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Evaluation of RK39 Rapid Immunochromatographic Test (ICT) For Diagnosis of Visceral Leishmaniasis: Hassan Ii University Hospital of Fez Field Study

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Original Research Article

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Abstract: Visceral Leishmaniasis is a vector-borne parasitic infection caused by a parasite of the genus Leishmania. This disease, which is more frequent between children than adults, knew during these last few years in Morocco a significant surge especially in the region of Fez. The direct examination, based on the detection of the parasite or its nucleic acid, is undoubtedly the key to a definite diagnosis, along with serological techniques that were proven to be efficient and could also aid the diagnosis. This study highlights the benefit of rK39 rapid tests in visceral Leishmaniasis diagnosis through the retrospective of 75 patients suspected to have Visceral Leishmaniasis. We shall also compare our results with those of other authors in different countries.

Keywords: Visceral leishmaniasis rapid immunochomatographic test, rK39, ICT, IT-Leish, sero-diagnosis.

INTRODUCTION

Visceral Leishmaniasis could lead to life-threatening infectious and hemorrhagic complications. When the diagnosis of leishmaniasis is suspected (fever, splenomegaly, pancytopenia, etc.), the use of a rapid diagnostic test allows a quick diagnosis orientation, especially in children where the main differential diagnosis evoked is leukemia [1]. These tests will in fact have to be supported with other tests to confirm the diagnosis.

MATERIALS AND METHODS

This is a retrospective study on 75 patients suspected to have Visceral Leishmaniasis and who have been subject to rapid diagnosis test and other tests allowing the detection of this pathology.

In fact, in addition to the ICT rK39, the serological tests used were ELISA and sometimes Western Bolt in case of discordance in the results of the two serological tests. After the serological diagnosis, a confirmation by myelogram was necessary to detect Leishmaniasis bodies on medullary blood. The diagnosis was performed in the parasitology-mycology department of Hassan II University Hospital in Fez.

We used the IT-Leish® as ICT using Ag rk39. The clinical information as well as the rest of the biological assessment were collected from the files of the patients, that have been admitted in various units of the medical center.

RESULTS

A total of 75 cases have been subject to this study. These patients presented clinical and biological signs consistent with the diagnosis of visceral Leishmaniasis, and a serology with a rapid diagnostic test in the foreground, which, as the name suggests, allowed to rapidly orient the diagnosis, pending further investigations, including the myelogram.

After the ICT rK39, 96% of patients benefited from a serology through ELISA in order to detect the presence of anti-Leishmaniasis antibodies (ELISA could not be done for the other cases as the kit was used up). In 86.1 % of cases, the results of the rapid test and those of ELISA matched: both tests we positive for 19 samples and negative for 43 other patients. However, in 13.9% of cases, the results of the two tests were different with a positive rapid test and a negative ELISA for 9 patients, and the opposite for one patient. In these cases of divergence in results of rapid test and that of ELISA, we sometime resort to Western Bolt in order to avoid ambiguity in serology result, awaiting the myelogram.

66.6% of the patients benefited from a myelogram allowing to confirm or disprove the diagnosis by looking for Leishmaniasis bodies on the medullary smear. For the rest, these are patients with a negative Leishmaniasis serology and whom the rest of the paraclinical tests revealed other conditions and discarded that of Leishmaniasis.

In fact, for the patients who benefited from myelogram, the May-Grunwald-Giemsa bone marrow aspiration has allowed the detection of Leishmaniasis bodies under amastigote forms in 98.6% of positive serology cases. In just one case, the myelogram did not objectify the presence of Leishmaniasis bodies because of the high hemodilution of the sample. The diagnosis was based only of epidemio-clinica and serological arguments. The positive clinical and biological evolution after the instauration of treatment was in favor of the diagnosis.

In another case, the ICT rK39 was negative but the spinal smear examination revealed the presence of several Extra and intra Leishmaniasis macrophage bodies. The clinical information collected from this patient has demonstrated the presence of a primitive immunodeficiency that explains these serological results.

Table-1: IT-Leish® ICT and ELISA results in Leishmaniasis diagnosis in our study

ELISA / IT-Leish Rapid Test	Positive	Negative
Positive	19	9
Negative	1	43

Table-2: Intrinsic value of evaluated tests

	Sensitivity	Specificity
The rK39 ICT	96.4%	100%
ELISA	67.8%	97,7%
Myelogram	98%	100%

DISCUSSION

Epidemiology of Leishmaniasis

The two most frequent types of visceral Leishmaniasis are represented by L.donovani at the level of Indian and east-African families and L.infantum at the Mediterranean [2], Center-Asiatic & American periphery [3]. And rarely, other types are found, like L.tropica in the Middle East and Latin America.

