Diagnostic value of the commercial MycoDot™ test in the diagnosis of active pulmonary tuberculosis in Nepalese population

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ABSTRACT

Background: A rapid, technically non-demanding and inexpensive diagnostic test is needed to improve the early detection and better management of active pulmonary tuberculosis especially in resource limited areas. **Objective:** To assess the diagnostic value and usefulness of the commercially available MycoDot™ (DynaGen Inc., Cambridge, MA, USA) in the diagnosis of active pulmonary tuberculosis in Nepalese population. **Materials and Methods:** A prospective study was done from January 2008 to August 2008 on a group of 120 sputum smear positive adult patient group aged 17 to 78 years, confirmed cases of active pulmonary tuberculosis (with TB) were enrolled and sera samples collected from them. The second group comprised of 105 healthy controls with no history of pulmonary tuberculosis (without TB) and sera samples collected from them. The MycoDot™ serologic test was done on the sera samples collected from both the study groups according to the manufacturer's instructions. **Results:** Of the sera collected from 120 sputum smear positive cases (with TB group), 48 sera specimens were MycoDot™ test positive (sensitivity = 40%; 95% CI 31.68–48.94). Healthy controls (without-TB group) – 8 sera samples were found to be positive by the MycoDot™ test and the remaining 97 sera samples tested negative by this serologic test (specificity = 92.38%; 95% CI 85.68–96.09). The value for % false positive was 7.61% and for % false negative was 60%. The positive predictive value and the negative predictive value was 85.71% (95% CI 73.22–93.20) and 57.4% (95% CI 49.56–64.88), respectively. **Conclusion:** The MycoDot™ test with its rapidity (completed within 20 min), easy – to – perform format and no expensive instrumentation required – had high specificity but a relatively low sensitivity in the study group subjects and thus could be used only as an additional test in the diagnosis of active pulmonary tuberculosis and to be interpreted judiciously alongwith clinical findings.

Keywords: Immunodiagnosis, MycoDot™ test, Sensitivity, Specificity, Pulmonary tuberculosis

INTRODUCTION

The diagnostic arsenal for the early detection and better management of pulmonary tuberculosis (TB) remains limited. The standard tests for the laboratory diagnosis of active pulmonary tuberculosis continues to be sputum smear microscopy (acid – fast staining) and culture. Ziehl – Neelsen (ZN) and auramine – rhodamine staining are the only rapid screening methods for acid – fast bacilli (AFB), but they have low sensitivity (approximately 10^4 bacteria per ml of specimen are required for a positive result) and the need for a repeat sputum specimen. Collecting a good sputum specimen is crucial for quality sputum microscopic examination. It needs to be ensured that it's a deep cough specimen and not just saliva. The sensitivity is poor for paucibacillary disease e.g. pediatric and HIV – associated tuberculosis.[1] Culture is the gold standard for TB diagnosis and is more sensitive and specific, but the results require growth of the mycobacteria for up to 8 weeks. Broth based rapid and automated TB culture methods such as Septichek AFB,[2] radiometric (BACTEC),[3] mycobacterial growth indicator

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tubes (MGIT),[4] FASTPlaque TB assay[5] have been developed but still they take at least 2 weeks for the results to be declared and are expensive. Nucleic acid amplification tests[6] such as polymerase chain reaction (PCR), reverse transcriptase PCR (RT – PCR), ligase chain reaction (LCR), and commercially available COBAS AMPLICOR PCR system[7] (Roche Diagnostics) had shown tremendous potential to be routinely used in the laboratory diagnosis of tuberculosis but still have limited utility in resource limited areas as they are quite expensive and technically demanding to perform on a routine basis.

Immunodiagnostic methods especially those using purified antigens[8] have shown high specificity in recent times. Currently, there are dozens of distinct new commercial antibody detection test kits are available[1] that detects whether the individual has raised antibody levels against a particular protein antigen(s) of the *Mycobacterium tuberculosis*. Lipoarabinomannan (LAM) which is one of the dominant antigen of mycobacteria has been taken into consideration for its putative role in the serological diagnosis of tuberculosis.[9] LAM is a polysaccharide antigen present in the cell wall of all mycobacteria. Purified LAM from *M. tuberculosis* in its native acylated state was first used for the serodiagnosis of leprosy[10] and later for the serodiagnosis of tuberculosis.[11] Sada et al.,[11,12] measured the anti-LAM IgG antibodies in the sera of patients with pulmonary, miliary and pleural tuberculosis and in tubercular lymphadenitis patients with ELISA and achieved a high degree of specificity (91%) and sensitivity (72%) and found no significant difference in the levels of antibodies between the polar forms of tuberculosis i.e. pulmonary and miliary tuberculosis. Since then, several reports have been published investigating and detecting the anti - LAM antibodies as a marker in the laboratory diagnosis of active tuberculosis,[13,14] pulmonary tuberculosis in Tanzania,[15] Ghana[16] and Bangladesh.[17]

