Bangladesh J Med Microbiol 2007; 01 (01): 21-24

Bangladesh Society of Medical Microbiologists

Original article

Comparison of Western Blot Technique with Microscopy and Culture for Diagnosis of Tuberculosis

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Abstract

Diagnosis of tuberculosis is usually done by microscopy for AFB and other tests. But each test has different limitation. This study was carried out to compare western blot technique with microscopy and culture for diagnosis of tuberculosis. Sputum and other samples were collected from 112 TB patients for bacteriological diagnosis by microscopy and culture for AFB. Serum samples were collected from 112 TB patients and 50 control subjects for detection of antibody response by western blot. For western blot analysis, *Mycobacterium tuberculosis* sonicate antigen extract was fractionated by electrophoresis on polyacrylamide gel. Western blot analysis revealed four polypeptides against which antibody response of study population were observed. The sensitivity of western blot was 73.21% which was higher than that of microscopy (63.39%) and culture (57.14%) for diagnosis of tuberculosis. The specificity of western blot was 92%.

Key words: Emerging resistance, *M. tuberculosis,* Common bacteria

Introduction

Tuberculosis (TB) remains as a major cause of morbidity and mortality worldwide. It is the world's leading cause of death from a single infectious agent.[1] Each year nearly one percent of the world's population is newly infected with TB, 5 to10 percent of them become sick or infectious at some time during their life.[2] In Bangladesh of National TB Control Programme (NTP), early case detection and treatment remains the corner stone for effective control of TB.[3] Currently, the definite diagnosis of TB is based on microscopy with Ziehl-Neelsen's (Z-N) acid fast stain and laboratory culture of *M. tuberculosis* on Lowenstein-Jensen (L-J) medium.[4] However, Z-N staining has less sensitivity (40-60%). Culture techniques are complex and time consuming. Moreover, it is difficult to obtain clinical specimens from children and patients with closed cases of pulmonary disease and specially in extra-pulmonary tuberculosis where more expensive and invasive procedures are required.[5]

So, there is a need for a rapid, sensitive and specific test other than microscopy suitable for routine use. A serodiagnostic test of tuberculosis could be of particular value either in patients with typical pulmonary or extrapulmonary tuberculosis, in whom a bacteriological confirmation is difficult to obtain.[6] Many researchers around the world have used western blot technique for detection of specific humoral immune response to mycobacterial antigens in patients with tuberculosis.

The present study was carried out to detect the antibody response to *M. tuberculosis* polypeptides using western blot technique among tuberculosis patients and to compare it with microscopy and culture for diagnosis of tuberculosis.

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Methods

One hundred and twelve TB patients and 50 control subjects were selected from out patient department of Bangabandhu Sheikh Mujib Medical University and Institute of tuberculosis control and research, Chankharpul, Dhaka. The study was done during the period from January, 2003 to December, 2003. Among TB patients, 89 had pulmonary tuberculosis and 23 had extrapulmonary tuberculosis. Among extrapulmonary tuberculosis cases, 8 were tubercular lymphadenitis, 8 cases were endometrial TB, 6 were pleural TB, and 1 case was renal TB. Among control subjects, 25 were disease controls, having pulmonary diseases other than TB and 25 were normal healthy subjects having no clinical sign of respiratory or any other disease. Samples for microscopy and culture for identification of AFB were collected according to site of infection, for example sputum from pulmonary TB cases, lymph node biopsy material from tubercular lymphadenitis, endometrial curettage from endometrial TB, pleural fluid from pleural effusion and urine from renal TB patient. A sample of each biopsy material was sent for histopathological examination and the report was recorded properly. Tuberculin status of the patients were assayed and recorded. For Western-blot analysis, blood was collected from all patients and control subjects for detection of anti-mycobacterial antibody. For preparation of antigen for Western-blot analysis, *M. tuberculosis* H37 Rv strain was collected from tuberculosis research centre, ICDDR'B, Dhaka. The laboratory works were performed in the department of Microbiology & Immunology, BSMMU and RTI & STI Laboratory, ICDDR'B, Dhaka.

Microscopy and culture for AFB:

For bacteriological diagnosis of tuberculosis, all sputum and other appropriate samples were processed for microscopy and culture for AFB. Sputum samples were digested and decontaminated by Petrof's method, biopsy materials were homogenized in sterile grinder, pleural fluid and urine were centrifuged under aseptic condition. Then the processed samples were used for microscopy for AFB using Zeihl-Neelsen stain and culture in Lowenstein-Jensen media. Reporting of microscopy and culture was done according to standard method.

Western blot analysis:

Antigens for Western blot analysis were prepared from *M. tuberculosis* H37 Rv strain growing on Sauton's medium for 6 weeks. Then the bacilli were sonicated, centrifuged and the supernatant containing cell wall protein was used as antigen. The antigen was separated by electrophoresis on sodium dodecyle sulphate polyacrilamide gel (SDS-PAGE) and the separated polypeptide bands from gel were electrophoretically transferred onto nitrocellulose membrane. Nonspecific binding sites of membranes were blocked by 5% skimmed milk. Then the membranes were incubated with test sera of study population followed by peroxidase conjugated anti-human IgG and the reaction was developed using choronaphthol and hydrogen peroxide as substrate. The results of immunoreactions were analyzed by counting the bands in each strip.

