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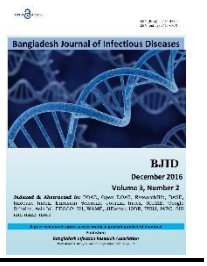
Original Article
Bangladesh Journal of Infectious Diseases
June 2019, Volume 6, Number 1, PP. 12-15
ISSN (Online) 2411-670X, ISSN (Print) 2411-4820
DOI: https://doi.org/10.3329/bjid.v6i1.42660



Comparison of KAtex, Bone Marrow Aspiration and DAT for the Diagnosis of Visceral Leishmaniasis

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[Received: 7 March 2019; **Accepted:** 20 May 2019; **Published:** 1 June 2019]

Abstract

Background: Newly developed KAtex test can be used as a non invasive tool for diagnosis of Kala-azar. **Objectives:** The aim of the present study was to compare KAtex, Bone marrow aspiration and DAT to diagnose VL. **Methodology:** This cross-sectional study was carried out in the Department of Microbiology at Dhaka Medical College, Dhaka, Bangladesh in collaboration with the Department of Parasitology, Institute of Epidemiology, Disease Control and Research (IEDCR), Dhaka, Bangladesh for a period of one year. Clinically suspected Kala-azar (VL) cases of different age and sex attending IEDCR, Dhaka from different Kala-azar endemic areas of Bangladesh were selected for this study. Patients having fever for more than 2 weeks, with or without splenomegaly, having history of loss of body weight following onset of fever were clinically suspected as Kala-azar cases. Microscopy and culture was performed in bone marrow (BM). KAtex was performed with urine sample. Agglutination of sensitized latex indicated presence of *Leishmania donovani* antigen in urine and thereby visceral leishmaniasis. No agglutination indicates absence of antigen in urine. DAT was done with serums of all cases. **Result:** Among 130 clinically suspected VL cases, 70 (53.85%) cases were BM positive and 60(46.15%) cases were BM negative. All the 70 BM positive cases were positive by KAtex and DAT. Among 60 BM negative cases, 15 were positive by KAtex and 23 were positive by DAT. The sensitivity of KAtex was 100.0% and specificity was 75.0%. The sensitivity of DAT was 100.0% and specificity is 61.6%. **Conclusion:** In conclusion, KAtex test is a good diagnostic tool for the detection of VL in comparison with DAT. *[Bangladesh Journal of Infectious Diseases, June 2019;6(1):12-15]*

Keywords: VL; KAtex; Bone marrow; Kala-azar; Direct agglutination test; DAT

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Conflict of interest: There is no conflict of interest to any of the authors of this article.
Funding agency: The study was not funded by any authority.
Contribution to authors: Dr. Ishrat Sharmin contributed from protocol writing upto article write up. The rest authors revised and corrected the paper.
How to cite this article: Sharmin I, Quamruzzaman AKM, Parveen R, Yusuf MA, Khanam RA. Comparison of KAtex, Bone Marrow Aspiration and DAT for the Diagnosis of Visceral Leishmaniasis. Bangladesh J Infect Dis 2019;6(1):12-15

Introduction

Visceral leishmaniasis (VL) is commonly known as Kala-azar[1]. It is a chronic febrile disease caused by *Leishmania donovani*[2]. The demonstration of the parasite (LD bodies) in the aspirates of the spleen, liver, bone marrow, lymph nodes is the only way to confirm VL conclusively[3].

Sensitivity of bone marrow aspirate smear is estimated to be 70.0% or lower[4]. Sensitivity of lymph node aspirate is estimated to be 50.0% in Sudan and sensitivity of splenic aspirate exceeds 90.0%[5]. But these invasive procedures are time consuming which carries risk of hemorrhage and requires expert persons. It may be false negative if the parasite density is low[6].

Antigen detection is more specific than antibody based immunodiagnostic tests. This method is also helpful in the diagnosis of disease in cases where there is deficient antibody production[7]. Currently a latex agglutination test named as KAtex has been described for the detection of urinary antigens in VL[8]. This test is positive in active cases and it is positive within one week of infection[9]. KAtex becomes negative one month after completion of treatment. KAtex is simply to use, results are available within 2 minutes, it does not require any electric appliances and is thus feasible in the rural health centres. Collection of urine is acceptable to the patients. Testing of an antigen has moreover a potential for monitoring response to treatment where the antibody based tests are of no help[9].

Serological tests for the detection of antibodies such as DAT have been developed in the pursuit of an alternative to demonstration of parasite. The direct agglutination test (DAT) has excellent diagnostic accuracy[10]. DAT is a sensitive, specific and simple test but its main disadvantage is that, it cannot readily distinguish between active disease, sub clinical infections or past infections[8]. Ab detection tests remain positive long after successful treatment, so can't be used to observe prognosis. Therefore, this study was undertaken to compare KAtex with BM aspiration and DAT to establish KAtex as a useful test for diagnosis of VL patients.

Methodology

This was a cross sectional study carried out in the Department of Microbiology, Dhaka Medical College, Dhaka in collaboration with the Department of parasitology in IEDCR from July 2006 to June 2007 for one year. Clinically suspected kala-azar (VL) cases of different age and sex attending IEDCR from different kala-azar endemic areas of Bangladesh were selected for this study. Patients having fever for more than 2 weeks, with or without splenomegaly, having history of loss of body weight following onset of fever were clinically suspected as kala- azar cases. Blood, Urine and bone marrow aspirations were taken with full aseptic precaution. Urine was collected in a sterile dry test tube for KAtex test. Bone marrow (BM) aspiration was done for microscopy and culture was done in N.N.N medium at 24^0c for 1-3 week. Data was collected in a pre designed data sheet. Then data were entered in computer and analyzed by using SPSS program. Following proper aseptic precaution blood was collected from all cases. Serum was separated by centrifugation. DAT was done with serums of all cases. Titre of ≥1:3200 was regarded as positive. Cross linking of the cells or large particles by antibody directed against surface antigens leads to agglutination[11].

