

Prevalence of *Visceral Leishmaniasis* in hospitalized patients as an increasing public health problem

Abstract

Introduction: Globally, *Visceral Leishmaniasis* an increasing public health problem, as evidenced by markedly increasing incidence rates and multiple outbreaks in all continents. *Visceral Leishmaniasis* is probably the most widespread and prevalent zoonotic disease in the world. World Health Organisation estimates the worldwide prevalence to be approximately 12 million cases, with annual mortality of about 60 000. The size of population at risk is about 350 million.

Methods: Sera of 150 patients were analysed by indirect enzyme linked immunosorbent assay (ELISA). Serum was collected from patients with VL before the onset of treatment. Serum was separated from 1mL and stored at -20°C.

Results: For two years from January 2011 to December 2012 in our laboratory were examined 150 cases (hospitalized patients for IgG antibodies. The mean age was 15.44 with minimum 1 and maximum 69, standart deviation 16.577. The city of Durrës represents 40.7 % of all cases. The prevalence for antibodies IgG was 17.3%. There was no significant relationship between antibodies IgG and sex, age and residence of patients.

Conclusion: The prevalence of Leishmania in our patients is high but was not found an association regarding residence, age's group and sex.

Keywords: *Visceral Leishmaniasis*, ELISA, antibodies IgG, hospitalized patients, zoonotic

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Abbreviations: VL, *Visceral Leishmaniasis*; WHO, World Health Organization; CL, cutaneous leishmaniasis

Introduction

Visceral Leishmaniasis was a parasitic infection primarily caused by *Leishmania donovani* in the Old World, and by *Leishmania amazonensis* in the New World.¹ Broadly speaking, the leishmaniasis can be divided into two larger groups of diseases: *Visceral Leishmaniasis* (VL) and cutaneous leishmaniasis (CL)^{2,3} and are primarily endemic in tropical and sub-tropical regions. The leishmaniasis are believed to be endemic in 88 countries⁴ on five continents like Africa, Asia, Europe, North America and South America.

World Health Organization (WHO) estimates 350 million people are at risk. An estimated of 2 million new cases (1.5 million cases of CL and 500 000 of VL) occur annually, with about 12 million people currently infected.⁵ Leishmaniasis caused by *L. infantum* is usually considered to be a zoonosis, although congenital infections have been reported even in Europe⁶ and in those country this disease appears to be spreading northward from its traditional foci. VL also called as Kala-Azar⁷ is a chronic illness that is characterized by irregular bouts of fever, anemia, hepatosplenomegaly and leucopenia, swelling of the spleen and liver and progressive substantial weight loss. In its more severe forms, the disease can cause serious disfigurement as well as death if left untreated. Leishmaniasis is popularly caused by several species of flagellated protozoan parasites. The vector is the female sand fly, and the dog is the reservoir in most of the regions. The causative organism was first isolated in 1903.⁸ In vertebrates, the amastigote form parasites are found intracellular in the reticulo endothelial system. This form is a flagellate, round, and 2-4µm in

diameter (Leishman-Donovan body). The promastigote form found in the vector is flagellate, 15-20µm long and spindle shaped.¹ VL was rapidly becoming an important opportunistic infection in areas where it coexists with HIV, in southern Europe, where 25-70% of adult VL cases are associated with coexistent HIV, and 1.5-9.5% of individuals with HIV from newly acquired or reactivated VL.⁹⁻¹¹

Materials and methods

This study was conducted at the Medical clinic "Hamdi Sulcebe", with Colaboratory of University Hospital Centre "Mother Tereza", Tirana, Albania.

In this study, sera of 150 patients were analyzed for the presence of antibodies IgG for VL by indirect ELISA technique. Microtiter strip wells are precoated with *Leishmania* antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HPR), labelled Protein A conjugate is added. This conjugate binds to the captured *Leishmania* specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) Substrate Solution, which gives a blue reaction product. The intensity of this product is proportional to the amount of *Leishmania* specific antibodies in the specimen. Sulfuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance alternative 450nm is read using ELISA microwell plate reader. Serum was collected from patients with VL before the onset of treatment during January 2012 to December 2012. Serum was separated from 1mL and stored at -20°C. All these individuals had no past history of VL. These patients were recruited and treated at Hospital of Infectious Disease and Hospital of Pediatric. The serological data, thus obtained were analyzed with SPSS 16 softwares.

