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COMPARISON BETWEEN BUFFY COAT SMEAR AND DAT FOR THE DIAGNOSIS OF VL IN SUDAN

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ABSTRACT

Visceral Leishmaniasis is considered by WHO as one of the leading diseases in the world and is present in 88 countries worldwide. It affects poor communities in peripheral areas. It infects the internal organs of the body.,fatal if not treated. It is caused by the parasite *L. donovani* in the old world and *L. infantum* in the new world. Correct diagnosis is necessary to treat the disease. Many diagnostic techniques are known, but none is agreed upon worldwide. This study aims to compare two techniques for the diagnosis of VL in Sudan. 37 patients were included in this study. Buffy coat smears were prepared from peripheral blood of these patients and were searched for the presence of the parasite. Serum from these blood samples was used to preform DAT technique. The sensitivity of buffy coat smear was 37.8% and that of DAT was 100% .When MacNemar test was conducted, it was found that DAT is better than buffy coat smear for the diagnosis of VL. We concluded that DAT is a better diagnostic technique and could be adapted for diagnosis of VL in Sudan.

Key word: Visceral leishmaniasis, parasitological diagnosis, serological diagnosis, DAT, buffy coat smear, sensitivity.
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INTRODUCTION

The Leishmaniasis is a group of diseases caused by the obligate intracellular protozoa of the genus Leishmania (WHO, 2019). The WHO considers it one of the six entities on its list of the most important diseases (WHO, 2007). There are three forms of the disease being:cutaneous Leishmaniasis infecting the skin,mucocutaneous infecting the oronasal and pharyngeal cavities and visceral Leishmaniasis infecting the internal organs of the body,visceral Leishmaniasis is the most severe form of all forms which is fatal if left untreated (WHO, 2004; WHO, 2008).

Visceral Leishmaniasis (VL) is a serious health problem affecting an estimated 200,000-400,000 person annually, throughout tropical and subtropical regions. Ninety percentages of the new cases comes from six countries: Bangladesh, Brazil, Ethiopia, India, Sudan and South Sudan. (WHO, 2015). 50,000 people are killed by VL worldwide. VL affects most impoverished people in peripheral areas worldwide. In Sudan, Alvar estimated 30,000 cases of which 20,000 originate from north Sudan (Burki, 2009). VL is caused by *Leishmania donovani* in old world and *Leishmania infantum* in both the old and new world (WHO, 2004).

One of the reasons for the high burden of VL worldwide is problems associated with the sensitivity and specificity of current available diagnostic tests. Diagnostic tests for VL need to be extremely sensitive (as the disease is fatal) and specific (as treatment drugs are toxic) (WHO, 2004; Chappuis *et al.*, 2007). These tests should also be simple (Guerin *et al.*, 2002). Parasitological diagnosis is the gold standard methods of diagnosis of VL because of its high specificity (WHO, 2004; Srivastava *et al.*, 2011). Samples are obtained from different parts of the body. The sensitivity of parasitological methods ranging from 93% in spleen to 53% for lymph node smear. The accuracy of these methods depends on the expertise of the technicians or physician and the quality of the reagents used (Chappuis *et al.*, 2007; WHO, 2010).

Serological methods had emerged for diagnosis of visceral Leishmaniasis to overcome the limitation of parasitological methods. One of the mostly used method is DAT. DAT in most research papers had shown good results (Kilic *et al.*, 2008). DAT had shown to be highly specific, sensitive, inexpensive and simple test (Chappuis *et al.*, 2007; Srivastava *et al.*, 2011; Rajesh and Sanja, 2012). DAT performance is not influenced by neither the region nor by the Leishmania species (Chappuis *et al.*, 2007).

The golden standard method in Sudan for diagnosis of VL is detection of the parasite in either bone marrow smear or lymph node smear. Both methods need an experienced expert to conduct them. Buffy coat smear is simpler to preform but is not considered a standard diagnostic method in Sudan.

The current study aims to compare the performance of DAT (aqueous antigen) and buffy coat smear for the diagnosis of VL in Sudan.

MATERIAL AND METHODS

A hospital based study was conducted collecting blood samples from Khartoum Teaching hospital. Thirty seven suspect patients were included in this study. All of the studied patients were examined clinically by a physician for symptoms and signs of VL. The clinical criteria included fever for two weeks or more, splenomegaly and/or lymphadenopathy. 5 mL of peripheral blood was collected from each patient. Three ml were used to prepare the buffy coat smear. The remaining two ml of blood was used to conduct DAT technique. These 2 mL were left to clot before the serum was collected in a container and stored at -20° C until use.