Result

Table 1 shows Bone marrow samples, urine samples and blood were collected from 130 clinically suspected kala–azar cases, 70 cases were bone marrow positive and 60 cases were bone marrow negative. Among 130 cases, 85(65.38%) were KAtex positive and 93(71.54%) cases were DAT positive.

Table 1: Comparison of Bone Marrow examination with KAtex and DAT

BM Culture	KAtex Positive	DAT Positive
Positive (n=70)	70(100%)	70(100%)
Negative (n=60)	15(25%)	23(38.33%)
Total (n=130)	**85(65.38%)**	**93(71.54%)**

Table 2 shows among 130 cases, 85(65.38%) were KAtex positive and 45(34.62%) cases were KAtex negative.

Table: 2: Comparison of KAtex with Bone Marrow Culture

KAtex	BM Culture		Total
	Positive	Negative	
Positive	TP 70	FP 15	85
Negative	FN 0	TN 45	45
Total	**70**	**60**	**130**

Table 3 shows that among 130 clinically suspected Kala-azar Cases, 70 Cases were bone marrow

positive and 60 cases were bone marrow negative. Among, 130 cases, 93(71.54%) were DAT positive and 37 (28.46%) case were DAT negative.

Table 3: Comparison of DAT with Bone Marrow

DAT	BM Culture		Total
	Positive	Negative	
Positive	70	23	93
Negative	0	37	37
Total	**70**	**60**	**130**

Table 4 shows sensitivity of KAtex is 100% and specificity of KAtex is 75%. Sensitivity of DAT is 100% and specificity of DAT is 61.6%.

Table 4: Sensitivity and Specificity of DAT and KAtex for diagnosis of VL

Variables	KAtex	DAT
Sensitivity	100.0%	100.0%
Specificity	75%	61.6%

Discussion

The study has been carried out to evaluate the performance of KAtex in the diagnosis of Kala-azar cases and to compare these results with those obtained by BM microcopy and culture and DAT. In this study, among 130 clinically suspected Kala-azar cases, 70(53.85%) cases are BM positive and 60(46.15%) cases are BM negative. Sensitivity of BM aspirate smear is estimated to be 70.0% or lower[4]. This coincides with the result in this present study.

In the present study, among 130 cases, KAtex was positive in 85(65.38%) cases and DAT was positive on 93(71.54%) cases, all the 70 BM positive cases were KAtex positive. Among 60 BM negative cases 15 (25%) were KAtex positive. In a study done by Nahar[12] results of BM aspirates and KAtex were compared. In that study all the 68 (100%) BM positive cases were KAtex positive. Among 82 BM negative cases 12 (14.63%) were KAtex positive in that study. The result of KAtex positivity in BM negative cases in present study is higher than that of Nahar[12]. This is might be due to the fact that in this study, tests were done on freshly collected urine samples. In the study done by Nahar[12], KAtex was done on urine samples collected from Mymensingh which were brought to Dhaka.

In another study done in Sudan, all the 15 (100%) smear positive cases were KAtex positive. Among 47 smear negative cases 6 (12.76%) were KAtex positive and 41 (87.23%) were KA tex negative[1]. The results of KAtex positivity among smear negative cases in Sudan is lower than the present study which may be for the reason that they used both fresh and frozen urine samples.

In the present study, among 130 cases, DAT was positive in 93(71.54%) cases. All the 70 (100%) BM positive cases were DAT positive. Among 60 BM negative cases 23 (38.33%) were DAT positive. In a study done in Sudan in 1990, out of 65 smear negative patients 18 (28%) were DAT positive[13]. Among those 18 cases, eight were tested with the leishmanin skin test, six give a positive result suggesting past or sub clinical infection[13]. In another study DAT was positive in all the 20 (100%) BM positive cases and DAT was also positive in all the 7 (100%) BM negative cases[14]. DAT positivity among BM positive cases in the present study was similar to the study of El-Safi[14]. DAT positivity among BM negative cases in the present study was less than the study of El-Safi because in their study BM was done only from the DAT positive cases.

The sensitivity of KAtex is 100% and specificity of KAtex is 75% in this present study. Vilaplana showed 100% sensitivity and 96% specificity for KAtex[15]. In a study in Sudan showed 100% sensitivity and 87% specificity for KAtex. In separate study on 52 samples from Yemen, sensitivity of 86% and specificity of 100% for KAtex were reported[1]. In another study conducted by EL- Safi from Sudan showed 95.2% sensitivity and 100% specificity for KAtex[8]. In the present study, results of KAtex is compatible to other studies.

The sensitivity of DAT is 100% and specificity of DAT is 61.6% in this present study. In a study, done by Harith and others showed 100% sensitivity and 72.60% specificity for DAT[16]. In a hospital based study conducted in Sudan in 1990, DAT showed 94% sensitivity and 72% specificity[4]. The results of present study is similar to other studies.

Conclusion

In conclusion KAtex test for the detection of leishmania antigen in urine can be used as a non invasive tool for diagnosis of Kala-azar which has a high sensitivity in comparison with DAT.

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