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Research Article

CONTRIBUTION OF THE RAPID DIAGNOSTIC TEST IN THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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Abstract

The diagnosis of visceral leishmaniasis (VL) in patients with fever and large spleen relies on the detection of Leishmania parasites in tissue samples and on serological tests. Parasitological techniques are invasive, require sophisticated laboratories, are time-consuming or lack precision. Recently, rapid and easy to perform diagnostic tests have become available. This study aims to discuss the epidemiological profile of visceral leishmaniasis in the region of Marrakech and to highlight the importance of biological diagnosis in the identification of the disease, by comparing the contribution of RDT with the usual tests.

Keywords: Visceral leishmaniasis, RDT.

INTRODUCTION

Visceral leishmaniasis, also known as Kala Azar, is a parasitic disease caused by the multiplication in the reticulo-histiocytic system of a protozoan of the genus Leishmania. It is transmitted by the phlebotomy bite, and its reservoir is the dog. It affects preferably young children, while its occurrence in immunocompetent adults is rare. It is a serious form of the disease because it is fatal if left untreated (WHO). This study aims to discuss the epidemiological profile of visceral leishmaniasis in the region of Marrakech and to highlight the importance of biological diagnosis in the identification of the disease, by comparing the contribution of RDT with the usual tests.

MATERIALS AND METHODS

This is a retrospective descriptive and analytical study conducted in the laboratory of medical parasitology mycology of the Military Hospital Avicenne Marrakech over a period of 5 years, from January 2016 to December 2020. The diagnosis of VL was based on the rapid diagnostic test (RDT), the microscopic examination of the medullary smear stained with MGG in search of leishmania bodies, and serology by ELISA and or Western-Blot reaction. The samples were collected in the pediatric department. Epidemiological, clinical, biological and therapeutic data were obtained from the medical records of all patients hospitalized for a clinical case suggestive of visceral leishmaniasis, having leishmania bodies on the myelogram and/or a positive leishmaniasis serology.

RESULTS

This study collected 41 cases of visceral leishmaniasis, during a period of 5 years from January 2016 to December 2020. The average age of our patients was 4.68 years, the most affected age group was under 2 years, the sex ratio M/F was 1.75. The majority of our patients were from the Marrakech-Safi region with a rate of 53%.

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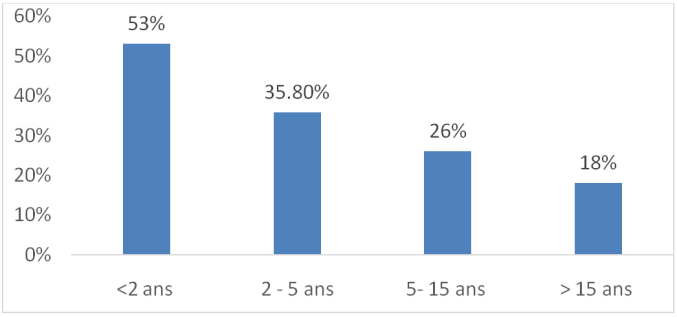

Figure 1. Age distribution of patients

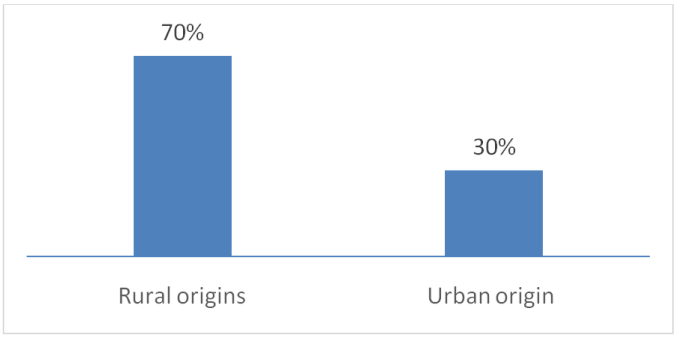

Figure 2. Distribution of patients by origin

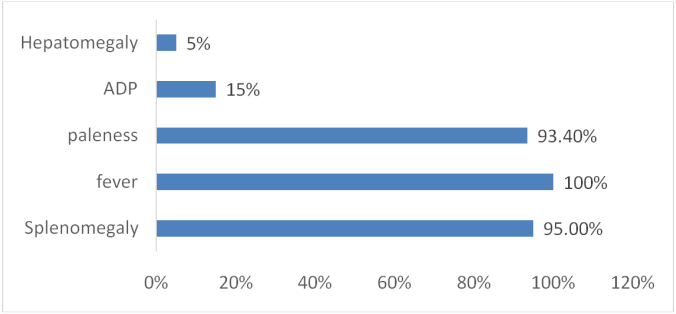

Figure 3. Signes cliniques retrouvés chez nos patients

Biologically, anemia was almost constant in 97% of cases, thrombocytopenia was noted in 65% of cases and leukopenia was found in 10%.