In Morocco, the Leishmaniasis Infantum represents 90% of the cases [3]. The main endemic centers are represented by the regions of Nador, Al Hoceima, Taza, Taounate & Fez [1].

Rapid Diagnosis Tests

Definition & principle

According to the World Health Organization, a rapid diagnosis test is an "accurate, simple, inexpensive test, easy to interpret, stable in extreme conditions, requiring little or no special prior treatment and few biological samples" [4].

Thanks to these properties, the rapid diagnosis test does not only allow the orientation of the diagnosis in a few minutes, but it can also be adopted in the patient's bed, where there is no laboratory or specialized staff [5].

The Leishmaniasis ICT are Immunochromatographic tests sensitized by recombining antigen, the rkE16 or the rk39, allowing the detection anti-Leishmaniasis antibodies [6].

We give the IT-Leish ® as an example of commercialized ICT, being the one used in our study. It-Leish® is an immunochromatographic test, in the form of a dipstick or cassette, sensitized with rk39 recombinant antigen. The serum is deposited in a tray containing a conjugate. When the antibodies are present in the patient's serum, they react with the rk39 Ag in the dipstick, where the redline would appear, and accordingly, the reaction is positive. On the other hand, when the reaction is negative, only the second line of control appears. Whereas the test that does not show any reaction on the control zone is invalid, whether or not the test zone is marked [6, 7].

Rapid test performances

The performance of the rapid test is evaluated by the intrinsic values represented by the sensitivity and specificity. Thus, sensitivity is defined as the ability of the test to reveal visceral Leishmaniasis patients, eliminating false negatives, that is, sick patients that the test was unable to identify. However, specificity is the ability of the test to detect non-visceral Leishmaniasis, eliminating false positives.

In our study, the rapid test turned out positive in 96.4% of patients with visceral Leishmaniasis, and negative in all Visceral Leishmaniasis-free patients. The sensitivity is, therefore, at 96.4% and the specificity is at 100%. In other words, there is only one false negative and no false positive.

Regarding sensitivity, our results are similar to those found in Switzerland [7] and in Bangladech [8],

where the sensitivity rate was respectively 97 and 95%. Also, in a prospective study conducted at Ibn Sina Hospital in Rabat concerning 49 patients hospitalized for suspected visceral Leishmaniasis, the sensitivity of rk39 was 96.3% higher than that of the Immunofluorescence Antibody Test: IFAT (92.59%) and that of the ELISA (85.19%) [9].

On the other hand, lower rates were noted in southern France and Brazil [10] (with respectively 91.8 and 85.7%), and the best results were noted in India by S. Sundar [11] where the sensitivity has reached the rate of 100%.

The specificity of the rk39 rapid test was in the present study at 100%. These same results have been found in several studies in Brazil, Iran, Italy and India [10, 11]. That is to say that out of 100 actual negative subjects, the rapid test was able to confirm all subjects without false positives. On the other hand, the specificity found in India was 95%. This rate was nevertheless considered sufficient when the definition of suspected cases (more than 2 weeks of fever and splenomegaly) is respected [7].

Two studies conducted by WHO [12] and the meta-analysis of F. Chappuis & coll [5] concluded that the performance of ICT depends on the choice of the used reagent that must take into consideration the different circulating strains in each region.

Limits

In a study conducted in Switzerland on HIV-positive patients, the sensitivity of IT-Leish® was 54% [7]. The conclusion from this study is that the rapid test, being a serological method based on the detection of antibodies, should not be used in immunocompromised patients. In our study, this concept was found in one of our patients with a primitive immunodeficiency as a cause of immunodeficiency, and in whom the ICT was negative despite the presence of Leishmaniasis in the marrow.

Also, in case of suspicion of relapse of visceral Leishmaniasis, the ICT rK39 is irrelevant, because antibodies persist until 24 months after successful treatment [7, 13].

CONCLUSION

Visceral Leishmaniasis is a public health problem in Morocco, particularly in the region of Fez, which is a significant endemic center.

In this context, rapid diagnostic tests are an important novelty in visceral Leishmaniasis diagnosis, allowing rapid, simple and budget diagnosis.

These tests may be adopted in first intention if the clinical signs are suggestive, considering the excellent results reached in this study, which was carried out in an endemic zone.

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