The Myanmar Health Sciences Research Journal, Vol. 18, No. 2, 2006

Aldehyde test (Formol-Gel test) in the diagnosis of kala-azar (Visceral leishmaniasis)

**Khin Saw Aye, **Khin Myat Tun, *Yin Min Htun, *Yin Thet Nu Oo, *Tin Zar Maw & ***Lwin Lwin Moe*

*Immunology Research Division
**Department of Medical Research (LM)
*** Clinical Research Division
Department of Medical Research (LM)

Visceral leishmaniasis is a severe disease, which is endemic in 88 countries including 17 developed nations. In endemic areas, recent migration pattern of people, vector (sandfly) and reservoir (dogs) have led to the urbanization of visceral leishimaniasis. This study aimed to find out the role of Formol Gel Test (Aldehyde test) in the diagnosis of kala-azar in rural areas, where the diagnostic facilities are scarce. Three- hundred samples of subjects from the villages of Maung-Daw Township, Yakhine State including 45 Immunochromatographic Test (ICT) strip test positive cases and as a control, 300 blood samples from National Blood Bank (NBB) were subjected to Aldehyde test (AT). Fifty-five (18.3%) cases from Yakhine State and 9 (3%) samples of NBB were found to be AT positive. Among 55 AT positive cases, 40 cases (73%) were ICT positive and 15 (27%) were negative. Among 45 ICT positive cases, 40 (89%) were also AT positive and 5 (11%) were AT negative. Sensitivity and specificity of AT were 87.5% and 93.8%, respectively. There was a significant association between the two tests of ICT and AT ($p<0.001$). Aldehyde test is simple, rapid and cheap for diagnosis of visceral leishmaniasis. Although negative Aldehyde test does not exclude Kala-azar infection, a positive result in clinically suspected cases in an endemic area is an applicable diagnostic tool for visceral leishmaniasis.

INTRODUCTION

Visceral leishmaniasis (VL) is a severe disease with high mortality and endemic in 88 countries including 17 developed nations [1,2]. A serious problem in much of the world including Brazil, China, East Africa, India, and areas of the Middle East, leishmaniasis is also endemic in the Mediterranean region including southern France, Italy, Greece, Spain, Portugal and Northern Africa. In areas where leish-maniasis is endemic, recent migration patterns of people, vectors (sandfly) and reservoirs (dogs) have led to the urbanization of VL [3]. In Southern Europe, VL has become the leading opportunistic infection in AIDS patients [4,5].

The demonstration of Leishman-Donovan bodies (amastigotes) in spleen, bone marrow or lymph node aspirate provides the definite diagnosis of kala-azar. These procedures have some limitation [6,7] because these are not always practicable in rural settings. Specific serological tests are complex, require trained man-power and a well-equipped laboratory. People of low socio-economic group residing in the rural area are often the victims of kala-azar. They are usually beyond the reach of well-equipped laboratory facilities. In such circumstances,

the Aldehyde test (AT) (Formol-Gel test), which is nonspecific, is simple to do and less costly. This communication assesses the role of this test in the diagnosis of kala-azar in a part of the world where the diagnostic facilities are scarce.

MATERIALS AND METHODS

One thousand subjects living in the villages of Maung-Daw Township, Rakhine State were tested for the visceral leishmanias is antibody in human serum by using *Kalaazar Detect* rapid test (InBios International, Inc., USA) in April, 2004. The *Kalaazar Detect* rapid test for VL is a rapid immunochromatographic (ICT) strip assay for the qualitative detection of antibodies to members of *L. donovani* in human serum.

Three-hundred cases including 45 ICT positive cases were performed the aldehyde test. The aldehyde test was done by adding one drop of commercial formalin (40% formaldehyde) to 50 ul of serum (WHO manual on VL Control) [8]. Solidification with complete opacity of serum within 10 minutes was taken as strongly positive, within 20 minutes was moderately positive and 30 minutes as weakly positive reaction. In a negative test, the serum remains unchanged or whitening and gelling only occurred after 30 minutes.

RESULTS AND DISCUSSION

In 1,000 subjects from Maung-Daw Township, Rakhine State, 45 cases (4.5%) were positive in VL antibody by using ICT. For comparison of the results of AT and ICT, three- hundred cases including 45 ICT positive cases from Yakhine State and 300 blood samples from National Blood Bank (NBB) as a control were subjected to Aldehyde test (AT). Among 55 AT positive cases, 40 cases (73%) were ICT positive and 15 (27%) cases were negative. Among 45 ICT positive cases, 40 (89%) were also AT positive and 5 (11%) were AT negative (Table 1). Sensitivity and specificity of Aldehyde test are 87.5% and 93.8% respectively. There was a significant association between the two tests (P<0.001). AT and ICT can, therefore, be regarded as equally dependable diagnostic tests for clinically suspected kala-azar patients.

