed until the diagnosis of the cases may also be related to the low income. Although most of the cases (80%) who applied to our hospital were from our city (Adana), 1/5 of them were from neighboring provinces and applied to our hospital because the diagnosis could not be made in the first health institution.
In this study, it is seen that VL cases show a steady endemicity throughout 17 years. Besides, VL cases admitted more frequently between February and September during the year (Figure 2).
The prognosis of 25 adult patients examined in this study is good with only one mortal case with late presentation. However, for most of the cases, the diagnostic process is long, laborious and costly. Although the treatment is successful in most of the cases, there is the possibility of side effects and relapse, and the necessity of evaluating the patients with a control BM aspiration and biopsy, which also cause the follow-up period to be prolonged. Although there are few drug options used in the treatment of VL in our country, according to the findings of our study, liposomal amphotericin B is a good option as an effective molecule with a low side-effect profile. Cost seems to be the most important problem for liposomal amphotericin B, but the short treatment time reduces the cost relatively.
Fever and chill were the most frequent symptoms, and all patients had these complaints for different time periods. Splenomegaly was the most important finding in either physical examination or on radiologic investigation. Both anemia and leucopenia were detected in all patients sometime in the clinical course which leads the patients to the internal disease specialists. Also, thrombocytopenia and even pancytopenia is common (96%). Although many different laboratory tests could be measured in abnormal range, among several different tests, the reversed albumin/globulin ratio, high inflammatory tests such as CRP or ESR are very frequent. Although all these tests are nonspecific markers of the systemic inflammatory disorders, they are frequently used for basal evaluation and to monitor the treatment response of the patients especially for several infectious diseases. Higher liver function tests (ALT, AST, total and direct bilirubin) and higher LDH levels were frequent in most of the VL patients however renal function tests were in normal range. All these routine tests must be evaluated carefully, since they are not specific for any situation, but clinician must be aware for VL especially in endemic geographic regions. Specific diagnostic tests for VL should be run immediately whenever VL is suspected.
Most of the cases identified in this study initially applied to the Internal Medicine Clinic and were diagnosed late after long examinations. In addition to general medical education programs, both family physicians' and internal diseases specialists' education programs should contain more detailed and comprehensive VL lectures enriched with cases with fever and pancytopenia.
It would be appropriate to establish a systematic epidemiological follow-up and feedback program for leishmaniasis and reservoirs occur in humans and animals in Turkey.

SUMMARY / CONCLUSION

VL is an important public health in urban area in Turkey. Due to climate change and economical issues, changes in leishmaniasis epidemiology can also be expected in the future.

The most frequent symptoms and findings are high fever and chills, splenomegaly, leucopenia, thrombocytopenia, pancytopenia in VL cases. When laboratory tests reveals the chronic inflammatory disorders in the patients with symptoms and physical examination findings matching with VL, diagnostic tests should be run immediately and persistently in the endemic regions for leishmaniasis.

VL cases were mostly male and from rural parts of the region. VL case admissions to the healthcare facility are usually late because of the long incubation period and nonspecific symptoms.

Clinicians, especially internal disease specialists must be aware for VL in patients who have protracted fever, pancytopenia and hepatosplenomegaly in leishmaniasis endemic regions.

Detection of the amastigotes in bone marrow is still major tool for diagnosis. Since cultivation of leishmaniasis is not widely available, newer diagnostic tests for *Leishmania* spp. could be helpful for early diagnose.

Epidemiological programs to monitor leishmaniasis, particularly VL, could be helpful.