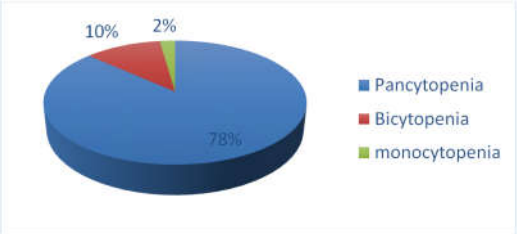

Figure 4: Anomalies of the haemogram

Myelograms were performed in all patients and confirmed the diagnosis in 87.8% of cases. The 5 cases with negative direct examination were caught and confirmed by serology.

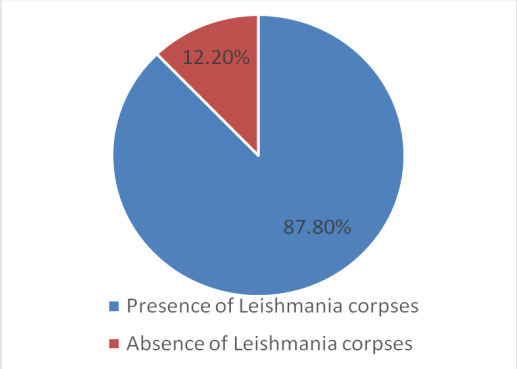

Figure 5. Contribution of the myelogram

In some cases of patients with positive direct examination, a comparison of the performance of the serological techniques showed that no control by the ELISA technique in patients with positive direct examination was negative. Contrary to the RDT, whose control in 2 patients, who had a parasitized medullary blood, came back negative with positive ELISA. Our results also confirmed that the ELISA technique has a better sensitivity than the RDT.

DISCUSSION

Since March 31, 1995, leishmaniasis has become a notifiable disease. This has allowed a more accurate estimation of their incidence and an establishment of the epidemiological profile of the disease in Morocco [2]. In Morocco, visceral leishmaniasis is caused by Leishmania infantum with Phlebotomus perniciosus as vector and the dog as reservoir. Phlebotomus usariasi has been demonstrated as a vector of L. infantum in the province of Taounate [3]. Humans are only accidentally infested and therefore constitute a parasite dead end. It is the most serious form of leishmaniasis and is fatal if left untreated. VL is mainly distributed in the north of the country and the main endemic foci are represented by the regions of Nador, Al-Hossima, Tetouan, Taza, Taounate, Sidi-Kacem, Meknes and Fez. The annual incidence of VL in Morocco has increased from 30 cases per year in the early 1990s to approximately 100 to 150 cases per year, mostly children, with a peak of 170 cases in 2006. [5] This increase is due on the one hand to the presence of risk factors that favor the increase in the density of animal reservoirs (mainly dogs) and vectors responsible for the disease, in particular poor hygiene conditions, and on the other hand to the proximity and availability of means of diagnosis and treatment and the awareness of the population [5]. This parasitosis is usually encountered in children under 5 years of age, due to the immaturity of their immune system.

In adults, the clinical expression of the disease is rare and the frequency of symptomatic forms makes the control of the disease difficult. The rare cases found in adults generally occur in a context of immunosuppression, whether viral, such as acquired immunodeficiency syndrome (AIDS), or iatrogenic, induced by certain drugs such as corticosteroids, immunosuppressants and antimitotics. In this case, the infection has an opportunistic character [3,4]. The clinical triad usually encountered in visceral leishmaniasis includes an irregular fever, sometimes called "mad fever", pallor, indicating anemia, and splenomegaly. These signs are most often associated with an altered general condition and possibly with hepatomegaly, which is generally present in one out of two cases, most often indicating an advanced form of an advanced form. Adenopathies are more rarely found when L. infantum is involved. This classic picture is most often progressive over a period ranging from a few weeks to several months [6,7,8].