Table1. Results of Aldehyde Test by Immunochromatographic Test (n = 300)

AT	ICT		
	Positive	Negative	Total
Positive	40 (73%)	15 (27%)	55
Negative	5 (2%)	240 (98%)	245
Total	45	255	300

AT = Aldehyde Test
ICT = Immunochromatographic Test

$$\text{Sensitivity} = \frac{\text{True positive} - \text{False negative}}{\text{True positive}} \times 100 = \frac{40-5}{40} \times 100 = 87.5\%$$

$$\text{Specificity} = \frac{\text{True negative} - \text{False positive}}{\text{True negative}} \times 100 = \frac{240-15}{240} \times 100 = 93.8\%$$

Among 300 cases of the control group (non kala-azar patients), there were negative to AT in 291 (97%) cases and only 9 (3%) were positive (Table 2). The findings of AT between kala-azar and non kala-azar patients were significantly different (P <0.01).

Table 2. Aldehyde Test in kala-azar and normal control

Cases	Positive	Negative	Total
Kala-azar	55 (18.3%)	245 (81.7%)	300
Normal control	9 (3%)	291 (97%)	300

Immunochromatographic strip test (ICT) for the diagnosis of VL was evaluated in a case control study in Bangladesh, parasitological confirmation was done by demonstration of *Leishmania donovani* bodies in bone marrow or splenic aspiration. ICT strip test for VL was done in all cases and control, sensitivity and specificity of ICT were 96.6% and 98.3%, the predictive value for negative results was 98.3% and for positive

results was 96.6% [9]. The ICT findings of Mymensingh Medical College showed 100% sensitivity with 92.3% specificity and sensitivity of AT was 92.1% and 99.5% specificity [10].

AT has been regarded as a non-specific test because false negative results can be encountered in conditions where immunoglobulins are greatly increased for some reasons such as multiple myeloma, leprosy, trypanosomiasis, chronic malaria and chronic liver diseases [11]. However, in conditions like chronic liver diseases, malaria, leprosy and trypanosomiasis, globulin levels do not usually raise high enough to produce a strongly positive AT [12]. Multiple myeloma is a disease where AT may be strongly positive but it seldom produces diagnostic difficulties. There are some other conditions which may be confused with kala-azar clinically such as haemolytic anaemia, chronic myeloid leukaemia, enteric fever, and infective endocarditis, but the AT is negative in all those conditions.

Although, a negative AT does not exclude kala-azar (as it requires 3 months to become positive) a strongly positive result in clinically suspected cases in an area where kala-azar is endemic, is very helpful in giving diagnosis.The Aldehyde (Formol-Gel) test is still of value in circumstances where complex serological test can not be done, ICT is not available, and where the performence or interpretation of both bone marrow or splenic aspirate tests for amastigote detection is problematic. It is simple to perform, involves negligible cost and can be done in any rural setting by the clinicians.

REFERENCES

1. WHO. Control of the Leishmaniasis, World Health Organization, *Technical Report Series* 1990. No.793.
2. Marsden PD. Selective primary health care: strategies for control of disease in the developing world. XIV. Leishmaniasis, *Rev. Inf. Dis.* 1984; 6:736-744.
3. Jeronimo SMB, Oliveira RM, Mackay S. *et al*. An urban outbreak of visceral leishmaniasis in Natal, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1994; 88(4):386-368.
4. Berenguer JS, Moreno E, Cercenado JCL, Bernaldo de Quiros A. Garcia de La Fuenta, Bouza E. Visceral leishmaniasis in patients infected with human immunodeficiency virus (HIV). *Annals of International Medicine* 1989; 111:129-132.
5. Houghton RL, Petrescu M, Benson, DR., Skeiky YA, Scalone A, Badaro R, Reed SG & Gradoni L. A cloned antigen (recombinant K39) of *Leishmania chagasi* diagnostic for visceral leishmaniasis in human immunodeficiency virus type 1 patients and a prognostic indicator for monitoring patients undergoing drug therapy. *Journal of Infectious Diseases* 1998; 177**:**1339-1344.
6. Maru M. Clinical and laboratory features and treatment of visceral leishmaniasis in hospitalized patients in North-western Ethiopia. *American Journal of Tropical Medicine and Hygiene*. 1979; 28: 15-18.
7. Chulay JD & Bryceson ADM. Quantitation of amastigotes of *Leishmania donovani* in smears of splenic aspirates from patients with visceral leishmaniasis. *American Journal of Tropical Medicine and Hygiene* 1983; 32: 475-78.
8. WHO. Manual on Visceral leishmaniasis Control. Formal-gel test for leishmaniasis. WHO/LEISH/96.40.
9. Sarker CB, Moman A, Jamal MF, Siddiqui NI & Talukder SI. Immunochromatographic (rK39) strip test in the diagnosis of visceral leishmaniasis in Bangladesh. *Mymensingh Medical Journal* 2003; July: 12 (2); 93-7.
10. Shamsuzzaman AK, Hossain MA, Musa AK, Hasan MU & Dhar DK. A preliminary report on culture of *Leishmania donovani* in Mymensingh Medical Collage and evaluation of new immunochromatography test (ICT). *Mymensingh Medical Journal* 2003; January: 12 (1); 51-4.
11. Manson-Bahr PEC. Leishmaniasis. In: Manson-Bahr PEC, Apted FIC eds. *Manson's Tropical Disease*, 18th edn. London. 1982; 93-115.
12. Levinson EL, Macfate JH, eds. *Clinical Laboratory Diagnosis*, 7th edn. London. 1969; 1044.