Results

During January 2011-December 2012 in our Laboratory were examined 150 sera for Leishmaniasis. The sera have come from University Hospital Centre "Mother Theresa", Infectious Disease Unit and Pediatric Unit to Medical clinic "Hamdi Sulcebe". All cases were hospitalized patients with clinical sign like Fever, Anemia, Lymphadenopathy, Splenomegaly, Hepatomegaly, Anorexia, Vomiting, Diarrhea, Malnutrition, Cough, etc.

Clinical and serological findings

Of the 150 (100) individuals examined, a total of 55 (36%) presented with some degree of splenomegaly: out of these 55 individuals, only 23 (41%) presented with other classic signs and symptoms such as pallor, weight loss, and fever. About 3% of them were patients with HIV-AIDS. Symptomatic VL is a frequent disease among human immunodeficiency virus type 1 (HIV-1)-infected patients from the Mediterranean basin.¹¹⁻¹³ The overall antibody prevalence IgG by ELISA was 17.3% (26 positive cases) (Figure 1).

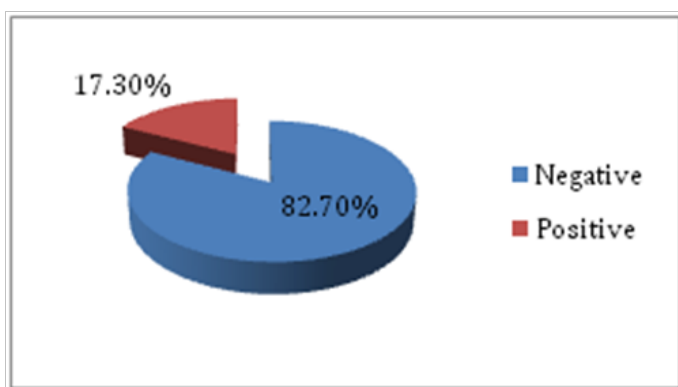


Figure 1 The positivity of cases for antibody IgG with ELISA-methods.

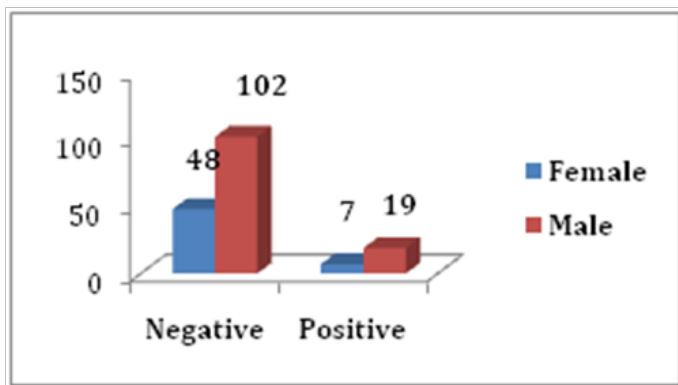


Figure 2 Number of cases divided by sex.

Out of all patients, 102 (68%) of them were males and 48 (32%) were females (Figure 2). There was no significant difference in gender with the disease. The difference in prevalence of positive cases among females (26.9%, 7 of 48) and males (18.6%, 19 of 102) was not statistically different.

The mean number of patients examined were 15.44 with mode 3 with the standard deviation (Std)=16.57. There was no significant difference in age with the disease. The data are grouped in three groups. Based to data calculated we can say that: the first group were

the pediatric age less the 15 (<15years old). This is the predominant group in this study with 105 (70%) cases in total. The second group was the active group 16-50 years old with 35 (23.3%) cases in total, and the third group was more than 51 (>51years old) with 10 (6.7%) (Figure 3).

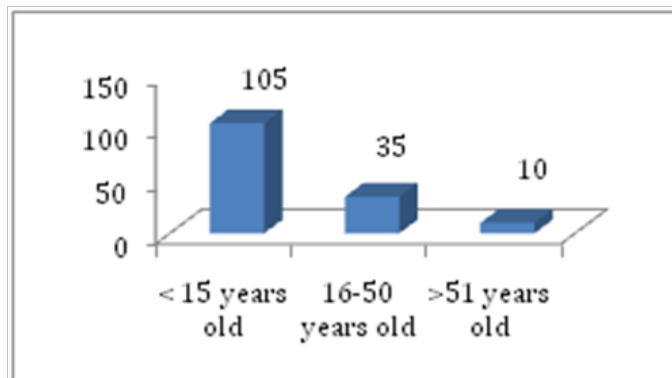


Figure 3 Number of cases divided by age determination.

The patients were from different city of Albania. The higher number of cases examined were from Durres city 61 (40.6%) of cases in total, Tirana city with 34 (22.6%) cases, 21 (14%) cases for Fier city and with 1 (0.66%) of cases resulted this number for patients from Shkoder city. There was no significant difference between places from the patients living and disease (Figure 4).