In adults, the clinical expression of the infection is variable, and can be asymptomatic, which is the most frequent case, or oligo-symptomatic, sometimes resolving spontaneously. In children, the clinical picture of VL is often complete and therefore easy to diagnose [9]. In AIDS patients, VL is particularly difficult to diagnose and in 10 to 15% of cases, it is an atypical form, with the usual localizations (digestive, pulmonary, cutaneous) that appear to be more frequent. The clinical picture seems rarer and splenomegaly in particular may be missing [10]. The biological signs of orientation are a more or less pronounced pancytopenia associating anemia, leukoneutropenia and thrombocytopenia, as well as an inflammatory syndrome: very accelerated globular sedimentation rate, hyperprotidemia and polyclonal hypergammaglobulinemia. In our experience, pancytopenia is found in 90% of cases, especially in pediatric forms [1,11,12,13]. Although the specificity of microscopic examination is high, its sensitivity varies according to the tissues collected: it is 93% to 99% for the spleen, 53% to 86% for bone marrow and 53% to 65% for lymph nodes [1]. Direct examination of bone marrow is the gold standard and first-line method for the diagnosis of visceral leishmaniasis [14,16].

The marrow sample is obtained by puncture of the iliac crest most often; Sternal puncture is exceptionally performed in children. The marrow material is then spread on thin smear slides, fixed and stained with May-Grunwald Giemsa (MGG). Microscopic reading is done at 1000x immersion. The amastigote forms appear as small rounded or oval bodies, with a clear cytoplasm, a purple-red nucleus and a darker purple punctiform or bacilliform kinetoplast. The bodies may be grouped in clusters or scattered in the stroma[32]. Leishmaniasis can also be looked for in peripheral blood after leukoconcentration [17]. This is a technique of concentration of blood leukocytes studied by centrifugation. The blood sample taken on anticoagulant (EDTA) is brought into contact with a saponin-based hemolyzing solution. As soon as the hemolysis is complete, a sample is recovered, the volume of which is adapted to the number of white blood cells provided by the hemogram, centrifuged at 2000 rpm for 12 minutes in a cytospin cytocentrifuge, and then thin smears stained with MGG are taken. This technique is described by most authors as a diagnostic tool mainly for immunocompromised patients [36]. In our study, myelograms were performed in all patients and confirmed the diagnosis in 87.8% of cases.

The myelogram did not show leishmanial bodies in 5 patients, in whom leishmanial serology was positive. It should be noted that this examination may be negative due to poor handling of the smear (blank puncture, defective staining) or operator error. In immunocompetent patients, visceral leishmaniasis generates a sufficiently strong humoral immune response to justify its investigation. Elisa (enzyme linked immunosorbent assay) is increasingly used because it is automated. Its sensitivity and specificity vary according to the antigen used. the Ld-ESM antigen from the promastigote forms of Leishmania donovani, but many other antigens are being other antigens are developed [15]. No ELISA test in patients with a positive direct examination was negative. A very strong diagnostic presumption is also based on the positivity of rapid tests that use strips sensitized with a recombinant antigenic protein. The patient's antibodies react with the antigen on the strip, causing the black line to appear on the strip. A positive result is characterized by the appearance of two bands: the antibody-antigen reaction and the control. These diagnostic tests are convenient, have good sensitivity and specificity, especially in the immunocompetent [15]. While the RDT, which had a parasitized marrow blood, came back negative with positive ELISA, in 2 patients.

WHO recommends that all patients with suspected malaria should be diagnosed without delay by parasitological diagnosis before treatment is administered. Rapid diagnostic tests for malaria (RDTs) can significantly improve the quality of management of malaria infections, especially in remote areas with limited access to good quality microscopy services. Malaria RDTs detect specific antigens (proteins) produced by malaria parasites in the blood of infected individuals. Some RDTs detect monospecific infections (either P. falciparum or P. vivax), others detect mixed infections (P. falciparum, P. vivax, P. malariae, and P. ovale), while others distinguish between P. falciparum and non-P. falciparum infections, or specific species. Blood is usually collected by finger prick and results are available within 15 to 30 minutes. While there are variations among commercially available RDT products, the principles of the tests remain similar [16,19]. RDTs are relatively easy to perform and interpret, provide rapid results, require limited training, and allow for community diagnosis of malaria [16,19]. The sensitivity and specificity of all RDTs are sufficient to replace diagnostic services or to expand diagnostic coverage in uncomplicated P. falciparum malaria. Tests using the anti-HRP-2 antibody may be more sensitive but are less specific than tests using the anti-LDHp antibody, although the differences are minimal. The HRP-2 antigen persists even after effective treatment and therefore cannot be used to detect treatment failure [16].

Conclusion

The WHO has established a strategy for the control of leishmaniasis, which is based on early diagnosis and rapid treatment; control of reservoir hosts and Phlebotomus vectors.
The first line diagnostic tool of certainty is the direct examination of the different smears of medullary blood. RDTs are becoming more and more important in the screening and diagnosis of visceral leishmaniasis because of their simplicity, rapidity and practicality at the patient's bedside, which give them many advantages and possibilities.

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