The 3 mL of blood was transferred into a vacuotainer containing EDTA before being centrifuged at 3000 rpm for 5 minutes. This centrifugation separates the blood into three layers: the plasma layer, buffy coat layer and red blood cell layer. Under aseptic conditions, a sterile pasteur pipette was used to collect the buffy coat layer which was used to prepare a smear in a slide. The slide was stained using Leishman stain

and examined under the microscope for the presence of L.D bodies (amastigote). The amastigotes were identified by clearly visible nuclei and kinetoplast. The serum was brought to room temperature before being used to perform the DAT technique. The antigen was kindly provided by Prof El-Harith for the conduction of the test. The diluent was prepared according to El Harith *et al.* (1995). 0.1 mL of gelatin and 0.1g of urea were added to 100 mL of normal saline. The mixture was then heated in a water bath at 56° C for 10 minutes to allow the gelatin and urea to dissolve in the normal saline. 0.4 ml of 2-mercaptoethanol (Sigma Co No 625) was added to the solution and mixed well by gentle shaking. The DAT technique was performed as described by El Harith *et al.* (1988). 50 µL of the diluent was placed in each well of the 96 v-shaped microtitre plate with exception of column no 2 in which 100 µL of the diluent was added. Row A was used as the positive control in which 1 µL of the positive control serum was added to well 2A. Column 1 of all raws was used as the negative control in which no serum was added. For B-H,1 µL of each test sera were added to the wells in column 2. Then using a multi-channel pipette, two fold serial dilution was made. The plate was incubated for an hour at 37°C. Finally the antigen was added to each well including that of the negative control. The plate was then left overnight and on the next day, the result was read visually against a white surface such a white sheet of paper. The end point was read by localizing a clear sharp edged blue spot identical to the one observed on the negative control well. A titre of 3200 was considered positive result for the test. The obtained data was analyzed using N-PAR MacNemar test to determine the significance of the difference between buffy coat method and the DAT technique. Sensitivity was calculated to compare the efficiency of the studied techniques.

RESULTS AND DISCUSSION

This study showed that 14 samples were positive by buffy coat technique and 23 samples were negative by buffy coat technique. The sensitivity of the technique was found to be 37.8%. All of the 37 samples were positive by the DAT technique indicating a sensitivity of 100%. MacNmar test was conducted to compare the results of buffy coat smear technique with the DAT technique. Table 1 showed that 10 samples were positive and 5 samples were negative by both techniques.

		DAT		Total
		+ve	-ve	
BC smear	+ve	10	0	10
	-ve	22	5	27
Total		32	5	37

Table 1: A comparison between buffy coat smear and DAT techniques of 37 suspect patients of VL (MacNmar'test).

The DAT was positive in all buffy coat cases. 22 samples were DAT positive but buffy coat negative. The DAT is significantly more sensitive than buffy coat smear technique (p=0.000). The sensitivity of buffy smear was found to be 37.8% which is in consistent with results reached by Diro *et al.* (2017) who reported a sensitivity of 19.5% and Rohrs (1964) a sensitivity of 35%. Shamsuzzaman *et al.* (2007) used the method of density gradient centrifugation using mono-nuclear cell separation fluid "Lymphoprep"and the sensitivity raised to 92.98%. This low percentage could be attributed to the method used for the collection of the buffy coat layer from the blood. Locally, contradictory results were recorded for the use of this technique. Archibald *et al.* (1937) in two districts (Fung and Kapota) found that the sensitivity was zero as no parasites were found in the smears. Henderson and Hygiene (1937) found the sensitivity to be 1%. Marshall (1911) obtained a sensitivity of 86.6%. The sensitivity of DAT was found to be 100%.This result is in consistent with the results reached by Shalayel *et al.* (2015) who reported a sensitivity of 100% and Bern *et al.* (2000) reported a sensitivity of 100%. Abdallah *et al.* (2004) reported a sensitivity of 91.0%. Using N-PAR MacNamar test, the DAT performed better than buffer coat smear technique (p=0.00) which advocate its use as a diagnostic technique over buffy coat smear technique. To my knowledge the performance of these two techniques were not compared.

CONCLUSION

This study demonstrated the superiority of DAT over buffy coat smear technique and advocates its use as a diagnostic test for detection of visceral Leishmaniasis in Sudan.

CONFLICT OF INTEREST

Author has no conflict of interest.

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