To the best of our knowledge, there is no report available from Nepal till date, investigating the value of detection of anti - LAM antibodies in the laboratory diagnosis of tuberculosis. This study was done to determine the value of detection of anti - LAM IgG antibodies by the commercially available MycoDot™ test in the rapid diagnosis of active pulmonary tuberculosis in the Nepalese population.

METHODOLOGY

STUDY SETTING: This prospective study was carried out at the tertiary care hospitals in Kathmandu, Nepal from January 2008 to August 2008. The institutional research and ethical committee approved the study and informed consent was obtained from the study subjects prior to enrollment in the study.

STUDY POPULATION: The study group comprised a total of 225 individuals. A group of consecutive 120 sputum smear microscopy positive by ZN stain, clinically and radiologically confirmed cases of active pulmonary tuberculosis (with TB), aged 17 to 78 years were enrolled in this study. Additionally, 105 healthy control group individuals, with no history of tuberculosis (without TB) - these were clinically, radiologically and sputum smear microscopically (ZN stain) negative for pulmonary tuberculosis were included for investigation as negative control.

SPUTUM SMEAR MICROSCOPY: The sputum samples from both the study groups were collected, decontaminated and concentrated by the N-acetyl-L-cysteine (NAC) and 2% NaOH method.[18] These were subjected to microscopic observations after ZN staining.

SEROLOGIC TESTS: Simultaneously sera samples were collected from both the study groups and were processed immediately. The MycoDot™ (Dyna Gen, Inc., Cambridge, MA, USA) serologic test was performed according to the manufacturers instructions. Briefly, 10 ul of diluted serum (1 : 10 in the sample diluent supplied with the kit) was poured and incubated onto the plastic combs bound with LAM antigen for 6 min. at room temperature. The combs were washed in the diluent rinse buffer by moving the combs back and forth across the wash tray 10 times to remove non-specific antibodies and were then incubated for 10 min. in the signal generating reagent and gently rock the comb back and forth 8–10 times and rinse as earlier. Allow the comb to air dry and record the results as recommended by the manufacturers instructions. A coloured spot as intense or more intense, than the weakest positive spot (cut-off) on the reference comb is to be considered a positive reaction. A spot less intense than the weakest positive spot on the reference comb or no spot at all is a negative reaction. The MycoDot™ test performed by the laboratory personnel were blinded to the clinical status of the patients or the results of ZN staining of the sputum samples tested. The positive and negative assay controls, supplied with the kit, were regularly performed.

STATISTICAL ANALYSIS: Diagnostic test 2×2 contingency tables were made. Sensitivity, specificity, positive and negative predictive values were calculated. All parameters were estimated with 95% confidence interval using the Stata 10.1 statistical software package (Stata Corp. College Station, Tx).

RESULTS

Of the 120 sera tested from patients who were sputum smear positive (ZN stain), clinically confirmed active pulmonary tuberculosis, 48 sera samples had reacted positive for anti-LAM IgG antibodies. The 72 sera samples from the remaining patients investigated were found negative by this test. The 105 sera samples tested from the healthy control group with no history of pulmonary tuberculosis, 8 reacted positive for anti-LAM IgG antibodies and 97 sera samples reacted negative. % false positive was 7.61% and % false negative was 60%. Screening test results by diagnosis of MycoDot™ test and the diagnostic accuracy parameters of sensitivity, specificity, positive predictive value and negative predictive value with 95% confidence intervals are shown in Table 1. A comparative data of the MycoDot™ test results obtained in this study and by different workers are shown in Table 2.