A positive and a negative control sera were used in each test. When a serum reacted with all the polypeptides in the strip as reacted the positive control serum, it was categorized as complete response. When a serum reacted with any one or more but not all the polypeptides, it was categorized as partial response. Both complete and partial responses were regarded as positive response. When a serum failed to react with any of the polypeptides, it was categorized as negative response.

Results

Microscopy for AFB was positive in 71 (63.39%) TB cases, 60 (67.42%) of them were pulmonary TB and 11 (47.83%) were extrapulmonary TB. Culture for AFB was positive in 56 (62.92%) pulmonary and 8 (34.72%) extrapulmonary, in total 64 (57.14%) TB cases. (Table I)

Table I: Results of microscopic examination and culture for AFB among TB patients

TB patients	Microscopy +ve	Culture +ve
Pulmonary TB (n=89)	60 (67.42)	56 (62.92)
Extrapulmonary TB (n=23)	11 (47.83)	08 (34.78)
Total (n=112)	71 (63.39)	64 (57.14)

Figures within parentheses indicate percentage

Western blot analysis revealed four antigenic polypeptides of *M. tuberculosis.* Out of 112 TB patients, 82 (73.21%) cases showed positive antibody response to the polypeptides of which 27 (24.10%) cases showed complete and 55 (49.11%) showed partial response. Three (12%) cases of 25 disease control and 2 (8%) of healthy control subjects showed only partial response. (Table II)

Table II: Results of antibody response to *M. tuberculosis* polypeptides of 65kDa, 45kDa, 38kDa & 19kDa (western blot)

Study subjects	Positive response			Negative response
	Complete	Partial	Total	
TB patient (n=112)	27	55	82 (73.21)	30 (26.79)
Non-TB disease control (n=25)	0	3	3 (12.0)	22 (88.0)
Healthy control (n=25)	0	2	2 (8)	23 (92.0)

Figures within parentheses indicate percentage.

Sensitivity and specificity of the three methods for diagnosis of tuberculosis were calculated. The Western blot technique shows the highest sensitivity of 73.21%, followed by microscopy (63.39%) and culture (57.14%). Specificity of microscopy and culture was not evaluated as sputum was not available from control subjects.

The antibody response (81.25%) among microscopy &/or culture positive pulmonary TB was higher than microscopy and culture negative group (56%). Similarly, the antibody response among microscopy &/or culture positive extrapulmonary TB patients also showed higher response (75%) than that among microscopy/culture negative patients (63.64%). (Table III)

Table III: Result of antibody responses of different groupes of TB patients to *M. tuberculosis* polypeptides

TB patients	Positive antibody response		
	Complete	Partial	Total
Micro/cul+ve pulmonary TB (n=64)	19 (29.69)	33 (51.56)	52 (81.25)
Micro & cul -ve pulmonary TB (n=25)	4 (16.0)	10 (40.0)	14 (56.0)
Micro/cul+ve extra-pulmonary TB (n=12)	3 (25.0)	6 (50.0)	9 (75.0)
Micro & cul -ve extra-pulmonary TB (n=11)	1 (9.09)	6 (54.55)	7 (63.64)
Total (n=112)	27 (24.10)	55 (49.11)	82 (73.21)

Figures within parentheses indicate percentages

micro-microscopy, cul-culture

Discussion

In this study, we evaluated the performance of western blot technique to detect antibody response of TB patients to *M. tuberculosis* polypeptides. Serum samples of tuberculosis patients and control subjects were tested for IgG response to *M. tuberculosis* polypeptides. Out of 112 tuberculosis patients, 24.11% showed complete response and 49.10% showed partial response; 12% of 25 disease control and 8% of 25 healthy control subjects showed only partial positive response. No control sera reacted with all the 4 polypeptides.
In this study, the sensitivity of western blot for diagnosis of tuberculosis was 73.21% and specificity was 92% for the healthy control subjects. The results of this study correlate with other studies. Franco *et al* reported both complete (against all 4 polypeptides) and partial responses by 76% TB patients and only partial response (positive response against a single band) by 8% healthy control subject.[1] Diagbouga *et al* found 57% positive response among TB patients and 0%, 2% and 7% positive response among tuberculin-negative, tuberculin-positive and patients with nontuberculous respiratory disease control group respectively.[7] In several studies, positive antibody responses in patients with tuberculosis were found 60-90% with specificities of 91-100%.[8,9,10,11]

The higher rate of seropositivity for the microscopy/culture positive TB patients compared to that for the microscopy/culture negative TB patients may be due to more bacillary load exposed to immune system leading to more antibody production. The cause of negative response among microscopy/culture positive patients of both pulmonary and extrapulmonary TB might be due to intracellular nature of the bacilli resulting in less or no exposure to the immune system, or it may be due to immunosuppressive condition of the patients. The results of antibody response of this study correlate with other studies. Franco *et al* found 76% and 58% positive response among smear positive and smear negative TB patients respectively.[1]

In this study, 73.21% TB patients' sera showed positive antibody response against *M. tuberculosis* polypeptides. Most (89.02%) of the positively responding sera recognized multiple bands (4, 3 or 2 bands) and only 10.98% sera recognized single band.

In conclusion, the main diagnostic benefit of the test rests in the rapid detection of approximately 70% of smear negative pulmonary and extrapulmonary tuberculosis. However, serology cannot replace sputum microscopy; a positive result

could potentially aid in clinical decision making in selected symptomatic cases.

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