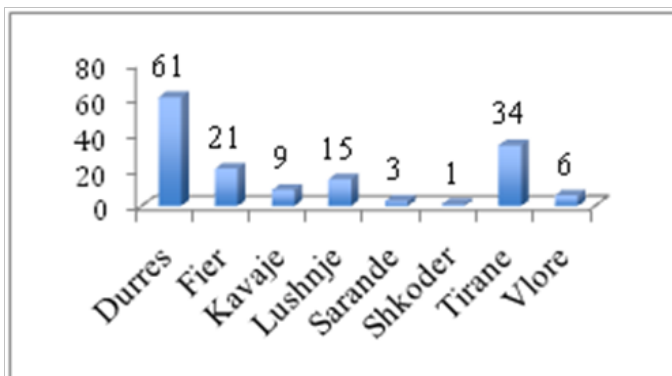


Figure 4 Number of cases for each city.

Discussion

In this paper, we studied the prevalence and distribution of VL over two years (period from January 2011 until December 2012). The incidence of zoonotic VL caused by *L. infantum* in humans is relatively low (0.02-0.49/100,000 in the general population) with an average of about 700 clinical cases reported each year in southern Europe.^{14,15} Increasing evidence suggests that elevated rates of asymptomatic *L. infantum* carriers are an indicator of the intense Leishmania circulation in southern Europe. Infection prevalences, as high as 10-47% in particular age groups, were recorded in healthy individuals from endemic foci of France, Greece, Italy and Spain by traditional and molecular methods.¹⁶

The vast majority of patients were autochthonous cases and this is convincing evidence for the presence of local transmission of VL in rural and urban areas. The prevalence in our study for VL in hospitalized patients resulted 17.3%. This prevalence is high for this small sample analyzed by our part. In this study, we have applied the ELISA method

in clinical routine and sero-epidemiologic surveys. Most foci in the Old World have a Mediterranean climate and sand fly vectors that can hibernate- usually *Phlebotomus* (*Larroussius*) species- and so there is a risk of the disease spreading from southern to northern Europe with climate change.¹⁷ The spread of leishmaniasis may be enhanced by globalisation, climatic change and other conditions which allow the parasite and its vectors to spread in space and time.¹⁸ In Albania there were favorable environmental conditions for local transmission of the disease. Our results showed that most cases were probably infected during the warmer months of the year (June-October) when phlebotomine sandflies are active.

Sub clinical forms of the disease may be more common in females than males. In some rural areas, the rate of active Kala-Azar cases in males may be higher than females.¹⁹ In our study, men were the most predominant analyzed cases. The prevalence of positivity divided by sex were 73.1% (19/26) for male and 26.9% (7/26) for female. No association was shown in this study between sex and the disease.

According to some authors, the ratio between children and adults with leishmaniasis in the Mediterranean region is 7:3 and the average age of the affected children is under four years.²⁰ However, in recent years, about a half of leishmaniasis cases in Europe have occurred in adults, following the appearance of the human immunodeficiency virus (HIV) infection and the increased number of patients receiving immunosuppressing treatments due to transplantation, malignancies or other underlying conditions.²¹ Based on our data, 70% of all analyzed cases were under 15 years old, 23.33% were 16-50 years old and 6.66% were more than 50 years old. So the ratio between children and adults were the same with the other study in Mediterranean region. The peak number of cases was in children 1-2 years old and the seropositive rate decreased with increasing of the age of the children. Some early studies in the world have shown a seropositive rate of about 50% in the age group 1-2 years and 96% of seropositive cases in children up to 8 years old.^{19,22}

However, Out of 150 diagnosed cases, found in this study, fever, anemia, splenomegaly, anorexia, vomiting, diarrhea, malnutrition were the predominant clinical features. These signs and symptoms are the same as those found in other clinical studies.^{19,23}

Our study is limited because of the relatively small sample size, but the results of this study are interesting and, at the same time, confounding. However, a larger sample should be studied, to investigate the situation in depth before conclusions can be reached.

Conclusion

VL is a grave public health problem in the world and particularly in Albania. Health authorities are unlikely to be resolved this disease by the current strategies. Understanding the transmission dynamics of VL could lead to sustainable prevention and control measures. A community-based VL control or suppression program could be formulated on educating residents of this endemic area about the risk associated with house construction and the proximity of domestic animals to one's living quarters. Alternative methods of vector control, other than the conventional indoor spraying of houses with residual insecticide (which can be prohibitively expensive) should be considered.

Acknowledgments

None.

Conflicts of interest

The authors declare there are no conflicts of interest.

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