DISCUSSION

This study showed that the MycoDot™ test had an excellent specificity (92.38%; 95% CI 85.68–96.09) but a low sensitivity (40%; 95% CI 31.68–48.94). Low sensitivity could be explained as the true - TB group patients may be suffering from other diseases – impairing their humoral immune response of the IgG 2 subclass – thereby lowering their IgG levels to an undetectable levels.[19] The response of the IgG 2 subclass is stimulated by polysaccharide antigens and therefore by LAM itself. Since LAM is a glycolipid common to all the bacteria belonging to the genus *Mycobacterium*, the test evaluated in this study could be positive also in patients infected by mycobacteria other than *Mycobacterium tuberculosis* (atypical mycobacteria); at least in those without the acquired immune deficiency syndrome (AIDS).[11,13]

The results further showed a quite low false positive rate of 7.61% - this result is consistent with the high specificity obtained in this study. A high positive predictive value (85.71%; 95% CI 73.22–93.20) showed the test could be used to detect the positivity for tuberculosis in suspected patients. Detection of anti-LAM IgG antibodies by MycoDot™ test was evaluated by several workers.[13–17] These studies had showed a high degree of specificity (84%–97.5%) but the sensitivity was low to moderate (26%–76%). Limitations of this study being – the sputum smear negative but suspected cases of pulmonary tuberculosis could have been included as a study group. This would have provided the real value of the MycoDot™ test in the diagnosis of pulmonary tuberculosis. The study included only the adult cases, inclusion of paediatric patients group would have revealed more information about this test kit utility.

Diagnosis of tuberculosis is complex and presents unique challenges among the infectious diseases. A systematic analysis of the humoral immune response of tuberculosis patients has shown that the profile of antigenic proteins of *M. tuberculosis* recognised by antibodies differs at different stages of infection and disease progression i.e. early exposure, active disease, co-infections and

Table 1: Screening test results by diagnosis: MycoDot™ test (reference standard-sputum smear microscopy positive by ZN stain)

Screening test result	Diagnosis		Total	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
MycoDot™ test	With TB	Without TB					
Positive	48	8	56				
Negative	72	97	169	40	92.38	85.71	57.41
Total	120	105	225	(31.68–48.94)	(85.68–96.09)	(73.22–93.20)	(49.56–64.88)

95% CI- 95% Confidence Interval, PPV-Positive Predictive Value, NPV-Negative Predictive Value

Table 2 : MycoDot™ test results obtained in this study and by different workers: A comparative data

Author (year – study)	Study/Patient group	Reference standard / Smear status	Patient country	Sensitivity (%)	Specificity (%)
1. Lawn (1997)	Pulmonary TB	Smear/Smear positive	Ghana	56	97.5
2. Somi (1999)	Adult pulmonary TB	Culture/Smear positive	Tanzania	26	84
3. Okuda (2004)	Active pulmonary TB	Culture/Smear positive	Japan	76	97
4. Shamsuzzaman (2006)	Active pulmonary TB	Smear/Smear positive	Bangladesh	72.92	93.94
5. This study	Active pulmonary TB	Smear/Smear positive	Nepal	40	92.38

latent infection.[20–23] Furthermore the specificities of the antibodies produced differ among patients. The diverse antibody response to *M. tuberculosis* may be governed by HLA types.[24] It had been shown that the cell wall antigen composition of the tubercle bacilli differ among the isolates.[25] Thus, the choice of antigen(s) that is specific for the indication would be a critical factor for the success to be an accurate diagnostic test. Patients with sputum-smear positive pulmonary tuberculosis are the most active in generating and spreading aerosols containing live tubercle bacilli, but their detection is often delayed, thus perpetuating the transmission of the infection and disease in the population. Reducing the transmission of tuberculosis is of key importance for achieving its continued decline and the aims of serological screening should shift from clinical to public health priorities.[26]

MycoDot™ test has proved to be a very specific for the detection of anti - LAM antibodies in tuberculosis patients. It is a rapid assay and the results are available in 20 min. It could be performed without sophisticated instrumentation and with minimal training of the laboratory personnel. This easily performed assay may be useful for the presumptive diagnosis of tuberculosis in adult population in remote regions in resource limited areas where the routine screening facilities for pulmonary tuberculosis is not available or in areas without access to more sophisticated diagnostic procedures, including mycobacterial culture.

CONCLUSION

The anti-LAM IgG immunoassay (MycoDot™ test) was highly specific but had a low sensitivity for the diagnosis of active pulmonary tuberculosis in adult population. It could be used for the presumptive diagnosis of active pulmonary tuberculosis in conjunction with other established methods of laboratory investigation (including chest X-ray, erythrocyte sedimentation rate, C-reactive protein, serum adenosine deaminase levels etc.) and the results to be interpreted with care. Better in-vitro diagnostic tools are needed for early case detection of tuberculosis patients for reducing transmission and the effective management of active pulmonary tuberculosis.

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