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RAPID NON - INVASIVE DIAGNOSIS OF KALA - AZAR

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A Research Report :

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ABSTRACT :

RAPID NON – INVASIVE DIAGNOSIS OF KALA – AZAR

Objective :- To determine whether rK – 39 Leishmania dipstick is an acceptable test in the diagnosis of Kala-azar (KA).

Design :- Retrospective review of hospital case records of KA patients admitted during poush – Chaitra 2057.

Setting :- Tertiary care – Sukraraj Tropical & Infectious Disease Hospital, Teku.

Subjects :- Admitted patients with the diagnosis of KA .

Main outcome Measures :- Sensitivity & Positive Predictive value of rK – 39 Lesihmania dipstick.

Results :- The rK – 39 Leishmania dipstick achieved a high sensitivity (96%) and high positive predictive value (95%). The dipstick is a simple, reliable and a robust technique.

Conclusion :- The rK – 39 Leishmania dipstick is an acceptable test of choice in the diagnosis of KA and may be of great utility especially in the endemic districts where invasive methods are neither applicable nor appropriate.

I. Introduction :-

Kala - azar (KA or Visceral Leishmaniasis VL) is endemic throughout the central and eastern terai region of Nepal bordering the Indian state of Bihar. 1 Although prolonged febrile splenomegaly, anemia/pancytopenia and hypergammaglobulinemia are suggestive of KA; yet diagnosis is definite on demonstration of *Leishmania donovani* (amastigotes) bodies in splenic or bone marrow aspirate^{2,3}

But splenic puncture may lead to potentially fatal haemorrhage and bone marrow aspirate has a low sensitivity yield. 4 .

Beside, invasive diagnostic method are neither applicable nor appropriate in the endemic districts because of lack of trained manpower and inadequate facilities. 5,6 .

Lack of knowledge, unavailable simple and reliable diagnostic test and poverty - all contribute to late diagnosis & institution of an effective chemotherapy; thereby maintaining the potential human reservoir at high level in the community. 7 since invasive diagnostic method is an unacceptable test of choice in the endemic region, an alternative technique is a dire necessity. But, such method should be simple, reliable, and robust to qualify as an acceptable test of choice. The present study, evaluated recombinant K- 39 (rK-39) *Leishmania* test with splenic aspirate in hospitalized KA patients.

II. Method :-

This was a retrospective review of hospital case record of KA patients admitted during the months of POUISH - CHAITRA 2057. All hospital case notes of the 4 months with the diagnosis of KA were screened.

Eligibility :-

To qualify as an eligible case, the following criteria was graded as a complete case record.

- A. Compatible clinical features of prolonged (>2 weeks duration) febrile. Splenomegaly (\pm hepatomegaly) with anemia (\pm pancytopenia) and a negative malarial smear.
- B. Splenic aspiration was performed.
- C. The rK – 39 Leishmania test was done.
- D. Both the tests had to performed on the same day.
- E. Aspirate reading was performed in Central Public Health Lab, Teku and rK – 39 test read in the hospital lab. Antimony relapse and Kala –azar treatment failure cases (KATF) were included.

Exclusion :-

Case records deemed incomplete (as defined) and case records with co- infection were excluded from the study.

Principle of rK – 39 dipstick :-

The rK – 39 antigen is striped onto nitrocellulose paper and a drop of whole blood is placed onto a sample pad with immunolabeled gold. Human I_gG binds to labeled gold and migrates up the strip, If antibodies to rK – 39 are present in serum, they bind to the rK – 39 antigen strip giving a visual positive test result.

The result is read within 10 minutes. A positive control visual band is also available on the dipstick.

The rK – 39 yielded a sensitivity of $(57/59) = 96.6\%$

III. Result :-

A total of 80 provisional diagnosis of KA patients were admitted during POUSH – CHAITRA 2057. We excluded 16 case records from the study because 12 were deemed incomplete and 4 had documented co – infection (2 – Pul. Tuberculosis, 1 – chronic renal failure and 1 - HB_s Ag +ve with Jaundice).

Among 64 eligible cases 59 were splenic aspirate and positive. The rK – 39 test was positive in 60 patients. While 2 patients were aspirate and rK – 39 negative.

Among 59 aspirate positive cases, the rK – 39 was negative in two patients. While 3 aspirate negative case were rK – 39 positive.

Table 1 depicts the finding

rK – 39 Leishmania dipstick

	+			
SPLENIC ASPIRATE	+ 57	02	=	59
TOTAL =	<u>- 03</u>	<u>02</u>	=	<u>05</u>
	60	04	=	64

TABE : 1

+ = Positive = Negative

The rK – 39 yielded a sensitivity of $(57/ 59) = 96.6\%$

The positive predictive value was $(57/ 60) = 95\%$

All aspirate positive cases received chemotherapy. While one aspirate negative but rK –39 positive case received chemotherapy on strong clinical basis. Among the two aspirate and rK – 39 negative cases, one was diagnosed as Brucellosis (Brucella serology positive) while the other was diagnosed as Tropical splenomegaly syndrome.

IV. Discussion :-

Several serodiagnostic methods have been evaluated for KA including IFAT, DAT, ELISA.^{8,9}

These tests utilise crude antigen (whole promastigotes or lysate thereof) which are liable to cross react with antibodies from other diseases. The rK - 39 is a cloned, potent amastigote antigen shared by members of *Leishmania donovani* complex.¹⁰

The occurrence of K39 predominantly on amastigote (replicative and pathogenic form) and not in promastigotes (transmitted form) confer additional advantage of sensitivity and specificity. This study confirmed the highly sensitive nature of rK - 39 (>96% sensitivity) and high positive predictive value (95% positive pred. Value).^{11,12}

Therefore, presence of antibodies to rK - 39 with compatible clinical case definition correlates with active KA.

One aspirate positive (parasite. Density 1+, range 0-6+) that failed to sero-react to rK -39 had initial Amphotericin B therapy (total 10 mg/ Kg BD. Wt.) Low numbers of amastigotes may reflect relatively lower titres of antibody that may fail to sero-react which may have prognostic significance.

Three aspirate negative cases that were sero-reactive to rK -39 may partly reflect the nature of splenic yield (ie. NOT 100% sensitive) and the tedious time consuming process (at least 100 fields scan to declare negative). That is generally not feasible in a busy central lab. Only 1 case among the three received therapy on strong clinical basis.

The rK - 39 test is suitable in young children (high risk group) and in older patients with deranged hematological parameters (including prothrombin time) where splenic aspiration is not advisable.

The rK -39 Leishmania dipstick is a simple test that neither require special training nor equipment. The dipstick neither require specialized handling nor storage and any adverse effect of humidity is minimized with a reagent incorporated in the pack which makes it a robust technique.

This test is reliable because of high sensitivity and high positive predictive value hence suitable for, integration into the diagnostic algorithm esp, in the endemic district.

Acceptability would be high in the endemic districts because of :

- A. Painless and minimal hazard (non - invasive)
- B. Simple and robust technique (nature of dipstick)
- C. Rapid and accurate result (within minutes with high sensitivity & high positive predictive value).

Although rK - 39 is an ideal test, the finding of a hospital study is not representative of an endemic community. The study is limited by hospital bias and spectrum of illness varies from the asymptomatic, sub-clinical - self resolving, sub-clinical - progressive, to overt KA^{13,14}. Therefore, a careful community field based study is essential before introducing the test in the endemic region.

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